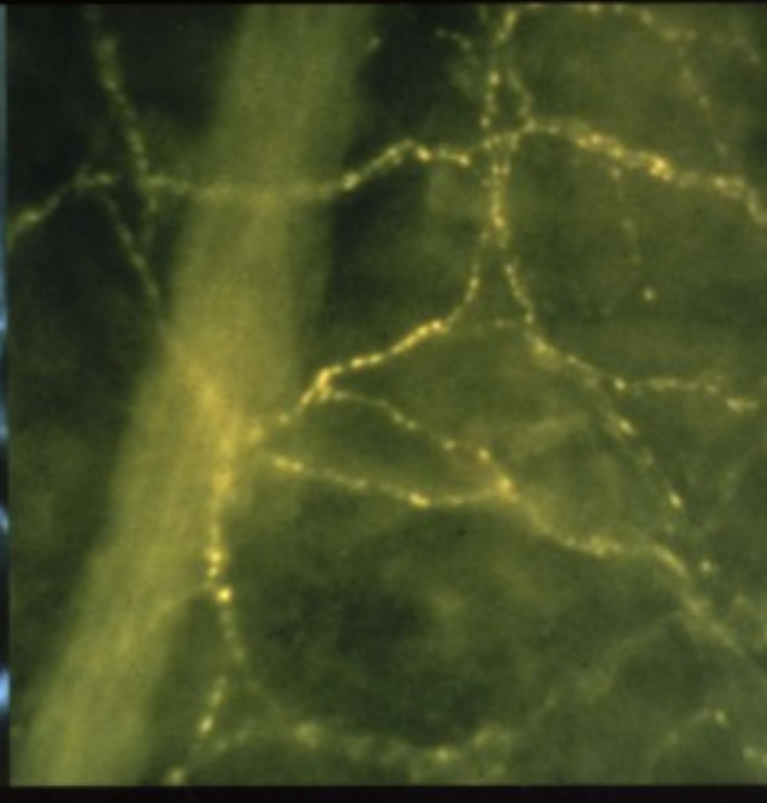
# cerebral vasospasm



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# FOREWORD

Cerebral vasospasm has been recognized for half a century as a cause of morbidity and mortality in patients with subarachnoid hemorrhage. Despite prodigious preclinical and clinical studies, the pathogenesis and the pathophysiology of this disorder are not fully understood. Effective measures for preventing and reversing the arterial narrowing and the ensuing ischemic neurological deficits have been elusive. Vasospasm continues to be the leading treatable cause of death and disability in patients with ruptured aneurysms.

In recent years, a vast body of knowledge relating to the underlying mechanisms and potential treatment for vasospasm has been accumulating not only from neurosurgical investigations, but also from research into atherosclerosis and hypertension, as well as basic smooth muscle cell physiology and pathology. This growing knowledge base is bringing us closer to a solution for vasospasm.

Vasospasm is a soluble problem, and the solution is long overdue. Progress has been retarded because the knowledge base required to solve the problem is, in large part, fragmented.

Macdonald and Weir's *Cerebral Vasospasm* is the definitive work on the subject; it is truly a magnum opus and contains essentially all of the information one needs or wants to know about vasospasm.

The importance of the work is that it brings together in one easily readable source information from disparate disciplines and sets the stage for the final assault on this perplexing problem. This book is the single most important factor in finding a remedy for vasospasm.

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# ACKNOWLEDGMENTS

We thank our colleagues who have worked with us on the challenge of vasospasm over the years: at the University of Alberta, Donald Boisvert, William Castor, Ming Chen, David Cook, Faye Davis, Lew Disney, Mamoru Doi, Ramon Erasmo, Francisco Espinosa, Max Findlay, A. Ghahary, Michael Grace, Yuji Handa, John Hansen, Hideaki Hara, Gregory Hariton, Ruth Henderson, Ken Hutchison, Kenji Kanamura, Hidetoshi Kasuya, Garner King, Christel Krueger, Jeffrey Lewis, Michael Marriott, T. P. Martin, John McIntyre, Gordana Maljkovic, Devidas Menon, Bruce Mielke, Jack Miller, Susan Norris, Tom Noseworthy, Michael Nosko, Francis Okwuasaba, Tom Overton, Kenneth Petruk, William Ritchie, Charles Rothberg, Tim Runzer, Kenichi Saito, Theodor Schnitka, Rick Schultz, David Secord, Joy Steele, David Steinke, Norman Stockbridge, Yoshihiro Takanashi, Takamura Tanabe, Tsotomu Tsuji, Bozena Vollrath, Toshiatsi Yamaki, J. D. Young, and John Zhang; at the University of Chicago, Chris Amidei, Mohamed Bassiouny, Graham Bell, V. Bindokas, James Brorson, Yong-Yuan Guan, Akihiko Hino, Lydia Johns, Chul-Jin Kim, Taro Komuro, Andrew Kowalczuk, George Lin, Linda Marton, Richard Miller, Shigeki Ono, Michael Sajdak, Bogdan Sima, Marcus Stoodley, Xiaoyu Wang, Bob Wollman, and Zhen-Du Zhang; and on various clinical trials, George Allen, Roy Battye, Ed Hall, Neal Kassell, and Gail Kongable. Mary Lou Weir prepared the text. Lydia Johns drew the illustrations. We appreciate the willingness of many others to let us refer to their contributions and illustrations. Finally, we thank Hilary Rowe and Joanna Dinsmore at Academic Press.

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# ABBREVIATIONS

| | |
|---|---|
| μl | microliter, $10^{-6}$ liter |
| μm | micron, micrometer, $10^{-6}$ meter |
| 5-HIAA | 5-hydroxyindoleacetic acid |
| ACE | angiotensin-converting enzyme |
| AA | arachidonic acid |
| ACh | acetylcholine |
| AComA | anterior communicating artery |
| ADH | antidiuretic hormone |
| AMP | adenosine monophosphate |
| ANF/P | atrial natriuretic factor/peptide |
| ANP-(LI) | atrial natriuretic peptide (like immunoreactivity) |
| aPTT | activated partial thromboplastin time |
| ATP | adenosine triphosphate |
| ATPase | adenosine triphosphatase |
| AVM | arteriovenous malformation |
| BBB | blood–brain barrier |
| BK | bradykinin |
| BPG | D-2,3-bisphosphoglycerate |
| BPMH | benign perimesencephalic hemorrhage |
| CaM | calmodulin |
| $Ca^{2+}$ | calcium |
| cAMP | adenosine 3′,5′-cyclic monophosphate |
| $CaO_2$ | arterial oxygen content |
| CAT | catalase |
| CBF | cerebral blood flow |
| CBV | cerebral blood volume |
| cGMP | guanosine 3′,5′-cyclic monophosphate |
| CGRP | calcitonin gene-related peptide |
| CI | confidence interval |
| $CMRO_2$ | cerebral metabolic rate for oxygen |
| CN | cranial nerve |
| CPK | creatine phosphokinase |
| CPP | cerebral perfusion pressure |
| CSF | cerebrospinal fluid |
| CT | computerized tomographic scan |
| D CLHb | diaspirin crosslinked hemoglobin |
| DAG | diacylglycerol |
| DBP | diastolic blood pressure |
| deoxy Hb | Hb[FeII], deoxyhemoglobin |
| DID | delayed ischemic deficit (clinical vasospasm) |
| dl | deciliter |
| DNA | deoxyribonucleic acid |
| DOPAC | 3,4-dihydrophenylacetic acid |
| DSA | digital subtraction angiogram |
| DWI | diffusion-weighted image |
| E | equilibrium potential |
| ECE | endothelin-converting enzyme |
| EDCF | endothelial-derived constrictor factor |
| EDRF | endothelial-derived relaxant factor |
| eNOS | endothelial nitric oxide synthase |
| EPI | epinephrine |
| ET | endothelin |
| FDP | fibrin degradation product(s) |
| g | gram |
| GCS | Glasgow Coma Scale |
| GDC | Guglielmi detachable coils |
| GHS | glutathione peroxidase |
| GOS | Glasgow Outcome Scale |
| GTN | nitroglycerine (glycerol trinitrate) |
| $H_2O_2$ | hydrogen peroxide |
| Hb | hemoglobin |
| $HCO_3^-$ | bicarbonate ion |
| HH | Hunt and Hess neurological grade |
| HO | heme oxygenase |
| HPLC | high-pressure liquid chromatography |
| HR | heart rate |
| HVA | homovanilic acid |
| Hyc | hydrocephalus |
| ICA | internal carotid artery |
| ICAM | intercellular adhesion molecule |
| ICH | intracranial hemorrhage or hematoma |
| ICP | intracranial pressure |
| IL | interleukin |
| $IP_3$ | inositol 1,4,5-trisphosphate |

| | |
|---|---|
| ITP | inosine triphosphate |
| IVH | intraventricular hemorrhage or hematoma |
| kDa | kilodaltons |
| l | liter |
| LDH | lactate dehydrogenase |
| LP | lumbar puncture |
| M | molar |
| MABP | mean arterial blood pressure |
| MCA | middle cerebral artery |
| metHb | Hb[FeIII], methemoglobin |
| $Mg^{2+}$ | magnesium |
| MHGP | 4-methoxy-hydrophenylglycol |
| min | minute |
| ml | milliliter |
| MLC | myosin light chain |
| MLCK | myosin light chain kinase |
| MLCP | myosin light chain phosphatase |
| mmHg | millimeters of mercury |
| MRA | magnetic resonance angiography |
| mRNA | messenger RNA |
| MRS | magnetic resonance spectroscopy |
| MW | molecular weight |
| $Na^+$ | sodium ion |
| $NAD^+$ | nicotinamide adenine dinucleotide (oxidized form) |
| NADH | nicotinamide adenine dinucleotide (reduced form) |
| $NADP^+$ | nicotinamide adenine dinucleotide phosphate (oxidized form) |
| NADPH | nicotinamide adenine dinucleotide phosphate (reduced form) |
| NE | norepinephrine |
| NO | nitric oxide |
| NOS | nitric oxide synthase |
| NPY | neuropeptide Y |
| $O_2^{\bullet -}$ | superoxide anions |
| $O_2$ | oxygen |
| OER | oxygen extraction ratio |
| $OH^{\bullet}$ | hydroxyl radical |
| oxyHb | Hb[Fe II]$O_2$, oxyhemoglobin |
| $P_aCO_2$ | arterial pressure of carbon dioxide |
| PAI-1 | plasminogen activator inhibitor-1 |
| $P_aO_2$ | arterial pressure of oxygen |
| Pcr | phosphocreatime |
| PDB | phorbol 12,13-dibutyrate |
| PET | positron emission tomography |
| PG | prostaglandin |
| Pi | phosphorous |
| $PIP_2$ | phophoinositol bisphosphate |
| PKC | protein kinase C |
| PL-$A_2$ | phospholipase $A_2$ |
| PLC | phospholipase C |
| PRP | platelet-rich plasma |
| PT | prothrombin time |
| RBC | red blood cell(s), erythrocyte(s) |
| rCBF | regional cerebral blood flow |
| RLC | regulatory light chain |
| RNA | ribonucleic acid |
| ROC | receptor-operated channel |
| SAH | subarachnoid hemorrhage |
| SBP | systolic blood pressure |
| sec | second |
| sGC | soluble guanylate cyclase |
| SNP | sodium nitroprusside, nipride |
| SOD | superoxide dismutase |
| SP | substance P |
| TBA | thiobarbituric acid reactive |
| TCD | transcranial Doppler ultrasonography |
| TNF | tumor necrosis factor |
| t-PA | tissue plasminogen activator |
| TX | thromboxane |
| VAC | voltage-activated channel |
| VCAM | vascular cell adhesion molecule |
| VIP | vasoactive intestinal polypeptide |
| VSMC | vascular smooth muscle cell |
| VSP | vasospasm |
| WBC | white blood cell(s), leukocytes(s) |
| WFNS | World Federation of Neurological Surgeons scale |
| Xe | xenon |

CHAPTER 1

# HISTORY



## I. Introduction

Cerebral vasospasm has dominated the interest of vascular neurosurgeons since its discovery, being recognized as a lethal complication of subarachnoid hemorrhage (SAH). Considering that this disease entity has been well known for less than half a century, we have made considerable strides in preventing and treating it. It is no longer quite as fearsome as it was in the early days of aneurysm surgery.

The record of our growing but still rudimentary knowledge of cerebral vasospasm is provided in the proceedings of a series of international conferences on the subject (Table 1.1).

**TABLE 1.1 International Meetings on Vasospasm**

**Jackson, Mississippi, USA—1972**
Smith, R. R., and Robertson, J. T. (Eds.) (1975). *Subarachnoid Hemorrhage and Cerebrovascular Spasm. The First "International" Workshop*. Thomas, Springfield, Il.

**Amsterdam, The Netherlands—1979**
Wilkins, R. H. (Ed.) (1980). *Cerebral Arterial Spasm. Proceedings of the Second International Workshop*. Williams & Wilkins, Baltimore.

**Charlottesville, Virginia, USA—1987**
Wilkins, R. H. (Ed.) (1988). *Cerebral Vasospasm. Proceedings of the III International Symposium in Charlottesville*. Raven Press, New York.

**Tokyo, Japan—1990**
Sano, K., Takakura, K., Kassell, N. F., and Sasaki, T. (Eds.) (1990). *Cerebral Vasospasm. Proceedings of the International Conference on Cerebral Vasospasm*. Univ. of Tokyo Press, Tokyo.

**Edmonton, Alberta, Canada—1993**
Findlay, J. M. (Ed.) (1993). *Cerebral Vasospasm. Proceedings of the V International Conference on Cerebral Vasospasm*. Elsevier, Amsterdam.

**Sydney, Australia—1997**
Dorsch, N. (Ed.) (1999). Cerebral Vasospasm VI. Proceedings of the VIth International Conference on Cerebral Vasospasm. Oslington Consulting (Publishing), Sydney.

**Interlaken, Switzerland—2000**

## II. Clinical Description

Echlin (1) described the status of vasospasm (VSP) prior to the advent of modern imaging technology:

> Since the middle of the nineteenth century, many observers have suggested on empirical grounds that spasm of vessels might play a role in epilepsy, migraine, temporary hemiplegia, hypesthesias, asphasia, and other transitory neurological phenomena. Despite such suspicions as to the nature of vasospasm, there was never any direct evidence of the phenomenon.

Gull (2) may have provided the first observation on delayed ischemic deficits (DIDs) due to vasospasm. He treated a 30-year-old female:

> While walking, she suddenly called out, "Oh my head" and put up her left hand. She vomited, and as her friend thought, fainted. After a brief interval she partially recovered, and was able to walk back to her residence with the support of two men. When admitted to the hospital at noon the following day, only a slight impression could be made by any attempt to rouse her. The right arm was quite paralyzed, the muscles flaccid; the right leg in the same condition.

On day 1 following the (SAH), her breathing was stertorous and swallowing was difficult. She was incontinent. On day 3, she appeared more sensible and was able to eat; when spoken to, she attempted to answer but was unable to articulate. On day 4 she had "rallied as to recognize her relative who visited her, and to say distinctly, 'My cousin.'" On the fifth day following the bleed, her symptoms became aggravated, her pupils fixed and dilated, and she died. There was a large SAH in the left sylvian fissure at postmortem examination. The left hemisphere showed massive softening. On the middle cerebral artery (MCA) were two small aneurysms, one of which had ruptured. There was some bloody serum in the ventricles.

Beadles (3) published a case history in 1907; the findings are also reminiscent of a DID on the basis of vasospasm. The patient was a 51-year-old male who had a sudden seizure with loss of consciousness. Four days after his hemorrhage, ophthalmoscopic examination revealed large hemorrhages in the fundus of the left eye. The patient had recovered and complained of considerable headache. He had no paralysis and had no further seizures. Eight days following his initial hemorrhage, his temperature began to rise and he became drowsy and completely lost all power in the lower extremities. He then passed into an unconscious state. Death, preceded by pyrexia and tachycardia, occurred 10 days following the SAH. At autopsy "profuse meningeal hemorrhage was found caused by rupture of a small aneurism of the anterior cerebral artery, not larger than a pea."

In 1975, Fisher (4) recorded that approximately one-third of patients who survived their initial SAH and whose state permited recognition of a new disturbance developed DID. These initially developed between days 3 and 13 (mean of 7 or 8 days). In cases in which they appeared to occur more closely to the SAH, there was usually a history of a hemorrhage preceding the one leading to admission. The DID usually took 2–4 days to reach its maximum but could peak as early as 24 hr from the onset. About 50% of the deficits were reversible, and improvement could occur over a few days to months. Although angiographic VSP occurred in approximately 40% of cases, it was not symptomatic unless it was of a very severe degree. About 75% of the deficits due to VSP involved the (MCA) territory. These included hemiparesis, monoparesis, motor or sensory dysphasia, agnosia, and apraxia. Usually motor symptoms predominated. In 25% of cases VSP produced symptoms related to the anterior cerebral artery territory, with a myriad of frontal lobe signs resulting. DID from the VSP was often a caricature of ordinary cerebrovascular syndromes. Nondominant lesions produced a general reduction in responsiveness. A recrudescence of fever and nuchal rigidity might be associated with DID.

The same year the statistical technique of multiple regression analysis was used on the data from 135 cases with SAH treated during a 5-year period at the University of Alberta (5). The hierarchy of associations of importance in predicting 2-month mortality rates was initial neurologic grade, grade at surgery, preoperative vasospasm, the presence of a large mass lesion, hypertension, a shorter interval to surgery, and greater age.

Saito and colleagues (6) in the late 1970s published an account of 44 consecutive cases of ruptured aneurysm in which VSP was present preoperatively or postoperatively. Computed tomographic (CT) scan demonstrated infarction in the territory of the spastic arteries in 71% of the patients with VSP. Permanent DID was always found to accompany VSP affecting one entire carotid system and the anterior cerebral artery of the opposite side. When VSP was restricted to one carotid system alone or bilateral anterior cerebral arteries alone, it was usually associated with only temporary symptoms. Infarction on CT scan and clinical symptomatology was always present when VSP involved the ascending branches of the middle cerebral artery. Over 4.5 years, 120 patients were seen with SAH due to ruptured aneurysms; VSP developed in 37%, 20% preoperatively and 17% postoperatively. VSP developed only in those cerebral arteries that were immersed in blood-stained cerebrospinal fluid. CT depicted low-density areas or infarction in the territories of the spastic arteries in 71% of the cases. The type and distribution of VSP assessed by angiography helped in determining the prognosis of the disease. VSP usually began at the the end of the first week post-SAH, reached a maximum between days 9 and 13 post-SAH, and lasted about 2 weeks.

## III. Pathology

In the mid-1940s Alpers and Forster (7) and Hammes (8) reported that the breakdown of red blood cells (RBCs) reaches its peak about the seventh day after SAH. Two decades later, Tourtelotte and coworkers (9) found that the cerebrospinal fluid (CSF) became clear by 10–20 days following SAH in over 80% of patients.

Robertson (10) studied 27 fatal cases of ruptured aneurysm in 1949. One of the cases that he described in detail was that of a 70-year-old female who had SAH documented by lumbar puncture. On day 5 post-SAH she developed hemiplegia, and on day 6 she became unconscious. The patient survived for 18 days prior to dying of a respiratory infection. At autopsy a ruptured anterior communicating artery aneurysm was found. The basal cisterns were stained yellow, and there was recent

blood clot under the olfactory bulbs. The cerebral vessels were patent. A patchy softening of the medial surfaces of both frontal lobes and the white matter below the left anterior horn was found. Infarction was also noted around the Sylvian fissure on one side. Robertson observed,

> Hence, it seems possible that the ischemic changes were due to temporary spasm of the supplying vessels. The changes in the territories of the anterior cerebral arteries may have been due to the compression of the arteries by the aneurysm, but it is more likely that the latter stimulated spasm of the vessels, even of vessels remote from the aneurysm.

He did not realize apparently that is was the encasing blood clot that caused the VSP since the clot had often been absorbed by the time of death. Infarction remote from the ruptured aneurysm, in the absence of intracerebral bleeding, was illustrated for the first time by Robertson (10) (Fig. 1.1). In a different analysis of 86 patients with aneurysms, he noted infarction in 30% of cases personally examined, whereas it was recorded in only 2 of a far larger number of cases examined without the brains first having been fixed in formalin.

## IV. Radiology

### A. Angiography

> Roentgen submitted his paper about a new kind of rays, which he called x-rays, on 28 December 1895 for publication in a not very important local journal in which it appeared almost immediately. Thus he gained priority over Philip Leonard, whose work on cathode rays was well known to Roentgen.
>
> —Guitierrez (11)

Cushing wrote a letter within 6 weeks of Roentgen's report: "Professor Roentgen may have discovered something with his cathode rays that may revolutionize medical diagnosis" (11).

It is fascinating that Egaz Moniz had a distinguished political career prior to beginning serious neurological investigation at what some would consider to be the advanced age of 51 (12). In June 1927, on Moniz's ninth attempt, he produced a cerebral angiogram of acceptable quality in a living person. The initial published account by Moniz (13) on "arterial encephalography" related to its importance in the localization of brain tumors. His achievements thereafter are legend (13). He also published

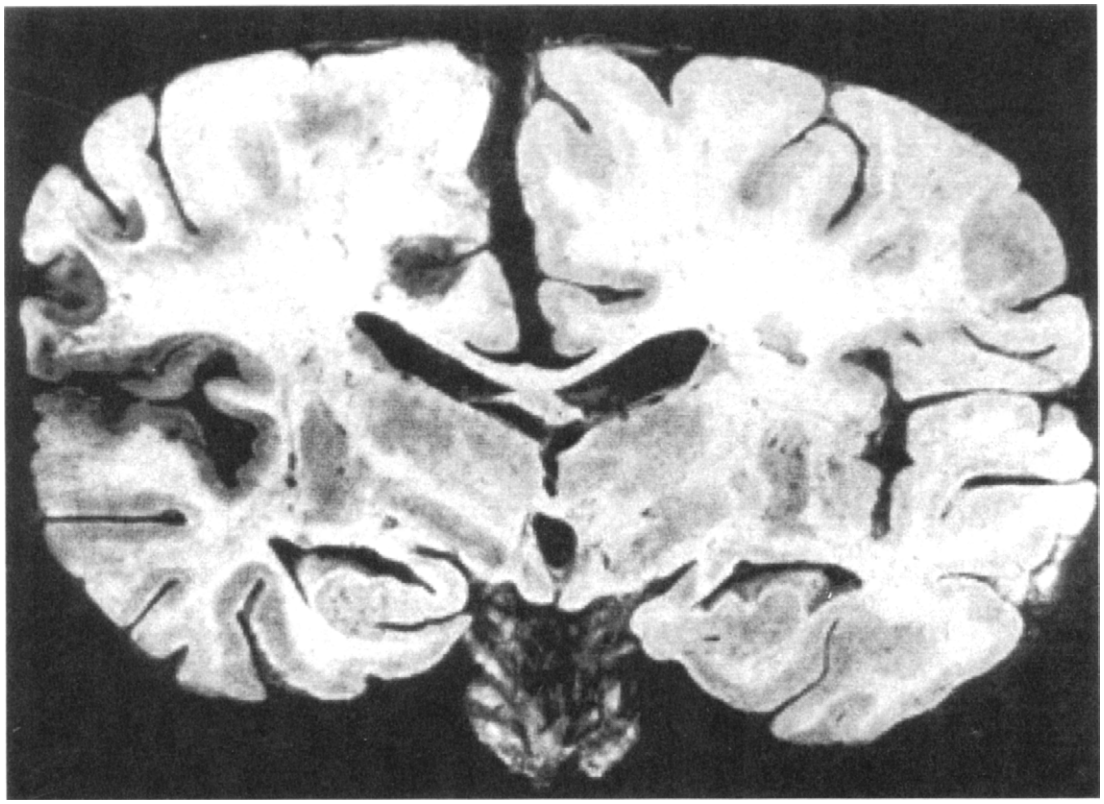

**FIGURE 1.1** "(Case 9)—(A) Aneurysm at junction of right anterior cerebral and anterior communicating arteries. Subarachnoid hemorrhage. (B) Cerebral infarct in the absence of intracerebral rupture" [from Robertson (10); reproduced with permission of Oxford University Press].

in 1933 the first case of an aneurysm to be visualized in life (Fig. 1.2).

Ecker and Riemenschneider (14) were aware that Villaret and Cachera had demonstrated that cerebral arterial spasm could result from experimental solid emboli and that systemic arterial spasm could result from the "crush syndrome" and inferred the presence of extracranial internal carotid artery VSP from penetrating wounds of the neck or blunt head injury. When Ecker presented his angiographic demonstration of intracranial VSP at the Cushing Meeting in 1951, the reaction of the audience was almost uniformly negative. Only one man in the front seemed to smile approval. Ectrer (15) noted,

> I addressed the rest of my remarks to him. In discussion, after some of the older men denied the existence of cerebral arterial spasm.... I went to the unknown smiling man in the front to thank him for his encouragement. He answered, still smiling, "I don't speak English."

Ecker and Riemenschneider (14) diagnosed VSP when a vessel was found to be of larger caliber on a subsequent angiogram than it had been initially, under apparently identical conditions (Fig. 1.3). In 6 cases of saccular aneurysm on the anterior circle of Willis, VSP was visualized. All of these patients had suffered from SAH. There were 23 other cases of aneurysm in which VSP could not be proven by repeat angiography. The authors found that VSP was generally maximal at the site of the aneurysm and most marked intracranially. In 10 cases of SAH from aneurysms in which angiograms were performed more than 26 days following SAH, no evidence of VSP was found. It was theorized that VSP might play a protective role in the production of intracranial aneurysmal thrombosis. Their key conjecture was that excessive VSP could produce unfavorable effects by impairing the circulation of the brain to an area nourished by the affected artery. Like Robertson, these authors hypothesized correctly that excessive VSP could produce unfavorable effects by lowering the blood flow to the area of brain nourished by the affected artery. The most important sentence in the history of VSP was theirs: "It is probable that arterial spasm plays an important role following spontaneous rupture of saccular aneurisms of or near the circle of Willis" (14). It took a decade or so before the widespread skepticism that greeted their observations was finally dissipated.

In 1978, Weir and associates (16) measured eight arterial points on 627 angiograms from 293 patients with aneurysms. A ratio between the sum of the vessel diameters in the subarachnoid space and the sum of the diameters in the base of the skull and neck was calculated and plotted against time (Fig. 1.4). Using this ratio, it appeared that intracranial constriction or VSP began

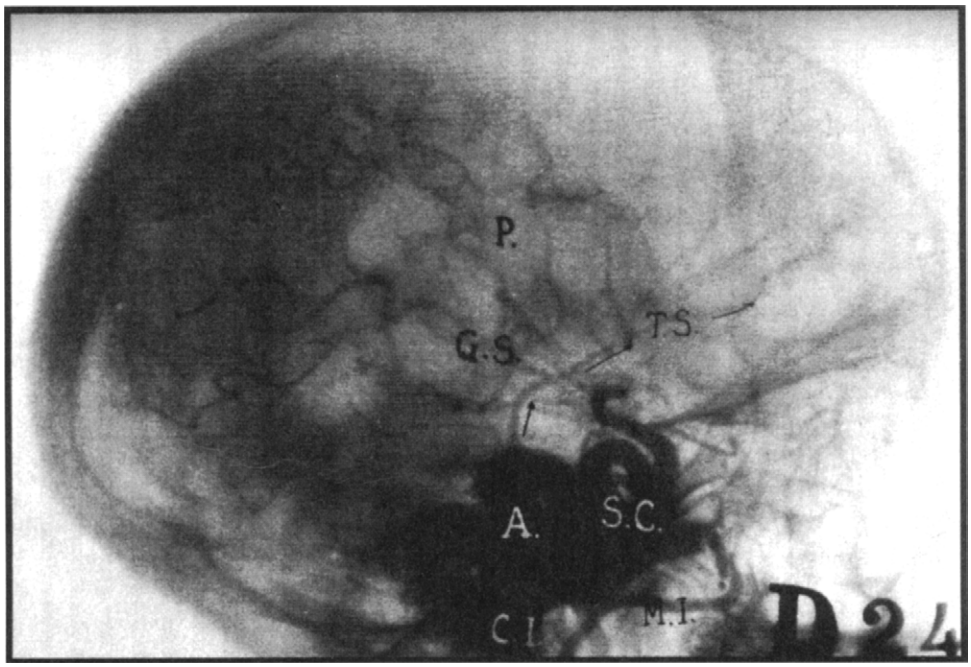


**FIGURE 1.2** "Anévrysme de la carotide interne. C.I., carotide interne avant son entrée de la cerveau. A., anévrysme; S.C., siphon carotidien; G.S., groupe sylvien; P, artère pericalleuse; T.S., artère temporale superficielle (dérivée de la carotide externe)" [from Moniz (13)].

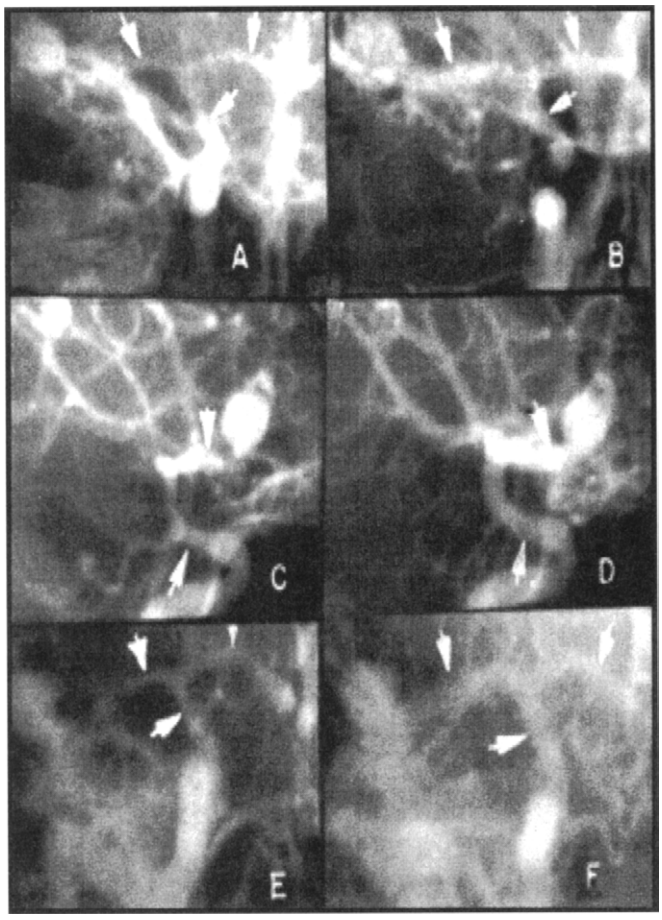


**FIGURE 1.3** "(Case 3)—Aneurism at bifurcation of right middle cerebral artery. Spontaneous subarachnoid hemorrhage Jan. 4, 1951. Arteriograms made after right carotid injection. Anteroposterior views: (A) January 15; (B) March 20. Lateral views: (C) January 15; (D) March 20; Chamberlain–Towne views: (E) January 15; (F) March 20. All films show the aneurism at the bifurcation of the right middle cerebral artery. The three views made in January all show constriction of the right internal carotid artery, its terminal branches, and the aneurism itself. Their spasm is absent from the films made in March" [reproduced with permission from *J. Neurosurg.* (14)].

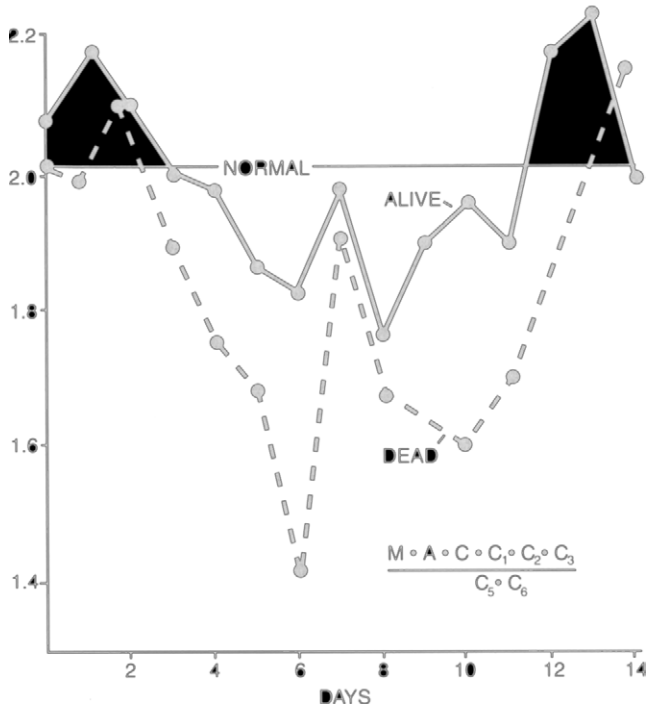


**FIGURE 1.4** "Curve for the reduction in ratio of the caliber of vessels in the subarachnoid space to those outside versus time. Based on data from 429 angiograms done posthemorrhage and preoperatively, there is more constriction in 120 angiograms on patients who died (*broken line*) than in 309 on survivors (*continuous line*)" [reproduced with permission from *J. Neurosurg.* (16)].

about day 3 after SAH , was maximal at days 6–8, and was gone by day 12. There was a tendency for patients in poor clinical grade to have a greater degree of constriction. The patients with the most VSP had significantly higher mortality than those with the least VSP. Ito (17) also measured arterial diameters in 189 aneurysm cases on the anteroposterior projection angiogram. The ratio of the sums of the intracranial diameters at three points to the cervical internal carotid artery diameter was calculated. The mortality rate was 18% in the patients with the least degree of VSP, 36% in patients with a moderate degree of VSP, and 41% in patients with the highest degree of VSP.

### B. Computed Tomography

Hounsfield (18) described his system for computerized transverse axial scanning tomography (CT) in 1973 (Fig. 1.5). X-ray transmission readings are taken through the head at a multitude of angles, and from these data absorption values of the material contained within the head are calculated on the computer and presented as a series of pictures of slices of the cranium. This system was initially 100 times more sensitive than conventional X-ray systems. Variations in soft tissues of nearly similar density could be displayed. Hounsfield wrote, "It is possible that this technique may open up a new chapter in X-ray diagnosis.... The increased sensitivity of computerized section scanning... thus enables tissues of similar densities to be separated and a picture of the soft tissue structure within the cranium to be built up." Within 3 years, Kendall and associates (19) carried out a study of 100 multiple aneurysm cases using CT which localized the site of the bleeding in 56% of the cases.

Katada and coworkers (20) noted a relationship between the amount of blood on CT and the development of VSP. Soon thereafter, 73 patients with SAH were

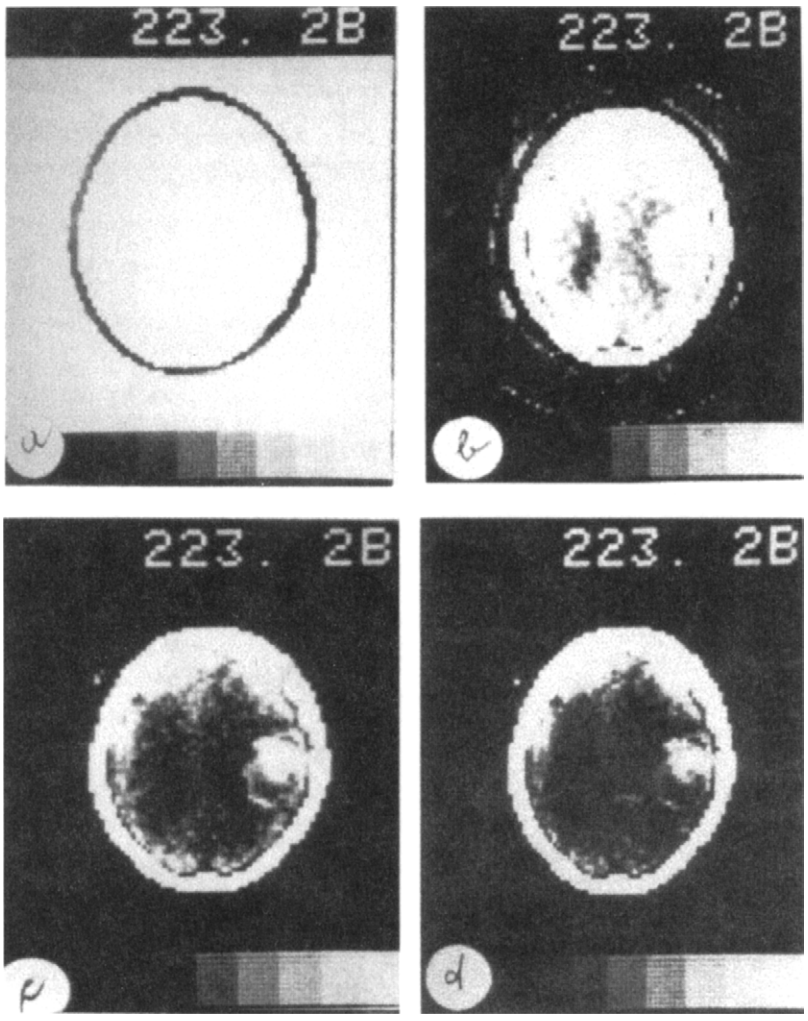


**FIGURE 1.5** "(a) Window level setting—100 units. (b) Setting 0 shows water in the ventricles. (c) Setting +15 units shows tumor and hemorrhage. (d) Setting +20 shows details of the hemorrhage" [reproduced with permission from *Br. J. Radiól.* (18)].

systematically studied by Takemae and associates (21) during the acute post-SAH stage, 39 of which underwent CT within 4 days; of these, 77% had high-density areas within the SA space. High-density areas disappeared between days 4 and 22 post-SAH on the repeat CT scans of patients treated conservatively. It was possible to predict VSP by examining the high-density areas on the CT scan within 4 days after SAH. VSP usually occurred between days 5 and 14. VSP developed in 83% of the patients with high-density areas in the basal cisterns and in 78% of the patients with blood clots within the Sylvian fissure. No VSP developed in patients without high-density areas on CT scan. When CT scan was performed more than 4 days post-SAH, there was no relationship between CT findings of blood and VSP. Also, high-density areas on the CT scan within 4 days post-SAH agreed with the distribution of VSP seen angiographically. Early surgery with removal of blood clots was recommended for those patients with high-density areas seen on CT scan within the first 4 days post-SAH. This important paper was published in a Japanese journal in 1978 (Fig. 1.6) and went largely unnoticed in the West.

Two years later, Fisher and coworkers (22) studied 47 cases of SAH. Only 1 of 18 patients in whom there was no subarachnoid blood developed severe VSP; 96% of patients with thick subarachnoid clot developed VSP. Every patient with severe VSP manifested delayed symptoms and signs. The authors concluded that blood localized in the subarachnoid space in sufficient amounts at specific sites is the only important etiological factor in VSP. Of 28 patients with no or mild SAH as judged by CT, 68% developed no worse than mild VSP. Abnormal

脳神経・30巻8号・1978年8月

急性期破裂脳動脈瘤のCT所見

—とくに脳血管攣縮と High Density との関連について—

竹前紀樹* 水上公宏 金弘
河瀬斌 荒木五郎**

***Abstract***

**COMPUTED TOMOGRAPHY OF RUPTURED INTRACRANIAL ANEURYSMS IN ACUTE STAGE —RELATIONSHIP BETWEEN VASOSPASM AND HIGH DENSITY ON CT SCAN—**

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**3) It is possible to anticipate whether vasospasm will develop by examining high density on CT-scan within 4 days after the onset. Vasospasm was observed on angiogram obtained between 5 to 14 days after the onset in 83% of the patients with high density in basal cisterns and 78% in Sylvian fissure, but no vasospasm developed in the patients without high density on CT-scan. On the other hand, there was no relationship between development of vasospasm and high density on CT-scan performed more than 4 days after the onset.**

**4) Location of high density (extravasated blood in subarachnoid space) on CT-scan performed within 4 days after the onset approximately coincided with the distribution of vasospasm demonstrated on angiogram.**

**5) Therefore, high density recognized on CT-scan performed within 4 days is considered to be essential for the development of vasospasm.**

FIGURE 1.6 "Computed tomography of ruptured intracranial aneurysms in acute stage. Relationship between vasospasm and high density on CT scan. Selections from the English abstract" [from *Brain Nerve* (21)].

contrast enhancement was not found to be of significance. All observations were initially controversial but have now gained widespread acceptance.

### C. Blood Flow Measurements

The time course of changes in cerebral blood flow following SAH were first delineated by Kågström and associates (23) in the mid-1960s. Spasm was associated with reduced blood flow in studies and was reported using radioactive tracers late in that decade (24).

Grubb and associates (25) carried out evaluation of 30 patients using a positron emission tomographic (PET) scanner in 1977 (Fig. 1.7). They demonstrated that patients with more severe neurological deficits or more severe degrees of VSP had the most marked depression of cerebral blood flow (CBF) and cerebral metabolic rate for oxygen ($CMRO_2$). In addition, they demonstrated a striking increase in cerebral blood volume (CBV) in such patients.

### D. Transcranial Doppler

Transcranial Doppler sonography (TCD) was introduced by Aaslid to assess spasm in the intracranial conductive vessels in 1982 (Fig. 1.8) (26). TCD is now routinely utilized at the bedside by most neurosurgeons treating SAH patients.

## V. Medical Aspects

### A. Hemodynamic Therapy

Denny-Brown (27) noted in 1951 that hypotension could have disastrous effects on patients with recent cerebrovascular accidents and therefore suggested it was logical to adopt measures to raise systemic blood pressure. He presented patients in whom dramatic clinical deterioration occurred when hypotension was superimposed on structural narrowing of major cerebral blood vessels. This clinical worsening was related to decreases in blood pressure that accompanied syncope, gastrointestinal hemorrhage, or sleep.

In 1976, Kosnik and Hunt (28) described 7 patients with SAH who developed delayed neurological deficits. In 5 of the 7 there was a marked improvement in their state following the raising of blood pressure, blood volume, and central venous pressure (Fig. 1.9). It was assumed that autoregulation was at least partly lost in such patients with a "cerebral hemodynamic crisis." Blood volume expansion was employed to augment the vasopressors in maintaining systemic hypertension. They used norepinephrine (16 mg in 500 ml of 5% dextrose in 0.45% saline) given intravenously through a central venous line. It was suggested that if ischemia and infarction were already well developed when this treatment was employed, the hypertension might be useless and might in fact accelerate brain swelling. Other hazards included the

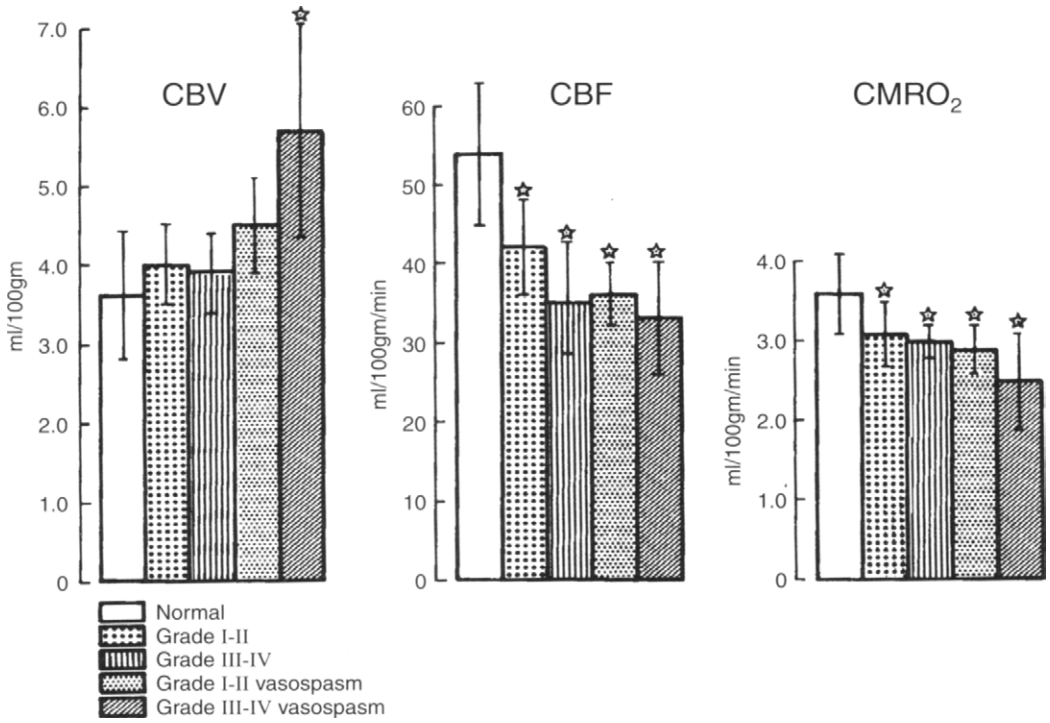


**FIGURE 1.7** "Mean values of CBV, CBF, and $CMRO_2$ in patients following a subarachnoid hemorrhage with and without cerebral vasospasm. Standard deviation of mean values is shown by vertical bars. Mean values significantly different from normal values are indicated by stars" [reproduced with permission from *J. Neurosurg.* (25)].

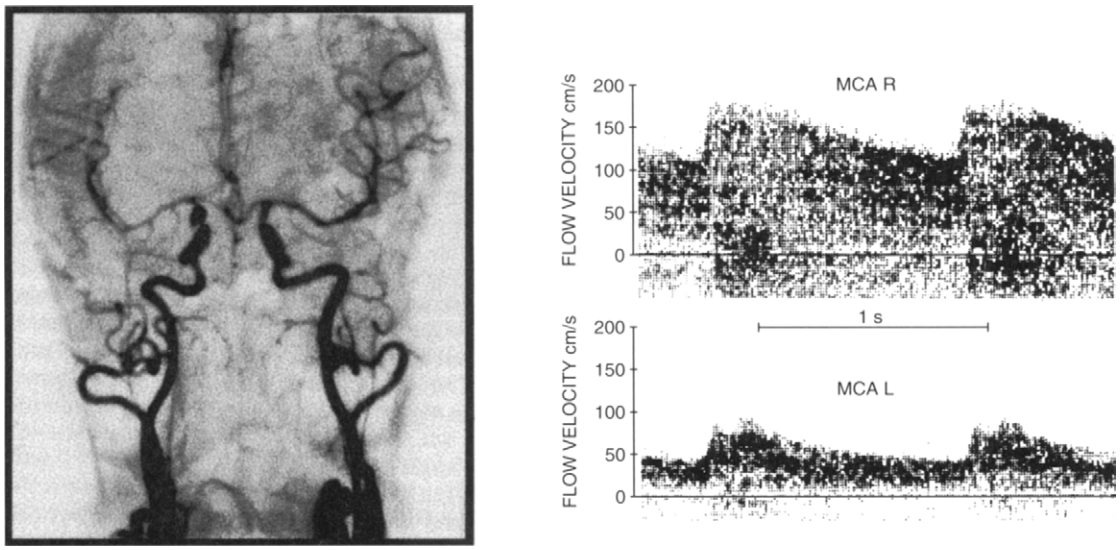


**FIGURE 1.8** "*Left:* Angiogram of a 46-year-old woman with aneurysm of the right internal carotid artery 7 days after subarachnoid hemorrhage. The right middle and anterior cerebral arteries were clearly spastic, while those on the left side had normal caliber. *Right:* Spectral display of the Doppler signals from both middle cerebral arteries (MCA R and MCA L) in the same patient. The flow velocity in the right MCA was markedly elevated (150 cm/sec) when compared to 58 cm/sec measured on the left side (within normal range)" [reproduced with permission from *J. Neurosurg.* (26)].

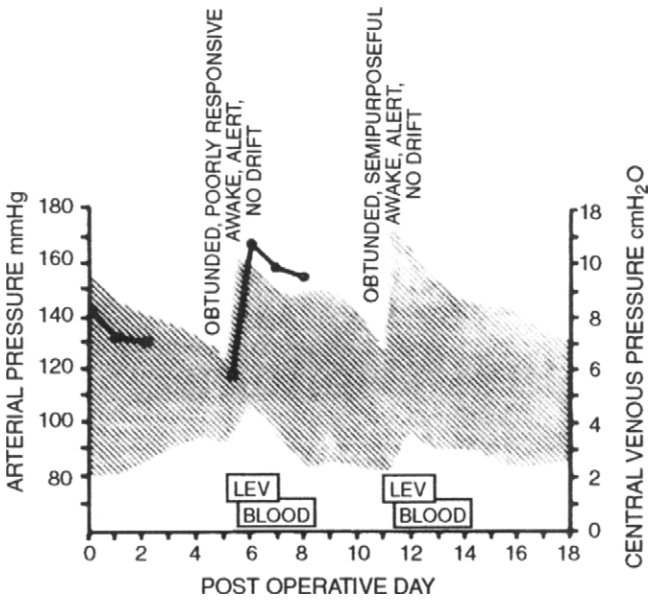


**FIGURE 1.9** "Postoperative course in case 3. This patient had two changes in his neurological status, both of which were resolved with pressure elevation" [reproduced with permission from *J. Neurosurg.* (28)].

nonspecific ones associated with an indwelling central venous line and the possibility of fluid overload and congestive heart failure. An important contribution to the understanding of VSP was the finding of Maroon and Nelson (29) in 1979 that red blood cell mass and total blood volume were significantly decreased in 15 patients following SAH. They listed the causes of contracted blood volume as bed rest, supine diuresis, peripheral pooling, negative nitrogen balance, decreased erythrocyte production, and iatrogenic blood loss. Their data supported the concept of intravascular blood volume expansion with erythrocytes and colloid to prevent and treat ischemia caused by VSP. Kassell and associates (30) presented data in 1982 from the largest group of patients treated with deliberate intravascular volume expansion and induced arterial hypertension. Neurological deterioration was successfully reversed in approximately 80% of cases. Causes of failure included preexisting infarction, progression of VSP, rebleeding, and the inability to induce hypertension. There were significant complications resulting from this, including aneurysmal rebleeding, pulmonary edema, dilutional hyponatremia, coagulopathy, pneumothorax, and myocardial infarction. These workers induced hypertension with dopamine and occasionally dobutamine. Pressure increases as high as 150 mmHg mean arterial pressure were employed. Of interest was the fact that they did not observe any increase in infarct size or conversion of a pale to a hemorrhagic infarct.

### B. Avoidance of Adverse Factors

In a large cooperative study reported by Nibbelink and coworkers (31) in 1975, antifibrinolytic medication was found to reduce rebleeding rates in unoperated aneurysm cases compared with historical controls. Unfortunately, this and other reports led to the general and rapid acceptance of the use of antifibrinolytic agents following aneurysm rupture.

The conclusive demonstration that the use of antifibrinolytic agents reduced rebleeding, but only at the unacceptable price of an increased rate of ischemic morbidity and morality, finally came in studies of the Cooperative Aneurysm Group and a European multicenter trial in the mid-1980s (32,33).

### C. Vasodilator and Neuroprotectant Medication

On the basis of a study by George Allen (34) in 1979 in which acute and chronic spasm in dogs was apparently reversed by the oral administration of the calcium antagonist nifedipine, and by pharmacologic evidence of its potent ability to antagonize vasoconstrictor agents *in vitro*, a subsequent human trial of the related drug nimodipine was organized. The results were published in 1983 (35). Allen and coworkers found that a deficit from vasospasm that persisted, was severe, or caused death before the end of the treatment of 3 weeks developed in 8 of 60 patients given placebo but only 1 of 56 given nimodopine. This was a significant difference (Table 1.2). This was the first multicenter, prospective, placebo-controlled, randomized trial of a medication which it was hoped would alleviate VSP.

## VI. Etiology

In 1925, Florey (36) used local mechanical and electrical stimulation to induce local spasm in the cerebral arteries of cats. The substance in blood eliciting the greatest meningeal response when injected intracisternally in dogs was present in RBCs (37). The supernatant fraction of autogenous RBCs incubated at body temperature for 4 days produced the greatest pleocytosis in CSF, and autologous hemolyzed RBCs produced the greatest protein elevation in CSF according to Jackson.

Echlin (1) presented convincing photographic evidence that marked acute spasm of the blood vessels of monkeys could be induced by the application of blood after the arachnoid had been opened (Fig. 1.10). Simeone and coworkers (38) demonstrated that a variety of chemical and physical stimuli could cause angiographic vasospasm in the primate. In this model, the puncture and subsequent

**TABLE 1.2 Classification of Patients with Deficits from Spasm, According to Neurologic Outcome**[a]

| Outcome | Treatment group | |
|---|---|---|
| | Placebo ($n = 60$)[b] | Nimodipine ($n = 56$)[b] |
| | No. of patients | |
| Normal | 6 | 8 |
| Mild or moderate | 2 | 4 |
| | Patient 1: 3/5 left hemiparesis | Patient 1: disoriented to city |
| | Patient 2: disoriented to year | Patient 2: 3/5 left arm |
| | | Patient 3: disoriented to city, 4/5 right leg, mild aphasia |
| | | Patient 4: 4/5 left hemiparesis |
| Severe | 8 | 1 |
| | Patient 1, 2, 3: death[c] | Patient 1: death[c] |
| | Patient 4: coma, decerebrate | |
| | Patient 5: no verbal response, 0/5 left hemiparesis | |
| | Patient 6: severe aphasia, 2/5 to 3/5 quadriparesis | |
| | Patient 7: 0/5 to 1/5 left hemiparesis | |
| | Patient 8: moderate aphasia, 2/5 right hemiparesis | |

[a] From Allen *et al.* (35). 
[b] Excludes five patients who violated protocol.
[c] All these patients had severe deficits immediately before death.

# Arterial Spasm from Subarachnoid Hemorrhage

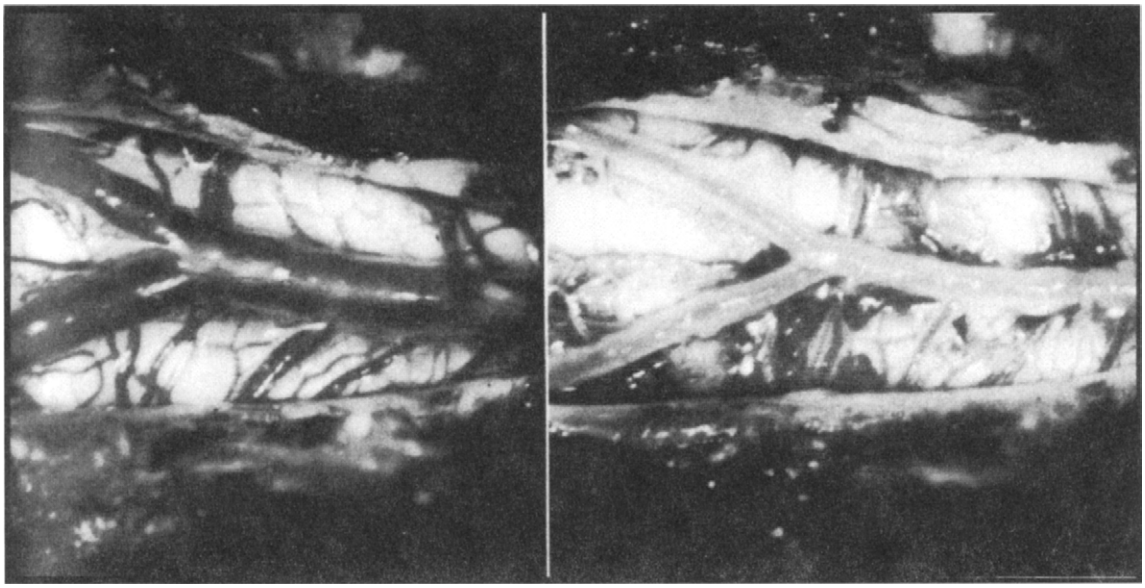

**FIGURE 1.10** "Monkey No. 313. (Left) Normal basilar and vertebral arteries. (Right) 1 1/2 minutes after irrigation of the vessels with 15 drops of fresh arterial blood. Note the marked spasm of the vessels" [reproduced with permission from *J. Neurosurg.* (1)].

withdrawal of small needles from the intracranial internal carotid artery caused prolonged angiographic VSP, which in some cases was observed to last as long as 4 days. In 1970, chronic VSP was angiographically demonstrated in primates following the injection of blood alone, without any damage to the arteries. The degree of VSP did not change with repeated hemorrhages (39).

Experimental evidence gathered during the 1970s supported the hypothesis that VSP was related to exposure of cerebral arteries to RBC breakdown products. In a dog basilar artery preparation, Miyaoka and colleagues (40) noted that fresh serum was vasoactive *in vitro* but was inactivated after 4 days of incubation. In contrast, mixtures of blood and CSF had progressively increasing vasoactivity following incubation, which was maximal at 7 days. Biochemical analysis revealed that the vasoactive property was present in polypeptides closely allied to oxyhemoglobin (oxyHb) or in oxyHb itself. Endo and Suzuki (41) found that prolonged incubation of a blood CSF mixture produced more severe and prolonged vasoconstriction than either fresh blood or spinal fluid. Osaka (42) also presented evidence that prolonged VSP was produced by a breakdown of RBC products. Fresh, intact RBCs were initially not vasoactive but caused vasoconstriction following incubation and lysis over several days. Platelet-induced vasoconstriction, on the other hand, did not persist over time.

The search for a reliable animal model that closely mimicked the human situation appeared to culminate successfully when Espinosa and colleagues (43) reported in 1984 that the microsurgical application of fresh autologous clot directly around the basal arteries in the primate would reliably produce severe, diffuse VSP subjacent to the clot and approximately 5% of such animals would develop delayed-onset hemiparesis or hemiplegia about 4 or 5 days after the surgical procedure.

## VII. Surgical Aspects

### A. Clot Removal

Mechanical removal of blood from the basal cisterns as a possible means of preventing arterial spasm in SAH was proposed by Johnson and colleagues in 1958 (44). In a remarkably prescient paper, Kennady (45) in 1967 reported the fate of RBCs injected into the subarachnoid space of dogs. He considered that significant amounts of blood could be removed following blood injection by irrigation of the subarachnoid space and that the efficiency of this removal could be increased by addition of "fibrinolysin" to the irrigation fluid. This was probably the primary observation that the addition of clot lysing agents in irrigation fluid facilitates the removal of subarachnoid clot. In the primate model developed at the University of Alberta the intrathecal application of recombinant tissue plasminogen activator (tPA) was subsequently shown to be efficacious in facilitating earlier removal of clot and in preventing severe VSP and DID (46).

### B. Timing of Surgery

Suzuki *et al.* (47) analyzed 413 cases of aneurysmal SAH admitted between 1971 and 1973. They found that excellent postoperative results were obtained when surgery was carried out in the first day or two following SAH. They suggested that operation carried out within 24 hr greatly lessened the possibility of the occurrence of postoperative VSP due to the removal of subarachnoid clot from around the basal arteries, which they suggested played a role in the production of VSP. Saito and coworkers (48) in 1977 noted that angiographic VSP usually developed about 4 days after SAH and subsided an average of 2 weeks following the onset. In this report, there were few deaths in patients operated on in the first 3 days following SAH and the highest death rate was for those operated on in the subacute (4–7 days) period after hemorrhage. In surgically treated patients, early VSP tended to be mild and was attributed to the removal of blood clot or blood-stained CSF at the time of surgery. An important observation was that 4–11 days elapsed between SAH and the onset of symptoms of VSP regardless of the timing of surgery. In the early 1980s, additional series reported evidence supporting the concept that early removal of subarachnoid blood clot would be beneficial in reducing the incidence of DID and development of infarction. Takahashi and associates (49) found a correlation between the results of early operation and CT findings. They advocated the placement of ventricular cisternal drains for the improvement of spinal fluid circulation and the removal of blood clot from the basal cisterns at the time of aneurysmal clipping. A similar surgical approach was taken by Mitzukami and coworkers (50), who in 1981 reported that in 90% of patients it was possible to remove subarachnoid clot from the basal frontal interhemispheric fissures, bilateral Sylvian stems, and Sylvian cisterns, as well as the anterior part of the insular cisterns in cases in which surgery was performed early. Subsequent angiography revealed no VSP or mild VSP in patients in whom the blood clot had been successfully removed, and no neurological deterioration occurred in these patients. In 8 patients in whom blood clot was not removed, permanent deterioration due to VSP occurred and the postoperative CT scans corroborated the presence of residual hematomas. Taneda (51) evaluated the

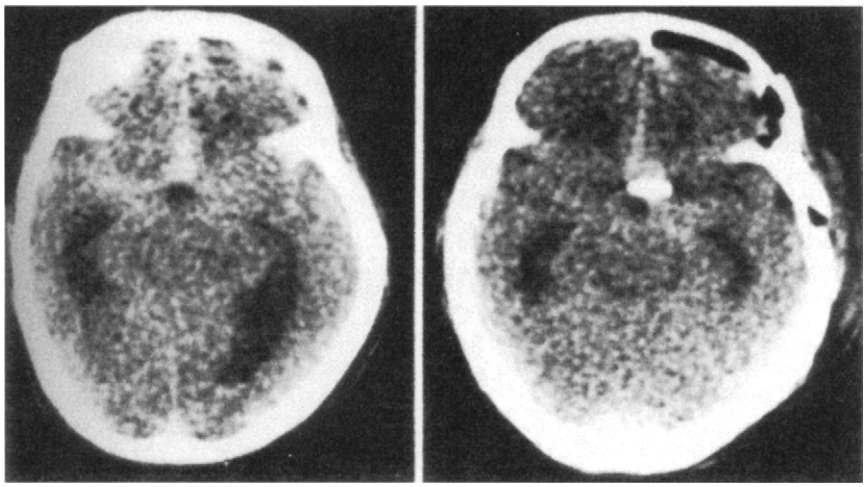

**FIGURE 1.11** "Computerized tomography scans. *Left:* Preoperative scan showing blood in the subarachnoid space on the day of rupture of an anterior communicating artery aneurysm. *Right:* Postoperative scan showing decreased blood in the subarachnoid space after removal of the clots via a right pterional approach. The white spot in the center of the brain is the aneurysm clip" [reproduced with permission from *J. Neurosurg.* (51)].

attempted removal of subarachnoid clot to prevent DID in a large series of patients (Fig. 1.11). It was concluded that an operation that effectively removed basal clot reduced the occurrence rate of severe DID. Like the other series, this one was not randomized. Interestingly, in 5 of 12 patients who had poor outcomes and who were operated on within 48 hr but did not have aggressive contralateral clot removal, the infarct was on the side opposite the operative approach.

### C. Angioplasty

Balloon angioplasty to reverse angiographic VSP was introduced in the Soviet Union in the early 1980s (Fig. 1.12) (52). It is an effective means of acutely and permanently increasing the arterial caliber of accessible arteries, but it is potentially dangerous and time is of the essence.

## VIII. Physiology

It is not surprising that the exact biochemical mechanism underlying the prolonged vasoconstriction of vasospasm is unknown since the nature of normal vascular constriction–relaxation is so incompletely understood. Most current concepts of smooth muscle contraction were initially gained by physiologists working with other forms of muscle. More than 100 years ago, while studying ventricular cardiac muscle contraction Ringer found that $Ca^{2+}$ was a requirement. Within a decade Fletcher and Hopkins showed that lactic acid was produced during muscle contraction. In 1930, Meyerhof demonstrated that lactic acid was generated during anaerobic glycogenolysis, which was the process that was subsequently found to provide the energy for extended contraction of skeletal muscle (53). Glycolysis, a sequential reaction converting glucose to pyruvate with the associated production of adenosine triphosphate (ATP), was elucidated by 1940 due to the efforts of many, including Meyerhof and Warberg (54). The energy for muscle contraction comes from glucose, which in turn is released from its storage form glycogen by a specific enzyme—a phosphorylase discovered by Carl and Gerti Cori, whose work gained them the Nobel Prize in 1947.

It was discovered in 1939 by V. Engelhardt and M. Lyubimoba that myosin was also an enzyme—an ATPase. When a solution of actin is added to a solution of myosin there is a large increase in viscosity. In the 1940s, A. Szent—Györgis (also a Nobel Laureate) showed that this reaction was reversible by adding ATP. He prepared threads of actomyosin that contracted when placed in a solution containing ATP, $K^+$, and $Mg^{2+}$. Actin alternately binds to and is released from myosin as ATP is hydrolyzed. The key role of ATP in energy exchanges in biological systems was realized by Lipman and Kalcher in 1941 (54). The energy for muscle contraction comes from the hydrolysis of ATP. ATP is a nucleotide consisting of adenine, a ribose, and a triphosphate unit. The active form is usually complexed with $Mg^{2+}$ or $Mn^{2+}$. Free energy is liberated when ATP is hydrolyzed to adenosine diphosphate and orthophosphate. The amount of ATP in skeletal muscle suffices to sustain contractile activity for

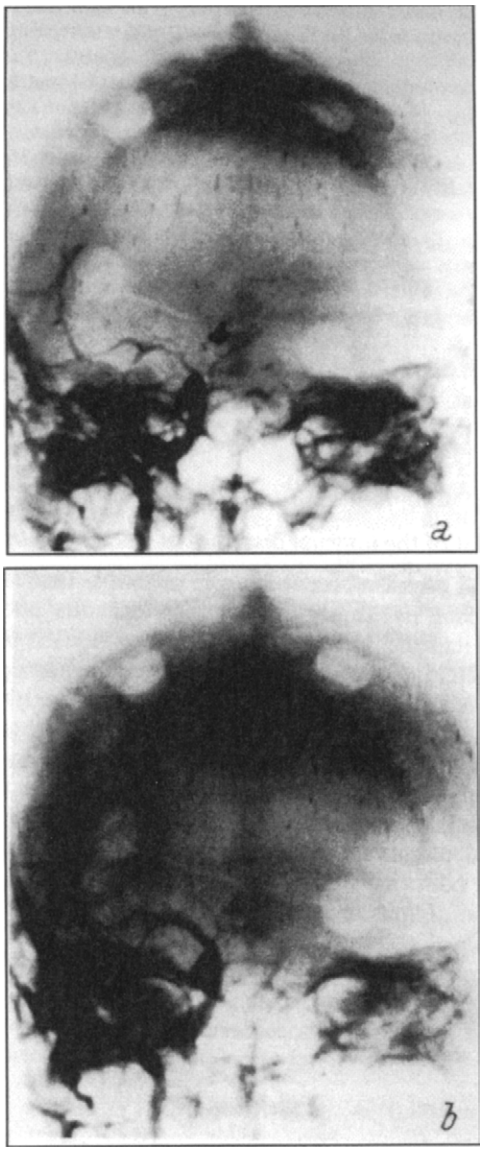


**FIGURE 1.12** "(a) Angiogram demonstrating ICA and MCA spasm in a patient operated on for an ACoA aneurysm. (b) Angiogram after vasodilatation" [from Zubkov *et al.*, 1984, *Acta Neurochir.* **70**, 65–69. Copyright © Springer-Verlag GmbH & Co. (52)].

only a fraction of a second. The reservoir of high-potential phosphoryl groups is in the form of phosphocreatine. Creatine kinase catalyzes the transfer of a phosphoryl group from the phosphocreatine to ADP to convert it to ATP and creatine. The reduction in energy charge of active muscles stimulates glycolysis.

The electron microscope was introduced to the study of muscle ultrastructure by Huxley (55). In the 1950s, two groups independently proposed the "sliding filament" theory of skeletal muscle contraction—A.F. Huxley and R.Niedergerke and H.E. Huxley and J.Hanson—which proposes that thick and thin filaments do not change length during muscle contraction but simply slide past one another (56,57). It has been known since then that the thick filament is mainly myosin and the thin filament is composed of actin and other proteins. The relative sliding of thick and thin filaments past each other is caused by "side pieces" or cross-bridges which project from the thick filament and interact with specific sites on the thin filament. The cross-bridge movements are constrained to a limited range. A molecule of ATP is split with each cross-bridge cycle.

Dozens, perhaps hundreds, of proteins in vascular smooth muscle cells regulate their structure and metabolism. Each interacts with others. Their actions are controlled by the reversible attachment and reattachment of phosphate groups under the influence of various enzymes which accelerate these interactions. In the 1950s, Fisher and Krebs studied how phosphorylation and dephosphorylation of proteins altered the three-dimensional structure of these folded chains of amino acids. The changes in configuration altered electrical charges and functional activity. Their studies were performed on muscle tissues and also won them the Nobel Prize in 1992 for work first published in 1956. They discovered that these phosphorylase enzymes are converted to an active form by the attachment of a phosphate group and the removal of the same group inactivates them. This phosphorylation–dephosphorylation was the switch that turned muscle contraction on and off. Enzymes called protein kinases catalyze the attachment of phosphates and protein phosphatases do the reverse. Many cellular processes are controlled by reversible phosphorylation. Almost one-third of cellular proteins exist in phosphorylated form and it has been estimated that 3% of all genes encode protein kinases and phosphatases (53).

Calcium ions ($Ca^{2+}$) were shown to be the activator of the contractile system by Weber and Wincur (58). The concentration of $Ca^{2+}$ controls contraction and relaxation by an allosteric mechanism in which the flow of information is from $Ca^{2+}$ to troponin to actin to myosin. The rise and fall of sarcoplasmic free $Ca^{2+}$ is the fundamental means of controlling smooth muscle contraction and relaxation (59).

It was not until the late 1970s that protein tyrosine phosphorylation was discovered and shown to be intimately involved with such diverse cellular processes as growth and differentiation. Numerous second messengers [$Ca^{2+}$, inosotol triphosphate, nitric oxide, cyclic adenosine monophosphate (cAMP), and cyclic guanosine monophosphate (cGMP)] are now known to be involved in sarcoplasmic enzymatic cascades. In that decade the primary role of myosin light chain phosphorylation in contraction was established. The low activity of $Mg^{2+}$ ATPase of smooth muscle actomyosin was shown to be

increased by phosphorylation of the regulatory light chain of myosin. The intracellular concentration of vascular smooth muscle $Ca^{2+}$ came to be quantitated by indicators and was found to be vital in controlling contraction–relaxation (60). The three-dimensional structures of actin and myosin were discovered. By the early 1980s, two new smooth muscle proteins were discovered, caldesmon and calponin, which inhibit the $Mg^{2+}$ ATPase of smooth muscle actomyosin.

Vascular smooth muscle cells can be stimulated by a host of hormones and transmitters, both physiological and pathological, which then activate an intricate cascade of signal transduction mechanisms. Responses can differ with the concentrations of the agonist applied and depending on the specific type, location, and condition of the blood vessel. For instance, the docking of an α-adrenergic agonist into a transmembrane receptor protein linked to a G-protein on the sarcoplasmic side of the membrane activates phospholipase C thereby increasing $IP_3$, which causes $Ca^{2+}$ to be released from intracellular stores. In 1984, it was shown in coronary arteries that both cGMP and cAMP could inhibit tension development (61). Of importance for the understanding of vasospasm was the realization that smooth muscle has the ability to maintain high force at low ATP cost (62). Concepts of filaments sliding past each other during contraction and then being locked in the contracted position with little or no ongoing energy expenditure such as occurs in rigor were introduced in recent decades with terms such as "catch" and "latch".

## IX. State of the Art

The Seventh International Conference on cerebral vasospasm held in June 2000 at Interlaken, Switzerland, was marked by an increasing number of studies on intracellular signal transduction pathways associated with vasoconstriction. A potential deterrent to their therapeutic value is that such systems are almost ubiquitous in other organs so that targeted delivery would be a problem. There were initial reports of adenovirus gene transfer. With the advent of cerebral microdialysis there were several accounts of chemical changes correlating with clinical vasospasm and possibly antedating it; these included a reduction in glucose and an increase in the lactate to glucose ratio. Glutamate is also elevated. The limitation of this technique is that it is invasive and the single monitoring catheter might miss the region of brain that ultimately becomes ischemic. On the other hand, dialysate may be a more sensitive source of biochemical information than ventricular CSF. There were several reports on fasudil hydrochloride, an inhibitor of multiple intracellular protein kinases, which has been administered by a variety of routes. Potential antivasospastic medications are now being given entrapped in lysosomes or compressed in pellets with a slow-release formulation. Experimental and clinical limitations of so-called triple H therapy were presented. There were a paucity of randomized prospective clinical trials. A recent study of nimodipine in traumatic SAH has not yet been analyzed. Potential therapies that have been recently investigated include electrical spinal cord stimulation, laser-induced pulsed-fluid wave vasodilation, and moderated hypothermia. We do not predict widespread adoption of any of these modalities. The technique of transcranial Doppler ultrasonography continues to be widely used as a surrogate for angiographic vasospasm, although its limitations as both a positive and negative predictor of delayed ischemia due to VSP continue to be documented. Despite the failure of huge clinical trials to demonstrate efficacy of drugs in the prevention of vasospasm and its sequella, numerous small clinical trials of drug therapies continue to be reported although it seems to us that efficacy in such series would be virtually impossible to demonstrate due to the low incidence of fatal VSP in any control arm. Proton magnetic resonance spectroscopy studies suggest that an increase in the choline to creatine ratio is a marker of SAH as is the *N*-acetylaspartate/creatine ratio. The technique of computerized tomographic angiography appears to be a means of diagnosis for patients with either no or severe spasm in proximal arteries. Cerebral circulation time as measured on digital subtraction angiograms shows a strong correlation with rCBF. It is suggested that the lumenal narrowing of large arteries works in concert with microcirculatory changes to produce delayed ischemic deficits. It is also reported that some of the morphologic changes in VSP may represent apoptosis. No grand, unifying hypothesis of VSP emerged at the meeting.

## X. Farewell Message

> In 1972, there was the Princeton Conference which in a sense was another conference on vasospasm. There, I and others had to defend the very existence of ischemia with vasospasm against neurologist, Clark Milliken, of the Mayo Clinic who accused us, the surgeons, of using vasospasm as a cover-up for our surgical mistakes. We won finally... in 1979... I recall two things; the first was Miller Fisher's four-grade classification of the distribution of blood from hemorrhage. It gave meaning for there was a direct relationship to the incidence and severity of vasospasm. ... And... we had the first glimpses that volume expansion and hypertension were effective.... There was early evidence that the calcium channel blockers might have a modifying effect. ... And now [1990], most of us have recognized a decline in the incidence of ischemia with vasospasm, almost certainly due to change in

surgical attitudes towards the former fluid restriction and hypotension. Now, maintaining and even expanding blood volumes, and even [giving] a little bit of hypertension from the beginning have reduced the incidence of significant ischemia... death and disability dropped... from 15% to 10% ,in the last five years.... We know now that cerebral arteries in persisting vasospasm become sick, with serious metabolic and structural derangement.... There must be little doubt in our minds that if the clot can be lysed within a day or two, safely, the patient can be freed from the calamity of vasospastic ischemia... we saw balloon angioplasty; what exciting possibilities! Do we dare have some hope that in the near future vasospasm will be prevented or even reversed by chemical means systemically? That will be best and safest. Next best will be the use of chemicals *in situ* as we have been seen with urokinase and tPA.... Last best will be the mechanical method, angioplasty.... I would caution that vasospasm is still around, it is still alive and living in every neurosurgical unit. Hence, my plea that our scientists not falter or lose interest in the search for a final understanding and solution.

—Charles G. Drake [63]

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CHAPTER 2

# EPIDEMIOLOGY



## I. Introduction

Vasospasm (VSP) is the most common cause of focal ischemia after subarachnoid hemorrhage (SAH). Of all types of ischemic stroke, it is unique in that it is to some extent predictable, preventable, and treatable.

About 1 in 10,000 people have an aneurysm rupture each year. Mortality and morbidity increase with the volume of hemorrhage and reflect the age and health status of the patient. Rebleeding is exceptionally adverse not only because it increases the volume of SAH but also because extension into the brain and ventricles becomes more likely. The chance of developing an aneurysm increases steadily with age. Most patients who die from aneurysmal rupture do so outside of hospitals or shortly after admission from the effect of the initial bleed or early rebleeding. Only those patients who survive past the first few days live long enough to potentially manifest symptoms from VSP.

The incidence of VSP is obviously less than the incidence of SAH since only some patients with SAH develop it. The incidence of VSP will depend on the type of patient a given institution receives and the way in which VSP is determined. The unqualified term VSP is usually used with reference to angiographically determined vessel narrowing. Clinical VSP is most often used synonymously with delayed ischemic deficit (DID). When used in another fashion, for instance, VSP based on increased middle cerebral artery (MCA) Doppler velocities, this should be specified.

At least two-thirds of patients having angiography between days 4 and 12 post SAH will have some degree of angiographic narrowing. The fraction developing neurological deterioration from this cause—a DID—varies with the assiduousness with which the patient is observed and the efficacy of prophylaxis but has historically been estimated at about one-third. Currently, between 5 and 10% of hospitalized SAH patients die from VSP. Patients in very good or very poor condition post-SAH are less likely to develop VSP than those in intermediate grades, the former because they have small volume SAH and the latter because they are more likely to die earlier from the initial episode. The key prognostic factor for VSP is the presence of thick, widespread subarachnoid clot which can be visualized on the computerized tomographic (CT) scan done in close proximity to the bleeding episode. The absence of blood on the initial CT scan means that VSP is very unlikely in the absence of rebleeding. Factors decreasing the duration of exposure to clot decrease the chance of VSP and consequently DID. Conversely, the use of antifibrinolytic drugs prolongs the exposure of arteries to clot and increases the incidence of VSP and DID. Poor admission clinical grade is associated with DID, presumably because they both indicate larger volumes of SAH. A definite relationship between age, hypertension, or sex and DID has not been established. It is likely that smokers are more prone to VSP and

DID. Factors unrelated to the development of VSP include season, geography, contrast material, and diabetes.

VSP, if severe enough to cause DID and infarction, clearly worsens the outcome. Patients without VSP do better than those with it. The development of severe VSP probably doubles the death rate (Table 2.1).

If surgery is performed in the first day or so it is sometimes difficult to differentiate deterioration from operative factors and the DID from VSP. Outcomes were generally worse when operations were preferentially performed during the peak period for VSP. Early surgery does not cause VSP and it permits more vigorous treatment should it develop. If thick clot is present an attempt at careful removal should be made. The amount of residual clot postoperatively is a prognostic factor for DID. Open operation exposes the patient to retractor pressure, venous sacrifice, temporary clipping ischemia, brain removal, and arterial injury. Studies have shown post operative decrease in cerebral blood flow (CBF), regional cerebral metabolic rate of oxygen ($rCMRO_2$), and oxygen extraction ratio (OER).

VSP as an independent variable is not as closely linked to outcome as other factors such as admission neurologic grade, extremes of age, massive intracranial hemorrhage (ICH) or intraventricular hemorrhage (IVH). Since VSP is a graded process it is expected that only the extreme cases will result in infarction in the absence of systemic hypotension, cardiac dysfunction, anoxia, and intracranial hypertension. The vulnerability of the brain to ischemia is also strongly influenced by preexisting hypertension and advanced age. The etiological relationship between VSP and infarction in fatal cases is no longer in dispute.

The general literature does not support the view that the incidence of DID from VSP has been decreasing. However, the placebo arms of very carefully studied, randomized clinical trials suggest that death and disability from VSP has indeed decreased in recent years. There is sparse information on VSP following coiling of aneurysms. Reported rates do not appear to differ substantially from surgical ones. Evidence exists that VSP may be reduced by clot removal either surgically or pharmacologically. There are also data suggesting that DID may be

**TABLE 2.1 Angiographic Vasospasm, Delayed Ischemic Deficit, and Outcome[a]**

| No. of references | No. of patients | Outcomes | |
|---|---|---|---|
| | | Incidence VSP | |
| | | At all times | 2nd week post–SAH |
| 187 | 25,233 | 45% | — |
| 23 | 1,362 | — | 67% |
| | | Incidence DID (all cases) | |
| 243 | 29,347 | 32% | |
| | | Posterior circulation aneurysm VSP | |
| 16 | 678 | 22% | |
| | | Outcome with VSP | |
| 21 | 3,123 | 41% (dead) | |
| | | Outcome without VSP | |
| 21 | 5,166 | 18% (dead) | |
| | | Outcome with VSP | |
| 16 | 2,016 | 42% (good) | |
| | | Outcome without VSP | |
| 16 | 3,998 | 68% (good) | |
| | | Outcome with DID | |
| 131 | 4,198 | 33% (dead) | |
| 87 | 2,857 | 33% (permanent deficit) | |
| 118 | 3,650 | 35% (good) | |

[a] Data from Dorsch, N. W. C. (1990) Incidence, effects and treatment of ischaemia following aneurysm rupture. *Cerebral Vasospasm. Proceedings of the 4th International Conference in Tokyo*. Univ. of Tokyo Press, Tokyo.

lessened by hypertension and hypervolemia as well as calcium antagonists. VSP may also be abolished by angioplasty.

## II. Incidence of Subarachnoid Hemorrhage

The incidence of a disease is the number of new cases of the entity per year in relationship to a given number of people at risk. The incidence of aneurysmal rupture is usually between 6 and 11 per 100,000 per year. Aneurysms comprise 75–80% of cases of SAH. The incidence of aneurysmal rupture has been fairly steady over the past 40 years. Most deaths from aneurysmal rupture occur in the first few days following rupture and then gradually taper off so that by 6 months approximately two-thirds of patients will have died if they are untreated. The rate of rerupture in untreated ruptured aneurysms thereafter is approximately 2 or 3% per year. The incidence of aneurysms rises progressively with age, declining perhaps in the 80s and 90s.

Between 1962 and 1963 the death certificates of 913,000 people under the age of 60 years were examined. Of cases who died from ruptured aneurysms, 12% died at home, 25% in local hospitals, and only 25% of the original population died in neurosurgical centers. In this population the incidence of ruptured aneurysms was 6 /100,000/ year (1). A review of 6 series from the 1950s and 1960s found that aneurysms caused 77% of SAH cases and incidences were 6, 6, 7, 9, 10, and 10.9/100,000/year (2). Of 136 hospital autopsies showing aneurysms, 40% were ruptured and 60% were unruptured (3). In 1977, 67,930 autopsies were reviewed, and the incidence of ruptured unoperated cerebral aneurysms was 0.3% and that of ruptured aneurysms 1.1% (4). In 51,360 autopsies from 8 series, 1.4% of cases had aneurysms (unruptured 0.3%, ruptured 1.1%) (5). In another 12 series of autopsies the incidence of aneurysms was 1.6% (0.2–9%) in 87,772 total cases (6).

Several studies used multiple regression analysis to determine the factors that affect outcome from SAH. Richardson's group studied features of use in predicting morbidity and mortality in the proximal ACA ligation treatment of ruptured anterior communicating artery (AComA) aneurysms (7). Morbidity and mortality increased with increasing age, female sex, increasing levels of hypertension, the presence of VSP, a decreased level of consciousness, and a greater degree of cross-filling with carotid compression. All of these patients had single A Com A aneurysms that had ruptured at least once. Between 1968 and 1973 a study of 2-month mortality rates in 135 SAH cases yielded the following hierarchy of associations: neurologic grade, 0.36; grade at surgery, 0.34; preoperative VSP, 0.28; mass lesion, 0.23; hypertension, 0.20; shorter interval to surgery, 0.10; age, 0.06 (8). A randomized trial between 1963 and 1970 included 187 cases treated with bed rest (9). Mortality rates at 90 days post-SAH were predicted by a short interval from SAH to treatment (0.25), medical condition (0.20), poor neurological grade (0.17), hypertension (0.13), and VSP (0.12). Similar results were obtained with respect to 14-day mortality from an analysis of 1114 cases from 1970 to 1977: neurologic status, 17.74; diastolic blood pressure, 11.66; interval to treatment, 9.44; VSP, 8.51; and medical condition, 5.51 (10). Two hundred and sixty-five cases treated between 1972 and 1977 were evaluated and showed associations with respect to 5-year mortality as follows: neurological grade, 0.65; age, 0.47; blood pressure, 0.31; interval to operation, 0.20; aneurysm side, 0.19 (11, 12).

Aneurysmal rupture produced ICH in 132 cases described in 1982. Mortality during the initial admission was predicted by the following factors: systolic blood pressure, 0.37; preoperative herniation, 0.35; grade at clipping, 0.33; grade at evacuation of clot, 0.27; diastolic blood pressure, 0.16; grade at initial CT scan, 0.14; size of ICH, 0.14; and VSP, 0.13 (13). A similar analysis of 91 cases with IVH had the following values: size of ventricles, 0.56; systolic blood pressure, 0.38; age, 0.37; diastolic blood pressure, 0.32; admission grade, 0.31; associated ICH, 0.17; location of aneurysm, 0.15; and location of ICH, 0.11 (14).

In middle Finland in 1976–1978 the incidence of SAH in a population of 241,000 people was 19.4 /100,000/year. Aneurysms caused 93% of SAH in the autopsied cases. The incidence rose with age from 1.6/100,000/year in 10-to 19-year-olds to 49.6/100,000/year for those over 70 years of age (15). In a New Zealand study of a population of 829,464 persons, there were 92 cases of SAH in a 1-year period. The age-standardized rate for men was 13.4/ 100,000/year and it was 15.8 for women (16). The combined morbidity and mortality in 119 aneurysmal SAH cases was 28%. Morbidity and mortality were related to poor neurologic grade and older and hypertensive patients. Race was not associated with an adverse outcome (17).

Fifteen percent of 1076 patients with aneurysmal SAH had been on antihypertensive treatment prior to the event. The hypertensive patients averaged 8 years older and were in poorer neurological condition with an increased frequency of atherosclerosis. At 2-year follow-up the hypertensive patients had a 59% overall mortality versus 42% for the entire group. Increased rate of mortality was particularly evident in the patients who were neurological grades I–II on admission (52 vs 22%). There was also an increased mortality rate in hypertensive patients who

rebled (100 vs 75%). The CT characteristics of these patients and the occurrence of VSP were not systematically investigated (18).

In a prospective series of patients who were initially poor grade on admission, 184 patients were studied in Canadian institutions in the late 1980s. A discriminant function analysis indicated that the relative descending importance of factors for outcome was whether the patient was treated surgically, neurologic grade on admission, age, initial systolic blood pressure, and aneurysmal size. Patients with thick-layer clot on the initial CT scan had fewer good outcomes and higher mortality than patients with thin layers of clot, although this did not achieve statistical significance. Death occurred in 36% of those with thin clot and 51% of those with thick clot on initial CT scans. Angiographic VSP was not associated with a poorer outcome in this group of patients, who were selected by virtue of initial poor grade. This was because so many of the patients had ICH or IVH or died quickly from the effects of their initial SAH. Angiograms were obtained on 135 patients on day 5 or later. Fifty-three percent showed severe diffuse VSP, while 12% showed moderated diffuse VSP. Thirty-two percent showed either mild or focal VSP, and only 3% showed no evidence of angiographic VSP. There was a trend for fewer good outcomes in patients with severe diffuse VSP but this did not reach statistical significance (19).

In 145 patients dichotomized into good and poor outcomes and studied using a multiple logistic regression model, it was shown that hypertensive white males with a history of intravenous drug abuse had a high risk of poor outcome following SAH (20). Among 3521 patients entering into a cooperative study, multivariate analysis showed that the most important prognostic factors were level of consciousness on admission, age, admission blood pressure, amount of SAH on CT scan, preexisting conditions, aneurysm site, and VSP (21). Three hundred and eight cases with SAH were evaluated. No patient had ICH or IVH. The duration and level of unconsciousness in cases of SAH without concurrent hematoma causing mass effect had a good correlation with the severity of SAH in the perimesencephalic cisterns (22). Four hundred and seventy-one consecutive patients with aneurysmal SAH were analyzed using logistic regression with stepwise forward selection of variables. On admission, poor outcome was predicted by low Glasgow Coma Scale (GCS), fluid restriction, age over 52 years, ictal loss of consciousness, or a large SAH. Delayed infarction was predicted by a large SAH or treatment with antifibrinolytic agents. The inclusion of the amount of SAH into the predictive model added little to the prediction of poor outcome in general but much to the prediction of delayed cerebral ischemia (23).

Two hundred and ninety-one SAH patients admitted within 4 days of bleeding were studied at 1-year follow-up. Twenty-nine percent had died and 10% were dependent. The risk of a poor outcome was predicted by clinical condition on admission, rebleeding, delayed cerebral ischemia (relative risk, 10.3; confidence interval, 95%, 4.2–25.4; $p < 0.0001$), surgery on an aneurysm, and heavy consumption of alcohol. Heavy drinkers had a more unfavorable outcome after rebleeding and delayed ischemia than did others. Interestingly, those with salicylates in their urine on admission had delayed ischemia with fixed neurological deficits less commonly than others (24). In a population study of the 1.3 million inhabitants of greater Cincinnati during 1988, the 30-day mortality rate for 80 cases of SAH was 45%. Almost two-thirds of these patients died within 2 days of onset. Twenty-one of the 22 deaths were due to the initial hemorrhage and 1 was from rebleeding. Nine of the remaining 14 deaths after Day 2 post-SAH were attributed to the effects of the initial bleeding (2 cases) or rebleeding (7 cases). A powerful predictor of 30-day mortality was the volume of the initial SAH. If the volume of SAH was 15 ml or less, only 3 of 29 patients died before 30 days. Two of these 3 deaths were from documented rebleeding and the third had a massive additional IVH. Delayed arterial VSP contributed to only 2 of 36 deaths. In this large representative metropolitan population it appeared that VSP was playing a minor role in mortality from SAH. The volume of SAH was calculated. The volume of SAH estimated depends on the degree of clotting, the real volume of SAH, and the initial volume of the subarachnoid space. Given the relative imprecision, SAH volume is nevertheless a useful predictor of outcome. This population study suggested late delayed arterial VSP contributed to only 6% of all aneurysmal SAH deaths. This study did of course contain many patients who did not survive to be admitted to neurosurgical services. The data from the study demonstrated that the volume of SAH increases rapidly with age, as does the mortality rate following SAH. The volume in operated patients averaged 10 ml compared to 28 ml for nonoperated patients. The operated patients were also younger (51 vs 59 years) and had better neurologic average grades (2.9 vs 4.2). This explains the difference in 30-day mortality—4% for operated versus 63% for nonoperated patients (25).

## III. Incidence of Vasospasm

Dorsch reviewed 187 references on VSP and found great variation in reported incidences of VSP and DID. The variation was attributed to differences in definitions

and in timing of angiography. When angiography was carried out in the second week after SAH, 67% showed VSP. Overall, 32% of patients developed DID. Analysis of a smaller number of reports on posterior circulation aneurysms revealed 22% developed DID (26). The average incidence of DID from 297 references containing 10,445 DID cases drawn from among 32,188 SAH patients was 32% (range, 5–90%). In reports in which there was a reasonably strict definition of DID (132 studies) the incidence ranged from 12 to 57%. In these strict studies there were 4068 cases of DID from 12,449 aneurysmal SAH cases (33%). About one-third of patients experience neurological deficits from VSP, and approximately one-third of these patients die from their deficits, one-third are left disabled, and one-third have favorable recoveries. Angiographic VSP is seen in two-thirds of patients with ruptured aneurysms when angiography is carried out between 4 and 12 days after SAH (26). In 1975, Fisher reported that one-third of patients who survived aneurysmal SAH developed delayed cerebral ischemia secondary to VSP (27).

In a Swiss population of approximately 1 million there were 8 patients (5.2% of 153) who sustained a significant morbidity or mortality from vasospastic ischemia and infarction (28). In a Swedish population of 6.93 million studied between June 1, 1989, and May 31, 1990, there were 325 ruptured aneurysms. Sixty-nine percent were admitted to neurosurgical units within 24 hr and surgery was performed in 85%. Twenty-three percent of cases were grade IV and 10% grade V; 5.8% or 19 cases had a poor outcome attributed to DID, giving an incidence of DID of 2.7 /1 million/year. In comparison, the percentages were 20% for initial bleeding, 7.2% for surgical complications, and 6.2 % for rebleeding (29).

One of the problems in the study of VSP is the relative inexactitude of classification systems. In a critical review by a Dutch group, all articles on SAH published in nine journals between 1985 and 1992 were assessed for the presence and precision of definitions used for classifying grade, rebleeding complications, delayed cerebral ischemia, hydrocephalus, and overall outcome. Of 161 articles reporting the initial condition, only 19% used the World Federation of Neurological Surgeons (WFNS) scale or the Glasgow Coma Scale. A specific outcome event was sufficiently defined in only 31% of instances, incompletely in 22%, and not at all in 47%. Precision in definition was more common in neurological than neurosurgical journals (30).

Over a 9-year period, 270 patients admitted within 7 days of SAH were studied. Thirty-four percent developed VSP, of which 15% showed ischemic symptoms only and 19% showed CT infarction. The incidence of symptomatic VSP was 12% in the GCS 15 group, 23% in the GCS 13–14 group, and 11% in the GCS 7–12 group. Vasospastic infarction occurred in 14, 23, and 26% of these GCS groupings, respectively. VSP was more common in older patients. The site of aneurysm on day of surgery had no apparent influence on the incidence of VSP or its outcome. VSP was considered the cause of poor outcome in one-third of the poor outcome cases (31).

Three hundred and four patients with ruptured aneurysms were categorized by the Hunt and Kosnik grade, WFNS scale, and the GCS. The incidence of symptomatic VSP was compared among the adjacent grades with each grading system. Symptomatic VSP was classified as being absent, transient, or permanent. There were significant differences between grades II and III in the Hunt and Kosnik classification and between grades I and II in the World Federation system. The incidence was not significantly different among the adjacent GCS scores or among patients with GCS scores of 14 who had either eye 3, motor 6, verbal 5 or eye 4, motor 6, verbal 4. Fisher groups 2 and 3 predominated in the GCS scores of 14 and 15 and group 4 in GCS scores 6–12. In this series the incidence of symptomatic VSP and hydrocephalus correlated with the Fisher CT classification better than the GCS or other grading systems. Presumably, both processes are proportional to the amount and distribution of SAH (32).

## IV. Timing of Angiography and Incidence of Vasospasm

The incidence of angiographic VSP depends on interval after the hemorrhage, with a peak incidence 6–8 days after SAH (range, 3–12 days). In addition to time after SAH, the other principal factors that affects the prevalence of VSP are the volume and distribution of subarachnoid blood. Current therapy for VSP has reduced the incidence of delayed cerebral ischemia.

Eight series of cases of aneurysms were reviewed in 1980, and 26% of the 1840 patients showed angiographic VSP. The relationship of the day of angiography to the day of SAH was often unstated in these earlier studies. The incidence of VSP will be low if studies are done in the first few days or more than 2 weeks after the SAH (33). In a massive literature review by Dorsch and King (34), more than 30,000 cases were found in reports in which VSP post-SAH was discussed. The overall incidence of angiographic VSP was 43%, but it was 67% when angiography was done at the time of expected VSP. There was no difference in incidence or time course of VSP between pre- and postoperative cases. VSP was the cause of death in about 10% of aneurysmal SAH cases and

disability in slightly more cases (34). Twenty-seven references specifically mentioning this gave a mean earliest onset of DID of 4.2 days. The mean time of onset for 481 patients was 7.7 days post-SAH. For 66 cases individually noted, the mean was 7.9 days. The peak of deficit was reached 1–4 days after onset. In cases of angiographic confirmation of clinical VSP the incidence of DID was 3781/12,246 or 31%. In the second week post-SAH the incidence of angiographic VSP was 67% (range, 40–97%). Symptomatic VSP both pre- and postoperative was shown to predict increased mortality and morbidity after aneurysmal SAH in a statistical analysis of 274 patients between 1968 and 1974 (35).

Fifty patients with ruptured saccular aneurysms were analyzed by Fisher and coworkers. Forty-eight were nonsurgical and 2 were observed postoperatively. The authors considered only neurological deficits developing more than 3 days following SAH. Fifty percent of their patients developed a delayed, new neurological deficit that was ischemic in origin. The most common day of onset for DID was the eighth day following SAH. Only 25% of patients who became mentally lucid within 24 hr of the hemorrhage developed a DID, compared with 60% of those who were confused or stuporous for a longer period. VSP did not seem to correlate with sex, age, hypertension, or treatment for hypertension. In 70% of patients with symptomatic or severe VSP, the vessel returned to normal caliber in approximately 28 days. Of 31 patients with severe VSP, 81% developed DID. None of their 19 patients with no or very mild VSP developed DID. Only 1 of their 25 patients who developed DID did so beyond the 13th day from SAH (36).

The placebo-treated group of a multicentered clinical trial of 296 patients was analyzed. Early admission angiograms were studied. Thirteen percent of cases had angiographic VSP at admission. Admission was within 48 hr of SAH. This VSP was associated with lower GCS score and higher serum $Na^+$ level (138 mmol/L liter). Ultra-early angiographic VSP was associated with an increase risk of delayed onset, symptomatic VSP (odds ratio, 2.5; 95% confidence interval, 1.2–5.4), and poor outcome at 3 months (odds ratio, 2.8; 95% confidence interval, 1.2–6.3) after adjusting for other variables. The risk of symptomatic VSP was not influenced by surgery performed within 48 hr of SAH. Poorer outcome was more likely to occur in patients with ultra-early angiographic VSP who did not undergo early surgery. Early surgery did not aggravate the risk symptomatic VSP and poor outcome. The ultra-early angiographic VSP observed might of course have been a consequence of an earlier unrecognized SAH and not an unusual early response to the SAH precipitating admission (37).

## V. Prognostic Factors for Vasospasm

### A. Blood on CT Scan

Takemae *et al.* (38) first showed that it was possible to predict VSP by finding high-density areas in the subarachnoid space on CT scans done within 4 days post-SAH. In their material, VSP developed in >80% of patients with high-density areas in the basal cisterns and in the Sylvian fissures. No VSP developed in patients without high-density areas on the initial CT scans. When the initial CT scan was performed more than 4 days after SAH, there was no relationship between CT findings and VSP (38). The distribution of the high-density areas on CT scans within 4 days after SAH agreed with the distribution of VSP demonstrated angiographically. These authors recommended early surgery with removal of blood clots.

In a series of 50 patients with ruptured aneurysms, 50% developed DIDs that were attributed to cerebral ischemia (36). It is likely that these patients were treated with various combinations of hypovolemia, hypotension, and antifibrinolytic drugs, all of which increase the chances that angiographic VSP will produce cerebral ischemia. Fisher *et al.* (39) found that every patient with severe angiographic VSP manifested delayed symptoms and signs. These authors concluded that blood localized in sufficient amounts in the subarachnoid space at specific sites is the only important etiological factor in VSP. For patients with no or mild subarachnoid clot in the initial CT scan, there was only about a 30% chance of developing severe or moderate VSP. The development of abnormal meningeal contrast enhancement has not been shown to correlate strongly with the subsequent development of symptoms or infarction (39). The amount and location of subarachnoid hematoma visualized on a CT scan performed within 4 or 5 days of SAH was categorized into four groups: grade I, no blood detected; grade II, diffuse, thin layers of SAH less than 1 mm thick; grade III, localized clots and vertical layers of blood more than 1 mm thick or more than 5 × 3 mm in the longitudinal or transverse planes; and grade IV, intracerebral or intraventricular hemorrhage, with little or no subarachnoid blood. Only 1 (5.6%) of 18 patients experienced cerebral VSP when no SAH or only diffuse, thin SAH was shown on CT. Similarly, patients in the group with ICH and IVH rarely developed cerebral VSP. However, diffuse, thick SAH (Fisher grade III) was associated with severe cerebral VSP in 23 (96%) of 24 cases, and all patients were symptomatic. The most severely affected arteries were those coursing through the thickest subarachnoid clots (39). The findings of this study were later confirmed prospectively. The predictive power of this

classification was prospectively verified (40). Very large studies have confirmed these early observations (41) (Table 2.2).

Multivariate statistical techniques are required to demonstrate which factors independently predict the development of VSP. The occurrence of VSP in proximity to the ruptured aneurysm is probably due to the increased amount of blood that is deposited around the aneurysm. Others have argued that rupture of the aneurysm with disruption and hemorrhage into a portion of the arterial wall accounts for the increased incidence of VSP near the ruptured aneurysm. The low incidence of VSP in patients with aneurysms that rupture into the brain or the ventricles but who have the same arterial wall rupture argues against the latter hypothesis.

Forty-one cases with thick subarachnoid clot were analyzed. None had concurrent ICH or IVH. Twenty-three cases had acute surgery and 18 were treated conservatively. Of the latter group, all except 2 died from primary brain damage. The other 2 died from other complications. Of the 23 surgically treated patients, 13% recovered fully and 39% died. Factors associated with outcome were neurological grade, type of brain stem responses, response to the rapid administration of 20% mannitol (300–900 ml), and the length of time from the last bleeding episode to the time of surgery. In such cases

**TABLE 2.2 Logistic Regression Equation for Prediction of Focal Ischemic (FID) Deficits after Subarachnoid Hemorrhage[a]**

| Variables | Coefficient | SE | *p* value |
|---|---|---|---|
| CT-normal | −0.493 | 0.161 | 0.000 |
| CT-SAH thick layer | 0.317 | 0.082 | 0.000 |
| Antifibrinolytic use | 0.238 | 0078 | 0.001 |
| CT-SAH diffuse | 0.172 | 0.078 | 0.027 |
| Consciousness | | | 0.026 |
| Alert | −1.06 | 0.354 | |
| Drowsy | 0.392 | 0.230 | |
| Stuporous | 0.241 | 0.159 | |
| Orientation | 0.220 | 0.099 | 0.026 |
| Constant | −1.34 | 0.205 | |
| | | | |
| Codes | | | |
| CT-normal | −1 = absent | 1 = present | |
| CT-SAH thick | −1 = absent | 1 = present | |
| Antifibrinolytic use | −1 = no | 1 = yes | |
| CT-SAH diffuse | −1 = absent | 1 = present | |
| Consciousness level | | | |
| Alert | 0 = no | 1 = yes | |
| Drowsy | 0 = no | 1 = yes | |
| Stuporous | 0 = no | 1 = yes | |
| Orientation | −1 = oriented | 1 = impaired | |

Example: Probability of FID in patient with thick SAH layer, antifibrinolytic use, drowsy consciousness, and impaired orientation:

$$P(\text{FID}) = \frac{1}{1+e^{-(-1\times-0.493+1\times0.317+1\times0.238+-1\times0.172+1\times0.392+1\times0.220-1.34)}}$$

$$= \frac{1}{1+e^{-(0.148)}}$$

$$= 0.537$$

[a] From Adams, H. P., Kassell, N. F., Torner, J. C., and Haley, E. C. (1987). Predicting cerebral ischemia after aneurysmal subarachoid hemorrhage: Influences of clinical conditon, CT results, and antifibrinolytic therapy. *Neurology* **37**, 1586–1591.

no relationship was demonstrable between grade immediately prior to the surgery and the outcome in these serious cases (42).

It seems likely that there are common factors between SAH and ICH. It is possible to measure the volume of ICH much more accurately than by use of our usual qualitative assessments of the volume of SAH. Thirty-day mortality was 44% for 188 cases with ICH. Half of the deaths occurred within the first 2 days of onset. It was found that the volume was the strongest predictor of the 30-day mortality for all locations of SAH. Patients with an ICH volume of 60 ml or more and a GCS score of 8 or less had a 30-day mortality of 91%. Those with a volume of under 30 ml and GCS of 9 or more had a predicted 30-day mortality of 19%. Only 1 of 71 patients with a volume greater than 30 ml was functioning independently at 30-day post-ICH (43). The tamponading counter pressure with parenchymal bleeding is probably effective more rapidly than with bleeding into the subarachnoid space where there is a potentially compliant cranial and spinal subarachnoid space. When SAH volumes were measured quantitatively by the same group the mortality rates were about one-eighth for volumes <10 ml and one-half for volumes 10–50 ml, and all patients with volumes above 50 ml died (Fig. 2.1) (25).

The importance of blood on CT scan for the development of VSP was confirmed in a study of 283 patients (all treated with nimodipine), of whom 33% developed symptomatic VSP. The four independent predictors of symptomatic VSP were as follows: thickness of subarachnoid clot odds ratio 4.1; early rise in transcranial Doppler Ultrasonography (TCD) velocity (MCA velocity >110 cm/sec on or before day 5 post-SAH), odds ratio 1.9; GCS <14, odds ratio 1.8; and anterior cerebral or internal carotid site of aneurysm, odds ratio 1.9. A risk index based on a combination of factors was only slightly more effective in predicting symptomatic VSP than clot thickness alone (68 vs 62%) (44).

### B. Hypertension

In a cooperative study the incidence of DID increased in all patients where admission systolic blood pressures were less than 180 mmHg if they were treated by antihypertensive drugs. This was particularly obvious in the patients with the lowest blood pressure on admission. For patients admitted with pressures greater than 180, the rate of focal ischemic deficits was slightly less if they were treated with medication (45).

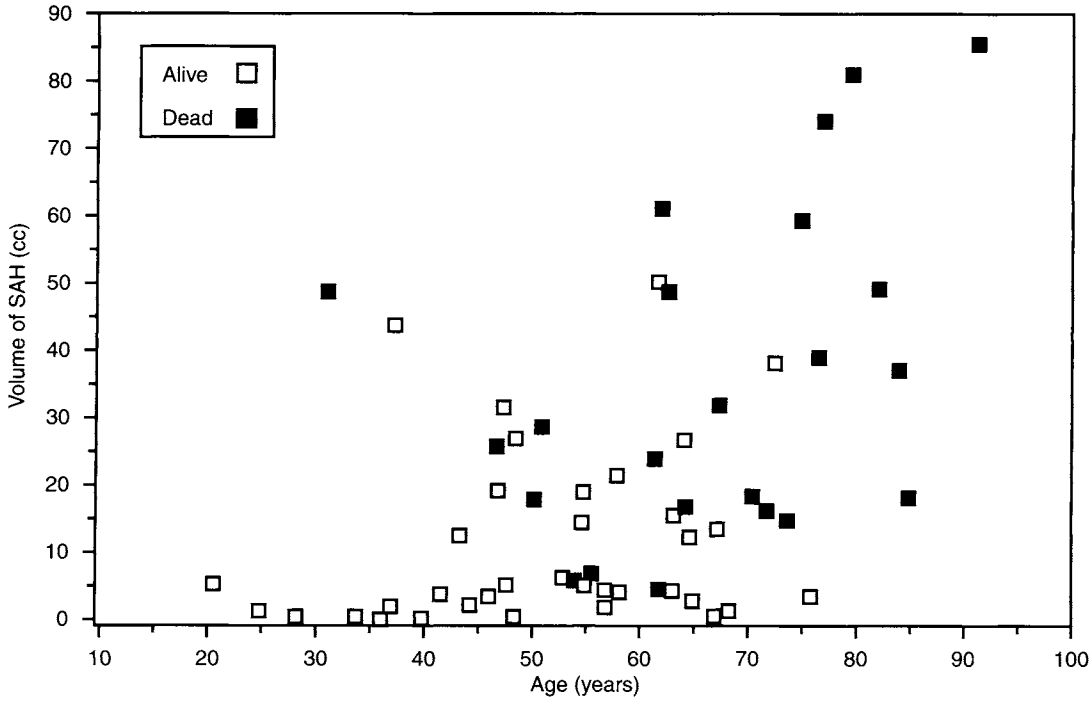


**FIGURE 2.1** Scatterplot showing relation of age and volume of subarachnoid hemorrhage (SAH) to 30-day mortality (59 cases with computed tomographic measurements) [reproduced with permission from Broderick, J. P., Brott, T. G., Duldner, J. E., Tomsick, T., and Leach, A. (1994). Initial and recurrent bleeding are the major causes of death following subarachnoid hemorrhage. *Stroke* **25**, 1342–1347].

### C. Anatomical and Systemic Factors

Late ischemia is more likely to develop in patients who have preexisting structural impairment of circulation such as a carotid stenosis or occlusion. The presence of small communicating or leptomeningeal arteries is an additional adverse prognostic factor. The involvement of end arteries such as the MCA supplying cerebral regions may result in a dense ischemic core surrounded by a penumbra of brain between the normally perfused and the nonperfused tissues. Adverse prognostic indicators also include spontaneous or deliberate dehydration, arterial hypotension, prolonged anesthesia, increased intracranial pressure, poor cardiac output, cardiac arrhythmias, anemia, and hypoxia (46).

Many other phenomena, such as IVH, acute hydrocephalus (Hyc), abnormal EEG, increased levels of cerebrospinal fluid (CSF) fibrin-degradation products, decreased blood volume, increased serum complement, early increases in MCA flow velocity, or decreases in CBF, have been associated with an increased risk of VSP after SAH (47,48). Some factors may be independent predictors of VSP risk but their value has not yet been proven, and they may simply reflect the relationship between more subarachnoid blood, more brain damage, and a sicker patient. The site of the aneurysm has little value for predicting VSP other than the fact that SAH, and hence VSP, is usually worst near the ruptured aneurysm.

### D. Clinical Grade

A general relationship exists between a worse clinical grade and increasing amounts of SAH. As a result, patients in higher clinical grades with depressed levels of consciousness after SAH are more likely to experience VSP if they survive long enough (48). However, clinical grade by itself provides little information predicting VSP or DID besides that provided by CT scan. In one series, transient clinical VSP was more common in poorer clinical grade cases, but permanent deficits did not significantly differ between grades (32) (Table 2.3). In this study Fisher grade II scans occurred most frequently in GCS score 15 cases, Fisher grade III scans in patients GCS 12–14, and Fisher grade IV scans in GCS 6–12 (lower GCS were not studied) (32) (Table 2.4).

Data on two hundred and seventy patients having surgery within a week after SAH during a 9-year period were accumulated. Clinical VSP was diagnosed on the basis of ischemic neurologic deterioration with or without CT infarction. Outcomes were assessed 6 months postoperatively. Thirty patients with GCS less than 6 were excluded from the VSP analysis. Thirty-four percent of patients developed VSP, 15% showing ischemic symptoms only and 19% showing CT infarction. DID occurred in 12% of GCS 15 cases ($n = 117$), 23% of GCS 14–13 ($n = 70$), and 11% of GCS 12–7 ($n = 53$). CT infarction

**TABLE 2.3 Vasospasm and Grading by the Hunt and Kosnik and the World Federation of Neurological Surgeons (WFNS) Systems[a]**

| System | Transient vasospasm (%) | Permanent vasospasm (%) | Total cases |
|---|---|---|---|
| Hunt and Kosnik | | | |
| Grade 1 | 4 | 19 | 27 |
| Grade II | 9 | 14 | 115 |
| Grade III | 30 | 17 | 117 |
| Grade IV | 42 | 18 | 45 |
| Total | 21 | 16 | 304 |
| WFNS | | | |
| Grade I | 7 | 14 | 98 |
| Grade II | 24 | 22 | 120 |
| Grade III | 32 | 11 | 19 |
| Grade IV | 44 | 16 | 64 |
| Grade V | | 33 | 3 |
| Total | 21 | 16 | 304 |

[a] Data from Hirai, S., Ono, J., and Yamaura, A. (1996). Clinical grading and outcome after early surgery in aneurysmal subarachnoid hemorrhage. *Neurosurgery* **39**, 441–447.

**TABLE 2.4 Preoperative Fisher Computed Tomography Classification of Subarachnoid Hemorrhage and Glasgow Coma Scale (GCS)[a]**

| GCS score | Grade 2 (%) | Grade 3 (%) | Grade 4 (%) | Total cases |
|---|---|---|---|---|
| 15 | 54[b] | 39 | 7 | 98 |
| 14 | 38 | 61[b] | 1 | 71 |
| 13 | 15 | 49[b] | 36 | 67 |
| 12 | | 50[b] | 50[b] | 12 |
| 11 | 10 | 20 | 70[b] | 10 |
| 10 | | 55[b] | 55[b] | 11 |
| 9 | | 27 | 73[b] | 11 |
| 8 | | 25 | 75[b] | 8 |
| 7 | | 23 | 77[b] | 13 |
| 6 | | 33 | 67[b] | 3 |
| Total | 30 | 45 | 25 | 304 |

[a] Data from Hirai, S., Ono, J., and Yamaura, A. (1996). Clinical grading and outcome after early surgery in aneurysmal subarachnoid hemorrhage. *Neurosurgery* **39**, 441–447.

[b] Most common Fisher grade for this GCS.

due to VSP occurred in 14, 23, and 26% of these GCS groupings, respectively (49). A close correlation between the clinical grade based on GCS and the incidence of VSP diagnosed either clinically or by CT evidence of infarction was evident. In this Japanese series, VSP was more common in the older age group. The site of aneurysm or the day of surgery had no apparent influence on the incidence of VSP or its outcome. Poor outcome attributable to VSP occurred in 12%, which was one-third of the cases of poor outcome from all causes.

One hundred and twenty-five patients operated for ruptured aneurysms underwent surgery at a mean of 2.1 days post-SAH. Sixteen of these cases manifested symptomatic VSP at a mean of 7.4 days post-SAH (range, 5–12). The 16 patients with DID were compared to 57 patients selected to have the same age and average day of surgery. The volume and location of subarachnoid clots were evaluated by serial CT scans. Only 1 patient had angiographic evaluation. Of the 16 patients with symptomatic VSP, the subarachnoid clots were localized, thick, or associated with ICH in 15 and thin in 1 at the time of admission. Fifteen of these patients showed continued CT evidence of subarachnoid clot during the period of clinical VSP. Most of the clots were near the ruptured aneurysms but in 3 cases were located in remote areas not accessible during the surgery. The clinical manifestations of VSP in these 3 patients correlated with the side of the clot and not the side of the operation. Eight patients had CBF measurements that showed appropriate reductions in the territory of the involved artery. The striking feature here is that the patients with clinical VSP showed the continued presence of subarachnoid clot in 94% of cases, whereas most of the 57 patients without symptomatic VSP showed early postoperative disappearance of the clot (50).

The volume and rapidity of blood entry into the subarachnoid space probably determines the duration of initial loss of consciousness and this parameter has been shown to be a powerful determinant of delayed cerebral ischemia. The univariate hazard ratio for loss of consciousness >1 hr compared to a lesser time was 6.0 (95% CI, 3.0–12.0). This striking increase in the risk compared to 3.4 for a large amount of subarachnoid blood in CT, 2.9 for a grade IV or V WFNS grade, and 1.4 for any risk factor for atherosclerosis (51).

### E. Antifibrinolytics

It was suggested, even in the early 1970s, that antifibrinolytic treatment might exacerbate cerebral ischemia post-SAH. The incidence of DID in patients treated with antifibrinolytics was 407/1307 or 31% in treated patients and 153/799 (19%) in controls in the subsequent literature (26).

Between 1981 and 1983, 934 patients were studied as part of an international cooperative trial on the timing of aneurysm surgery. Patients were admitted within 3 days post-SAH. Six-month mortality was 24.9% for patients given antifibrinolytic drugs and 18.5% in patients not so treated. Of 594 patients treated with antifibrinolytic drugs, 42.3% of those who developed focal ischemia were vegetative or dead versus 17.5% for those who did not develop focal ischemia. The respective data for the 340 patients not treated with antifibrinolytic medication were 27.3 and 18.3%. The patients who did not develop focal ischemia therefore had the same vegetative and death rate regardless of whether or not they had been treated with antifibrinolytics. On the other hand, prior treatment with such drugs greatly worsened the prognosis if focal ischemia did develop. Patients with diffuse or thick, focal SAH on CT scans had a significantly greater chance of developing ischemia regardless of whether or not they received any antifibrinolytic drugs. The development of cerebral ischemia was evident in about 1 patient in 4. The likelihood of good recovery was halved and mortality rate doubled when ischemia occurred. Mortality after SAH approached 40% among patients with ischemic complications. A logistic regression equation for prediction of DID ranked the variables, indicating a higher likelihood of ischemic deficits as being a drowsy level of consciousness (0.392), CT-SAH thick layer (0.317), antifibrinolytic use (0.328); stuporous (0.241), and CT-SAH diffuse (0.172). The likelihood of DID developing was decreased by an alert state or a normal CT scan. The predictive value of a focal, thick collection of SAH on the CT was reliable whether or not antifibrinolytics were used (41) (Table 2.5).

Patients who were drowsy or stuporous on admission and were treated with antifibrinolytic drugs were significantly more likely to develop ischemia than were patients not on antifibrinolytic drugs. Forty-two percent of patients with diffuse blood on the initial CT scan who were on antifibrinolytic drugs developed ischemia compared to 29.6% not so treated. When focal thick collections of blood were seen on the CT the percentage with ischemia was even higher—47.4 versus 38.5% in untreated patients (52).

In the first 2 weeks post-SAH using life-table techniques the databank of the timing of aneurysms study showed that with antifibrinolytics there was a statistically significantly higher ($p = 0.008$) incidence of DID, predominantly caused by VSP. There was a 42% increase in the risk of developing focal ischemic deficit in the group treated with antifibrinolytic agents ($p = 0.03$). The addition of antihypertensive therapy to the antifibrinolytic therapy increased the probability of developing focal ischemic deficit in the first 14 days from 30 to 38%

**TABLE 2.5 Influence of the Results of CT Findings on the Frequency of Ischemia among 934 Patients[a]**

| | Antifibrinolytic drugs (N = 594) | | | | | No antifibrinolytic drugs (N = 340) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CT findings present | | CT findings absent | | | CT findings present | | CT findings absent | | |
| | *n* | % with ischemia | *n* | % without ischemia | *p* value | *n* | % with ischemia | *n* | % without ischemia | *p* value |
| Normal | 83 | 13.3 | 500 | 39.0 | 0.000 | 32 | 9.4 | 299 | 27.8 | 0.04 |
| Hydrocephalus | 84 | 42.9 | 499 | 34.1 | NS[b] | 39 | 25.6 | 292 | 26.0 | NS |
| IVH | 100 | 29.0 | 483 | 36.6 | NS | 48 | 29.2 | 283 | 25.4 | NS |
| ICH | 87 | 46.0 | 496 | 33.5 | 0.03 | 51 | 29.4 | 280 | 25.4 | NS |
| SAH | 462 | 40.3 | 121 | 16.5 | 0.000 | 272 | 27.9 | 59 | 16.9 | NS |
| Diffuse | 307 | 41.7 | 276 | 28.3 | 0.001 | 162 | 29.6 | 169 | 22.5 | NS |
| Focal, thick | 152 | 47.4 | 431 | 31.1 | 0.000 | 91 | 38.5 | 240 | 21.2 | 0.002 |
| Focal, thin | 97 | 36.1 | 486 | 35.2 | NS | 72 | 19.4 | 259 | 27.8 | NS |

[a] From Adams, H. P., Kassell, N. F., Torner, J. C., and Haley, E. C. (1987). Predicting cerebral ischemia after aneurysmal subarachnoid hemorrhage: Influences of clinical condition, CT results, and antifibrinolytic therapy. *Neurology* **37**, 1586–1591.
[b] NS, not significant.

($p < 0.05$). The risk seemed to be greatest for patients who were admitted with normal systolic blood pressure. Perhaps the antifibrinolytics increased the dose of vasoconstrictor agonist by preserving the clot and the antihypertensive blunted the homeostatic hypertensive response that would have countered the angiographically evident VSP (45).

An apparently definitive randomized, double-blind, placebo-controlled trial of antifibrinolytic therapy in SAH found that, compared with placebo-treated patients, tranexamic acid-treated patients had an increased incidence of DID (24 vs 15%, respectively). Bleeding occurred in 24% of control and 9% of treated patients. There was no significant difference in outcome between the groups at 3 months (53).

### F. Age and Sex

Between 1982 and 1986, in a study of 25 patients aged 65 years or older and 118 younger patients, all had early surgery performed The clinical manifestations of VSP were evaluated at 3 months post-SAH and classified as transient or permanent. When classified by age there were no differences in the extent of SAH, the incidence of VSP on angiography, or DID. However, the incidence of permanent DID was significantly higher in the older group (82% of all patients with DID 30.2% in the younger group) (54). Inagawa found no difference in the incidence of angiographic VSP and DID between 76 patients with SAH who were younger than 60 years and 69 patients older than 60 years (55). On the other hand, several investigators found that elderly patients developed slightly but significantly less VSP than younger patients, although they tolerate ischemia less well and were more prone to develop infarction (54,56).

In a contemporary German series treated with early surgery and intravenous nimodipine, the incidence of asymptomatic and symptomatic VSP post-SAH significantly decreased after the age of 60 years. The clinical grade on admission and concomitant systemic diseases were much more commonly related to DID and poor outcomes in elderly patients than angiographic VSP (57). One hundred and twenty-nine patients over age 70 who were admitted between 1972 and 1992 were studied. Good recovery increased from 38 to 43% in grades I–II and from 0 to 23% in grade III, respectively, in the early and later times of this study. Postoperative symptomatic VSP continued to be a major cause of mortality and morbidity (58). One hundred and forty-two patients had surgery within 3 days post-SAH. Forty-two percent of patients over 65 years of age deteriorated. Angiographic VSP established by measurement negatively correlated with age. In patients over age 65 the severity of VSP was not related to the reversibility of symptoms or the outcome. At the time of clinical deterioration, associated systemic complications such as cardiac failure, hypoxia, and electrolyte imbalance occurred in 18% of patients under age 50, 38% of patients between age 50 and 64, and 50% of patients over age 65. It was suggested that since the etiology of postoperative deterioration—even when

angiographic VSP is present—is multifactorial, particular care should be taken in elderly patients before using hypervolemic therapy because of associated cardiorespiratory problems (59).

Advanced age is a prognostic indicator for poor outcome. Nine hundred and six patients in the nicardipine trial were analyzed after being categorized into five different age groups. Twenty-three percent of patients were older than 60 years. Age was related to the presence of thicker subarachnoid clot on admission CT scans. Hypertension was among the preexisting medical conditions that increased significantly with age. The incidence of life-threatening complications increased with advancing age. No age-related differences were found in the overall incidence of VSP, although symptomatic VSP was more frequently reported in older age groups ($p = 0.01$). Overall outcome using the Glasgow outcome scale was significantly poorer after the age of 60 years (60).

Of 1076 patients with aneurysmal SAH, 674 were females. Angiographically demonstrable VSP was significantly more common in females ($p < 0.05$). A significantly poorer outcome ($p < 0.05$), despite the higher rate of internal carotid artery aneurysms, which have a significantly better prognosis compared to aneurysms at other sites, occurred in females (61).

### G. Smoking

Twenty-one premenopausal women with coronary artery VSP were investigated in a case control study. Only cigarette smoking was significantly more prevalent among the coronary spasm cases. Among the affected cases 62% were smokers, and in the controls only 18% were smokers. These figures were remarkably similar to those obtained for cerebral VSP (62). Various factors were analyzed using multivariate analysis for patients with SAH. A significant association was demonstrated between symptomatic VSP and cigarette smoking (odds ratio 4.7, 95% CI 2.4–8.9, $p = 0.03$). Cigarette smoking increased the risk of symtomatic VSP after aneurysmal SAH (63). Nearly 3500 patients enrolled in five different prospective studies organized by the Neuroclinical Trials Center of the University of Virginia were analyzed with respect to smoking. Of 1346 nonsmokers, 49.3% had angiographic VSP compared to 54.2% of the 2090 smokers. This gave an odds ratio of 1.22 , 95% confidence interval of 1.07–1.40, and a $p$ value = 0.005 (64).

### H. Physiological Parameters

CBF was measured in the first week post-SAH for 46 good grade patients. The mean initial CBF in patients who did not subsequently develop cerebral ischemia was 49 ml/min/100 g, which was higher than the figure of 42 for patients who subsequently developed ischemia. Patients with a CBF <50 ml/min/100 g and diffuse SAH on initial CT scan had a very high incidence (78%) of DID despite having a good clinical grade at the time of the initial CBF measurement (65). Twelve patients had aneurysm clipping on the day of admission, at which time they had Fisher grade III SAH CT scans. All patients had serial Xe CT – CBF studies postoperatively. Angiographic VSP developed in all patients. DID developed in 4. The critical level for hemispheric CBF associated with neurological deficits from VSP was about 20 ml/100 g/min. Therapeutically induced hypertension improved the neurologic deficits due to VSP in 4 patients but in 1 patient aphasia persisted. In 8 patients without symptomatic VSP, studied at four successive time intervals, the hemispheric CBF ranged between a low of 32 and a high of 41 on the operated side. During induced hypertension the corresponding flows rose to between 33 and 45. In the patients who developed symptomatic VSP the flow on the craniotomy side ranged between 28 and 40 and with induced hypertension rose to between 34 and 43. The flows were also reduced on the contralateral side, ranging between 32 and 38 before hypertension and 36–42 following hypertension (66).

Eight patients were studied pre- and postoperatively by position emission tomography (PET) scan. There was a significant increase in mean OEF values for all eight patients but not a significant change in CBF, $CMRO_2$ or cerebral blood volume (CBV). The increase in OEF was caused by a decrease in $O_2$ content which was due to a postoperative decrease in Hb. The OEF increase was not thought to be a direct effect of craniotomy. In two patients there was an increase in postoperative rCBF and $rCMRO_2$ in areas subject to retraction. It was concluded that the regional effect of craniotomy on cerebral circulation and metabolism was not marked in cases operated with microsurgical technique (67).

The central conducting time was studied in 67 patients with SAH. These values were measured on admission and 1day postoperatively. Central conducting time on admission was not predictive for the development of VSP. Increased central conducting time and interhemispheric differences at the time of admission indicated a worse prognosis. An increase in the actual central conducting time was statistically significant only when severe grades of VSP, as measured by TCD, were present (6.7 msec and MCA velocity 200 cm/sec) (68).

### I. Hydrocephalus

Of 87 patients studied post-SAH, 74% showed angiographic VSP on an angiogram done within 30 days

post-SAH. Sixty-two percent of the patients had both Hyc and VSP and 22% had neither. Thirteen percent had VSP but no hydrocephalus. Hyc and VSP were significantly associated ($p < 0.01$). Presumably both phenomena relate to the volume of the initial SAH (47). Forty-seven Fisher grade I and II patients had an incidence of acute Hyc, delayed VSP, and chronic Hyc of 29, 7, and 14%. In the Fisher III patients, the percentages were 70, 64, and 58% ($p < 0.01$), respectively. About one-third of the patients showed both acute Hyc and acute VSP. These three features were significantly associated (69).

## VI. Factors Unrelated to Vasospasm

There is no documented relationship between atherosclerosis, diabetes mellitus, hypertension, sex, and VSP. There was a good correlation between the extent of SAH and the development of VSP in 50 patients. Analyzed with respect to the influence of the amount of nonionic contrast medium on postoperative VSP and outcome, the volume of contrast medium did not seem to exercise any influence (70). Data from 3521 SAH patients studied in the cooperative aneurysm study between 1980 and 1983 showed that 685 developed some grade of VSP as defined symptomatically or angiographically. This was a 19% total incidence of VSP. Cyclic analysis demonstrated a strong seasonal occurrence for the incidence of SAH with a peak in February in the Northern Hemisphere. Cerebral VSP incidence, after controlling for SAH occurrence, exhibited only a small peak to trough ratio of 1.15 relative to the larger 1.74 ratio seen with unadjusted VSP data. VSP was therefore unrelated to seasonal or latitudinal variation (71). Of 150 patients with SAH, 22 had diabetes mellitus. The preexistence of this condition did not significantly influence the outcome when examined in conjunction with other chronic diseases and epidemiology factors (72).

## VII. Effect of Vasospasm on Outcome

Two hundred and sixty-five aneurysm patients were operated at a mean age of 47 years. They were treated in the mid-1970s usually by delayed operation. The postoperative mortality rates were 8% in patients who had no VSP, 12% in those with localized VSP, and 40% in those with generalized VSP (11,12). Seven of eight series reviewed in the 1980s demonstrated a higher mortality when VSP was present than when it was absent (8). Taking all cases and not adjusting for other known prognostic factors, death occurs in 18% of SAH patients without VSP and 42% with VSP. Without treatment it was estimated that one-third of patients who develop delayed ischemia die, one-third have a permanent deficit, and one-third recover (48). In an exhaustive review of the clinical literature on VSP, between 1960 and 1992, 1100 sources provided 60,000 patients. About one-third of patients with SAH from aneurysms developed DID; of these, about one-third died. Therefore, VSP was responsible for the death of 1 patient in every 10. In addition, about the same number were left permanently disabled. No consistent trend in the incidence of vasospastic ischemia was evident over time. When angiography was carried out between days 4 and 11 after SAH, two-thirds of patients showed VSP (73). Twenty-five studies were analyzed with respect to the influence of VSP on death rates: When VSP was present it was 1067/3483 or 31%, and when no VSP was present it was 1015/6096 or 17%. The common odds ratio (95% CI) = 3.28 (2.94–3.66). It is therefore beyond a shadow of a doubt that VSP is a factor tending to increase the death rate (34). Twenty-one studies gave a good outcome in 3515/5019 cases or 70% of those with no VSP. On the other hand, only 44% (1051/2368) of patients with VSP had a good outcome. For patient without VSP the common odds ratio (95% CI) = 3.05 (2.73–3.40) for a good outcome. One hundred and six reports showed a death rate of 1009/3277 (30%), permanent deficits in 1132/3327 (34%), and good outcome in 1186/3327 (36%) when VSP was diagnosed. There has been a trend towards lower mortality rates from VSP between the 1970s and early 1990s.

An idea of the natural history of VSP and DID can be gained by examining the placebo groups in recent prospective studies. In one study of 97 initially poor-grade patients who had repeat angiography carried out on day 7, the incidence of VSP was 95%; 56% had DID and 53% had infarction from VSP. The mortality rate in these initially poor-grade cases was 39%. VSP was considered to be the primary cause of death in one-third of cases and a contributing factor in more than half (74). Among 3521 patients entered into a prospective study within 3 days of aneurysmal SAH, VSP caused death in 7.2% and disability in 6.3%. The initial effect of the hemorrhage caused death in 7% and disability in 3.6%, rebleeding caused death in 6.7% and disability in 0.8%. These patients were treated between 1981 and 1984 (21). In a subgroup analysis of 722 patients with aneurysms in all clinical grades there was a 35% incidence of DID, 13% died or were disabled from VSP, and 5% died of VSP alone (75).

In 531 consecutive cases of ruptured aneurysms, 329 were operated with an operative mortality of 4.3%. The main cause of death in these cases was VSP (64%) and surgical complications. The mortality rate in the nonoperated group was 41%. In this group the causes of

TABLE 2.6 Influencing Factors and Discriminant Functional Analysis[a]

| Standardized canonical discriminant functions (mortality in op group) | | Standardized canonical discriminant functions (morbidity in op group) | |
|---|---|---|---|
| **Function** | **Coefficient** | **Function** | **Coefficient** |
| H&H grade at op | 0.549 | H&H grade at op | 0.606 |
| Symptomatic VSP | 0.339 | Symptomatic VSP | 0.339 |
| Op time | −0.305 | Fisher grade | −0.289 |
| HH grade on admission | 0.241 | Hydrocephalus | −0.267 |
| Hydrocephalus | −0.140 | Hypertension | 0.242 |
| Fisher grade | −0.138 | HH on admission | 0.241 |
| Age | −0.135 | Op time | 0.109 |
| Hypertension | −0.029 | Age | 0.097 |
| Predictability of mortality: 80.63% (81.2% of those who survived, 75.8% of those who died) | | Predictability of morbidity: 79.94% (79.7% of those who recovered well, 85.7% of those who had poor outcome) | |

[a] Data from Lee, S.H., Han, D.H., Yang, H.J., Kim, H.J., Sim, B.S.,and Choi, K.S. (1990). Analysis of morbidity and mortality in subarachnoid hemorrhage. In *Cerebral Vasospasm* (K. Sano, K. Takakura, and N.F. Kassell, Eds.), pp. 55–56. Univ. of Tokyo Press, Tokyo.

death were rebleeding, VSP, and the effects of the initial ictus. Symptomatic VSP was related particularly to Fisher's SAH grade III and Hunt and Hess grade but not particularly to aneurysmal location, age, or hypertension in this series (76) (Table 2.6). In another prospective study of patients who had good neurological grades on admission, 137 control subjects showed a 28% rate of DID, and an even higher rate (50%) showed evidence of infarction on CT scans. Nine percent of patients died; of these, 75% had VSP (46). Between 1989 and 1993, 275 patients were admitted to a Swedish neurosurgical department. Seventy-one percent were admitted within 24 hr post-SAH. Nimodipine was used in 84% of patients and a good neurological recovery occurred in 59%. Mortality was 21% and morbidity 20%. The most common cause of morbidity and mortality was damage from the initial bleed (23%). Morbidity and mortality from DID were less frequent than either surgical complications or rebleeding. Five percent of patients had an unfavorable outcome due to DID. The final outcome was strictly correlated with the initial clinical condition with only 2 of 51 grade V patients making a good recovery. The amount of SAH was also strictly correlated with outcome. Patients with a history of hypertension had no difference in outcome versus normotensive individuals. A posterior circulation aneurysm increased the mortality rate (77).

## VIII. Influence of Surgery on Vasospasm

Heros and associates studied a series of 86 patients with ruptured aneurysms, in which there were 11 preoperative deaths. Most of these patients were angiogrammed prior to death, and severe VSP was present in every patient in whom this was done. When autopsy was performed they all showed ischemic infarction. Postoperative ischemia attributable to VSP developed in 14 patients, and 4 of these died. As a result of a search of literature they believe that 30–50% of patients will develop VSP after aneurysm rupture. They place the postoperative incidence of VSP at 40–65%. The incidence of VSP in their experience was not affected by age, hypertension, arteriosclerosis, diabetes, size of aneurysm, location of aneurysm, or the use of intraoperative hypotension (78).

In 1979 Sano and Saito surveyed 443 aneurysms that had been treated between 1971 and 1978. Of the surgically treated patients, 82% were leading useful social lives. There was a 5% postoperative mortality rate. Patients operated on the day of SAH or in the next 2 days showed better results, with lower morbidity and mortality rate from VSP. Fatal postoperative VSP occurred most commonly in patients operated on between days 4 and 7 after SAH. Sano and Saito recommended continuous cisternal, ventricular, and epidural drainage following the clipping of aneurysms in the acute stage. Only 15% of their 443 patients developed postoperative VSP. VSP lasted 8–24 days, with a mean duration of 14 days. Of patients with VSP, 46% recovered and were working. Thirty patients had operations carried out while VSP was present. Half of these were operated on an average of 4.5 days post-SAH, and 60% of these were well and working; the other half were operated on an average of 7.4 days following SAH, and 80% were well and working. Operative mortality and

morbidity were closely related to the admission grade of the patient. Postoperative VSP that was symptomatic had an onset a mean of 6.8 ± 1.7 days from SAH, whereas preoperative symptoms from VSP had an onset 7.7 ± 2.6 days from SAH. The range of onset was 4–16 days for both groups. Of 68 patients with preoperative VSP, 52% did well, 24% had a fair result, and 25% died. Of 27 patients with postoperative VSP, 23% died. The authors recommended operating on patients with angiographic VSP as soon as their level of consciousness began to improve, which was usually a week or more from the onset of symptoms. This was crucial evidence that early operation per se did not induce VSP (79).

Takemae and colleagues studied the relationship between VSP and high density on CT scan. High-density subarachnoid clot was seen in 77% of cases scanned within 4 days of SAH. It disappeared on average between 4 and 22 days post-SAH in 10 patients that were managed conservatively. VSP developed between 5 and 14 days in 83% of those patients showing high-density blood in the basal cisterns and in 78% of those with high densities in the Sylvian fissure. No VSP developed in patients without such high density on the CT scan. These authors considered that such cisternal clot was essential for the development of VSP. Early operation was recommended in cases with such high densities within 4 days of SAH in order to remove as much arachnoid clot as possible and thereby prevent or minimize the development of VSP (38).

Hunt found that the incidence and severity of VSP in the first 3 weeks post-SAH were not statistically related to the presence or absence of operative intervention. Patients experiencing VSP between days 4 and 21 post-SAH comprised 68% of the unoperated cases and 67% of the cases operated on in the first week. All of these patients were either grades I or II on admission (80). He wrote;

"We still do not operate in the face of severe vasospasm or neurological deficit.... Nevertheless, more and more patients are being operated on early in that 'window in time' when spasm, edema, and infarction have not yet occurred."

Ljunggren and coworkers cared for 219 consecutively treated patients of whom 54% made a good recovery and 31% died. Of the 53 nonsurgical patients, only 11% made a good recovery and 70% died. Emergency evacuation of significant ICH was performed in 30 patients: 30% made a good recovery and 50% died. Of 81 patients who were grades I–III and had operation within 48–60 hr after SAH, 74% made a good recovery and 10% died within a month of surgery. Twenty-one percent of those operated on had an immediate uneventful postoperative course, with the delayed onset of ischemia 4–13 days after SAH. The mortality rate was 10% [approximately half the patients who developed VSP died of ischemia (81)].

The results of early operation for aneurysms in 45 cases were analyzed by Takahashi and coworkers. Of 12 patients operated on within 24 hr after SAH, 92% showed a good result and 8% a fair result; there was no mortality, and VSP occurred in 33%. Of 13 patients operated on between days 2 and 7 post-SAH, results were 46% good and 15% poor, with a 38% mortality rate. Fifteen percent developed VSP. Of patients operated on between days 8 and 14, 100% had a good result and 7% developed VSP. In cases in which there was no blood on the preoperative CT scan, there was a 9% postoperative mortality rate. When blood was seen adjacent to the ruptured aneurysm, the postoperative mortality rate was 17%. In 11 patients in whom there was significant cisternal blood, there was a 0% postoperative mortality, but 50% of the patients developed transient neurologic deficits (82).

One hundred consecutive ruptured aneurysm patients were admitted between 1983 and 1986 to a German unit to be operated upon by six neurosurgeons. Microsurgical techniques were employed. In 5 patients a parent or perforating artery was known to be occluded intraoperatively. Premature aneurysm rupture occurred in 30 patients. Twenty-eight percent of the patients were in neurological grades IV and V and 71% were in Fisher CT grade III. Ninety-six percent were operated within 48 hr. The subarachnoid cisterns were rinsed intraoperatively with $2.5 \times 10^{-5} M$ nimodipine solution. All patients were subsequently given this drug 2 or 3 mg/hr postoperatively for 2 weeks. Fifteen patients had a primary deterioration immediately postoperatively. CT scan revealed infarctions in 23%, the infarction was already visible in the earliest postoperative CT scans in 17. Nine infarcts were locate in the basal ganglia and were asymptomatic in 7 cases. Seven patients had hemispheric lesions, which were symptomatic in 5. Interestingly, in only 1 case was VSP alone considered responsible for the CT-visualized infarction. Twelve patients showed a later secondary deterioration and 4 of these were attributed to VSP, but this was characterized by only a transient mental deterioration. In 4 additional cases VSP and other adverse events such as hypotension and postoperative hematoma combined to cause a permanent deficit in 1 case. Of 10 patients treated with hypertension postoperatively for the occurrence of DID, 7 recovered completely. The overall management morbidity and mortality in this group of 100 patients was 18%. Only 2 patients had fatal DID, but in 1 case this was due to a secondary occlusion of basilar artery branches on day 5 post-SAH and in the other it was due to decompensated VSP after an episode of long-lasting severe systemic hypotension (83). This important series emphasized that delayed neurological symptoms are not necessarily the consequence of symptomatic VSP. Two-thirds of their patients with DID had VSP plus

**TABLE 2.7 Timing of Aneurysm Surgery—Planned Surgical Interval and Relative Significance of Focal Ischemic Deficits[a]**

| | Planned surgical interval (days from subarachnoid hemorrhage) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0–3 | 4–6 | 7–10 | 11–14 | 15–32 | Total | Significance |
| Complications | | | | | | | |
| Focal ischemic deficits (%) | 27 | 29 | 32 | 31 | 33[b] | 29[c] | $p = 0.43$ |
| Hydrocephalus (%) | 13 | 7 | 11 | 15 | 26[b] | 13 | $p < 0.001$ |
| Brain swelling (%) | 12[b] | 12[b] | 10 | 10 | 5 | 11 | $p = 0.011$ |
| Rebleeding (%) | 6 | 9 | 13 | 14 | 22[b] | 10 | $p < 0.001$ |
| Hematomas (%) | 10 | 9 | 9 | 10 | 13[b] | 10 | NS[d] |
| Iatrogenic arterial occlusion (%) | 2[b] | 2[b] | 1 | 0 | 2[b] | 2 | NS |
| Number of cases | 1595 | 372 | 623 | 433 | 247 | | |

[a] Data from Kassel, N. F., Torner, J. C., Haley, E. C., Jr., *et al.* (1990). The International Cooperative Study on the Timing of Aneurysm Surgery: II. Surgical results. *J. Neurosurg.* **47**, 37–47.
[b] Planned surgical interval associates with highest % of this complication.
[c] Most common complication.
[d] NS, not significant.

other causes, such as edema, preexisting infarctions, and complications unrelated to VSP.

The International Cooperative Study in the timing of aneurysm surgery found that focal ischemic deficits did not occur in any particular planned surgical interval. In contrast, rebleeding and hydrocephalus occurred more commonly when delayed surgery was planned and brain swelling occurred when very early surgery was planned (84) (Table 2.7). The time to onset of DID tended to be earlier in cases having earlier actual surgery. Deficits began in the days 0–3 period in 8% of cases compared to 35% in the days 7–10 interval. There was also a tendency for the deficits to have their onset in the time interval in which surgery was actually performed (85) (Table 2.8). During this study information was obtained on the efficacy of early surgery for clot removal. Analysis of postoperative CT scans in such patients found that 54% still had thick or diffuse SAH blood remaining postoperatively which was related to high rates of ischemic deficits. In this study, performed in the early 1980s, cumulative focal ischemic deficit rate was 30% in the 2 weeks following SAH. Eighty percent of the events were attributable to VSP, but other causes included Hyc, brain swelling, hypotension, and operative complications. The incidence of DID did not vary significantly by actual surgical interval in the first 2 weeks. Preoperative and postoperative CT scans done within 3 days of surgery were obtained on 592 patients operated in the first 3 days post-SAH. A shift in

**TABLE 2.8 Time of Onset of Vasospasm—Related Deficits by Surgical Interval[a]**

| | | Time to deficit (days) | | | | | |
|---|---|---|---|---|---|---|---|
| Surgery interval (days) | N | 0–3 | 4–6 | 7–10 | 11–14 | 15+ | Total |
| *Actual* | | | | | | | |
| 0–3 | 1478 | 3.1%[b] | 5.8% | 7.0% | 1.8% | 0.3% | 18.0% |
| 4–6 | 353 | 0.0% | 7.4%[b] | 8.8% | 1.7% | 0.3% | 18.2% |
| 7–10 | 367 | 1.4% | 1.1% | 7.6% | 4.1% | 0.8% | 15.0% |
| 11–14 | 269 | 0.4% | 1.9% | 2.6% | 6.7%[b] | 1.5% | 13.1% |
| 15+ | 455 | 3.1%[b] | 6.2% | 9.2%[b] | 4.2% | 2.6%[b] | 25.3%[c] |
| Total | 2922 | 8.0% | 22.4% | 35.2%[d] | 18.5% | 5.5% | |

[a] Data from Torner, J. C., Kassell, N. F., and Haley, E. C. (1990). The timing of surgery and vasospasm. *Nuerosurg. Clin. North Am.* **1**, 335–347.
[b] Highest percentage of VSP-related deficits by actual surgery interval occurring in this time interval to deficit onset.
[c] Actual surgical interval with highest percentage of VSP-related deficits.
[d] Most common time interval for onset of VSP-related deficit.

the distribution of CT SAH presence and thickness was observed. Postoperatively, about one-third of the patients had no SAH evident and one-fourth showed only a thin layer. A significantly lower rate of ischemic deficits was observed for patients with only thin or no SAH remaining. After early surgery 46% had reduced clot densities compared to thick clots observed at admission. Those patients with thick and diffuse clots remaining had a 50% chance of developing focal ischemic deficits attributable to VSP (86).

The effect of early surgery removal of clot on the prevention of DID was evaluated in 82 patients with preoperative symmetrical blood clot on CT scans. Thirty-three percent of these patients developed DID on the operated side and 12% on the nonoperated side. CBF was significantly decreased in the hemisphere on the operated side compared to the nonoperated side. DID developed in 69% of those with preoperative thick layer clot compared to only 31% of those with thin layer clots. Neurological grades II–IV patients developed DID in 59% of cases compared to 41% in those in better neurological grades. Of patients with thick layer clots who had successful reduction in clot by surgery, 46% developed DID on the operated side and 17% on the nonoperated side. Of those in whom significant thick layer clot remained, 56% developed DID on the operated side and 25% on the nonoperated side. This suggested that there was some efficacy in the clot reduction at operation. On the other hand, for patients whose preoperative CT scans showed only a diffuse thin sheet of blood there was no difference in the incidence of DID between those in whom clot was cleared and those in whom it was not (17% in both). The operated side showed a lower mean hemispheric CBF but the differences were not impressive ($52.8 \pm 2.7$ vs $55.5 \pm 3.3$ ml/100g/min) (87).

Between 1981 and 1988, 330 SAH patients had early surgery via the pterional approach within 4 days post-SAH. Of this group of aneurysms there were 13 AComA aneurysms subjected to early surgery and 10 were observed during the natural course. There were no right and left differences in the amount of subarachnoid clot in the preoperative CT scans. Symptomatic VSP was defined as hemiparesis with a reduction in angiographic diameter of major trunks to less than two-thirds of their admission diameters. The 10 patients subject to observation only developed a right-sided focus of VSP in 6 hemispheres and a left-sided focus in 5. In the early operation group VSP developed in 12 hemispheres ipsilateral to the pterional approach and only 2 hemispheres contralateral to the operative approach (88).

One hundred and fifty patients with aneurysmal SAH were divided into those operated in the first 3 days ($n = 116$) or after day 20 or not operated at all ($n = 34$). The incidence of angiographic VSP was not different between the two groups—95% for early surgery and 88% for delayed or no surgery. The incidence of DID in those operated early was only 18% compared to 44% for the patients treated with delayed surgery. Patients in better neurological condition who were operated early (grades II and III) developed symptomatic VSP in 13% and low-density areas on CT in 10%. These rates were significantly lower than those for patients in similar grades who were operated late or not at all (50 and 36%, respectively). In patients in poor neurological condition (grade IV) the timing of operation did not affect the incidence of either DID or CT evidence of infarction (89).

The effect of clot removal on VSP was studied on 104 patients who had surgery within the first 3 days, were neurological grades I–IV, had CT evidence of SAH only, and had angiograms done before or on day 2 and between days 7 and 9. The relationship of interhemispheric fissure SAH on CT and angiographic VSP in the distal anterior cerebral artery was studied as well as the relationship of Sylvian stem SAH and angiographic VSP in the proximal MCA. Both the pre- and postoperative SAH grades correlated with the subsequent development of low-density areas in the territories of the appropriate arteries. The reduction in cisternal blood assessed by CT scan did not relate directly to the reduction in VSP. For AComA postoperative DID occurred in 25% of cases operated by the interhemispheric route compared to 50% of cases operated via the pterional one (90). This suggested that the clot removal had been more complete by the interhemispheric approach, thereby reducing VSP in the vessels at greatest risk. There was no significant difference in the severity of VSP in the anterior cerebral artery territory between the side of approach and the opposite side so that these investigators concluded that the effect of clot removal was not very significant.

The timing of aneurysm study suggested an overall DID rate in the first 2 weeks post-SAH of 30%. This was attributed to VSP in approximately 80% of cases. The rate of DID was related to the density of blood seen on the initial CT scan. In this study focal ischemic deficits were diagnosed on the basis of clinical criteria. The etiology was verified by angiography in 45% of cases, CT scan in 58%, neurologic status change in 88%, and other tests in 5%. The time of onset of DID was analyzed by the surgical interval. For each surgical time interval, this was the most common time in which all focal ischemic deficits began. The actual surgical time span was also the interval in which VSP-related deficits most commonly had their origin. This is certainly consistent with the possibility that the surgical insult is a contributing factor to "VSP"-induced DID. Torner and colleagues concluded that the relationship of time of surgery and VSP is influenced by

time course of VSP, the effectiveness of surgical clot removal, and the choice of medical management to prevent rebleeding and treat VSP (91).

In 295 patients managed between 1986 and 1988 in Glasgow, surgical mortality rate was only 4%. Seven percent of the patients had unruptured aneurysms and only 9% were in neurological grades IV or V. Seventy-one percent were admitted within 2 days of SAH; however, 42% of the patients were operated after the 10th day post-SAH. Of 14 variables tested against outcome, 4 were significantly associated with a poor outcome: grades III–V, ICH on admission CT, development of DID, and postoperative hematoma requiring surgery. Twenty-three percent of the 275 patients with SAH developed angiographic VSP; of this group, 78% had a favorable outcome and 21% died. The posterior circulation site for an aneurysm, preoperative hypoxia, and Hyc requiring shunting were also associated with poor outcome, but the strength of the association failed to reach significance. When VSP was associated with a hematoma ($n = 21$) only 62% had a favorable outcome compared to the 86% favorable outcomes in the 42 patients with VSP but no hematoma (92).

Fifty-six patients having surgery at different times post-SAH with pre- and postoperative angiography had a multiple regression analysis performed using arterial diameters during VSP as the dependent variable and prognostic factors for VSP, such as time when surgery was performed, as independent variables. An image analyzer measured intra- and extracranial arterial diameters from 56 patients whose 108 angiograms included 187 different carotid angiograms. A ratio of the intra- to extracranial carotid artery diameters was used to quantify the degree of VSP and to correct for differences in magnification between studies. The variables that predicted angiographic VSP were age, poor clinical grade, and preoperative arterial ratio. Poor outcome was not predicted by any of these. Interestingly, angiographic VSP was not predicted in this study by the Fisher grading scale. Despite the fact that the 34 patients operated on in the first 3 days had worse risk factors (38% clinical grades III and IV and 62% Fisher grade III), only 15% showed infarction due to VSP and only 12% had a bad outcome. For the 20 patients operated on between days 4 and 12, only 10% were clinical grades III and IV and only 25% showed Fisher grade grade III, but 20% showed infarction due to VSP and 15% had a bad outcome (93).

Patients developing ischemia post-SAH can be divided into those who have early surgery and awaken from anesthesia with a new deficit and those who develop "true" delayed ischemia. In a British series there was a excess of preexisting vascular disease in the postoperative ischemic patients (94). There is a tendency for patients operated on during the time of VSP (days 4–12 post-SAH) to have a higher risk of developing DID (56). However, a multivariate statistical analysis of the factors predisposing to the development of VSP did not find that the time of surgery affected the development of VSP (56). Current understanding of the etiology and pathogenesis of VSP suggests that surgical manipulation of arteries should have minimal effect on the severity of VSP and the increased risk of cerebral ischemia in patients operated on during VSP is due to other factors.

In 1990, 64 patients were operated on in the first 3 days post-SAH by a German group. Forty-two patients were not treated with the assistance of temporary clipping and 22 were treated. Mean temporary clipping time was 5.4 min (range, 2–18 min). The percentage of patients in each group showing increased velocity by TCD was not significantly different: no temporary clip, 45%; temporary clip, 41%. The incidence of DID was less in the group not treated with temporary clipping (−36 vs 45% with temporary clipping). Bad outcomes occurred in 31% of the patients not treated with temporary clipping and 43% of those treated with it. The postoperative complication rates (excluding DID) were 28 and 23% and the death rates were 7 and 5%, respectively. One could interpret these results as indicating that temporary clipping might have a slightly adverse influence on VSP but that it might be used in more difficult aneurysms. Observations during surgery indicate that manipulation of arteries and temporary clipping may precipitate acute vessel spasm. This spasm, however, is short lived and probably does not contribute significantly to DID. Manipulation of vasospastic monkey cerebral arteries did not increase VSP 24 hr later (95).

Study of TCD ultrasound velocities on patients operated at different times after SAH suggested that surgery had no effect on the development or severity of VSP (97). The distinction between preoperative and postoperative VSP is unimportant; however, the time of surgery in relation to SAH is important. The natural history of VSP, and not any effect of surgery, determines the degree of arterial narrowing.

PET studies were performed on four patients before and after right frontal craniotomies for clipping of ruptured anterior circulation aneurysms. The preoperative studies were conducted the day before surgery and postoperative studies were conducted on days 6–17 postoperative. No patient had Hyc or ICH. There was a 45% reduction in $rCMRO_2$ (1.87–1.04 ml/100g/min) and a 32% reduction in rOEF (0.41–0.28) in the region of retraction but no change in the opposite hemisphere. rCBF was unchanged in all regions. The reduction in $CMRO_2$ and the OEF indicated a primary reduction in metabolism and uncoupling of flow and metabolism (luxury perfusion) (98).

Twenty patients who underwent selective amygdalo hippocampectomy for epilepsy using the transsylvian approach were studied with serial TCD. Seventy percent of these cases showed ipsilateral /bilateral increase in TCD velocities by more than 50% from the baseline. These velocity changes were not associated with any morbidity or mortality. It was assumed that the transsylvian approach is associated with significant hemodynamic change (99).

Seven patients had surgery performed within 9–29 hr post-SAH using the standard pterional approach. A meticulous attempt to remove clot was performed. Comparisons were made between the hemispheres with surgical intervention and those without, in terms of the incidence of DID and cerebral infarction on CT scans, degree of angiographic VSP, and CBF. This was a relatively small series of patients and long-term postoperative CT evaluation was not documented so that it is conceivable that a trend in favor of clot removal producing less VSP may have been missed (100). Angiographic VSP was quantified by measuring the ratio of diameters of intracranial arteries to extracranial arteries. A significant reduction in CBF was observed during the early postoperative period in the basal frontal lobe of the surgical side. This CBF reduction was thought to correspond to the region of brain retraction. It was concluded that the effect of clot removal could be offset by the negative effect of early surgery since early surgery with clot removal seemed to have little effect on the course of chronic VSP. Critical aspects of this study were the selection of patients with midline aneurysms and the use of a quantitative method of assessing the symmetrical distribution of SAH. Anterior communicating aneurysms probably require greater retraction than aneurysms at other sites in the anterior circulation when the pterional approach is used. The study did not support extensive clot removal during early surgery at the expense of potential damage to vital structures (100).

Thirty-two patients with ruptured AComA aneurysms had CT scans within 2 days post-SAH and unilateral pterional approaches within 3 days post-SAH. The patients showed bilaterally symmetrical clot without ICH. There were no postoperative complications. CBF studies were performed by SPECT with $^{123}$I-IMP. Postoperative regional hypoperfusion due to brain retraction was frequently recognized in SPECT scans without the development of infarction. rCBF showed a continuous fall during the first 4 weeks postictus before rising. The rCBF in the vicinity of the surgical approach was significantly lower, particularly in the postoperative period of 3–7 days. There was a significant association between the decrease of cisternal blood in the interhemispheric systems and cistern of the lamina terminalis but not in the Sylvian fissure or distal insular cisterns after surgery and the degree of local VSP and local fall in CBF during the VSP time interval. This was evidence for the effectiveness of direct clot removal by early surgery, although it was suggested that the beneficial effect of clot removal could be masked by the adverse effect of brain retraction (101).

## IX. Relative Significance of Vasospasm

The influence of VSP on mortality is not as strong as the apparent influence of the initial neurologic grade reflecting as it does the severity of the pathophysiological insult to the brain during the hemorrhage. It is also of lesser importance than severe hypertension. It does not show up as an important independent predictor of mortality in the presence of ICH; presumably in this circumstance most of the blood goes into their brain rather than into the subarachnoid space. It is associated with poor outcome in IVH cases but less so than acute ventricular dilatation and clot volume.

Prognostic factors for rebleeding in patients with surgically untreated PComA aneurysms were assessed by Richardson and colleagues in 1966 (102). Their patients all had a single PComA aneurysm that ruptured at least once in the 8 weeks preceding admission. Patients were then treated with 6 weeks of bed rest and progressively mobilized to be observed for between 6 and 18 months. Rebleeding episodes were verified by the history, lumbar puncture, and autopsy evidence. Thirteen potential prognostic factors were analyzed. A discriminative function was developed as follows: $z = 1.5 \times (\text{sex}) + 1.25 \times (\text{hematoma}) + 0.8 \times (\text{size}) + 0.7 \times (\text{spasm})$, where sex equals 1 for males and 2 for females, hematoma equals 1 if absent and 2 if present, size equals 1 if 9 mm or less and 2 if 10 mm or greater, spasm equals 1 if absent and 2 if present. When the $z$ value is greater than 6, it was very likely that the patient would rebleed. The overall accuracy of this equation with their natural material was 79%. The presence of VSP increased the chances of rebleeding: 79% rebled when VSP was present and 53% when VSP was absent. This was perhaps the earliest use of a discriminative function equation in neurosurgery.

Between 1968 and 1973, a study of 2-month mortality rates in 135 cases yielded the following hierarchy of associations: neurologic grade, 0.36; grade at surgery, 0.34; preoperative VSP, 0.28; mass lesion, 0.23; hypertension, 0.20; shorter interval to surgery, 0.10; and age, 0.06 (35). Favorable outcomes occurred in approximately 42% of all patients with cerebral VSP compared with 68% of all patients without it. The occurrence of VSP doubled the death rate after SAH (26, 48). The risk of DID was analyzed in 176 patients admitted within 72 hr post-SAH.

It was best predicted by the amount of subarachnoid blood, the amount of intraventricular blood, and whether antifibrinolytic drugs were used, regardless of clinical condition or Hyc. Interestingly, the site of DID was not related to the location of the SAH. DID was diagnosed if there were new focal signs or a decrease in the level of consciousness or both and if CT after admission showed a hypodense lesion compatible with the clinical signs or at least no lesion other than ischemia and infarction that could explain the signs (103).

In an American series of patients studied between 1980 and 1990 using multivariate logistic regression analysis of predictive factors related to symptomatic VSP, the following were significantly related: age under 35, amount of SAH, and clinical grade. By univariate analysis the factors were age under 20, amount of SAH, and clinical grade. When patients were analyzed by either good or bad outcome it was only in the poor outcome group that the amount of SAH and clinical grade correlated with VSP. Factors not related to development of clinical VSP were sex, aneurysm location, incidence of complications, use of calcium channel blockers, time to surgery, length of stay, and outcome (104).

In 295 patients with aneurysms treated in the late 1980s the overall mortality rate was 9%. Not all of these aneurysms had produced SAH. The factors that were significantly associated with poor outcome were poor neurological grade on admission, the presence of a hematoma on the initial CT scan, DID, and the development of postoperative hematomas. Two-thirds of the patients who developed a DID (nearly one-third of whom had recent SAH) made a good recovery (92).

## X. Vasospasm and Cerebral Infarction

VSP produces cerebral ischemia and infarction by hemodynamic mechanisms. There is seldom complete arterial occlusion or embolization. Conventional fluid dynamics and the Hagen–Pouiseulle relationship (that describes the flow of Newtonian fluids through rigid tubes) indicate that resistance to blood flow is related to the length of the stenosis and the viscosity of the blood and inversely related to the radius of the tube to the fourth power. Therefore, the level of CBF to the brain distal to a vasospastic artery, which determines whether infarction will develop, is related to the severity and length of stenosis, blood viscosity, as well as to factors that alter flow proximal to the stenosis, such as blood pressure, cardiac output, and intravascular volume. The oxygen and glucose content of the blood, the extent of collateral and anastamotic flow, preexisting arterial hypoplasias and atherosclerotic narrowings, administration of brain protectants, variations in brain temperature, and therapies for VSP will also influence the development of cerebral infarction. There are numerous other causes for cerebral infarction after SAH and aneurysm surgery, and it is not surprising that it has been difficult to document a direct and constant relationship between VSP and cerebral infarction in every case. In most series, there is a significant correlation between severe VSP and development of infarction in the territory of the spastic artery. Twenty-nine patients dying from aneurysmal rupture without having been treated surgically were examined. A significant relationship between the presence and degree of VSP and ischemic brain damage was found. Although ICH probably increased the risk of infarction associated with VSP, hematomas did not increase the incidence of ischemic brain damage (105). Saito *et al.* found that the CT scan demonstrated infarction in the territory of the vasospastic arteries in about 70% of patients with VSP. DID resulting in death was always found to accompany VSP affecting one entire carotid system and the anterior cerebral artery of the opposite side. When VSP was restricted to one carotid system or both anterior cerebral arteries alone, it was usually associated with only temporary symptoms. Most patients who lose consciousness with the initial subarachnoid hemorrhage show high-density subarachnoid blood (106).

There were initial problems in realizing the connection between infarction and VSP because of confounding effects of surgical intervention and the variable findings of radiological studies. In large autopsy series of patients dying post-SAH, between one-fifth and one-third can be anticipated to show infarction in the territory of distribution of vessels that are encased with very thick clot. In a series of 83 autopsies on aneurysm patients, the incidence of infarction was 19% in those dying in the first 3 days, 48% in patients surviving between 4 and 14 days, and 70% in those dying after that time period (107).

Thirty-seven percent of 176 post-SAH patients developed DID. Hypodense lesions on CT were single focal in 11%, multiple focal in 12%, or diffuse in one or both hemispheres in 4%. Eighteen percent had asymmetric decrease in ventricular size. Of 18 autopsied cases, only 6% had a purely single vascular territory lesion. DID after SAH was thought to be a multivascular of diffuse process in most patients (108).

Of 265 neurological grades I–III cases followed for a mean of 1.4 years after SAH and surgery, the rates of infarction at follow-up in cases not receiving nimodipine were as follows: no or localized clot on the first CT scan, 44%; thin layer, 42%; thick layer, 55%; and severe bleeding, 91%. Thirty-nine percent of the entire nimodipine-treated group had late infarcts versus 56% of the placebo-treated group. A logistical regression analysis of

risk factors predicting cerebral infarction showed severe bleeding in initial CT scan (29.45), thick layer of blood on CT scan (2.60), hypertension (3.06), and acute operation (2.17). Of 78 patients with postoperative moderate or severe VSP, 65% had infarction in the follow-up CT scan. Patients with infarction averaged 46 years of age versus 42 years for patients without. Of those with prior hypertension, 64% developed infarction versus 46% for the normotensive group (46).

## XI. The Incidence of Vasospasm over Time

There was no clear reduction in reported incidence of VSP or DID in the decades from the 1970s to the early 1990s. Dorsch and King's review found an incidence of DID of approximately 40% with a range of approximately 20–60% (34). On the contrary, it was suggested that the

> overall mortality in patients with ruptured aneurysms who reached the hospital has declined from approximately 40% in the 1960s to 25% in the 1970s, to 20% in the 1980s, and to approximately 15% currently.... Vasospasm as a cause of death and disability has decreased from approximately 35% in the 1970s, to 20% in the 1980s, to 10% currently.
>
> —Neil Kassell, Fifth International Conference on Cerebral Vasospasm, 1993.

In one of the large cooperative studies reported in 1993, there were 457 patients in a placebo group composed of all neurological grades. Fifty-one percent had angiographic VSP, 46% developed DID, and 18% died. Four percent of deaths were attributed solely to VSP and 10% to multiple causes including VSP. Most of these patients were managed in the microsurgical era, and contemporary methods for fluid replacement and blood pressure control were used (109).

In a Japanese series, when patients were divided by time period into the first or second half (1981–1989) the overall incidence of VSP was almost identical in the two time periods. However, CT evidence of infarction occurred in 24% of patients in the early time period and only in 15.2% of those in the later one. The tendency toward decreased severity of VSP in the later term was most remarkable in patients whose GCS scores were between 13 and 14. The decreased incidence of VSP producing infarction was believed to be a contributing factor to the decreased mortality and the increased rate of good outcome in the later half (31).

A retrospective 10-year review of 224 good-grade patients with anterior circulating aneurysms studied between 1983 and 1993 showed a progressive increase in the number of favorable outcomes in three successive time periods, from 75 to 87 to 94%. This salutary experience was attributed to improvement in critical care techniques in the management of VSP. Angiographically confirmed VSP was observed in 117 patients (52%). Thirty-nine patients (17%) developed DID. The severity of the SAH demonstrated by preoperative CT did not predict the development of symptomatic VSP, although the admission clinical grade was associated with DID. Angioplasty was performed on 22 symptomatic patients, of whom 96% demonstrated a clinical response with favorable outcome. Only 2 of the 224 patients died of VSP (0.9%). Numerous clinical and radiological characteristics were evaluated for prognostic significance. The amount of SAH on admission initial CT scan showed a significant association with outcome, as did the presence of low-density areas on the postoperative CT scans. However, VSP was not one of the variables to have prognostic significance in multivariate analysis in these good-grade patients. Prophylactic hypervolemia was used throughout this series. Calcium channel blockers were used during the second time period and the routine use of angioplasty for the treatment refractory VSP was introduced in 1989. When angioplasty was not used, 77% of cases with symptomatic VSP achieved a favorable outcome in contrast to 97% when it was used (110). A review from the University of Washington of 159 poor-grade patients was performed. Fifty-four percent of the grade IV patients and 24% of the grade V patients experienced a favorable outcome. Eighty-nine clinical and radiological variables were analyzed with respect to prognosis. After uni- or bivariate analysis VSP was not identified as a significant variable in predicting outcome. Among the 69 poor-grade patients with SAH who died, VSP was the cause of death in only 1 case (0.6%). This unit has been the most aggressive in North America in the early use of angioplasty in the setting of angiographically verified VSP (111).

Five hundred and seventy-one Japanese patients with aneurysmal SAH were studied between 1972 and 1992. Individuals over 70 years of age were excluded. These patients were divided into four groups according to the date of treatment. During the four different time intervals the mortality rate for grade III patients at 6 months post-SAH fell progressively from 28 to 11% as the average time from SAH to surgery fell from 18 to 1.5 days. Patients in neurologic grades I and II had a very low incidence of symptomatic VSP regardless of the treatment era. VSP as a cause of death in neurological grades I–III patients showed no consistent trend ranging from 0 to 100% as a cause of death. As a cause of disability the incidence of VSP in the four time periods was 44, 52, 47, and 38%. The grade III patients appeared to show a progressive reduction in symptomatic and permanent VSP over the last three time periods. A significant number of grade III patients with modified Fisher CT scores of 3.1–3.4 were disabled by VSP. When they were initially treated

conservatively, about one-fourth developed DID. The decrease in the incidence of VSP was attributed by the authors to their regimen of early surgery, cisternal drainage followed by urokinase injection, intravascular volume expansion, and thromboxane $A_2$ synthetase administration (112).

## XII. Vasospasm and Nonaneurysmal Subarachnoid Hemorrhage

Although most cases of cerebral VSP follow aneurysmal SAH, there can be other causes (48) (Table 2.9).

### A. Nonaneurysmal Subarachnoid Hemorrhage

Fifty consecutive patients with nonaneurysmal SAH were analyzed. Twenty-three patients had blood visible on the CT scan but only 4% developed DID, and all of these patients made a good recovery. Only 4% of these cases were grades IV or V. Only about half of the patients in this series had blood demonstrated on CT scan in the interfrontal or Sylvian fissures or basal cisterns (113). There were probably a few missed aneurysms since 1 patient who initially had blood in the basal cistern died of a rebleed. It is difficult to agree with the conclusion that the mere presence of blood in the subarachnoid space is not a sufficient cause for delayed ischemia. It is probably more correct to state that it is not the sole cause of delayed ischemia. In their review of eight series of SAH of unknown cause followed up between 1 and 13 years the total mortality ranged between 0 and 27% with three-fourths of the series reporting mortalities of under 15% and rebleeding rates under 10%. Other causes of SAH, such as head injury or unknown etiologies, may also be associated with cerebral VSP (48). Giombini *et al.* (114) found angiographic evidence of VSP in 7 (12%) of 58 patients with SAH of unknown cause. In 65 cases of perimesencephalic nonaneurysmal SAH, no patient experienced DID (115).

**TABLE 2.9 Diseases Associated with Cerebral Vasospasm and the Approximate Incidence of Cerebral Vasospasm in the Disease**[a]

| Disease | Angiographic incidence (%) |
|---|---|
| **Associated with SAH** | |
| Ruptured aneurysm | 60 |
| Ruptured AVM | 12 |
| SAH of unknown etiology | 12 |
| Traumatic SAH | <10 |
| Postoperative SAH | Rare |
| **Not associated with SAH** | |
| Unruptured aneurysm | Rare |
| Pituitary adenoma | Rare |
| Other intracranial neoplasms | Rare |
| Bacterial meningitis | Uncommon[b] |
| Tuberculous meningitis | Common[b] |

[a] From Macdonald, R. L., and Weir, B. (1997). Cerebral vasospasm and delayed cerebral ischemia. In *The Practice of Neurosurgery* (G. T. Tindall, P. R. Cooper, and D. L. Barrow, Eds.), Vol. 2. Williams & Wilkins, Baltimore.

[b] Arterial narrowing usually is caused by vasculitis, which is a different process from cerebral vasospasm after aneurysmal SAH.

### B. Arteriovenous Malformations

Arteriovenous malformations (AVMs) are traditionally listed as a cause of SAH. However, Aoki reviewed the CT features of 50 patients with ruptured AVM and found SAH in only 2 cases (4%) (116). Not surprisingly, angiographic VSP complicates only 12% of AVM cases, and DID occurs even less often (117). In a different series VSP was confirmed in 31% of 13 patients with SAH from AVM. In previous reports the incidence of VSP following rupture of an AVM ranged between 8 and 12%. Massive subarachnoid clot was considered to be the key factor in causing post-AVM rupture VSP (118).

### C. Other Causes

Cerebral VSP has been reported after surgery in the basal cisterns for unruptured aneurysms, pituitary adenomas, and various other neoplasms. Most cases were reported during the pre-CT era. It is impossible to exclude the possibility that an unrecognized SAH occurred postoperatively. Purulent tuberculosis and other types of meningitis may be complicated by narrowing of cerebral arteries, although vessel narrowing in infectious conditions is probably caused by inflammatory vasculitis, which is a different histological process than VSP after aneurysmal SAH.

Suwanwela and Suwanwela used angiography for diagnosis in 350 patients with moderate to severe head injuries. Cerebral VSP was detected in nearly 19% of the angiograms performed 1–19 days after the patients sustained head injury and was believed to cause symptoms in some cases. The CSF was bloody in most patients with VSP (119). CT scans show that patients with head injury often have SAH, although thick blood clots in the basal cisterns are less common than after aneurysmal SAH. This infrequency of thick clots accounts for the lower incidence of VSP in patients with head injuries. A TCD study of 30 patients with head injuries found that MCA

velocities increased in 8 patients (27%), usually between Days 4 and 12 after injury (120).

## XIII. Endovascular Coiling and Vasospasm

Sixty-nine patients who were neurological grades I–III underwent occlusion of their aneurysms using Guglielmi coils within 72 hr of rupture. Symptomatic VSP was defined as the onset of neurologic deterioration verified by angiographic or TCD studies. Symptomatic VSP occurred in 23% of 69 patients. Admission grade and amount of blood on the initial CT scan were both associated with the incidence of subsequent VSP. Two of the 69 patients died of VSP (121).

One hundred and fifty-six patients treated within 72hr of SAH by either clipping or coiling were analyzed. Twenty-six percent suffered ischemic infarction and this rate was correlated with poor neurological grades, higher Fisher CT grade, higher MCA TCD velocities, more repeat hemorrhages, the occurrence of DID, and endovascular treatment ($p = 0.02$). While the infarction rate was 38% for endovascular treatment versus 22% for surgery, this may be partly explained by the fact that 67% of the endovascular treatment group had a Fisher grade IV pattern versus only 25% in the surgically treated group. It is therefore unproven (although certainly possible) that the failure to remove subarachnoid clot contributed to the higher infarction rate observed with endovascular treatment. When only CT Fisher grade III and Hunt and Hess grades I–IV cases were analyzed, the difference in infarction incidence between the two treatment groups failed to reach statistical significance (122).

Symptomatic VSP and TCD velocities >120 cm/sec were studied by multivariate analysis of predictors in 244 patients. So defined, VSP occurred in 22% of surgically treated and 17% of endovascularly treated patients. There was no significant differences in frequency of VSP between surgical and endovascular treatments. VSP decreased with age >50 years and poorer neurological grade, and it increased with hyperglycemia occurring in the intensive care unit. Sequella at 6 months post-SAH increased with VSP, poor neurological grade, and treatment complications (123).

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CHAPTER 3

# HEMATOLOGY



## I. Introduction

For chronic VSP to develop, a patient must survive for several days following the deposition over seconds to minutes of somewhere between 1 and 250 cc of arterial blood into the subarachnoid space. Clearly, a fibrin plug must quickly close the rent in the aneurysmal dome to prevent immediate death. In some patients the volume of blood in the subarachnoid space is so small that clotting does not occur. This is evident at early operation when pinkish or reddish cerebrospinal fluid (CSF) is found around the basal arteries without the formation of actual clot, whereas in other patients a thick, tenacious, jam-like reddish-blue clot is evident within hours of the hemorrhage. The clot becomes beefier in consistency and more tenaciously adherent to the vessels which it surrounds during the days following SAH. It is our impression that such cases are particularly susceptible to VSP. The key to the VSP mystery may lie in complex biochemical cascades associated with clotting, disintegration of enmeshed red blood cells (RBCs), and the fibrin lattice, inflammatory responses to the clot, and processes of phagocytosis and repair (Table 3.1).

Whether VSP develops must depend in part on the quantity of spasminogen(s) released on the abluminal side of the involved vessels. The RBC obviously disintegrates in the alien subarachnoid space more quickly than it would in the blood. The mean survival time is reduced to approximately 1 week from 4 months. Passage of intact RBC out of the subarachnoid space in humans is unlikely to occur. Disruption of the RBC membrane may occur because of osmotic forces and/or because of phagocytosis, which is triggered by the loss of protective or the addition of targeting molecules on the RBC surface. It is likely that the cause of VSP is oxy- and/or deoxyHb and/or heme since they are the vasoconstrictors produced in by far the greatest amounts as hemolysis proceeds. There is evidence that these substances can permeate the vascular wall from the adventitial side through to the endothelium. How extracellular Hb causes contraction of the vascular smooth muscle cells is unclear; possibilities include blockage of tonic vasodilating influences, direct opening of $Ca^{2+}$ channels, stimulation of the production of vasoconstrictors such as endothelin, blockage of the production of vasodilators such as prostacyclin, or the production of free radicals. Free iron does not have an obvious role in the genesis of VSP, although evidence exists that iron chelation may prevent chronic VSP. The genesis of VSP is dependent on the presence of RBC and not platelets, leukocytes, or plasma proteins. Increasing fibrinolysis (by using t-PA) dissolves the fibrin scaffolding holding the RBC in close contact with the vessel wall and reduces the degree of VSP; antifibrinolytics (such as ε-amino caproic acid) have the opposite effect. Markers of coagulation and fibrinolysis tend to be present in greater amounts in patients with VSP compared to those without, presumably reflecting greater clot volumes.

Blood clot in the subarachnoid space incites an inflammatory response—headache, fever, nuchal rigidity, systemic leukocytosis, as well as pleocytosis within the CSF. Monocytes in the blood migrate into the CSF, where they adhere to and subsequently engulf and break down the RBC. Increased permeability of the blood–CSF

**TABLE 3.1 Potential Spasmogens Released after SAH and Their Possible Role in Vasospasm[a]**

| Spasmogen or process | Possible role |
|---|---|
| *Erythrocytes and contents* | |
| oxyhemoglobin (deoxyhemoglobin) and breakdown products such as hemin, iron, bilirubin, and globin chains | Vasoconstriction, promote free radical reactions, block NO vasodilation, increase ET release, block perivascular nerve effects, alter eicosanoid release |
| Products of free radical reactions stimulated by hemoglobin oxidation | May cause vasoconstriction |
| Adenosine nucleotides | Vasoconstriction |
| Other cystosolic proteins | Unknown |
| Erythrocyte membranes | Provide lipid for lipid peroxidation, unknown |
| *Platelet contents* | |
| Serotonin | Possible vasoconstriction early after SAH |
| Adenosine nucleotides | Vasoconstriction |
| *Leukocytes and inflammatory mediators* | |
| Leucocytes | Vasoconstriction |
| Eicosanoids | Increased vasoconstriction by prostaglandins and thromboxanes, decreased vasodilation by decreased $PGI_2$ |
| Cytokines (interferons, tumor necrosis factors, interleukins, macrophage-derived cytokines, growth factors, chemokines, monokines) | Increased inflammation, possible vasoactive effects |
| *Products of coagulation cascade* | |
| Fibrin degradation products | Increased vasoconstriction due to other spasmogens |
| Fibrinogen | Unknown |
| Thrombin | Unknown |
| *Other serum proteins* | Unknown |

[a] From Weir, B., Stoodley, M., and Macdonald, R. (1999). Etiology of cerebral vasospasm. *Acta Neurochir.* **72**, 27–42. Copyright © Springer-Verlag GmbH & Co.

barrier may permit the increased passage of immuoglobins into the CSF.

## II. Blood

Blood is an inhomogeneous fluid composed of formed elements suspended in a complex colloidal fluid known as plasma.

### A. Cellular Elements

White blood cells (WBCs) consist of granulocytes, lymphocytes, and monocyte/macrophage cells. The major forms of mature polymorphonuclear granulocytes are neutrophils, eosinophils, and basophils. The various leukocytes are differentiated by their morphology and the staining qualities of their granules, nucleus, and cytoplasm. The types are neutrophils (59%), eosinophils (2.7%), basophils (0.5%), lymphocytes (4%), and monocytes (4%) (1). Blood cells are produced in the bone marrow. In the circulating blood are found monocytes, neutrophils, eosinophils, basophils, reticulocytes, RBC, platelets, and T cell and B cell lymphocytes. When monocytes move from the intravascular space to other tissues they develop into macrophages. The platelets, also known as thrombocytes because they participate in clotting, are fragments of megakaryocytes.

### B. Plasma

Plasma is composed of water (90%), protein (7%), and organic and inorganic solutes (3%) (2). Protein concentrations in plasma are much higher than those within

interstitial fluids such as CSF. This protein separation creates an osmotic force that tends to keep fluid in the circulatory system. Major plasma proteins are albumin, globulin, and fibrinogen. Albumin makes up 55% of plasma proteins, molecular weight (MW) is 69 kDa, and concentrations range between 4 and 6 g/100 ml plasma. Globulins make up 30% of total proteins and are larger, with MW ranging between 80 kDa and 200,000. Plasma concentrations are 1.5–3 g/100 ml plasma. The largest protein molecule is fibrinogen, which makes up 7% of plasma proteins and ranges in weight from 350 to 400 kDa. The plasma concentration is 0.2–0.4 g/100 ml plasma.

Albumin, the small protein, preserves osmotic pressure in the vascular system and is involved in metabolic transport. γ-Globulins are immunoglobulin antibodies. α-Globulins and β-globulins transport metal irons such as iron and copper as well as lipoproteins. Fibrinogen is converted into fibrin during blood clotting. The complement proteins (C1–C9) function in nonspecific host defenses and help initiate inflammation. After blood clots, a yellowish fluid (serum) is expressed that resembles plasma except that it lacks fibrinogen and other clotting factors.

The blood–brain barrier (BBB) normally excludes the high MW serum proteins from the CSF so that levels of coagulation proteins in normal CSF are only 1–5% of what they are in the blood (3, 4). With damage to the BBB, serum proteins including the coagulation cascade components can enter into the CSF in greater than normal concentrations. CSF is usually devoid of plasminogen activator and fibrinolytic activity. The leptomeninges may be a source of plasminogen activators (4). After SAH, some t-PA may enter the CSF from the blood, although it circulates only at very low levels.

## C. Erythrocytes

### *1. Structure*

RBCs are round, anucleate, biconcave cells which stain pink with Wright's or Giemsa stain. RBCs were first described in the seventeenth century. Crystalline Hb was isolated in the mid-nineteenth century. RBCs number approximately $5.2 \times 10^6$ / μl blood in men and 4.6 in women, the Hb content is 15.7 and 13.8 g/dl, and the hematocrit ratio is .46 and .40, respectively. Normal RBCs have a diameter between 7.2 and 7.9 μm and a maximum thickness of 2.6 μm.

The average RBC is estimated to live 4 months and to be subject to a trip of 300 miles thru the circulation. It is able to fulfill its physiological role by virtue of its high tensile strength, flexibility, and concave shape. RBCs possess no organelles. They are filled with the protein Hb. The cytoplasm also contains soluble enzymes capable of both glycolysis and hexosemonophosphate shunt functioning which produce ATP. The cytoskeletal proteins include ankyrin, band 4.1 and band 3 proteins, spectrin, and actin, all of which are involved in maintenance of the shape and pliability of the RBCs. Blood is sometimes grouped based on carbohydrate determinants on the external surface of the RBCs.

The RBC membrane is composed of lipids and proteins. The water content is 721 mg/ml, with the total protein being 371 mg/ml; about 18 mg/ml is made up of non-Hb protein found in enzymes and the cellular stroma. While Hb is an obvious candidate as a spasmogen because of its high concentration, the numerous other components of RBCs possibly play a major or adjunctive role (Tables 3.2 and 3.3).

### *2. Metabolism*

$O_2$-binding transport and delivery do not require active metabolic expenditures by the RBCs. However, energy sources must suffice for RBCs over their 120-day life spans and must also maintain (i) iron in its divalent form ($Fe^{2+}$); (ii) electrolytes in concentrations against gradients imposed by high plasma $Ca^{2+}$ and $Na^+$ and low plasma $K^+$; (iii) sulfhydryl groups of enzymes, Hb, and membranes in an active, reduced form; and (iv) normal cell shape.

RBCs can generate energy as ATP by the anaerobic, glycolytic (Embden–Meyerhoff) pathway as well as produce reducing power as NADH by the same pathway and as NADPH by the hexosemonophosphate shunt. The RBCs must also maintain Hb in the reduced ($Fe^{2+}$) state and maintain osmotic equilibrium despite its high internal concentration of Hb. Since RBCs have no nucleus or ribosomes they cannot synthesize proteins, repair themselves, or divide. Lacking mitochondria, they cannot obtain their energy by aerobic glycolysis (5). Glycogen does not normally accumulate in RBCs because synthesis and utilization are balanced. RBCs require constant access to glucose to maintain energy metabolism. There is a carrier-mediated transport mechanism. Normally, 90% of glucose is metabolized anaerobically and 10% by the aerobic pathway. The important products of anaerobic glycolysis are NADH, ATP, and 2,3-BPG. In early glycolysis, two molecules of ATP are used and ultimately a maximum of four are produced. For each molecule of glucose metabolized, two molecules of NADH are generated. There are 11 enzymes in the glycolytic pathway. The viability of the RBCs is reduced if the activities of the glycolytic enzymes are impaired. The pentose–phosphate pathway uses intermediate products of glycolysis to

**TABLE 3.2 RBC: Types of Constituents[a]**

| |
|---|
| Protein |
| Hemoglobin (oxyhemoglobin, methemoglobin, carboxyhemoglobin) |
| Nonhemoglobin protein |
| Insoluble protein stroma |
| Enzyme proteins |
| Lipids |
| Phospholipids |
| Fatty acids (palmitic, oleic, linoleic, other) |
| Cholesterol |
| Other |
| Nucleotides |
| Adenosine triphosphate |
| Adenosine monophosphate |
| Uridine |
| *N*-acetyl glucosamine |
| Others |
| Amino acids and other nitrogen-containing compounds |
| Glutamine |
| Taurine |
| Aspartate |
| Alanine |
| Glutamate |
| Uric acid |
| Urea |
| Others |
| Coenzymes and vitamins |
| Nicotinic acid |
| Others |
| Carbohydrates, organic acids, and metabolites |
| Ribonucleic acid |
| Fructose 2,6-biphosphate |
| 2,3-biphosphoglycerate |
| Glucose |
| Others |
| Electrolytes |
| Potassium |
| Chloride |
| Phosphorus |
| Sodium |
| Magnesium |
| Others |

[a] Modified from Beutler, E. (1995). Composition of the erythrocyte. In *William's Hematology*, 5th ed. McGraw-Hill, New York.

reduce NADH. RBCs cannot use NADH for energy directly, but NADH serves as a cofactor in the reduction of oxidized glutathione that, because it is the principal reducing agent in the RBCs, is the most important protection against oxidative attack that degrades Hb to metHb (6).

**TABLE 3.3 RBC: Quantity of Constituents Possibly Related to VSP[a]**

| | |
|---|---|
| Hemoglobin | 330 mg/ml[b] |
| Nonhemoglobin proteins | 9.2 mg/ml |
| Protein from enzymes | 2.9 mg/ml |
| Fatty acids | 2 mg/ml |
| Phospholipids | 2.98 mg/ml |
| Ribonucleic acid | 1.36 mg/ml |
| Fructose 2,6-biphosphate | 48 μmol/ml |
| 2,3-Biphosphoglycerate | 4.17 μmol/ml |
| Adenosine triphosphate | 1.35 μmol/ml |
| Adenosine diphosphate | 0.22 μmol/ml |
| Urea | 4.12 μmol/ml |
| Reduced glutathione | 2.23 μmol/ml |
| Nicotinic acid | 0.11 μmol/ml |

[a] Modified from Beutler, E. (1995). Composition of the erythrocyte. In *William's Hematology*, 5th ed. McGraw-Hill, New York.

[b] Hb is seldom expressed in molar terms due to uncertainty regarding its polymeric state. If it is assumed to be 16,000 the g/dl is multiplied by 0.62 to convert it to mmol/liter, if 64,000 the conversion factor is 0.155 [from Lehman, H. P. (1976). Metrication in clinical laboratory data in SI units. *Am. J. Clin. Pathol.* **65**, 2].

### *3. Plasma Membrane*

The RBC consists of a plasma membrane surrounding a solution of protein and electrolytes (7). The RBCs are biconcave disks that are elastic and capable of considerable shape change on passing through the microcirculation. When suspended in hypotonic solutions, they swell. When depleted of their ATP, RBCs become spiculated. These crenated RBCs have regularly spaced projections. This is a prelytic stage.

The membrane structure of the RBCs is a matrix formed by a double layer of phospholipids. The lipid molecules in the bilayer are oriented with the nonpolar groups directed toward one another forming a hydrophobic interaction. The hydrophilic polar-head groups are on the outside, where they interact with the aqueous environment in both the plasma and the cytoplasmic surfaces. Globular proteins float within the lipids of the membrane. Some proteins transverse the lipid bilayer only once, whereas others have multiple transmembrane sections. On the inner or cytoplasmic side of the membrane are multiple attachment points of the cytoskeletal proteins. Some transmembrane proteins are covalently linked to lipids; glycosylphosphatidylinositol-anchored proteins do not span the entire membrane but instead have phospholipid tails that attach to the membrane. RBC membranes are of high tensile strength and flexibility. A protein meshwork laminates the internal side of the

RBC membrane to the internal cellular structure. Phospholipids and cholesterol comprise 95% of the membrane. Membrane proteins have been divided into integral proteins embedded in the lipid bilayer and peripheral proteins of the submembrane skeleton. RBC surfaces have many neuraminic acid residues that result in a negative surface charge. RBC surface antigens reside on glycolipids or on externally exposed portions of transmembrane proteins.

The majority of the membrane lipids are phospholipids or unesterified cholesterol (5). The major compounds are phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, and phosphatidylserine. Eighty percent of phosphatidylethanolamine and phosphatidylserine lie in the inner monolayer, while choline-containing phosphatidylcholine and sphinogomyelin lie in the outer layer. Total phospholipids make up about 3.1 mg/$10^{10}$ RBCs, with the corresponding figure for cholesterol being 1.3 and glycolipids 0.1. The most common saturated fatty acids in the RBC phospholipids are palmitic and stearic. Of the unsaturated fatty acids, oleic, linoleic, and arachidonic make up more than 40%.

As many as 40 different proteins have been isolated from the RBC membrane (7). Major membrane polypeptides with MW greater than 100 kDa include spectrin $\alpha$ and $\beta$, ankyrin, and the anion channel. Smaller polypeptides under 75 kDa include protein kinase, glucose transporter, actin, G-3PD, and glycophorin A. Of the cytoskeletal proteins in the cytosol, the most common are those of the spectrin–actin type. RBC membranes act as partial barriers to the penetration of solutes. Nonpolar substances diffuse at a rate proportional to their solubility in organic solutes. Polar solutes cross at specialized sites. RBCs have many specialized transporting proteins, including an anion transporter, several cation transporters, a glucose transporter, a urea transporter, and a water channel. There are at least 50 enzymes in the RBC membrane or bound to it. Certain ones may occur both free in the cytosol and in association with the membrane. ATP is not only generated by membrane-bound enzymes but also utilized by other membrane-bound molecules, the most important of which is adenyl cyclase which converts ATP to cAMP, protein kinases, and ATPases. Protein kinases are enzymes that phosphorylate other proteins in the presence of ATP by forming phosphoserine or phosphothreonine bonds. A variety of structural proteins and enzymes are ultimately phosphorylated. RBCs contain numerous protein kinases. The RBC membrane is nearly impermeable to monovalent and divalent cations, thereby conserving the high interior $K^+$ and low $Na^+$ as well as a very low $Ca^{2+}$ content. Two cation pumps maintain the low intracellular $Na^+$ and $Ca^{2+}$and high $K^+$ content and use ATP in the process. The $Na^+$ pump extrudes 3 $Na^+$ ions outwards for an inward passage of 2 $K^+$ ions. The calmodulin-activated $Ca^{2+}$ pump extrudes $Ca^{2+}$and maintains a very low $(Ca^{2+})i$ concentration. Anions are readily exchanged via the anion transport protein. There are numerous $H_2O$ channel proteins which permit fast movement of $H_2O$ molecules across the membrane. Glucose has its own transporter. Nucleotides and related compounds do not cross the normal RBC membrane.

#### *4. Cytoplasm*

RBC cytoplasm also contains a complex mix of lipids, phospholipids, nucleotides, fatty acids, amino acids, coenzymes, vitamins, carbohydrates, organic acids, and electrolytes (5, 6). Total lipids, which make up about 5 mg/ml (fatty acids 2 mg/ml), number at least 40. Common ones include palmitic (41%), oleic (19%), and linoleic (15%). Of long-chain aldehydes n-$C_{18}$ is most common (43%) and n-$C_{16}$ is next at 24%. Fatty acids of the neutral lipid fatty acids include 18:1 (29%) and arachidonic (8%). Total phospholipids make up about 3 mg/ml and include plasmalogen and cephalin. The phospholipids are composed of at least 40 fatty acids. The 15 described nucleotides comprise 1.53 μmol/ml RBC with ATP making up 1.35. Thirty-two amino acids and other nitrogen-containing compounds have been analyzed. Those with the highest concentrations are urea, reduced glutathione, glutamine, taurine, alanine, and glutamate. There are also a dozen coenzymes and vitamins found in RBCs, the one with the highest concentration being nicotinic acid. Of the carbohydrates, glucose is the most abundant and is in equilibrium with glucose in the plasma. 2,3-BPG is present in a concentration of 4.17 μmol/ml and fructose 2,6-bisphosphate is 48 μmol/ml. There is 1.36 mg/ml of ribonucleic acid. Of 24 known electrolytes the concentration of $K^+$ is 102, that of $Cl^-$ is 78, that of $Na^+$ is 6.2, that of $Mg^{2+}$ is 3, and that acid soluble phosphorus is 13.2 μmol/ml (8).

The insoluble portion of RBCs which remains after hemolysis is called stroma. There are about 230–300 mg of stroma/d. Stroma consists mainly of proteins and lipids, with a small amount of carbohydrate.

#### *5. Hemoglobin Structure*

Animal life has an almost universal requirement for $O_2$, and the most important substance for this process is the $O_2$-transport protein, Hb. It constitutes 90% of the dry weight of the mature RBC. Hb is a conjugated protein with a MW of 64 kDa. It is a roughly spherical molecule with a maximum diameter of 6.4 nm (Fig. 3.1). It consists of two pairs of similar ($\alpha$-like and $\beta, \gamma, \delta$) polypeptide chains, to each of which is attached a highly colored prosthetic group consisting of iron and protoporphyrin. The protein portion is called globin and the prosthetic group is called heme. The dominant adult Hb is Hb A ($\alpha_2\beta_2$), which makes up more than 96% of the total.

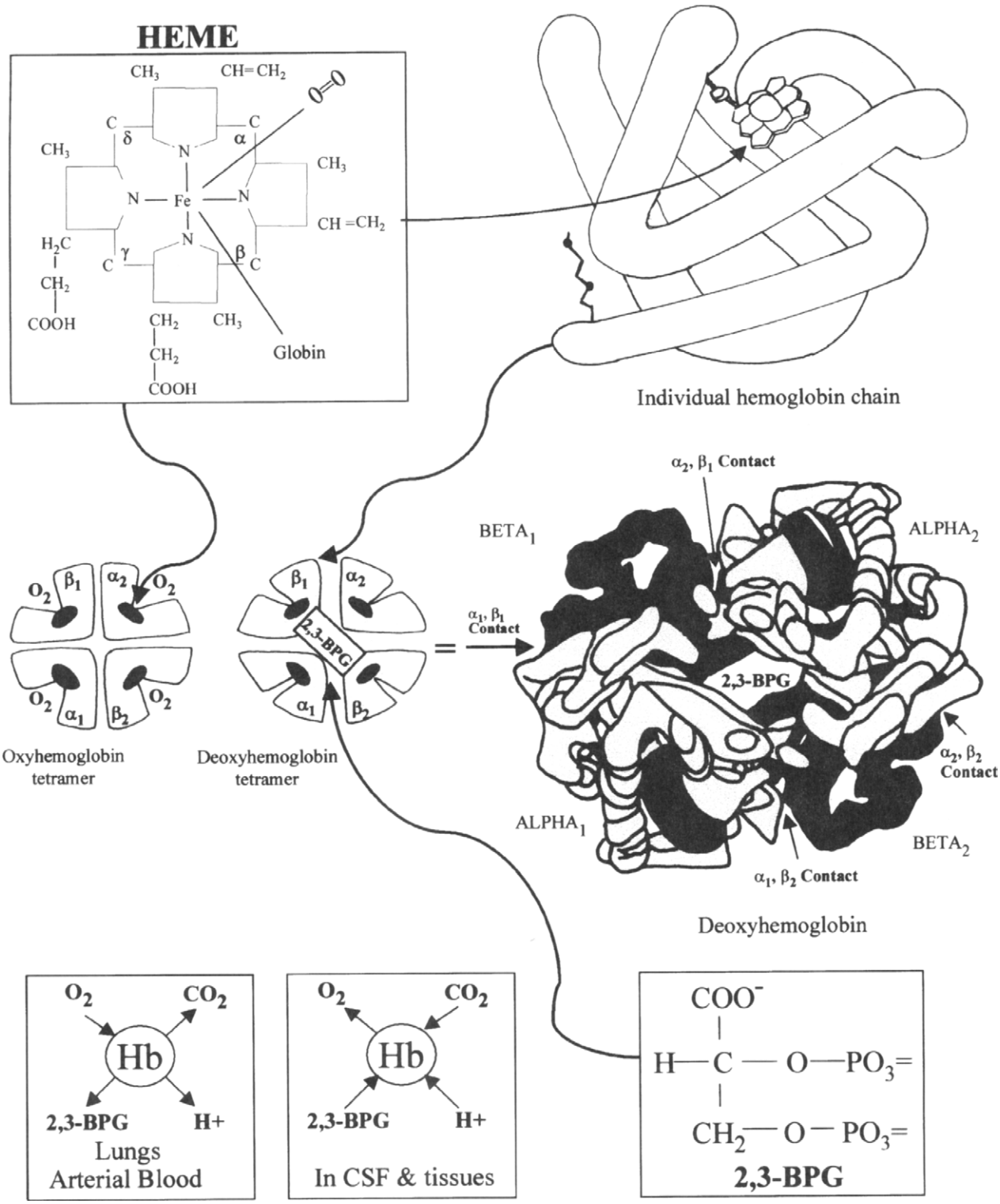


**FIGURE 3.1** The structures of heme, globin, hemoglobin, and 2,3-BPG.

Hemoglobins F and $A_2$ constitute the other 3.5% in normal persons (9).

RBCs contain a variety of colored proteins, such as Hb (deoxyHb and oxyHb), carboxyHb, and metHb, and small amounts of other forms of hemoglobin.

Heme is composed of a porphyrin ring and an iron atom. The iron atom has six coordinate bonds, four of which are formed between the iron and nitrogen atoms that are at the center of the porphyrin ring system. A fifth bond forms between a nitrogen atom contributed by a histidine residue in the globin (the proximal histidine) and the sixth bond is formed with $O_2$. The oxygenated form of Hb is stabilized by hydrogen bond between the $O_2$ and the side chain of a different histidine residue, the distal histidine. Functional Hb contains iron in the ferrous ($Fe^{2+}$) state. Oxidation to the ferric ($Fe^{3+}$) state renders the Hb incapable of binding $O_2$. The oxidized form of heme is known as hemin and the oxidized form of Hb is methemoglobin (metHb). When in the deoxygenated state the iron atom occupies a space slightly outside the heme ring plane. It moves into the plane of the ring when it is oxygenated and pulls the proximal histidine along with it. This movement is transmitted through the globin chain, resulting in increased affinity of other hemes for $O_2$(10,11). Upon oxygenation the positions of the Hb subunits shift relative to one another. In the $\alpha_1$ $\beta_1$ and the $\alpha_2$ $\beta_2$ pairs the bonding between subunits is strong. Each of these pairs acts as a unit in the transition between the two forms of Hb. The relaxed form is oxyHb and the taut form is deoxyHb. Each heme binds one $O_2$ molecule, and each HbA molecule binds four $O_2$ molecules. The $\alpha$ chains are the same in all human Hb, but there is variation in non-$\alpha$ chains depending on age and other factors. The amino terminal valines of the $\beta$ chain are important in 2,3-BPG – interactions, where the contact points between heme and the globin chains tend to be fixed. 2,3-BPG binds to the two $\alpha$ $\beta$ chains in the Hb molecule. There are no disulfide bonds in Hb. Three-fourths of the amino acids in the globin chains are in the helical arrangement. The polypeptide (globin) chains (9), which are part of the Hb molecule, differ from one another in amino acid sequence. The $\alpha$ chain contains 141 amino acids and the non-$\alpha$ chains 146. The oxygenated Hb molecule contains two $\alpha$ and two $\beta$ chains, four heme molecules, and four $O_2$ molecules attached to the heme. DeoxyHb lacks the $O_2$ and gains 2,3-BPG (10,11).

Heme is readily oxidized *in vivo* to hemin. The prosthetic group of Hb is heme (ferroprotoporphyrin IX). Heme is held inside a protein cage. In this nonpolar environment it is difficult to oxidize ferrous ($Fe^{2+}$) to ferric ($Fe^{3+}$), thereby permitting $O_2$ binding without oxidation. Hb in the deoxy state has a quaternary structure held together by intersubunit salt bonds, hydrogen bonds, and hydrophobic contacts. In deoxyHb, 2,3-BPG is situated in the central cavity between the two $\beta$ chains. On switching from the deoxy to the oxy structure there are changes within the subunits. The geometry of the heme in deoxyHb may be similar to that of isolated heme. DeoxyHb is in equilibrium with oxyHb + $H^+$ (a proton). Oxidized Hb (metHb) is generated in RBCs but is constantly reduced to $Fe^{2+}$Hb in the cell by the NADH–cytochrome $b_5$ reductase–cytochrome $b_5$ system. Hemin becomes hematin when dissolved in alkaline solution. Hb is synthesized in the developing RBCs in which mitochondria synthesize protoporphyrin. Iron is supplied from circulating transferrin, and globin chains are synthesized on ribosomes (10,11).

### *6. Hemoglobin Synthesis*

Hemoglobin accounts for about 90% of the dry weight of the RBCs. Three complex metabolic pathways are required to synthesize its three components: the protein globin, protoporphyrin, and iron (9,12–14). Heme is particularly important in controlling the rate of globin synthesis. Porphyrins are flat molecules with holes in the center which tend to stack up the other. All porphyrins are colored and have similar light absorption characteristics, with an intense absorption band of about 400 nm. All of them have a typical fluorescence. The biosynthesis of heme begins in the mitochondria with the combination of glycine and succinyl coenzyme A to yield $\delta$-aminolevulinic acid (ALA). The next two molecules of ALA fuse to yield porphobilinogen. Four molecules of porphobilinogen combine to yield hydroxymethylvaline. This in turn is rapidly converted to uroporphyrinogen III. Through a series of decarboxylation steps, coproporphyrinogen III results. Coproporphyrinogen III is transported back from the cytosol into the mitochondria and three additional reactions yield heme. This is the final combination produced by the union of $Fe^{2+}$ with protoporphyrin IX. Heme synthase is the enzyme that inserts $Fe^{2+}$ into protoporphyrin IX (15).

### *7. Hemoglobin Oxygen Transport*

The avidity with which Hb binds $O_2$ increases with increasing $O_2$ saturation. The binding of more proteins by deoxyHb than oxyHb produces a leftwards shift of the $O_2$ dissociation curve with increasing pH. Hb is approximately 50% saturated with $O_2$ (the $p_{50}$) at an arterial $pO_2$ of 26 mmHg. At this level of saturation of Hb, whole blood contains 11.49 ml $O_2$/dl of blood, with dissolved $O_2$ being 0.09 ml $O_2$/dl of blood.

The partial pressure of $O_2$ in the pulmonary alveoli is about 95 mmHg. After traversing the lungs the $pO_2$ is about 90. The value of $p_{50}$ is dependent on pH, temperature, and 2,3-BPG concentrations. There is an increasing

affinity for $O_2$ by Hb at lower temperatures. When $pO_2$ is lowered and lactic acid and $CO_2$ increase, the dissociation curve shifts to make more $O_2$ available to the tissues. The protein binding of deoxyHb follows $CO_2$ diffusion into the RBCs and $CO_2$ is converted to bicarbonate which then leaves the RBCs, although the proton produced stays bound to the deoxyHb. Increases in 2,3-BPG move the $O_2$ dissociation curve in the same way as the acidosis and fever. The reaction of Hb with $O_2$ and protons is very fast compared to its slow rate of dissociation from carbon monoxide and NO. OxyHb and carboxyHb lose their ligands on exposure to light.

MethHb is reddish-brown in color. Ferric subunits no longer transport $O_2$ and the increased $O_2$ affinity of $Fe^{2+}$ hemes accompanying the $Fe^{3+}$ hemes in tetramers also impairs $O_2$ delivery. Hb binds to carbon monoxide (CO) 200 times more strongly than it binds to $O_2$. Its half-life is approximately 4 hr. The release of $O_2$ by Hb is regulated by two main mechanisms: (i) the partial pressure of $CO_2$, which is mediated by a small decrease in the pH of blood in peripheral capillaries, and (ii) a mechanism mediated by 2,3-BPG. In the lungs Hb takes up $O_2$ and gives up 2,3-BPG, H+, and $CO_2$. In peripheral tissues the reverse changes take place. A decrease in pH (an increase in $H^+$) reduces the affinity of Hb for $O_2$ resulting in the release of $O_2$ from Hb. An increase in $pCO_2$ has the same effect. One gram of fully saturated Hb binds 1.39 ml of $O_2$. The degree of saturation is proportional to the oxygen tension ($pO_2$), which ranges between 100 mm Hg in the lungs to about 35 mmHg in veins. The relationship between $pO_2$ and Hb $O_2$ is described by the $O_2$ dissociation curve of Hb which is influenced by factors such as the internal RBC chemistry, pH, temperature, and concentration of 2,3-BPG. The avidity with which Hg binds $O_2$ is expressed in terms of the $O_2$ tension at which 50% saturation occurs ($p_{50}$). The dissociation curve shifts to the left when $O_2$ affinity increases, and therefore the value of $p_{50}$ is reduced. In the lungs $CO_2$ is released, pH rises, and the $O_2$ affinity curve shifts to the left so that $O_2$ is more readily taken up by Hb (9,13).

The concentration of phosphorylated compounds such as 2,3-BPG also affects the $O_2$ affinity of Hb. In the RBCs, 2,3-BPG is the principal phosphorylated compound and is produced by glycolytic intermediates. It contains two-thirds of the RBC phosphorous. It is almost exclusively found in RBCs. This rate of production is linked to concentrations of ATP and ADP. DeoxyHb binds to 2,3-BPG, which reduces its $O_2$ affinity and increases $O_2$ delivery to the tissues.

The high level of $CO_2$ in the tissues causes it to diffuse into RBCs, where it is rapidly catalyzed by carbonic anhydrase. Carbonic acid is formed, which then dissociates into bicarbonate and a proton ($H^+$). Bicarbonate is exchanged for $Cl^-$ in the plasma and $H^+$ binds to oxyHb, which facilitates the dissociation of $O_2$ from Hb. The opposite takes place in the lungs.

### *8. Hemoglobin Oxidation*

OxyHb in solution gradually undergoes autooxidation to become metHb ($HbFe^{3+}$). This occurs more rapidly at decreased pH, increased temperature, in the presence of metal ions and organic phosphates, and when Hb is partially oxygenated. The oxidation of Hb occurs in a stepwise fashion from fully reduced Hb to fully oxidized Hb (6):

$$Hb(O_2)_4 \rightarrow Hb(O_2)_3 + O_2$$

$$HbFe^{2+} + O_2 \rightarrow HbFe^{3+}O_2^- \rightarrow HbFe^{3+} + O_2^{-\bullet}$$

$$2HbFe^{2+} + O_2 + 2O_2^- + 4H^+ \rightarrow 2HbFe^{3+} + 3O_2 + 2H_2O$$

$$2HbFe^{2+} + 2H_2O_2 \rightarrow 2HbFe^{3+} \bullet H_2O + O_2$$

MetHb is formed in RBCs at the rate of 0.5–3% of Hb per day.

As $Hb(O_2)_4$ changes to $HbFe^{3+}$, the spectrum defining the relationship of light absorbence to wavelength changes with the disappearance of the 575-nm band and the appearance of a band at 631 nm. Hb has multiple derivatives, including deoxyHb, oxyHb, acid and alkaline metHb, cyanometHb, hemichromes, carboxyHb, and sulfHb. In the oxidative denaturation of Hb, the hemichromes initially formed can change back to metHb, but further denaturation results in the production of irreversible hemichromes and precipitated hemichromes or globin. Most metHb in RBCs is reduced through the action of cytochrome $b_5$ metHb reductase.

### *9. Free Radicals*

Molecular $O_2$ undergoes successive univalent reductions to produce a variety of reactive oxygen species (6) (Fig.3.2). These agents can cause oxidative denaturation of Hb and may damage other cellular components such as the lipids in the cell membrane. Superoxide anions ($O_2^{-\bullet}$) are the natural products of biological reactions. Once generated in aqueous solution, they can form additional toxic oxygen products:

$$O_2^{-\bullet} + O_2^{-\bullet} + 2H^+ \rightarrow H_2O_2 + O_2$$

In the presence of trace metals the extremely reactive hydroxyl radical (OH•) may be formed:

$$O_2 + H_2O_2 \rightarrow OH\bullet + OH^- + O_2$$

The hydroxyl radical (OH•) may be generated from peroxide in the presence of certain metals (Fenton reaction):

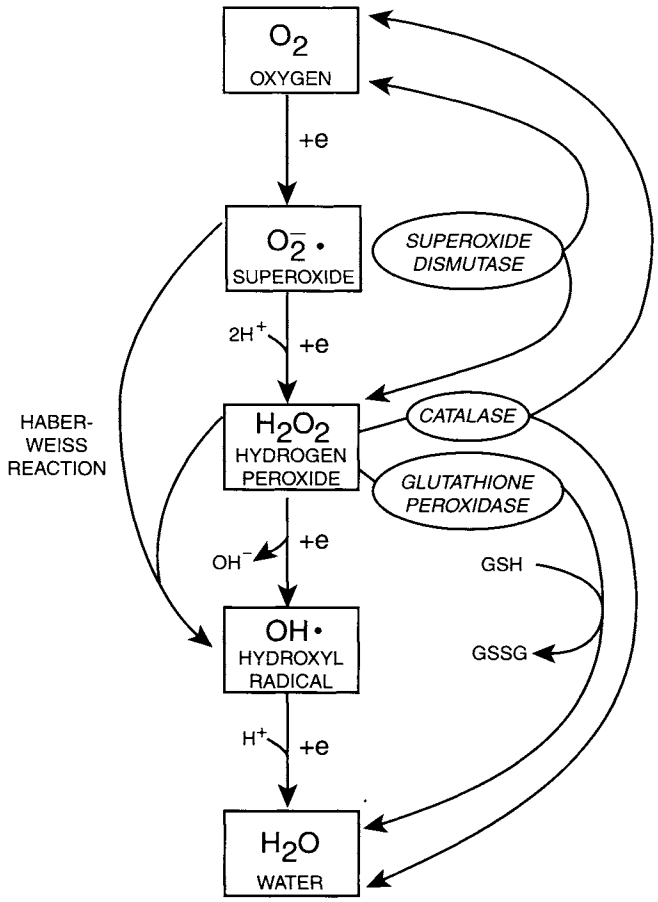


**FIGURE 3.2** Steps in the univalent reduction of oxygen and enzymatic pathways affecting the intermediates. The enzymatic pathways, shown on the right, provide the means for processing these intermediates without formation of the highly reactive hydroxyl radical. This potent oxidant can be formed by the reaction shown on the left if superoxide and peroxide concentrations are sufficient and if catalytic quantities of transition metals are present [reproduced with permission from Telen, M. J., and Kaufman, R. E. (1998). The mature erythrocyte. In *Wintrobe's Clinical Hematology* (G. R. Lee, J. Forester, J. Lukens, F. Paraskevas, J. P. Greer, and G. M. Rogers, Eds.), 10th ed. Williams & Wilkins, Baltimore].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\bullet$$

The body has protective enzymes to convert OH• to less dangerous peroxide. Superoxide dismutase (SOD) accelerates this reaction more than 100-fold. SOD is a copper–zinc-containing enzyme. Once $H_2O_2$ is formed, two additional enzymes catalyze its decomposition within the RBCs–GSH and catalase, which convert it to $H_2O$ and $O_2$. Glutathione is the major reducing agent in RBCs. MetHb reduction is achieved through a NADH-linked system. NADH generated in reaction to reduced cytochrome $b_5$ reduces the iron of metHb from the trivalent to the divalent form. RBCs also have a high concentration of carbonic anhydrase which maintains the equilibrium between $CO_2$ and carbonic acid and thereby aids in $O_2$ and $CO_2$ transport. Catalase, which decomposes $H_2O_2$ to $H_2O$ and $O_2$, is also present in RBCs. This enzyme and GSH are probably the greatest protection RBCs have against free radicals. SOD is also a potential protectant. RBC membranes also contain protein kinases which catalyze the transfer of terminal phosphate from ATP to various cytoskeletal protein acceptors. Of the proteolytic systems in RBCs, calpain can be activated by elevated $(Ca^{2+})i$ concentrations. ATP and guanosine triphosphate are the direct source of energy for many intracellular processes and are the precursors of cyclic nucleotides. Nicotinic acid nucleotides $NAD^+$ and $NADP^+$ are also essential to the biochemistry of the cell (8). In abnormal circumstances the RBCs can use substrates other than glucose, such as adenosine, fructose, and galactose, as well as other compounds as a source of energy.

### *10. Hemoglobin and Nitric Oxide*

Nitric oxide (NO) is a physiologic ligand for Hb. NO can stimulate guanylate cyclase, which catalyzes the formation of cGMP, a mediator of smooth muscle relaxation, platelet aggregation inhibition, and increased macrophage cytotoxicity. Hb reacts with NO very quickly and prevents guanylate cyclase activation by endogenous NO. Extravascular Hb may be a cause of vasoconstriction by binding NO, the natural vasodilator (16).

$HbO_2$ scavenges NO in a reaction yielding methHb. The infusion of free Hb causes hypertension because oxygenated heme reacts with NO in a reaction yielding metHb. This reaction can be prevented by the preliminary incubation of free Hb with *S*-nitrosothiols. Higher concentrations of NO bound to heme are found in veins than in arteries because Hb picks up NO on the deoxyhemes (16).

### *11. Degradation of Hemoglobin and Heme Oxygenase System*

Injection of nonviable RBCs or Hb into the bloodstream results in an increase in iron and bilirubin in the plasma within minutes and the rapid appearance of bilirubin in the bile. The relationship of Hb breakdown to bilirubin production was first proposed by Virchow more than 150 years ago, but the major enzymatic mechanisms for heme catabolism was only defined two decades ago (17). Heme oxygenase (HO) breaks heme into equimolar amounts of biliverdin, carbon monoxide, and iron. Three isoforms exist: HO-1 is ubiquitous and its mRNA and activity can be increased in response to a variety of stimuli such as heme, metals, and cellular stress; HO-2 is present in the brain and is not significantly

inducible; and HO-3 may be involved with heme binding. Heme is oxidized to the gene regulator iron, the antioxidant biliverdin, and the heme ligand CO. The biological functions of CO and NO are linked. NO and NO donors can induce HO expression. CO can act within vascular smooth muscle cells (VSMCs) to induce vasodilation. The microsomal HO system catalyzes the oxidative fission of the α-methene bridge of heme to yield equimolar amounts of biliverdin-IX-α, CO, and iron. The preferred substrate of HO is free heme or complexes in which heme is loosely bound to protein. It is inactive if heme is bound to its apoprotein (Hb or Hb haptoglobin complexes). The two forms may be distinct molecular species. For every 1 *M* of heme degraded, 1 *M* of biliverdin is formed with the consumption of 3 *M* of $O_2$ and 4 or 5 *M* of NADPH (18). Most free NO in the blood would be rapidly scavenged by Hb forming stable Hb [FeII] NO complexes. In human Hb, the β subunits contain highly reactive sulfhydril groups which may interact with NO. There is little reaction between *S*-nitrosothiols and heme of Hb. Arterial but not venous blood contains significant levels of S-nitrosylated Hb. Hb is therefore S-nitrosylated in the lungs and the NO group is released during arteriovenous passage in a dynamic cycle. The vasoactivity of *S*-nitrosoHb is promoted by the RBC export of *S*-nitrosothiols. This cycle may affect blood pressure and efficient $O_2$ delivery (19). Aortic rings were relaxed with ACh [which causes release of endothelial derived relaxant factor (EDRF)]. This was antagonized by Hb in which heme was blocked by cyanmet derivitization or by Hb in which the β-93 thiol groups were blocked by nitrogen ethylmaleimide. Native Hb was more effective than the modified versions (19). Free NO has no relaxant activity in the presence of Hb[Fe II]$O_2$ or Hb[Fe III]. S-nitrosylated Hb in both free form and within RBCs caused vasodilation and a hypotensive response when injected in rats. Hb is only partly nitrosylated *in vivo*. $O_2$ drives the conversion of NO-deoxyHb to *S*-nitroso-oxyHb.

In the absence of $O_2$, nitroxyl anion ($NO^-$) is liberated in a reaction producing metHb (20). NADPH and $O_2$ must be available (18)(Fig. 3.3). The production of heme from hemin is the first step in this multistep process. HO-1 occurs in all tissues including the brain. It is inducible by its substrates. Erythrophagocytosis causes a dramatic rise in HO-1 activity in tissue macrophages (21). HO-1 is a heat shock protein. In the first step, when HO-1 binds hemin in a 1:1 molar ratio, there is a reduction of the hemin to its ferrous form (heme). The heme oxygenase reaction is followed by a subsequent conversion of biliverdin by bilirubin reductase to bilirubin. The latter is lipophilic and virtually insoluble in water. It can cross the BBB easily. Normally, the plasma concentrations of bilirubin are less than 1 mg/dl (22).

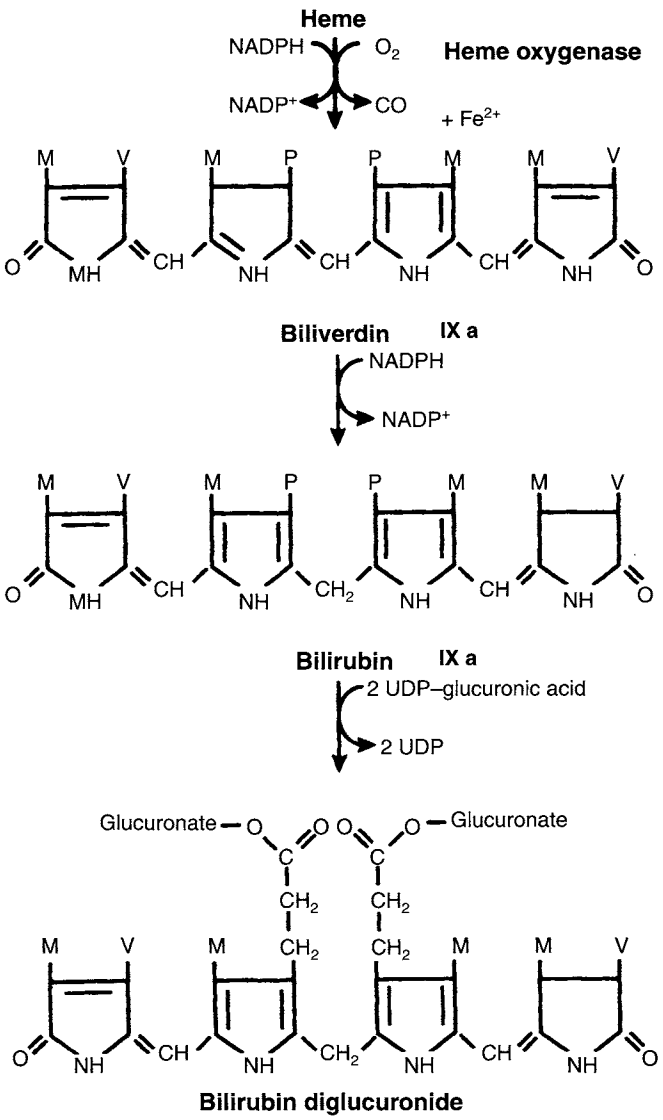


FIGURE 3.3 Heme degradation. Heme oxygenase cleaves the carbon bridge connecting two pyrrole rings resulting in the release of $Fe^{3+}$ and carbon monoxide and the formation of biliverdin. UDP, uridine diphosphate; M, methyl; V, vinyl; P, propionyl [reproduced with permission from Coffee, C. J. (1998). *Metabolism*. Fence Creek, Madison, CT].

Heme (ferrous protoporthyrin IX) is readily oxidized outside the cell body to hemin or ferric protoporphyrin IX, which has one residual positive charge so it is commonly isolated as a halide such as a chloride. Hemin is the form of heme normally present in the body. Since it is hydrophobic it tends to incorporate into the RBC membrane. When dissolved in alkaline solution it becomes hematin as the halide is replaced by a hydroxyl ion.

In normal metabolism of heme the contained iron is retained, mobilized, and reutilized, and the hydrophobic products of porphyrin cleavage are processed. Hb in

plasma has a short existence as it rapidly dissociates into its $\alpha\beta$ dimers. These bind to the plasma protein haptoglobin prior to removal from the circulation. In an effort to find a therapeutic $O_2$-carrying compound to treat shock, Hb has been modified. Diaspirin Hb (DCLHb) is produced by cross-linking bis-(3,5-dibromosalicyl) fumarate at the Lys 99 of the $\alpha$ chains. Polymerized DCLHb has a relatively long lifetime in the circulation compared to Hb (23).

### *12. Destruction of Erythrocytes*

As RBCs age, various changes take place during glycolysis that consist mainly of a decrease in the activity of multiple enzymes and phosphorylated intermediates (22). Enzymes involved in the pentose–phosphate pathway also diminish. Various other cellular constituents are also decreased. There tends to be an increase in $[Na^+]_i$ and $[Ca^{2+}]_i$ and a decrease in $[K^+]_i$. Other characteristic changes take place in the membrane, including a reduction in surface area and negative charge. There tends to be increased lipid peroxidation of the RBC membrane as well as specific alterations in the phospholipids. The activities of most intracellular enzymes decrease. There is also increase in metHb, $O_2$ affinity, and glycolated Hb. RBCs become more susceptible to osmotic or mechanical stresses and show increased viscosity and decreased density. Senescent RBCs become smaller. The diminished negativity of the RBC membrane reduces the repulsion of phagocytes. RBCs are exposed to repetitive oxidative injuries since they are $O_2$-carrying devices. Because Hb is subject to oxidative denaturation, intermediate products (hemichromes) interact with $O_2^{-\bullet}$ and $H_2O_2$ to create $OH\bullet$, which is even more damaging.

The mechanism by which senescent RBCs are withdrawn from circulation is unclear. Phagocytic cells of the spleen, liver, and bone marrow all contain HO which can degrade heme. Erythrophagocytosis is considered to be the usual mode of destruction of aged RBCs. Some may also be lost by fragmentation rather than being engulfed. Up to 90% of physiological RBC breakdown occurs without release of Hb into the plasma. RBCs are usually destroyed outside the vascular system by macrophages. Macrophages probably detect aged, senescent, or damaged RBCs by their decreased deformability and altered surface properties. RBC membranes have surface antigens with which antibodies combine (24). The surface can also be affected by complement components or by oxidation of the membrane. It is still not known with certainty what factors cause macrophages to sequester cells that have reached the end of their life span. It is similarly unknown why young RBCs in the CSF are phagocytized regardless of their age. In pathologic states when RBCs are destroyed intravascularly, the released Hb is bound to haptoglobin, a dimeric glycoprotein which binds Hb $\alpha$ dimers (Fig. 3.4). The haptoglobin–Hb complex is rapidly cleared from plasma by the liver, where the heme of Hb is converted to iron and biliverden by HO and biliverden is further catabolized to bilirubin. Free haptoglobin, in contrast to the Hb complexed haptoglobin, has a half-life in plasma of 5 days. Free heme released intravascularly is bound to the glycoprotein hemopexin and the complex is cleared within hours when it is converted to bilirubin in the liver. When RBCs are destroyed extravascularly they are engulfed by phagocytic cells and degraded within lysosomes into lipids, proteins, and heme (14). Free Hb may also be oxidized in the plasma to metHb, which can be bound by other proteins such as hemopexin and albumin. Heme is also removed from these proteins by hepatocytes. The liver cells take up these complexes by receptor-mediated endocytosis.

If the complement cascade is activated, a membrane attack complex forms on the terminal complement component embedded in the RBC membrane lipid bilayer. The complex acts as a cation channel. The intracellular negatively charged Hb, ATP, and 2,3-BPG attract $Na^+$ into the cell with a simultaneous $K^+$ loss. If compensatory $Na^+$–$K^+$ pumps are overwhelmed, increased intracellular $Ca^{2+}$ and $H_2O$ can cause lysis by colloidal osmotic forces (7). SAH, by affecting the blood–brain/CSF barrier, persumably allows C protein extravasation. After SAH in 15 patients the terminal complement complex concentration on days 0–2 was 210 ng/ml in CSF and 63ng/ml in plasma but was absent in control CSF. Terminal complement complex was reduced to 24 ng/ml on days 7–10 post-SAH. Incubation of normal human CSF *in vitro* also activated the terminal C pathway (25).

The marker of self which murine RBCs use to prevent macrophage engulfment appears to be CD47 (integrin-associated protein). CD47 on normal RBCs binds to the inhibitory receptor signal regulatory protein alpha on macrophages, notifying them that they are not to be destroyed by the macrophages. This is analogous to the way in which major histocompatibility complex I molecules protect cells by binding to natural killer cells inhibitory receptors that can activate src-homology phosphatases and inhibit natural killer cells (26).

### *13. Iron Metabolism*

Iron is an essential element in all living cells and participates in numerous metabolic pathways. Iron is easily reversibly oxidized and reduced. Iron has only a transient existence as a free cation and is instead bound to or incorporated within various proteins (10), including heme proteins, flavoproteins, and heterogeneous proteins

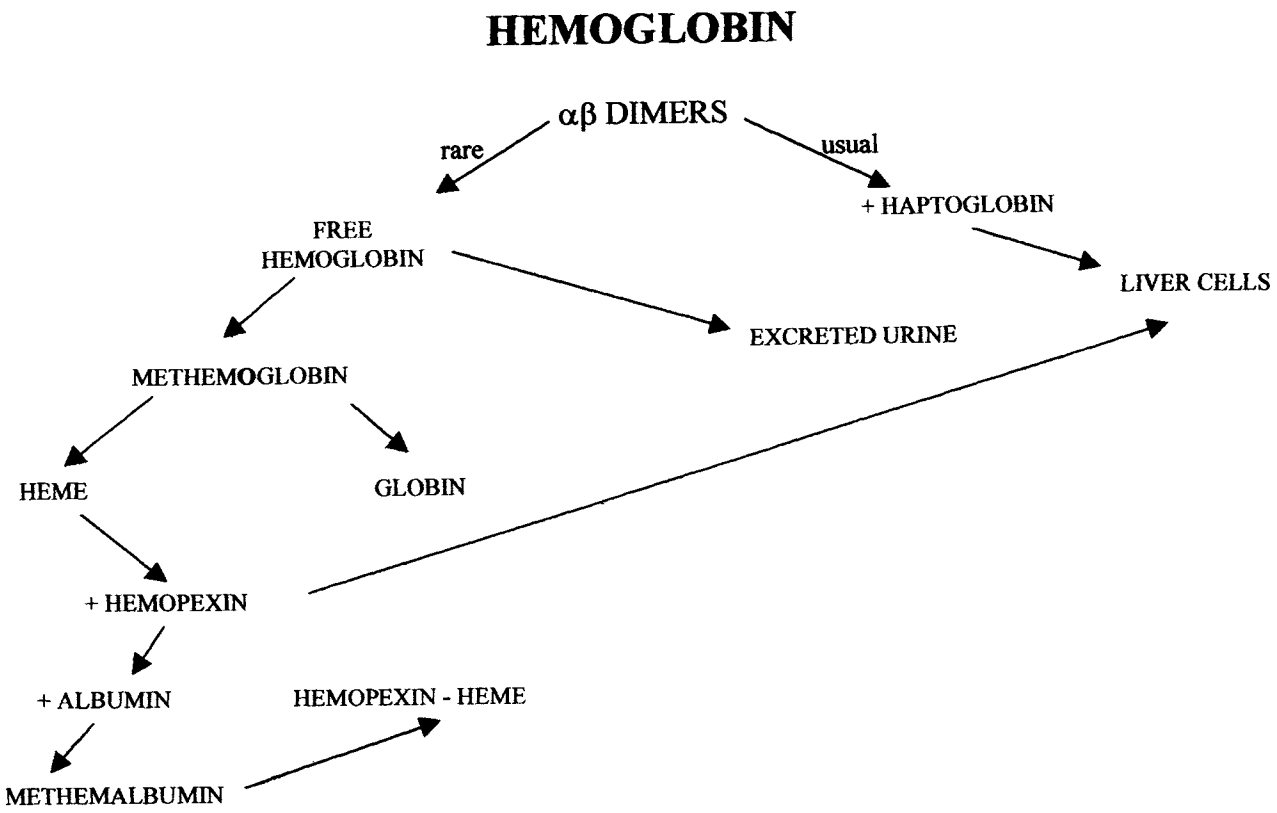


**FIGURE 3.4** Pathways for transport of hemoglobin and its constituents.

(Table 3.4). The heme proteins are Hb, myoglobin, the cytochromes, cytochrome oxidase, homogentisic oxidase, peroxidases, and catalase. A cytoplasmic enzyme aconitase is an intracellular regulator of iron metabolism. Of six identifiable iron compartments, the largest is Hb, which contains 2000 mg or 67% of total body iron. The next largest is storage iron consisting of ferritin and hemosiderin which comprises 1000 mg or 27% of total body iron. Ferritin is a water-soluble protein–iron complex of molecular weight 465 kDa. Its outer protein shell is made up of apoferritin, which has an iron–phosphate hydroxide core. A small portion of ferritin comes from dietary absorbed iron.

Iron in the storage compartment exists in two forms. First, ferrritin, which is a water-soluble complex of ferric hydroxide and the protein apoferritin. The latter forms a shell within which ferritin, hydroxyl ions, and $O_2$ are dispersed on the lattice. Ferritin is found in all body fluids and tissues. Second, hemosiderin, which occurs in monocytes and macrophages. It is insoluble in water and contains about 25% iron by weight. It can accumulate in clumps or granules under pathological conditions.

Iron is transported in plasma bound to the β-globulin transferrin (MW 80 kDa). This protein is synthesized in the liver and lasts about 1 week in the circulation. One transferrin molecule can bind two atoms of iron. It is usually about one-third saturated. Transferrin gains iron mainly from macrophages of the reticuloendothelial system. Iron is incorporated from plasma transferrin into developing erythroblasts and reticulocytes.

About one-fifth of iron within Hb is released within a few hours of phagocytosis of RBCs. The iron obtained by macrophages is passed into the plasma, where it is bound by transferrin and redistributed, with 80% being rapidly reincorporated into Hb. About 40% of the iron from phagocytosed RBCs reappears in new circulating RBCs within 12 days. Another 40% of such iron enters the storage pool as hemosiderin. In chronic inflammation phagocytic cells store iron for longer periods.

Hemin (oxidized heme) is a prominent breakdown product of Hb and occurs in high concentrations in CSF following SAH. In VSMCs from rat aorta grown *in vivo*, hemin was shown to stimulate NO synthase activity as inferred by the increased accumulations of nitrite and nitrate (the oxidative products of NO) in the culture medium in a dose- and time-dependent fashion. NO synthase inhibitors impaired this effect. It was hypothesized that the generation of hemin from metHb during

**TABLE 3.4 RBCs: Iron and Iron Proteins**[a]

| |
|---|
| % of tissue body iron 3221 mg |
| Hemoglobin (67%) 1 ml packed RBCs contain 1 mg iron |
| Storage compartment (27%) |
| Ferritin: water-soluble complex of ferric hydroxide and apoferritin present in all cells and tissue fluids |
| Hemosiderin: found in the monocyte – macrophage system, Kupfer cells in liver and spleen, water insoluble, 20–25% iron by weight accumulates in pathological condition |
| Myoglobin (4%) |
| Labile iron pool (80 mg) |
| Tissue iron compartment (8 mg) |
| Cytochromes, homogentisic oxidase, peroxidase, catalase, succinate dehydrogenase, NADH dehydrogenase, acyl-coenzyme A dehydrogenase, xanthine oxidase |
| Transport compartment (3 mg) |
| Transferrin (apoferritin plus iron) |

[a] Modified from Fairbanks, V. F., and Beutler, E. (1995). Iron metabolism. In *William's Hematology*, 5th ed. McGraw-Hill, New York.

hemolysis of a subarachnoid clot can stimulate an excessive and destructive production of NO from VSMCs (27).

Ferric iron-bound transferrin induces a rapid increase in the level of inositol phosphates in porcine cerebral arterial smooth muscle cells. This involves the phospholipase C pathway (28).

### *14. Iron Chelation*

It has been hypothesized that the elaboration of iron from the subarachnoid clot might enhance the generation of free radicals and lipid peroxidation. Iron-chelating compounds have been used for attempted prophylaxis of VSP. Deferoxamine is a bacterial siderophore isolated from a bacterium which selectively chelates free ferric ($Fe^{3+}$) iron but has no effect on iron bound to heme proteins or transferrin. Iron is unreactive to free radical-generating reactions after exposure to deferoxamine. *In vitro* studies have shown release of significant amounts of $Fe^{3+}$ iron in a time-dependent manner. In a rabbit model, pre-SAH treatment with deferoxamine (50 mg/kg/8hr) failed to prevent post-SAH–basilar artery reduction in diameter (29). In cultures of endothelial cells and VSMCs there was a dose-related disruption of the cytoskeleton, particularly the F-actin and vimentin filaments after exposure to oxyHb. The cytoskeletal injury was prevented by deferoxamine or albumin (30). In a rat femoral artery model, perivascular application of deferoxamine prevented significant arterial narrowing or structural change 7 days following the application of periadventitial autologous whole blood or platelet-rich plasma. The protective effect of deferoxamine was eliminated by presaturation of the deferoxamine with excess ferric iron prior to application. Deferoxamine chelated the ferric iron released from incubated whole blood *in vitro* over 7 days in a dose-dependent manner. $Fe^{2+}$ in solution is rapidly oxidized to $Fe^{3+}$ iron. Topical application of $Fe^{3+}$ on cerebral arteries produces only mild vasoconstriction, whereas $Fe^{2+}$ causes no narrowing (31).

In a rabbit SAH model the pharmacological responses of arteries removed 7 days post-SAH were examined. Contractions were increased in SAH animals compared to controls. Deferoxamine or sympathectomy did not reduce the hypersensitivity of the vasoconstriction in the SAH animals (32).

Because iron is in the $Fe^{2+}$ state when catalyzing the generation of free radicals or production of lipid peroxides, Horky *et al* (33) assumed that chelators of $Fe^{3+}$ or free iron would not be effective. Also, NO preferentially reacts with $Fe^{2+}$. In a primate model a chelator of iron in the $Fe^{2+}$ form that acts intracellularly and penetrates the BBB was examined in a primate model; this drug is 2,2′-dipyridyl. Cerebral VSP in the primate model was prevented by its continuous intravenous administration. The mean plasma iron concentration in the treated animals dropped from 115 μg/ml on day 0 to 2 μg/ml on day 7. The mean percentage transferrin saturation dropped from 36 to 1.2% during the same time. $Fe^{2+}$ catalyzes free radical production and lipid peroxidation by means of the Fenton reaction and related reactions. In the subarachnoid space $Fe^{2+}$ can react with organic hydroperoxides in a reaction similar to the Fenton reaction that also produces free radicals: $Fe^{2+} + ROOH \rightarrow Fe^{3+} + \bullet OR + OH^-$. Dipyridyl can induce production of HO, which catalyzes the degradation of heme to iron, biliverdin, and CO.

The antioxidant ascorbic acid had a similar protective effect to the iron-chelating agent deferoxamine in the rat femoral artery model. Concurrent study of a 21-amino steroid failed to show a beneficial effect (34). Deferoxamine acts mainly extracellularly, where it chelates free iron but not heme or ferritin-bound iron (35). The oxidation of oxyHb to metHb has successfully prevented vascular damage *in vitro* and VSP (36). Ascorbic acid may oxidize $Fe^{2+}$ to $Fe^{3+}$ in the subarachnoid space. It has been found to suppress the VSP produced by oxyHb (37).

## D. Endothelial Cells

When an aneurysm ruptures, platelets and the products of coagulation temporarily close the defect until permanent repair can occur. Transient vasoconstriction may also occur. The thrombus can subsequently be lysed by the fibrinolytic system. Thrombosis and fibrinolysis are

cooperative activities of the blood and the endothelial cell monolayer that lines all blood vessels.

### *1. Structure*

The endothelial surface in human bodies is composed of 1–6 $\times 10^{13}$ cells, weighing about 1 kg and covering an area of 1–7 square meters. The surface lines every organ acting as a gatekeeper and regulating blood flow. Cell culture perturbs endothelial cells from their normally low replication rate of 0.1%/ day to 1–10%/day. Endothelium acts to prevent physical disruption of the blood vessels. Its prolonged activation may lead to vascular disease. It is not a homogeneous organ. Little is known of the potential consequences of genetic differences in endothelial cell behavior from different individuals (38).

Endothelial cells measure 20 $\times$ 50 $\mu m^2$ in surface area (39). They rest on the subendothelium, which is an extracellular matrix secreted by them (Fig. 3.5). Cranial blood vessels have relatively tight junctions between endothelial cells. The junctions contain proteins such as plakoglobin and the cell adhesion molecule CD31. There are functional gap junctions between endothelial cells containing different connexins. Even the smallest blood elements (platelets, 2 $\mu m$) cannot pass through tight junctions. In reaction to adjacent inflammation, however, larger leukocytes can attach to the endothelial cells and create a passage between them (40).

Subendothelium is a basement membrane composed of collagen, elastin, microfibrils, laminin, mucopolysaccharides, fibrinectin, von Willebrand factor, vitronectin, thrombospondin, and sometimes fibrinogen and fibrin. In larger cerebral vessels the subendothelium is surrounded by the elastica (39).

### *2. Function*

The endothelial layer has properties that prevent blood from coagulating and platelets from becoming activated (39–42). Normally, platelets are exposed to prostacyclin and EDRF which keep them inactivated. The mechanisms by which vascular endothelium resist thrombosis include the negative surface charge; the production of prostacyclin, which inhibits platelet aggregation; the secretion of thrombomodulin, which with thrombin activates protein C, which in turn inactivates procoagulant factors Va and

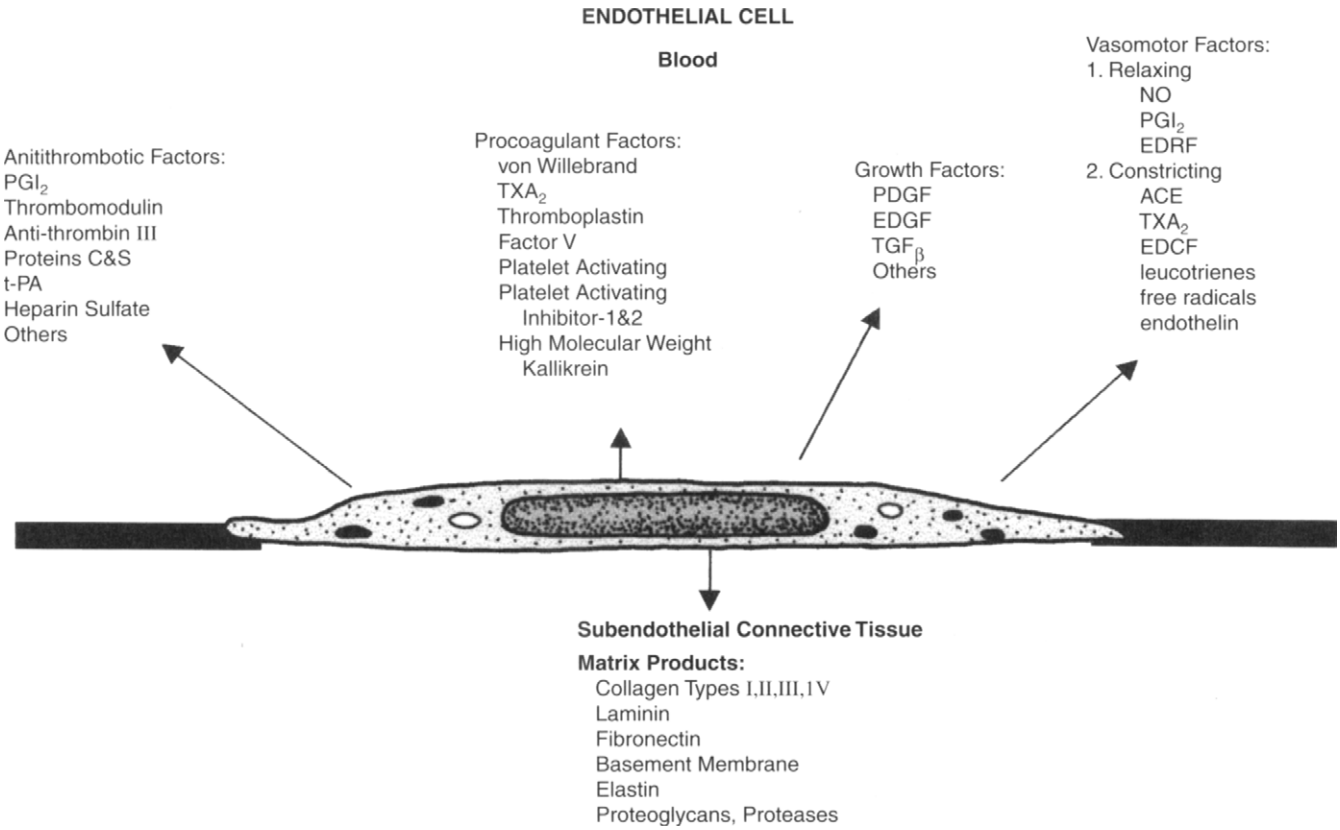


**FIGURE 3.5** The endothelial cell and its products.

VIIIa, the production of heparin sulfate, which increases the inhibitory potency of antithrombin III with respect to factors Xa and thrombin, and the secretion of t-PA, which activates plasminogen. Activated coagulation factors are inactivated by antithrombin III in the presence of its physiological activator and by activated protein C as well as other circulating protease inhibitors such as $\alpha_2$-macrogobulin. Endothelial cells can produce antithrombin III, which rapidly inactivates circulating thrombin. The resulting antithrombin III–thrombin complex is cleared both in the liver and by endothelial cells. Endothelial cells also produce proteins C, S, and thrombomodulin. These substances can inactivate coagulation factors by proteolysis. Protein C is activated by thrombin. Serum also contains a tissue factor pathway inhibitor. Endothelial cells have fibrinolytic poperties. They can produce plasminogen activators of the urokinase type (u-PA), which activate plasminogen in the fluid phase, and the tissue type (t-PA), which is active when in contact with plasminogen bound to fibrin. Endothelial cells normally contain only t-PA. During inflammation and leukocyte migration they can also synthesize u-PA. Thrombin can stimulate the release of t-PA and u-PA from human endothelial cells. Thrombin can also have an opposite effect by stimulating plasminogen activator inhibitor (PAI-1). Activated protein C stimulates fibrinolysis by binding to PAI-1. Endothelial cells are also stimulated to synthesize u-PA and t-PA by basic fibroblast growth factors and vascular endothelial growth factors.

Endothelial cells by multiple mechanisms control blood flow and pressure. These include systems that cause both vasoconstriction and vasodilation. They can secrete renin, which converts angiotensinogen to angiotensin, and produce angiotensin converting enzyme (ACE), which converts angiotensin I to the active vasoconstrictor angiotensin II. They also secrete endothelin (ET), an extremely potent vasoconstrictor, and also vasodilators such as adenosine and EDRF. Endothelial cells are intrinsically nonthrombogenic in the normal state. They secrete $PGI_2$, which is synthesized from AA and secreted into the adjacent blood. It is a potent vasodilator and platelet aggregation inhibitor. It synergistically inhibits platelet function with NO or a similar compound. $PGI_2$ has a half-life of less than 6 min in whole blood; its synthesis is stimulated by thrombin and hypoxia. The synthesis of the other potent vasodilator, EDRF, is stimulated by a variety of angonists, such as ACh, ATP, thrombin, NE, BK, 5-HT, and other factors. Like other nitrovasodilators, EDRF raises cGMP levels in vascular smooth muscle, thereby triggering relaxation.

Penetrating injuries of the vessel wall induce proliferation and the subsequent inward migration of VSMCs from the media and fibroblasts from the adventitia. These cells are mobile and can secrete elastase and collagenase as well as synthesize the components of connective tissue that enable them to remodel the vessel wall. Endothelial cells secrete platelet-derived growth factor abluminally, which induces smooth muscle cell division and migration. Thrombin also increases platelet-derived growth factor secretion. In an opposite type of reaction, endothelial cells can produce a heparin-like inhibitor of smooth muscle cell growth (41). Bloody CSF was found to have cytotoxic effects on bovine cerebral endothelial cells in culture (43).

## E. Platelets

### *1. Morphology*

Platelets are about 2 μm in diameter (about one-fourth the size of a RBC) and number 250,000–400,000 /$mm^3$. They are anucleated , disc-shaped fragments of megakaryocytes produced in the bone marrow (42,44). The shape is lentiform (Fig. 3.6). There is a clear, peripheral region called the hyalomere and a region containing purple granules called the granulomere visible after staining. The platelet is surrounded by a glycocalyx, a coat external to the plasmalemma. The adhesiveness of the glycocalyx can be enhanced by $Ca^{2+}$ and ADP (13).

There is an internal system of canals that open to the surface as indentations. Platelet membranes contain glycoproteins, glycolipids, absorbed plasma proteins, and mucopolysaccharides. Their membrane is a bilayer of phospholipids in which cholesterol, glycolipids, and glycoproteins are embedded. More than half of the platelet's phospholipids are in the plasma membrane, which contains the pumps that maintain ionic homeostasis. Platelet membranes are about 35% lipids and 8% carbohydrates. Phospholipids comprise 75%, neutral lipids 20%, and glycolipids 5%. Arachidonic acid (AA) is an important component of the various phospholipids. This provides a store of AA for conversion to thromboxane $A_2$.

### *2. Function*

Major platelet function includes (i) shape change, (ii) exposure of GPIIb/IIIa receptors, (iii) degranulation, (iv) surface change to a procoagulant state, (v) increase in $(Ca^{2+})i$ [normally $(Ca^{2+})i = 10^{-7}$ M, $(Ca^{2+})e = 10^{-3}$ *M*], and (vi) calcium-dependent phosphorylation of platelet proteins. They act as a storehouse for a variety of substances affecting vascular tone, fibrinolysis, and wound healing. Platelets function in blood coagulation by aggregating at breeches of the vessel wall and produce various factors that aid in clot formation. They are also responsible for clot retraction and contribute to clot removal (45).

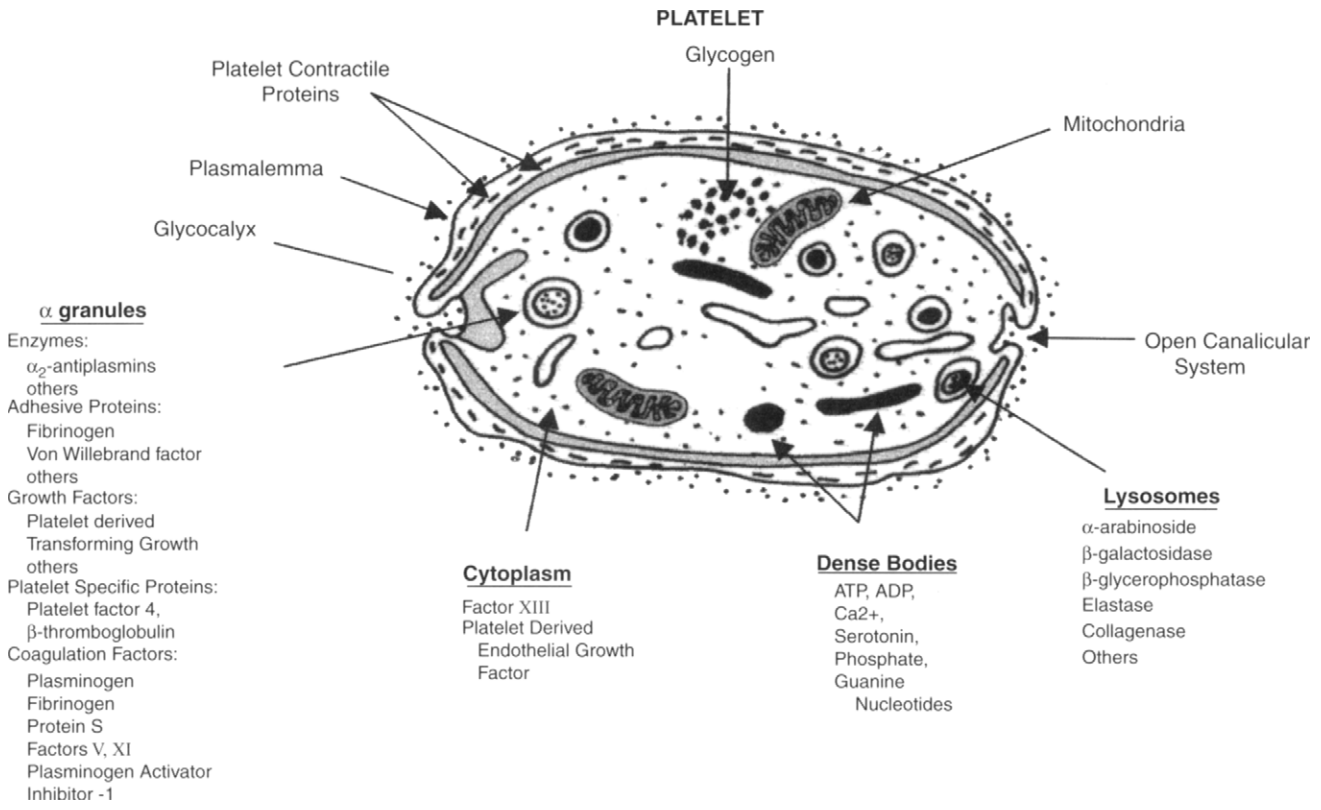


**FIGURE 3.6** The platelet and its contents and products.

Platelets are activated by multiple stimuli, including (i) substances in the milieu of arterial atherosclerotic plaques such as ADP, epinephrine, collagen, and thrombin, and (ii) mechanical stimuli such as shear forces. Internal pathways of platelet activation include the production of thromboxane $A_2$ via the arachidonic pathway. There is also a thromboxane $A_2$ independent pathway of aggregation. The final common pathway of platelet activation is the expression of platelet GPIIb/IIIa receptor, which binds to its primary ligand fibrinogen. Inhibitors of platelet aggregation include antithrombins, ticlopidine-type drugs, aspirin, thromboxane $A_2$ synthetase inhibitors, thromboxane $A_2$ receptor inhibitors, and glycoprotein IIb/IIIa receptor antagonist (46).

Thrombocytopenia is a platelet count less than 140,000/mm$^3$. Spontaneous hemorrhages usually do not occur until the platelets fall below 20,000–30,000/mm$^3$ (47,48). Post-SAH patients are at risk from thrombocytopenia because they are routinely exposed to anti-inflammatory drugs, antibiotics, antiepileptics, diuretics, and other medications such as heparin and cimetidine, all of which cause immune-mediate thrombocytopenia. In addition, calcium channel blockers and nitroprusside as well as radiographic contrast agents have also been known to cause platelet dysfunction (49).

### *3. Cytoskeleton*

The cytoskeletal elements of a platelet consist of membrane attachments, a microtubular system, and microfilaments (42,44,45). The glycoproteins of the membrane attachments include actin-binding protein, spectrin, and vimentin. The microtubular system within platelets is composed of the two polymers α- and β-tubulin. Their organelles consist of peroxisomes which have high catalase content and contribute to lipid metabolism, particularly plasmalogen synthesis. The platelet cytoskeletal proteins resemble those of vascular smooth muscle. They include actin (MW = 42 kDa). ATP is broken down to produce the energy for the polymerization of actin to produce the F-actin filaments which consist of two intertwined strands. Myosin (MW = 480 kDa) makes up 2–5% of platelet protein. Myosin light chain (MW = 20 kDa) in a phosphorylated state is required for ATPase activity. Myosin light chain kinase (MLCK; MW = 105 kDa) phosphorylates myosin light chain and activates actomyosin ATPase, which leads to contraction. Tropomyosin

(MW = 28 kDa) binds to the groove on actin filaments. Caldesmon (MW = 80 kDa) binds to actin, tropomyosin, myosin, and calmodulin and may have a role in actin filament bundling and actomyosin ATPase. Calmodulin (MW = 17 kDa) binds four $Ca^{2+}$ and activates MLCK. Additional cytoskeletal proteins include profilin, gelsolin, thymosin $\beta_4$, actin-binding protein, talin, α-actinin, and vinculin, which may link actin to membrane proteins.

Platelets change from their normal discoid shape to that of a sphere from which long filopodia extend several micrometers, ending in tiny points. This shape change probably depends on actin fibril formation.The initiator of actin polymerization may be increased cytosolic $Ca^{2+}$ or accelerated phosphoinositol metabolism. The contraction involving actin and myosin may stimulate granule secretion and later clot retraction. When blood initially clots, the fibrin mesh extends throughout the clot, solidifying all of the serum in a gel-like state. The clot retracts within minutes to hours, extruding a large fraction of the serum.

### *4. Cytoplasm*

The mitochondria are involved in oxidative energy metabolism (50). Platelet lysosomes are granules containing acid hydrolases. Lysosomes secrete their contents more slowly and incompletely than α granules and dense bodies. The dense bodies have high $Ca^{2+}$ content. They are also rich in 5-HT and adenine nucleotides. The most abundant granules are α granules, which number up to 80 per platelet and contain high concentrations of β-thromboglobulin, platelet factor 4, and proteoglycans. There are also multiple adhesion glycoproteins, coagulation factors, mitogenic factors (including platelet-derived growth factor), transforming growth factor-β, endothelial growth factor, and epidermal growth factor. Fibrinolytic inhibitors include $\alpha_2$-plasmin inhibitor and platelet activator inhibitor-1. Platelet factor 4 binds to heparin-like molecules on the surface of endothelial cells and neutralizes heparin. β-Thromboglobulin is chemotactic for granulocytes and enables them to undergo endocytosis. Fibrinogen is concentrated in the α-granules. Platelets have about 20% of the factor V present in whole blood. It is found in the α granules where protein S is also located. Transforming growth factor-β promotes the synthesis of extracellular matrix proteins, plasminogen activator inhibitor-1, and metalloproteinases. Platelet surface proteins include integrins, leucine-rich glycoproteins such as von Willebrand's factor, immunoglobulins, cell adhesion molecules, selectins, quadraspanin, seven transmembrane domain (G protein-linked) proteins including the thrombin receptor, the thromboxane $A_2$ receptor, the $\alpha_2$-adrenergic receptor, and the vasopressin receptor.

### *5. Metabolism*

Platelets gain their energy from glycogen stores as well as uptake of glucose from the surrounding medium. Platelet stimulation is accompanied by increased glycolytic activity and oxidative ATP production (50).

### *6. Aggregation*

In contrast to an intact endothelial layer, the subendothelium stimulates platelets to adhere (44). Platelets in the subarachnoid space presumably contact the nonendothelial cells which line it and possibly also collagen fibers, causing the platelets to degranulate and attach. Components active in platelet aggregation are the subendothelium and endothelial components such as von Willebrand's factor, platelet-activating factor, and thromboxanes. Components that are also active in coagulation include tissue factors V, 1X, and X, fibrinogen and fibrin, kininogen, factors XI and XII, prekallikrein, factor XIII, and vasoconstriction. Vasoconstriction alone cannot result in hemostasis without the participation of the coagulation system and platelets. Platelets have evolved to adhere to damaged regions of blood vessels where they link to one another and generate thrombin. This contributes to the hemostatic mechanism by producing a platelet plug that is then reinforced by the action of thrombin, converting soluble fibrinogen to fibrin strands that enmesh the platelets and RBCs at the site of injury. When an aneurysm ruptures, vasoconstriction, coagulation, and platelet plug formation are all operative. The response is proportional to the extent of tissue damage and the amount of tissue factor exposed, the age of the individual, the hematocrit, the size and location of the vessel, and the nature of blood flow through it. The strongest physiologic activators of platelets are collagen exposure, ruptured arteriosclerotic plaque, and thrombin. Shear stress and thrombolytic agents may also activate platelets. Platelet adhesion is stimulated by activation of the GPIIb/IIIa receptor, but as adhesion and aggregation proceed other processes are involved including the release of thrombin. Thrombin generation is facilitated by aggregated platelets through several mechanisms including exposure to activated factor V. Thrombin in turn activates platelets, leading to even more extensive degranulation and further coagulation. It initiates the deposition of fibrin strands that reinforce the platelet plug. Mitogenic and vasoactive agents released from platelets contribute to the inflammatory response. The activation of platelets is strongly countered by both prostacyclin and NO released from endothelial cells. Fast blood flow, WBC–platelet interactions, and inhibitors of thrombin generation can also inhibit platelet activation. Platelets accelerate thrombin formation by unclear mechanisms (45). The

fibrinogen receptor on the surface of the platelet (GPIIb/IIIa) is the dominant platelet receptor, numbering 80,000 per cell. Up to 40,000 are also present inside the platelet in the α-granule membranes and the lining of the canalicular system. This receptor shares the same basic structure as the other integrin receptors. It is a transmembrane proteins with four calmodulin domains that are able to bind divalent cations. When platelets are activated they bind fibrinogen relatively strongly. Other platelet receptors include those for collagen, fibronectin, laminin, vitronectin, and other unknown substances. Thrombin is the only clotting factor to activate platelets. It is a powerful stimulant of phosphatidylinositol and cAMP synthesis. $(Ca^{2+})i$ also increases, DAG is formed, and proteins are phosphorylated. Platelets also have receptors for α-adrenergic compounds. Upon activation these induce aggregation and degranulation but not shape change. Other effects include elevation of $(Ca^{2+})i$ and inhibition of adenyl cyclase. There is a synergism between epinephrine and other platelet activators. The principal sites for 5-HT storage are the dense granules of platelets. 5-HT in physiological concentrations can also elevate $(Ca^{2+})i$, activate phospholipase C, phosphorylate proteins, and induce mild aggregation. Platelet-activating factor is a phospholipid produced by the interaction of endothelium and certain white cells. Vasopressin also interacts with platelets to induce shape change, aggregation, and degranulation.

The platelet–endothelial cell adhesion molecule-1 (MW = 130 kDa) is a transmembrane glycoprotein of the immunoglobulin family, which includes CD32, CD102, and HLA class 1 molecules. P-selectin is found on the surface of α granules. Other membrane proteins include quadraspanins, CD36, lysosome-associated membrane proteins, and multiple other receptors. All the platelet agonist receptors so far characterized are linked to G proteins, which can associate with and activate platelet membrane phospholipase C. Aggregation can proceed quickly. Following the addition of thrombin, within 10 sec 80% of 5-HT stored in the dense granules is released. Elevation of $(Ca^{2+})i$ and phosphorylation of myosin light chain and the substrate of protein kinase C (PKC) all occur in less than 1 sec. The mechanisms by which platelets suddenly change their shape and aggregate at the site of vessel injury are likely to involve the increase in $(Ca^{2+})i$ and the phosphorylation of cytosolic proteins. The latter is probably brought about by changes in the membrane receptor GPIIb/IIIa to a high-affinity binding state. When agonists bind to it, the G protein α subunit activates phospholipase C, which then hydrolyzes phosphatidyl inositol 4,5-bisphosphate, which in turn forms DAG (which mediates PKC activation) and $IP_3$ formation. The binding of $IP_3$ to tubular membrane receptors results in $Ca^{2+}$ release to the cytoplasm and the activation of various proteins, including PKC and phospholipase $A_2$. The latter releases AA from membrane phospholipids. Estimates have been made that as many as 60 phosphoinositol products result from agonist stimulation. About one-third have been identified. There is a simultaneous induction of platelet coagulant activity and release of the contents of their α granules and dense granules. The contents of these granules are lysosomes, which become expressed on the surface inducing coagulant activity and shape change. The low concentration of the cytosolic $Ca^{2+}$ pool is regulated by the plasma membrane $Na^+$–$Ca^{2+}$ transporter and a slowly exchanging pool in the dense tubular system regulated by $Ca^{2+}$, $Mg^{2+}$-ATPase (50). Normally, the concentration within the tubular system is the same as that of the extracellular $Ca^{2+}$. Upon agonist stimulation there is a rapid elevation of cytosolic $Ca^{2+}$ with entry of $Ca^{2+}$ through a receptor-operated channel and release from the tubular system through another $Ca^{2+}$ channel. $IP_3$ triggers the extrusion of $Ca^{2+}$ from the dense tubular system. Elevation of the $(Ca^{2+})i$ activates several enzymes including phospholipase A which starts the AA cascade with activation of PLC hydrolysis. Aspirin and nonsteroidals medications can reduce $IP_3$-induced platelet aggregation and degranulation. $(Ca^{2+})i$ rise following exposure to an agonist lasts only seconds, but the sequential effects can be longer lasting such as the phosphorylation of myosin light chain through the action of MLCK.

Collagen directly increases platelet adherence, aggregation, and degranulation. This is true for both connective tissue and basement membrane collagen. Collagen probably activates a G protein. Thromboxane $A_2$/prostaglandin $(PG)H_2$ is produced by both blood vessel wall and activated platelets and triggers further release of AA, which is converted to thromboxane $A_2$ by the enzyme thromboxane synthase. Intermediate arachidonic metabolites have very short plasma half-lives.

Calpains are found in platelets. These calcium-dependent neutral proteases preferentially cleave cytoskeletal proteins such as actin-binding protein and talin. They may promote cytoskeletal reorganization following activation but are not involved early in the aggregation process. Calpains can also cleave PKC. It is hypothesized that calpains may have a role in the irreversible aggregation of clot retraction rather than the earlier events. Activation of PKC can also induce platelet aggregation and degranulation. Its effects are complex, neither solely activation or inhibition. There are at least eleven different proteins in the PKC family.

Phospholipase $A_2$ is present inside the cytoplasm of almost all cells and exists as multiple isozymes that release AA from surface membrane phospholipids (50). This action is regulated partly by $(Ca^{2+})i$. Following its release

from membrane phospholipids, AA is metabolized along several pathways. Via the cyclooxygenase pathway, short-lasting thromboxane $A_2$ is produced. It enhances platelet aggregation. Through the other major pathway, lipoxygenase enzymes convert AA into a variety of both stable and unstable metabolites such as hydroxyperoxyeicosatetranenoic acids, which in some cases may prevent collagen-induced platelet aggregation and arachidonate release. Tyrosine residues of intracellular proteins frequently become phosphorylated during platelet activation. This occurs on granular release of thrombin and another agonist. Platelets can also produce intracellular second messengers such as cAMP. In general, cAMP and cGMP inhibit platelet activation (50). Synthesis of RNA is directed by large, complex enzymes or groups of enzymes called polymerases. Translation is a ribosomal process in which a polypeptide chain is constructed according to the pattern dictated by the sequence of codons in mRNA.

#### *7. Vasospasm*

In a feline two-hemorrhage model of SAH, scanning electron microscopy of the basilar artery was performed. One hour after SAH no platelet adhered to the luminous surface, but in the time period 4–7 days post-SAH platelets were observed to adhere or aggregate on the luminal surface indicating an impairment of anti-platelet-aggregating activity of the endothelial cells (51). Platelet-activating factor was administered together with autologous blood to rabbits by intracisternal injection. Neurological deterioration and basilar artery constriction were apparently aggravated in a dose-dependent fashion. A reduction in basilar artery constriction was achieved using anti-platelet activating factor IgG. The neurological deterioration was also prevented by intracisternal administration of anti-platelet-activating factors (52). In a canine study, the use of the platelet aggregation inhibitor ticlopidine was not associated with any amelioration in basilar artery VSP, histological changes in the basilar artery, or any significant change in systemic whole blood coagulation time, prothrombin time, or partial thromboplastin time (53).

Studies of intravascular platelet and coagulation factors performed on human patients revealed systemic platelet hyperactivity and a hypercoagulant state following SAH. It is possible that changes in intravascular components could accelerate cerebral ischemia due to VSP by forming microthrombi, contributing to increased blood viscosity and reducing the deformability of RBCs (54).

#### *8. Fibrinolysis*

The lysis of clot is also influenced by platelets (45,50). Both profibrinolytic and antifibrinolytic effects of platelets are known. The profibrinolytic effects of platelets include release of t-PA and u-PA, the binding of unactivated platelets to plasminogen, and the acceleration of this binding by thrombin. The plasminogen-binding protein thrombospondin is found on the surface of platelets after activation, activation of plasminogen by t-PA is enhanced by platelets, and clot lysis is thereby promoted. On the other hand, platelets, have an antifibrinolytic effect. Plasminogen activator inhibitor-1 and $\alpha_2$-antiplasmin are present in platelet granules. Platelets also release a protein that stimulates other cells to release a fibrinolytic inhibitor. Platelets contain factor XIII, which can crosslink fibrin and retard its breakdown. However, by crosslinking $\alpha_2$-antiplasmin to fibrin, platelets have an antifibrinolytic effect. Platelet surface receptors can bind plasma factor XIIIa, which localizes it to the site of thrombus formation. The efficiency of fibrinolysis is diminished by platelet-induced clot retraction. Thrombolytic agents such as t-PA can activate platelets. Plasma can aggregate platelets but at low doses it can inhibit platelet activation and aggregation. Thrombolytic agents may generate thrombin and depress the prostacyclin increase which follows acute thrombosis. Thrombolytic agents also have platelet-inhibiting effects. Platelets can be disaggregated by t-PA because of selective lysis of platelet-bound fibrinogen. Platelet glycoprotein can be cleaved by plasmin. Fibrinogen degradation products may inhibit platelet aggregation.The effect of fibrinolytic agents on platelets is therefore complex. Stimulation of platelets by thrombolytic agents may actually prolong the time required for reperfusion and contribute to reocclusion following successful clot lysis.

### F. Neutrophils

#### *1. Structure*

Neutrophils have a diameter of approximately 9 μm and make up 60–70% of WBCs. The nucleus has three or four lobes (Fig. 3.7). The cytoplasm is loaded with azurophilic granules, which are small and stain light pink with Wright's or Giemsa stains and contain acid and alkaline phosphatase, collagenase, lactopherin, lysozime, and phagocytin (55, 56).

Normally, there are between 4300 and 10,800 WBCs/$mm^3$ (compared to 4.2–5.9 million/RBCs/$mm^3$) so there are approximately 400–1400 RBCs for every 1 WBC. Following SAH the ratio of RBCs to WBCs in the CSF decreases with the inflammatory response which destroys RBCs and introduces more WBCs into the CSF. Normal CSF has fewer than five lymphocytes or monocytes/$mm^3$.

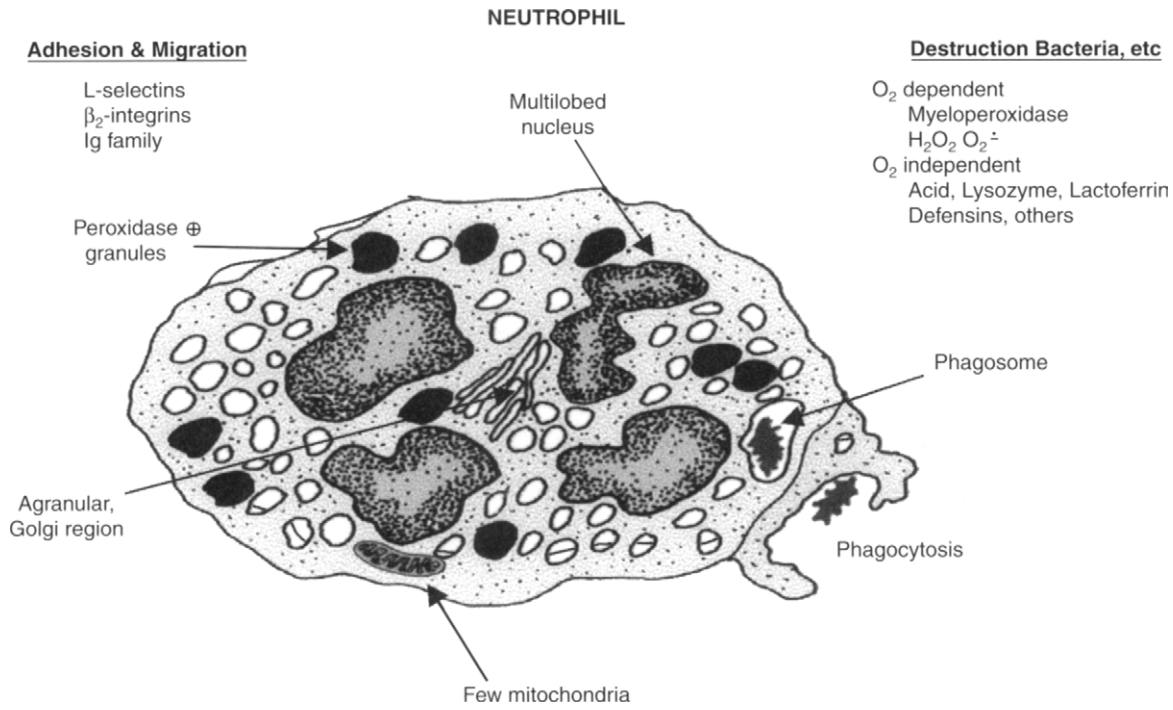


**FIGURE 3.7** The neutrophil and its contents and functions.

### 2. *Contents*

Water comprises 82% of the weight of WBCs. The mean $Na^+$ of mixed WBCs is 60 m*M* and the mean $K^+$ is 100 m*M*. Granulocytes contain 7.36 mg/$10^9$ cells of polysaccharides (57). Neutrophils contain more amino acids than the surrounding plasma. Unbound amino acids found in the highest concentrations are taurine, serine, glycine, alanine, glutamic acid, and glutamine. Six different phospholipids make up 35% of the lipid composition of neutrophils, with the remainder being composed of triglyceride, glycolipid, and cholesterol. Seven nucleotides have been demonstrated, with the most abundant being ATP (8800 nmol/$10^9$), AMP (6100 nmol/$10^9$), and ADP (1600 nmol/$10^9$). Neutrophils have all the types of RNA and cofactors required for protein and vitamin synthesis (57).

### 3. *Function*

Neutrophils have a half-life of 6 hr (58). They function to phagocytose, kill, and digest bacteria (59). They are capable of producing $H_2O_2$ during phagocytosis. Neutrophils protect against infection. At sites of infection or tissue damage they adhere to vascular endothelial cells and migrate through or between them. Neutrophils are attracted by such molecules as complement cleavage molecules, certain leukotrienes, and platelet-activating factors. Having left the circulating blood, few neutrophils return. Neutrophil–endothelial cell adhesion is brought about by a wide variety of proteins on both cell types, including multiple integrins, selectins, and Ig family molecules (55).

### 4. *Metabolism*

Neutrophils generate ATP via the glycolytic pathway and anaerobic pathways (60). The conversion of glucose to lactate is the principal means by which neutrophils produce energy. The rate-limiting enzyme of glycolysis is hexokinase. Phagocytosis does not affect the rate of glycolysis but ATP levels fall. Neutrophils can also metabolize glucose using the hexose monophosphate shunt. Of 21 glycolytic and related enzymes found in neutrophils, the most active are phosphoglycerate kinase, triosephosphate isomerase, glucosephosphate isomerase, and glyceraldehyde P.

The NADPH which generates microbiocidal oxidants is produced by the hexosemonophosphate shunt. Glycogen is present in large quantities in neutrophils. Oxidative metabolism is increased by phagocytosis, thyroid hormone, increased $CO_2$ levels, glucose concentration, and a variety of immune substances. Mature neutrophils have few mitochondria. Neutrophils can digest exogenous nucleic acids using ribonuclease and deoxyribonuclease enzymes normally present. Adenyl cyclase generates cAMP, and phosphodiesterase degrades cAMP.

Neutrophils synthesize phospholipids and neutral lipids. Neutrophils have the biochemical machinery to release AA from phospholipids. Cyclooxygenase- or lipoxygenase-catalyzed oxidation converts AA into a variety of lipid mediators. Cyclooxygenase produces $PGE_2$, $PGD_2$, and $PGF_2$, with $PGG_2$ and $PGH_2$ as intermediates. $PGE_2$ is a mediator of inflammation and $PGH_2$ converts into thromboxane $A_2$, an unstable vasoconstrictor. 5-Lipoxygenase oxidizes AA to 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid, which is subsequently converted to leukotriene $A_4$. Other leukotrienes produced have anti-inflammatory actions (60).

### *5. Acute Inflammation*

Inflammation consists of a complex series of events that protect the body against injury and infection (61). In the first minute to days after infection or injury there is a local hemodynamic and microvascular change that causes leukocytes to adhere to the endothelium and migrate, following which they are activated and release toxic products. This process is governed by a variety of compounds found in plasma or released from local cells. Following the acute phase there is a longer lasting infiltration by lymphocytes and monocytes, sometimes accompanied by proliferation of local fibroblasts and capillaries.

Normal neutrophil production rate is $0.85–1.6 \times 10^9$ cells/kg per day (58). Neutrophils are produced and stored in the marrow before release into the bloodstream, and within 6 hr of entering the circulation half of them have disappeared. The loss is thought to be from migration into tissue or departure through the gastrointestional tract and mucosal surfaces. Neutrophil production is stimulated by colony-stimulating factor, glycoproteins, certain interleukins (ILs), and stem cell factor. When macrophages and T lymphocytes are activated by inflammation they release colony-stimulating factor and cytokines which also cause endothelial and mesenchymal cells to release colony stimulating factor, ultimately producing more neutrophils.

The granules of neutrophils contain microbiocidal enzymes, antibacterial proteins, neutral serine proteases, metalloproteinases, acid hydrolases, and a variety of other enzymes, receptors, and binding proteins. The primary granule of the neutrophil is a reservoir for digestive and hydrolytic enzymes which are subequently delivered to the phagosome. Proteases in the azurophilic granule effect tissue destruction by being released during inflammation. They include serine protease, elastase, and cathepsin (58).

It is likely that the deposition of blood in the alien subarachnoid space causes local inflammation with release of inflammatory mediators such as histamine and PG and vasodilation of the adjacent vasculature in the pia-arachnoid and in the walls of the blood vessels running through the subarachnoid space. The first cells to migrate into the subarachnoid clot are neutrophils followed by other WBCs. Under the influence of a group of molecules called selectins, WBCs are rolled to a halt on the walls of blood vessels at the site of inflammation and subsequently insinuate across the vessel walls. WBCs, platelets, and endothelial cells will have their own particular selectins. Firm adhesion of neutrophils to the vascular endothelium is also influenced by the $\beta_2$ integrin family of molecules which interacts with endothelial intercellular adhesion molecules. Many endogenous and exogenous compounds can act as chemotactic agents for leukocytes. These include complement components, lipoxygenase pathway products, bacterial-derived chemotactic peptides, and endogenous cytokines. WBCs engulf and destroy foreign particles by a process of phagocytosis. First, the WBC binds to the target cell and subsequently engulfs it. Phagocytic cells kill microbes or destroy tissues by releasing a wide variety of $O_2$-dependent, arginine-dependent, or non-free-radical enzymes.

Regulatory proteins called cytokines are involved in acute inflammation. IL-1 causes fever and the acute-phase response. IL-1 is produced by monocytes, macrophages, and endothelial cells. The same cells produce nitrogen radicals and derivatives. Macrophages also produce complement in conjunction with endothelial cells. WBCs are a major source of $O_2$ radicals. They also release a variety of proteases and other proteins by degranulation. In addition, platelet-activating factor and arachidonic acid metabolites also come from WBCs (61).

The complement system consists of 18 plasma proteins which mediate chemotaxis, increase vascular permeability, activate phagocytosis, and have opsonic and cytolytic activity (61). This system is activated sequentially by proteolytic cleavages of its early components by one of two pathways, the classical pathway or a second one triggered by a variety of substances such as toxins and venoms. The clotting cascade generates fibrinopeptides that increase vascular permeability and attract WBCs.

Complement activation forms membrane attack complexes which insert themselves into the cells that initiate complement activation or into innocent bystanders cells. In the presence of autologous serum aged RBCs caused a large increase in membrane conductance of the VSMCs. This effect was prevented by heat inactivation of the serum. The effect was only seen with aged RBCs and not fresh ones. C8- and C9-depleted heterologous sera produced minimal effects, which could be changed into

the full effect by the addition of missing complement component. It was hypothesized that the membrane attack complexes, presumably designed to destroy the intruder RBCs in the alien subarachnoid space, might have deleterious effects on the innocent bystanding muscular smooth muscle cells (62).

Immunostain for leukotriene C4 was strongly positive in the intima and the adventitia of normal canine basilar artery. After SAH there was little change. Inflammatory cells characterized as neutrophils and macrophages were shown to infiltrate from the adventitia of the basilar artery to the periphery of blood clot and these were markedly immunoreactive for leukotriene C4. The neutrophils increased in numbers progressively as time passed after the SAH. It was hypothesized that the leukotriene C4 might be an etiologic agent for the VSP and that it could originate with the infiltrating neutrophils and macrophages. Neurons but not astrocytes or oligodendrocytes were also immunoreactive for leukotriene C4 (63).

In 103 patients the WBCs and platelet counts after SAH were significantly higher in those with symptomatic VSP at all time intervals compared to those without (64). The minimum platelet counts were similar in patients with and without VSP (65).

A study in rabbits found no histopathological (neutrophil-derived myeloperoxidase) or biochemical evidence for neutrophils in the genesis of VSP (66).

#### *6. Chronic Inflammation*

Chronic inflammation is associated with the infiltration of lymphocytes, plasma cells, monocytes and fibroblasts, angiogenesis, and deposition of collagen. Numerous mediators are involved in the attraction of such cells to a site of chronic inflammation. The chemokine β family is involved in the induction of monocyte and lymphocyte chemoattraction. There are very specific monocyte chemotactic proteins. Fibroblastic infiltration and angiogenesis are mediated by a variety of cytokines and growth factors. The cellular composition at sites of chronic inflammation is variable (61).

Some patients develop an intense fibrosis in the subarachnoid space following SAH, whereas others have almost a complete return to normal. The volume of the subarachnoid clot is probably a major determinant of outcome.

### G. Mast Cells and Basophils

#### *1. Structure*

Basophils have diameters of approximately 8 μm, number 50–100/mm$^3$, and are the rarest of the WBCs comprising 0.5–1%. The nucleus is S-shaped. The few granules are large and dark blue when stained. The cytoplasm contains eosinophilic chemotactic factor, heparin, histamine, and peroxidase. They have the longest life span of WBCs and may survive for months. The basophils which have IgE receptors on the plasma membrane mediate inflammatory responses (67).

#### *2. Function*

These cells generate ATP via the glycolytic pathway and Kreb cycle. Mast cells and basophils make up the subpopulations of basophilic leukocytes. The basophils circulate in the blood while mast cells normally migrate into connective tissues or serous cavities. Basophils are round cells with irregular short surface projection, secretory granules, and cytoplasmic glycogen aggregates. Mast cells are characterized by partially condensed chromatin, numerous cytoplasmic granules containing a crystalline structure, and regular thin surface projections. When they are activated mast cells and basophils undergo degranulation. Mast cells and basophils secrete histamine, platelet-activating factor, NO, AA metabolites, proteinases, and cytokines as well as other compounds. Mast cells and basophils can be differentiated by the characteristic surface phenotypic markers which include Fc receptors, various integrins, and cytokine receptors. Basophils and mast cells are involved in many allergic and inflammatory disorders (68). They are probably involved in allergic inflammation. Basophilic WBCs affect a wide variety of other cells by releasing histamine, AA metabolites, and various cytokines. Mast cells may have a role in regulating tissue perfusion and maintaining extravascular space homeostasis (67).

In a few human cases, following SAH appropriate histologic technique has demonstrated a marked increase in mast cells in the muscular layer. To demonstrate these cells autopsy must be performed no later than 6 hr postmortem. The number of mast cells appeared to be higher in the arteries closer to the aneurysm (69).

### H. Eosinophils

Eosinophils have diameters of about 9 μm, number 150–400/mm$^3$, and comprise 2–4% of all WBCs. They are characterized by granules which show a pronounced take-up of acidic dyes. Eosinophils have a wide capacity to synthesize various cytokines and are selectively attracted to sites of allergic reactions (70, 71). Cytokines derived from eosinophils include IL, interferons, growth factors, and chemokines. Eosinophilia characteristically occurs in parasitic infestations by worms. Eosinophilia also occurs in allergy-related diseases such as asthma (70).

## I. Monocytes and Macrophages

### *1. Structure*

Monocytes have diameters of about 12 μm, number 200–800/mm$^3$, and make up 3–8 % of the WBCs (72–74). Their plasma membrane is capable of forming pseudophodia and pinocytic vesicles. Their cytoplasm has many azurophilic granules (Fig. 3.8). Monocytes survive less than 3 days in circulating blood and become macrophages extravascularly (75). Macrophages are relatively large cells with a round to reniform nucleus that occupies less than half the cell diameter. Kupffer, epithelioid, and microglia cells are all examples of macrophages in different locations. They vary in size from 10 to 80 μm. There are mononuclear and phagocytic cells. Histochemically, they stain for lipase and myeloperoxidase. Macrophages can fuse or otherwise form multinucleate giant cells (75). The terms histiocyte and macrophage are synonymous (76). The location and function can affect their morphology. They may have a brush border of the macrophage with large numbers of microvilli. The cytoplasm has a large variety of ribosomes, mitochondria, and lysosomes. The nucleus can be fusiform or horseshoe-shaped. Within the cytoplasm can be seen ingested cellular debris within lysosomes. There is a prominent microtubular and microfilamentous system and actin–myosin-like proteins are present.

### *2. Function*

Monocytes and macrophages share pronounced phagocytic abilities and other characteristics (77). Monocytes are attracted to sites of inflammation and actively diapedese across vessel walls. Outside the vasculature they mature into macrophages with enhanced phagocytic capacities and increased enzymatic content. Macrophages also exist within various extravascular spaces and cavities. Macrophages can ingest RBCs in the subarachnoid space. They interact with antigens and neutrophils in the developing immune response and are able to destroy RBCs rapidly in the subarachnoid space that would otherwise have lived months in the circulating blood. It is considered that the microglia within the parenchyma of the brain are tissue macrophages. The macrophages involved in the cleansing of the subarachnoid space originate from lining cells of the subarachnoid space but may also pass into the subarachnoid space from adjacent vasculature. There is also the possibility that microglia could

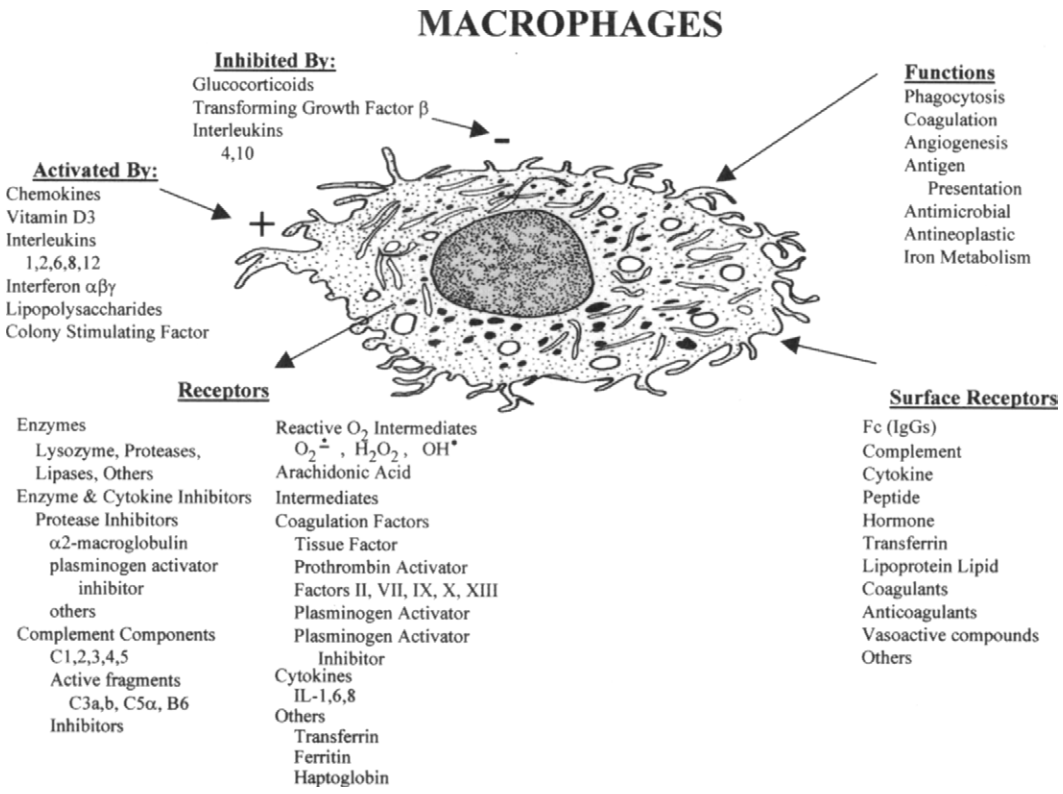


**FIGURE 3.8** The macrophage and its products, receptors, and functions.

migrate out of the brain into the subarachnoid space, although this seems less likely.

### *3. Metabolism*

Most of the metabolic energy of mononuclear monocytes is derived by glycolysis augmented by oxidative phosphorylation (78). Continual pinocytosis turns over the plasma membrane approximately twice each hour. Macrophages exist in a relatively quiescent state until stimulated. Stimulated macrophages can engulf complement coated RBCs, increase their synthesis and secretion of proteases and acid hydrolases, and increase the synthesis of complement.

### *4. Surface Receptors*

The surface of the macrophage has Fc receptors for IgG (78). Complement activation liberates numerous ligands which bind to phagocytic specific receptors. Complement receptor-1 is found on monocytes and macrophages. Monocytes and macrophages are antigen-presenting cells. They carry class II glycoproteins of the major histocompatibility gene complex. A specific marker for monocytes and macrophages is the CD68 antigen. Other surface receptors include cytokines, peptides, hormones, transferrin, lipoprotein lipids, coagulants and anticoagulants, fibronectin, and laminin. Surface receptors also exist for cholinergic and adrenergic agonists (72,73). Macrophages vary their output of biosynthetic and bioactive molecules depending on incoming chemical signals such as cytokines or bacterial lipopolysaccharides.

The wide variety of immunological and homeostatic responses orchestrated by macrophages is partly related to the hundreds of discrete surface receptors. Such receptors allow them to adhere to and engulf other cells, to ingest microorganisms, and to respond to cytokines and growth factors. The various glycoproteins called adhesins account for the ability of macrophage to adhere to surfaces. Surface IgG receptors promote engulfment and ingestion of immunoglobulin-coated particles. Macrophages secrete many cytokines, including IL1$\alpha$ and 1$\beta$, tumor necrosis factor-$\alpha$, and interferons-$\alpha$, -$\beta$, and -$\gamma$. Macrophages contain enzymatic machinery (NADPH oxidase) to generate superoxide free radicals and hydrogen peroxide (78). Many factors can attract monocytes, including: IL-1, thrombin, platelet-derived growth factor, C5a, collagen fragments, elastin, fibronectin, kallikrein, and plasminogen activator. When activated by inflammatory cytokines or receptor-mediated phagocytosis, activated macrophages initially respond with a respiratory burst releasing products of $O_2$ metabolism and proteolytic enzymes. These cause surrounding local cell death and matrix degradation. Within several days following activation, macrophages synthesize and release proteolytic enzymes. Macrophages can synthesize and secrete over 100 identified substances ranging from small ions to massive protein molecules. When the RBC is opsonized or coated with an IgG and comes in contact with the Fc receptor on the macrophage, it induces the assembly of a respiratory burst oxidase enzyme in the phagocyte. This oxidase generates a series of oxidants from molecular $O_2$ with the formation of free radicals. The RBC is enveloped by the phagocyte and is digested by its enzymatic machinery.

In the primate unilateral clot model, Handa and colleagues found a correlation between the degree of deposition of IgG in the media and the severity and time course of VSP. Their illustrations also suggested deposition in adventitia which could correspond with macrophage location (Fig. 3.9) (79).

Phagocytosis of immunoglobulin G-coated RBCs depresses several macrophage functions, including phagocytosis, respiratory burst capacity, and killing of bacteria. Hb-derived iron may interact with oxygen products of the macrophage respiratory burst. This does not occur when the RBCs are coated with complement or when RBC ghosts (lacking Hb) are used (80).

Following SAH, the spinal fluid shows an influx of WBCs within a few hours and a macrophage response beginning within a couple of days. Such cells are considered to originate from arachnoid trabeculations (81). The initial cellular response is of neutrophils, but within approximately 24 hr lymphocytes join the response (82). The leukocytosis in the CSF is proportional to the time of incubation of the RBCs prior to their injection into the subarachnoid space in animal models of SAH. Presumably, the hemolysis that occurs outside the body releases more Hb and other compounds which incite a greater inflammatory response. In some animals some of the RBCs injected into the subarachnoid space pass back into the systemic circulation via unknown lymphatic pathways or through the pacchionian granulations (83,84). The injection of RBCs and iron-containing compounds results in inflammatory changes in the meninges of dogs as well as the deposition of iron-positive material in ependymal lining cells (85). For 2–4 days after SAH the morphology of the arachnoidal villus remains normal, but a remarkable phagocytosis occurs 10 days or more after SAH with increased cellularity remaining as long as 3 months later (86). Macrophages produce enzymes such as plasminogen activator, elastase, collagenase, esterase, and lysozyme. The collagen-stimulating factor they produce presumably acts on leptomeningeal fibroblast target cells, causing them to lay down collagen and ultimately cause fibrosis (87). The CSF of humans shows

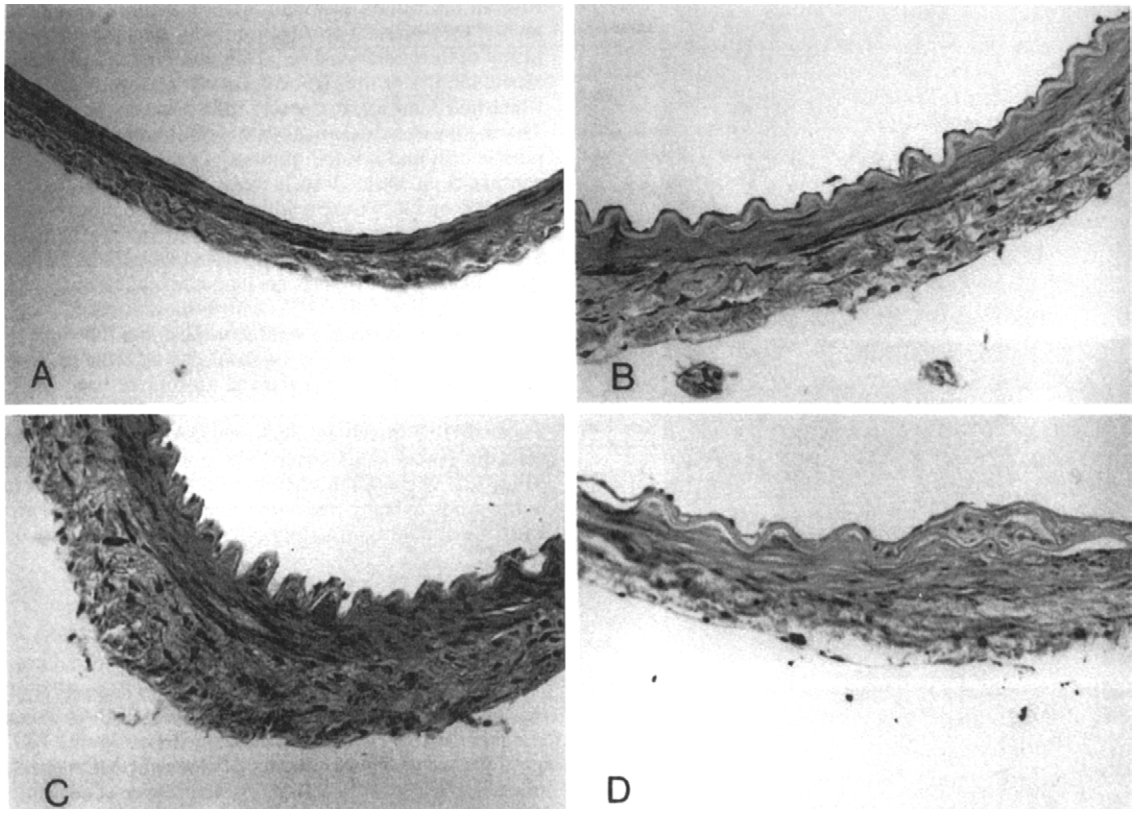


**FIGURE 3.9** Histopathological study with hematoxylin and eosin staining of the MCA on the clot side in the sham-operated group (A), the 3-day SAH group (B), the 1-week SAH group (C), and the 2-week SAH group (D). Deposition of IgG was observed in the media of the vasospastic artery, and the degree of deposition correlated with the severity and time course of VSP [reproduced with permission from Handa, Y., Kabuto, M., Kobayashi, H., Kawano, H., Takeuchi, H., and Hayashi, M. (1991). The correlation between immunological reaction in the arterial wall and the time course of the development of cerebral vasospasm in a primate model. *Neurosurgery* **28**, 542–549].

iron-positive cells as early as 1 week following SAH. Such cells increase to 8.5% of total nucleated cells in the CSF by 6 weeks after SAH and then decreases to 1% by 15–17 weeks. The iron-positive cells are all macrophages. They persist in the CSF long after the CSF has become clear, usually within 3 weeks of SAH. Cells stained with Perl's reagent identify intracellular $Fe^{2+}$ (88).

The induction of SAH in rats led to the inflow of macrophages and T cells to the subarachnoid space for 2–5 days after SAH. The peak time of appearance of T cells and macrophages was 2 days. The helper suppressor T cell ratio also peaked about 2 days after SAH. Immediately after SAH only a few leukocytes were observed in the subarachnoid space. Increased numbers of T cell lymphocytes and macrophages were found in the cerebral tissue within this same time frame (89). The time course of events after SAH in rats is probably accelerated and may well be different than that in humans.

## J. Lymphocytes

### *1. Structure*

Lymphocytes are small agranulocytes with diameters of about 8 μm, numbering 1500–2500/$mm^3$, and comprise 20–25% of WBCs (90, 91) (Fig. 3.10).

### *2. Composition*

Lymphocytes contain 71% $H_2O$. Cation content is 35 fmol/cell, of which 22–28 fmol/cell is $K^+$ and 7.9 fmol/cell is $Na^+$. Their $Ca^{2+}$ content is 580–800 pmol/$10^6$ cells. Cytosolic free $Ca^{2+}$ is very low. There is a relatively small amount of ribosome RNA compared to other cells but DNA content is the same. They have few lysosomes which contain a variety of enzymes such as acid hydrolases. The membrane consists of lipid and protein in equal amounts and a small amount of carbohydrates. Phosphatidyl choline is the main phospholipid. Various nucleotide enzymes are on the surface. Activated T lymphocytes

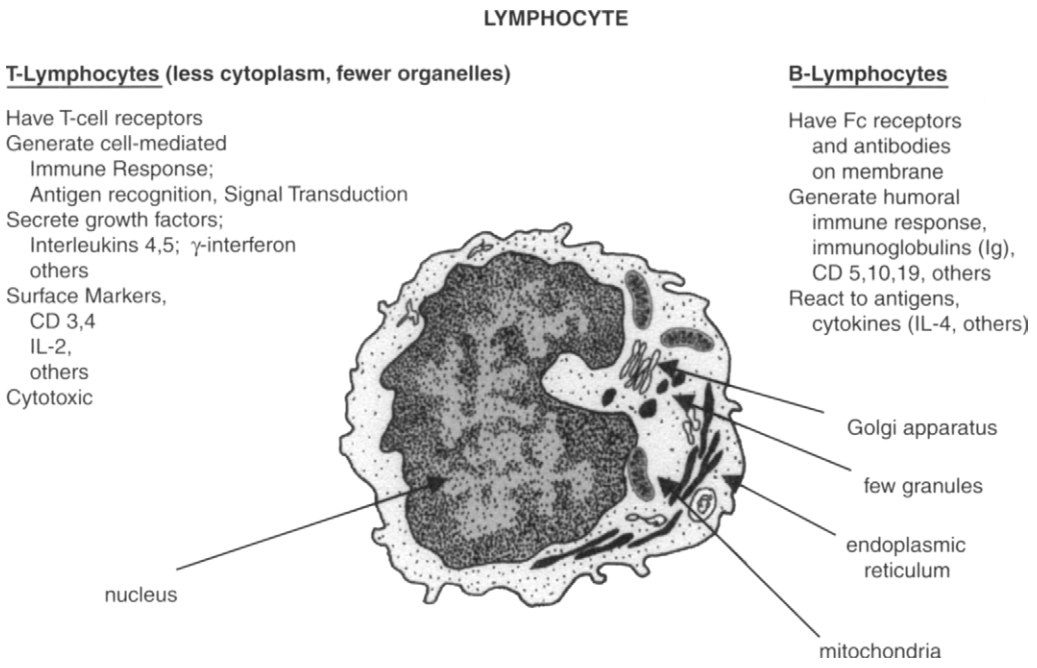


**FIGURE 3.10** The lymphocyte; T and B cell characteristics.

can release ATP. The plasmic matrix contains filaments and tubules containing tubulin, actin, myosin, filamin, and others. The mechanism exists to excise and repair DNA. The different cell surface receptors, depending on the stimulus, can trigger a cascade of protein kinase and phosphatases and nuclear transcriptional regulatory factors that control apoptosis, cell inactivation, proliferation, and differentiation (92).

### *3. Function*

T lymphocytes have T cell receptors (13). There are a few azurophilic granules in the cytoplasm. They can survive for several years and generate cell-mediated immune responses as well as secrete numerous growth factors. The B lymphocytes express Fc receptors and antibodies. There are a few azurophilic granules (Fig. 3.10). They do not survive as long as T lymphocytes (only a few months). B lymphocytes generate humeral immune responses. Agranulocytes also include null cells, which differ from T and B lymphocytes by their lack of characteristic surface determinants. They comprise about 5% of circulating lymphocytes. Other agranulocytes include hematopoietic stem cells and natural killer cells.

### *4. Vasospasm*

Of 13 cases of SAH, 9 developed VSP. There was a tendency for the latter cases to show decreased peripheral blood T lymphocytes, a depression of lymphocyte phytohemagglutinin, and concanavalin A response. Tuberculin anergy was found. These results suggested that a depression of cell-mediated immunity function might be associated with VSP (93). Patients with multiple SAH episodes provided lymphocytes that showed reduced adherence to fibroblast monolayers *in vitro*. Patients with single SAH episodes showed no difference from normals. It was considered that the influence of 2-CdA on the adherence of peripheral blood mononuclear cells was inversely related to the degree of immune system activation (94).

In a canine model of VSP the immunosuppressive agent FK-506 failed to effect the development of basilar VSP, although there was a suggestion of a slightly reduced lymphocytic infiltration around the basilar artery. Immunoglobulins (IgG, IgM, and C3) were present in the intima and the luminal side of the smooth muscle layer as well as brain capillaries. Their deposition may have resulted from increased vascular permeability in VSP (95). Similar results were obtained by a different group (96). Human lymphocyte antigen (HLA) types were studied in 45 SAH patients. HLA-Bw60 antigen showed significant increases in patients with delayed ischemic neurologic deficits resulting from VSP (97). The fraction of chromium-51, labeled peripheral blood mononuclear cells adhering to fibroblast monolayers was reduced in SAH patients with multiple hemorrhages compared to normal subjects. This can possibly be explained as a depletion of activated lymphocytes from peripheral

blood after SAH. The fraction of lymphocytes adhering was increased by 2-CdA (94).

$CD4^+/CD8$ ratio and T cell–extracellular matrix interactions were higher in patients after SAH and may play a role in VSP. No significant differences were found in major lymphocyte subsets between the patients receiving no dexamethasone after SAH and healthy donors (98).

## III. Coagulation

### A. Coagulation Pathways

Hemostasis depends on primary platelet plug formation and secondary fibrin formation. The entire coagulation cascade is activated by a complex of tissue factor and factor VIIa (13,99–104). The formation of excessive fibrin is opposed by a tissue pathway inhibitor (103,104). Blood coagulation is initiated when the integrity of the vascular tree is lost as in aneurysmal rupture and blood egresses into a nonvascular space such as the subarachnoid space. Hemostasis, or the cessation of the escape of blood, is achieved first by vasoconstriction and coagulation. Coagulation involves a complex interplay of platelets, coagulation proteins, and various plasma protease inhibitors. Blood coagulation is a result of a cascade in which an enzyme is generated at each step that then goes on to sequentially activate the next factor (Fig. 3.11). The initial response to injury is magnified. Various zymogen proteins become converted to proteases during coagulation. These include prekallikrein and factors IX–XII, VII, and II. Other proteins act as cofactors which speed up several reactions in the cascade. These include factors V and VIII and kininogen. Classically, the coagulation system has been divided into intrinsic (functioning to produce intravascular thrombin) and extrinsic (coagulation following tissue injury) pathways; although incorrect in that there are not two entirely separate pathways, it is still considered to be a useful concept to describe the two arms of the process which come together at the point where both series of enzymatic pathways generate activated factor X, referred to as Xa. Factor Xa then converts prothrombin to thrombin, which proceeds to cleave

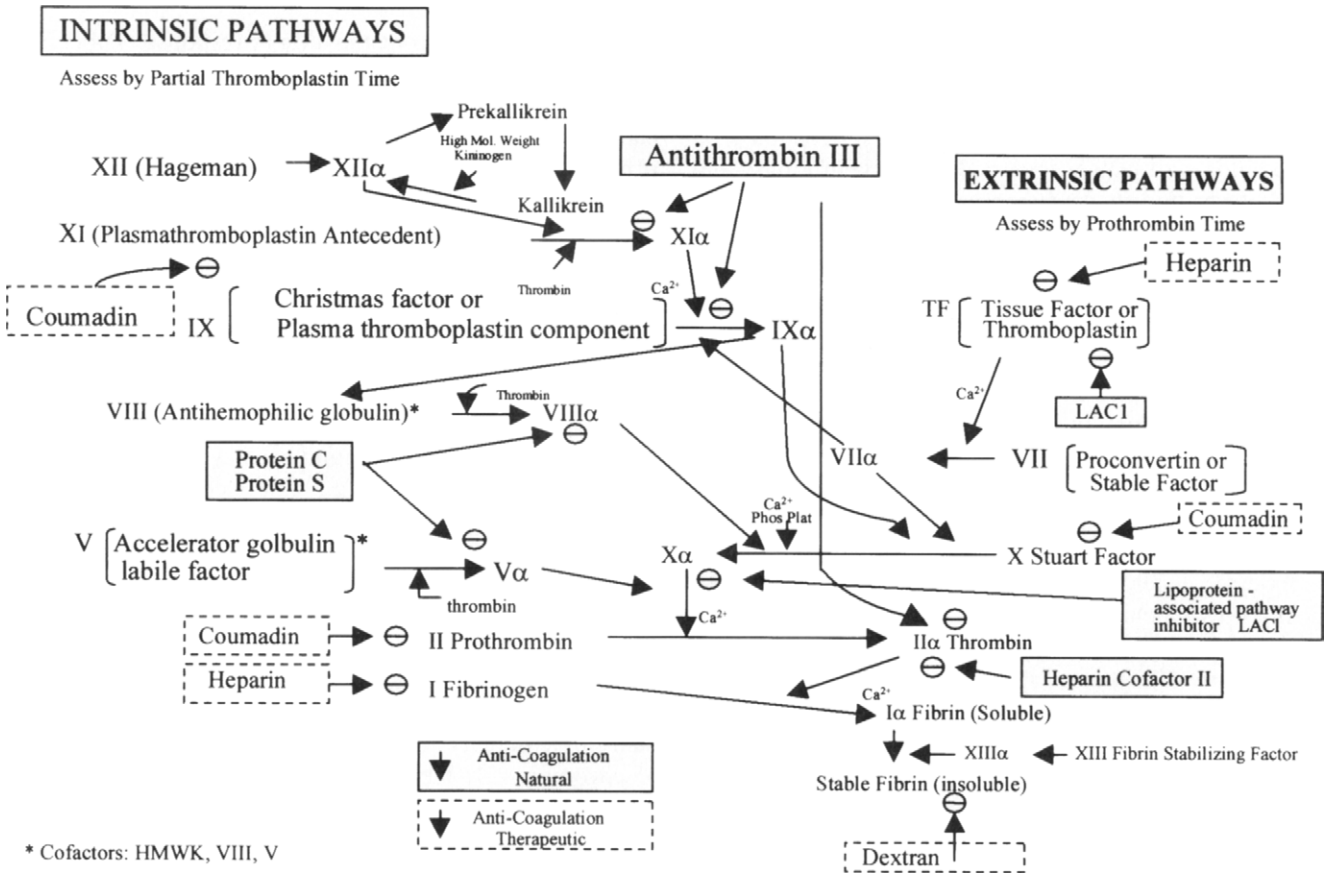


**FIGURE 3.11** Pathways of coagulation.

fibrinopeptides A and B from the fibrinogen molecule to produce fibrin. Thrombin also activates factor XIII, which crosslinks fibrin and stabilizes the developing latticework, thereby countering the fibrinolytic system.

When escaped blood contacts foreign tissues or exposed collagen not only is platelet aggregation initiated but also the coagulation cascade begins. Factor XII autoactivates to XIIa and this process is speeded up when factor XIIa converts prekallikrein to kallikrein. In the presence of kininogen factor, XIIa is much more rapidly activated. This is an early example of amplification.

Factor XI is activated on the same surface as factor XIIa. Factors XI and IX are then activated in sequence in the presence of $Ca^{2+}$. Factor IXa activates factors X (Stuart factor) in the presence of factor VIII (antihemophilic factor), $Ca^{2+}$, and phospholipid. This series of reactions comprises the "intrinsic" pathway. In the "extrinsic" pathway, tissue factor or thromboplastin is released from damaged tissue and reacts with factor VII to form a complex that, in the presence of $Ca^{2+}$, activates factor X. The brain is an extremely rich source of tissue factor. Activated factor Xa was recently shown to be inhibited by a lipoprotein-associated coagulation inhibitor, a protease inhibitor found in plasma. The interplay of the various factors in the coagulation cascade is extremely complex and probably incompletely understood.

The ultimate product of the coagulation cascade is crosslinked fibrin (Fig. 3.12). It is produced by the structural change in fibrin brought about by the interaction of factor XIII (fibrin-stabilizing factor). Fibrinogen consists of three pairs of polypeptide chains held together by disulfide bonds. Thrombin cleaves two types of peptides to produce fibrin monomers which rapidly polymerize various types of dimers and polymers in the minutes and hours after initial fibrin formation.

Coagulation factors that are used up in the course of coagulation include factors I, V, and VIII. Thrombin interacts with these three factors plus factor XIII. All of these factors increase during inflammation. Another different group of coagulation factors is the prothrombin group composed of factors II, VII, IX and X, which all require vitamin K for synthesis and $Ca^{2+}$ for activation. A third group of coagulation factors is the contact group, which includes factors XI, XII, and prekallikrein, which are not dependent on vitamin K for synthesis and are not calcium dependent (13).

Blood coagulation proteins have variable half-lives in plasma: factor VIII, 12 hr; fibrinogen, 3–5 days; antithrombin III, 2.5–4 days; prothrombin, 2–5 days, factor XI, 2.5–3.3 days; protein S, 1.75 days; factor X, 1.25 days; factor IX, 1 day; and the remainder less than 1 day (105). The survival of these coagulation

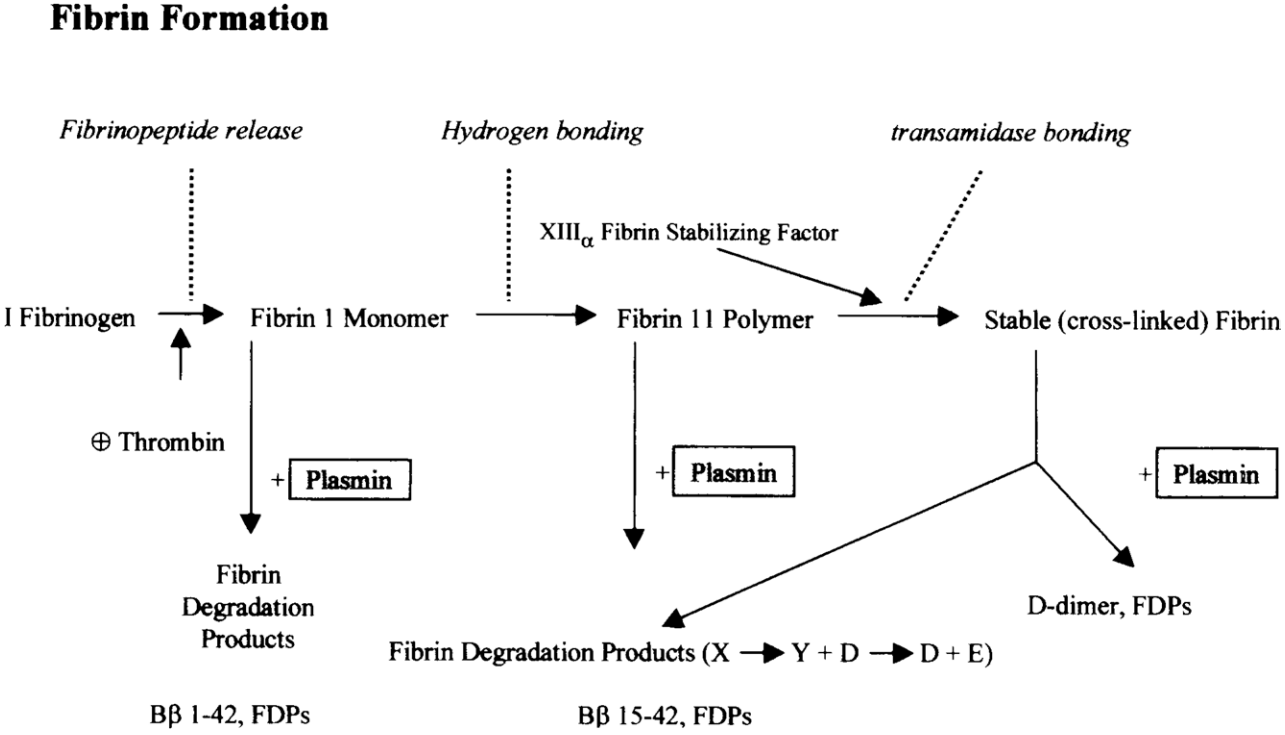


**FIGURE 3.12** Steps in fibrin formation and breakdown to fibrin degradation products.

factors within the CSF space would probably not be longer.

A case of ruptured aneurysm with hemophilia B has been reported. Factor IX was only 22.5% of control. The patient rebled postoperatively and died (106). Two SAH cases of women affected by congenital deficiency of factor VII who had SAH were reported (107).

In humans following SAH, IL-6 and platelet-activating factor increased for up to 14 days following SAH, IL-1β showed a transient increase only between 5 and 9 days post-SAH, and tumor necrosis factor-α remained unchanged. The highest levels of platelet-activating factor and von Willebrand factor as well as thrombin–antithrombin III complex were found in patients who developed delayed ischemic deficits from VSP. A hypercoagulable state following the SAH was associated with VSP as evidenced by these changes in internal jugular venous blood (108).

### B. Coagulation Inhibitors

There are also a group of plasma proteins that act to inhibit coagulation (Fig. 3.12) (102). These include $\alpha_2$-macroglobulin, antithrombin III, and $\alpha_1$-proteinase inhibitor. Antithrombin III inhibits factors XIIa, XIa, Xa, IXa, and thrombin. Its activity is greatly magnified by heparin.

The effects on thrombin are normally limited to the site of vascular injury because (i) thrombin is directly inactivated by plasma protease inhibitors, including $\alpha_2$-macroglobulin and $\alpha_1$-antitrypsin; (ii) thrombin–antithrombin complex formation prevents the escape of thrombin into the circulation; and (iii) thrombin is bound to the thrombomodulin receptor on the luminal surface of endothelial cells and this complex is unable to cleave fibrinogen.

CSF from patients following SAH has been analyzed for the presence of membrane-bound tissue factor and thrombin–antithrombin III complex. In the interval of 0–4 days post-SAH, levels were higher in poor-grade patients. High levels were predictive of VSP in the period 5–9 days post-SAH. Only the membrane-bound tissue factor correlated with outcome. Thrombin–antithrombin III complex did not correlate with VSP during the interval when it most commonly occurs, which suggested that thrombin activation would not be the cause of VSP (108). If thrombin–antithrombin III complex levels were greater than or equal to 25 ng/ml and plasmin-α-2plasmin-inhibitor complex levels were greater than or equal to 3 μg, only 25% of SAH patients had a good or fair outcome. With lower levels 83% had a good outcome. The levels of these complexes did not vary between patients with VSP or those without VSP (109).

Changes in blood coagulation and fibrinolysis were studied after SAH. The activated partial thromboplastin time, fibrin and fibrinogen degradation products, fibrinogen, and platelet aggregation rates were all found to be abnormal in the acute phase and related to outcome (110).

### C. Anticoagulants

The value of therapeutic anticoagulants is better established in the treatment of venous thromboembolic disease than arterial thrombosis (13,99,111). Bleeding occurring during oral anticoagulation is treated by discontinuance of the coumadin, intravenous vitamin K and concentrates of coagulation factors or fresh frozen plasma. Most common coagulation tests do not measure platelet activity since they are performed in cell-free systems. The prothrombin time (PT) is performed by adding $Ca^{2+}$ and thromboplastin to plasma which rapidly activates factor VII to form a clot. The partial thromboplastin time (PTT) is carried out by adding $Ca^{2+}$ and phospholipid to plasma. It is a test of the intrinsic coagulation cascade. Factor VII deficiency resulting from coumadin (Warfarin) is reflected in a prolongation of the PT. Heparin is monitored by the PTT because heparin enhances the efficiency of the main inhibitor (ATIII) of activated factors IX–XII (thrombin).

Kapp *et al.* (112) compared 112 consecutive patients with SAH receiving heparin and carotid ligation with patients receiving carotid ligation alone as reported by the Cooperative Study on Aneurysms. In the group receiving heparin, ischemic complications occurred in 6% compared to a 23% complication rate for the historical cooperative study control group. Mortality rates were apparently reduced from 16 to 10%. The use of historic controls severely limits the interpretability of this result (112). It is conceivable, however, that platelet microthrombosis was favorably affected.

### D. Fibrinolytics

The system that breaks down fibrin and protects the body from uncontrolled intravascular coagulation involves plasminogen, plasminogen activators, and $\alpha_2$-antiplasmin (113). Primary fibrinolysis occurs when plasmin is generated from plasminogen by an activator in the plasma. In distinction to this secondary fibinolysis is when plasmin is generated within a formed clot by an activator that is also present in the fibrin clot. Secondary fibrinolysis is the normal mechanism of clot degradation initiated by t-PA (Fig. 3.13).

Fibrinolytic agents lyse fresh thrombi (114,115). They have been used systemically for the treatment of pulmonary embolism or arterial thrombosis. Natural lysis of coronary or cerebral arterial thrombi occurs within 24 hr

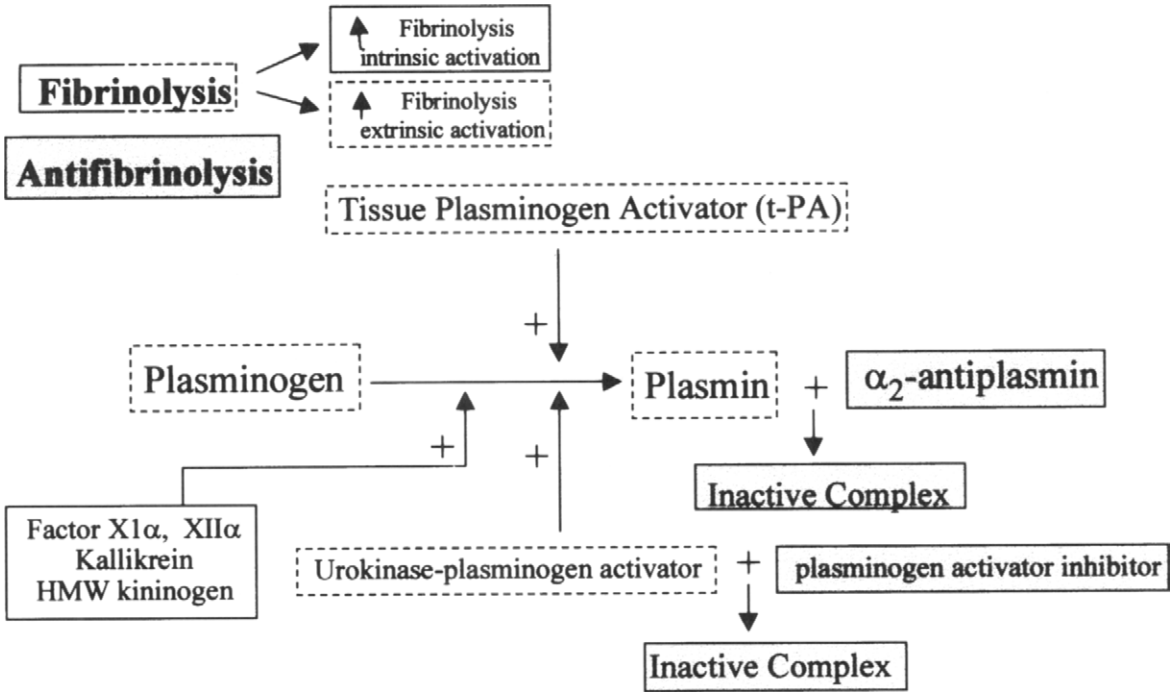


**FIGURE 3.13** Factors involved in fibrinolysis and antifibrinolysis.

in up to one-third of cases, but this is generally too slow a process to prevent infarction. t-PA has exceptionally high affinity for fibrin and theoretically can lyse thrombi with less systemic activation of fibrinolysis than u-PA or streptokinase (13).

The inactive form of plasminogen circulates in the blood. Coagulation causes the release of t-PA from endothelium and other tissues which then converts plasminogen to plasmin. Plasmin can dissolve fibrin clot and produce fibrin degradation products. Both plasmin and plasminogen activator are bound to fibrin during its formation, thereby ensuring the subsequent localization of the fibrinolytic response to the actual site of thrombosis. Any plasmin that diffuses away from the clot is inhibited by circulating $\alpha_2$-antiplasmin. The circulation of high quantities of plasmin is prevented since it would digest the circulating fibrinogen as well as fibrin localized within clot.

Prevention of VSP in experimental animals by rapid lysis and clearance of SAH by t-PA has been reported. Plasmin inactivators are not attracted to fibrin and tend to remain in circulating blood. Endothelial cells are the major source of t-PA and release in response to such stimuli as injury, ischemia, and vessel occlusion. Little free t-PA circulates in blood because it is rapidly bound to a circulating inhibitor protein and subsequently cleared by the liver. Urokinase-type plasminogen activators are produced by fibroblasts, endothelial cells, leukocytes, and platelets.

Large-volume SAH may activate coagulation from contact with collagen fibers in the arachnoid trabeculae and by release of thromboplastin from brain tissues and the meninges (116). Plasminogen enters the subarachnoid space with the arterial blood and is incorporated into the fibrin clot. A small amount of t-PA may accompany the SAH as well and more may be released from inflamed meningeal vessels, from breakdown of the BBB, and from deposited platelets coming out with the SAH and leukocytes associated with the inflammatory response (117–119). Fibrinolysis in the subarachnoid space after SAH is generally quite limited. That fibrinolysis does occur is demonstrated by the progressive presence of fibrin degradation products (120).

Fibrinolysis would not remove the RBCs but it might disperse them, thereby reducing the concentration of vasoconstrictor agonists on the blood vessel adventitia. The dispersing of RBCs might also permit faster phagocytosis by macrophages within the CSF pathway. It is unknown to what extent erythrocytes can leave the subarachnoid space by perineural or transpacchionian granulation routes. These are probably extremely limited if in fact they exist.

Recently, in patients with thick subarachnoid clot at high risk from VSP, t-PA has been employed locally

within the subarachnoid space to hasten the dissolution of the fibrin mesh holding the RBCs and presumably high concentrations of spasminogen directly up against the blood vessel adventitia. In primate experiments this was shown to prevent chronic VSP. Clinical studies have been suggestive but not definitive in support of this form of prophylaxis.

Thrombolytic agents act directly or indirectly as plasminogen activators. Plasminogen is the inactive proteolytic enzyme of plasma which binds to fibrin during thrombus formation. Fibrin-bound plasminogen is more susceptible to activation than is plasma plasminogen. Different plasminogen activators have unique properties that affect the rate and specificity of plasminogen activation, but ultimately all cleave a specific peptide of a plasminogen molecule. Agents with a high propensity to activate plasma plasminogen are less "fibrin selective" . The plasma half-lives of different plasminogen activators vary. The one-chain form of t-PA activator has a half-life of 5 min compared to 8 min for the two-chain form, and a higher dosage is required for induction of the lytic state. Thrombolytic agents include streptokinase (SK) acylated plasminogen streptokinase activator complex (APSAC), urokinase (UK), recombinant single chain urokinase plasminogen activator, and recombinant tissue-type plasminogen activator. t-PA has no known incidence of allergic side effect. When used intravascularly 150 mg of t-PA over a period of 6 hr is associated with a 1.6% incidence of ICH when used for coronary thrombolysis. Thrombolytic agents do not distinguish between the fibrin of the thrombus and the fibrin of the homeostatic plug so they are "double-edged swords." Plasminogen activation decreases certain clotting factors and impairs coagulation. They also cleave receptors on the surface of platelets to impair adhesion and aggregation. Membrane glycoproteins on the surface of the vessel endothelial cells may also be cleaved, which impairs platelet-fibrin adhesion. Structural proteins in the matrix of vessel walls may be affected to impair fibrin and cellular attachment. The result of these changes can be a poor response to vascular injury. Plasminogen activation results in cleavage of thrombospondin, fibronectin, and fibrinogen in the platelet aggregate of a hemostatic plug which results in disaggregation. In conjunction with the fibrinolysis of the fibrin within the plug leading from sites of previous vascular injury can occur. The dosage of tissue t-PA required to achieve coronary reperfusion in humans is 10-fold that required in animals and a lytic state is frequently produced. A lesser degree of hypofibrinogenemia is produced by t-PA than by SK at doses that result in equally successful reperfusion. In randomized trials, rapid lysis of 75% or more of the thrombus in proximal deep veins of lower extremities has been achieved in more than half the patients given a thrombolytic agent, which is much better than the 6% improvement with heparin alone. Thrombolytic therapy is currently employed for a wide variety of pathological venous and arterial thromboembolic conditions. The thrombolytic agents vary in relation to fibrin selectivity and half life in the circulation. Less fibrin-selective agents, such as SK, APSAC, and UK, have longer half-lives and therefore potentially cause more severe and sustained hypocoagulable states. Used for coronary thrombosis, t-PA must be infused for 3 or more hr in order to provide a continuous supply of fresh agent to the thrombus. The cost of drugs to treat myocardial infarction is $200 for SK and $2200 for t-PA (100 mg) or UK (3 million units) (121).

Fibrinolysis acts to maintain vascular patency by the proteolytic degradation of fibrin. Local control of fibrinolysis involves the coordinated interaction of enzymes, zymogens, and inhibitors. Both the coagulation and fibrinolytic pathways have common features such as the amplification of proteolysis by conversion of substrate proenzymes to active serine proteases and in sharing of regulatory proteins. For instance, factor XIIa participates in both intrinsic coagulation and intrinsic plasminogen activation, and antithrombin III is an inhibitor of both thrombin and plasmin. Plasminogen activators are serine proteases with restricted substrate specificity so that they catalyze the hydrolysis of the Arg 561–Val 562 bond in the zymogen, plasminogen, to produce plasmin. The latter has broad trypsin-like specificity so that the performance of fibrinolysis requires localization of plasminogen activation within the fibrin mesh. Fibrin-bound plasmin is protected from neutralization by the inhibitor $\alpha_2$-antiplasmin, and the endogenous activator t-PA has a high affinity for fibrin. Physiological thrombolysis is the local activation of fibrin-bound plasminogen in an inhibitor-free environment. Endothelial release of t-PA is increased by catecholamines, vasoactive agents such as ACh, 5-HT, bradykinin, histamine, vasopressin, and thrombin. Release of t-PA can be reduced by intracellular cyclic adenosine monophosphates (prostacyclin and prostaglandin $E_1$) and certain inflammatory agents. The determinacy of clearance of fibrinolytic agents depends on the mode of administration, tissue uptake, proteolysis, and circulating antiproteases. Responses to t-PA can be influenced by changes in the level of endogenous antiproteases such as plasminogen activator inhibitor-I and other proteins. t-PA was discovered in the mid-1940s (122).

A fibrinolytic system digests fibrin and removes fibrin clot once hemostasis is achieved. Lysis is due to the incorporation of fibrinolytic system components into the clot during its formation. They include activators and inhibitors of fibrinolysis. Normally, fibrinolysis proceeds slowly relative to coagulation. Plasminogen is central to

fibrinolysis, a zymogen that is converted via activators to the enzyme plasmin. Hemostatic substrates of plasmin include fibrin, fibrinogen, the thrombin-activated form of factors V and VIII C, and platelet membrane glycoprotein Ib. Activation of plasminogen can be initiated by t-PA, urokinase, and the contact system of coagulation. Fibrinolysis may be inhibited by specific inhibitors such as plasminogen activator inhibitor (PAI); $\alpha_2$-plasmin inhibitor ($\alpha_2$-PI), which inhibits plasmin; and cofactors such as fibrin, which promotes t-PA-induced activation of plasminogen and alterations in the production and release of plasminogen activator. Plasminogen is produced in the liver and is a glycoprotein with a plasma concentration of 20mg/dl. It is a two-chain, disulfide-linked molecule with a heavy and light chain. The heavy chain possesses the five kringles with associated lysin binding sites that are responsible for binding plasminogen and plasmin to fibrin, ∈-aminocaproic acid, $\alpha_2$-plasmin inhibitor, and other molecules. Plasmin is inhibited by a variety of serine protease inhibitors of which at least nine have been identified in human plasma. The half-life of t-PA in the circulation is 2–5 min due to hepatic clearance and binding to fibrin. Plasma concentrations of t-PA range between 5 and 10 ng/ml. Fibrinogen and fibrin are major substrates for plasmin. t-PA is difficult to measure because of its low levels in blood. If patients are receiving rt-PA normally they would be monitored by PT, APTT, thrombin time, fibrinogen, plaminogen and $\alpha_2$-plasmin inhibitor level, fibrin(ogen) split products, soluble fibrin, fibrinopeptide A, t-PA, and PAI (123).

Fibrinolytic agents dissolve stasis thrombi with variable efficiency in different species. Nonprimate clots are more resistant to dissolution by t-PA *in vitro*. t-PA is the fibrin-selective, intrinsic thrombolytic agent. It is produced by human tissue as a single-chain plasminogen activator and urokinase plasminogen activator.

The conversion of plasminogen to plasmin is stimulated by plasminogen activator such as t-PA and inhibited by plasminogen activator inhibitor. The breakdown of fibrin to fibrin degradation products is accelerated by plasmin and blocked by $\alpha_2$-PI. Plasminogen activators are present in most normal and neoplastic tissues. t-PA is a serine protease that has a high specific activity in converting plasminogen to plasmin through cleavage of a single peptide bond. Endothelial cells are the principal physiological source of t-PA but it can be produced from other tissues and tumors (113).

t-PA is synthesized as a single-chain molecule of 530 amino acid residues. It can be converted to a two-chain form by plasmic cleavage. The two-chain form has a greater binding affinity for fibrin. *In vivo* t-PA is released from endothelial cells by stimuli such as thrombin, exercise, venous stasis, and DDAVP administration (113).

Plasminogen has a plasma concentration of 24 $\mu M$ and half-life of 2.2 days. Plasmin has a similar molecular weight but is not normally found in plasma and its plasma half-life is 0.1 sec. t-PA has a MW of 72 kDa, one or two chains, a plasma concentration of only 2 ng/ml, and half-life of approximately 5 min. The single-chain form is rapidly converted to the two-chain form by plasmin in the presence of fibrin. Most t-PA circulates in complex with its inhibitor. $\alpha_2$-Plasmin inhibitor has a plasma concentration of 1 $\mu M$ and a plasma half-life of 3 days. All plasminogen activators share the ability to form plasmin from the inactive zymogen plasminogen. Plasmin interacts with inhibitors in such a way as to provide for intermittent activation at sites of fibrin deposition without initiating a systemic fibrinolytic state. Plasmin bound to fibrin is inactivated much less readily than free plasmin. Plasminogen activator inhibitor-I is present in plasma, platelets, endothelial cells, and the endothelial cell matrix. Fibrinogen has a molecular weight of 340 kDa and consists of three polypeptide chains. Thrombin cleaves fibrinopeptide A and B from fibrinogen (113).

When blood clots, fibrin forms a matrix and enmeshes RBCs, platelets, and WBCs. When clot is broken down plasmin breaks fibrin into several degradation products. Plasmin circulates in the blood as its inactive proenzyme–plasminogen, which is activated by plasminogen activators. The plasmin has a high affinity for fibrin and is incorporated into the fibrin blood clot as it forms (124).

The major human plasminogen activators are tissue type (t-PA) and urokinase type (UK). Like plasmin, t-PA has special affinity for fibrin and is partly sequestered in blood clot. Unless it is bound to fibrin, t-PA is relatively inefficient in activating free plasminogen but it can rapidly form plasmin from plasminogen which is already incorporated into the clot. Endothelial cells are a major source for t-PA. The BBB normally excludes high-molecular-weight serum proteins from the CSF so that the levels of coagulation proteins in normal CSF are only about 1–5% as high as those in the blood (4). Normal CSF is generally considered to lack plasminogen activators and fibrinolytic activity (4,117,118). With SAH plasminogen flows into the subarachnoid space with the arterial blood and is incorporated into the fibrin clot. Small amounts of t-PA enter with the SAH and more may be produced from inflamed meningeal vessels, breakdown of the BBB, platelets associated with the SAH, and WBCs associated with the subsequent inflammatory response. Fibrinolysis in the subarachnoid space after SAH is apparently limited (125); however, the appearance of fibrin degradation products in CSF post-SAH is proof that subarachnoid clot fibrinolysis occurs. It is unknown to what extent fibrin degradation products represent damage to the

blood–CSF barrier or true subarachnoid fibrinolysis (124).

### E. Antifibrinolysis

Following the formation of a fibrin-platelet plug in the rent in the aneurysmal dome through which blood escaped to form a clot in the subarachnoid space, there are two mutually contradictory therapeutic aims. One is to stabilize the clot and facilitate the fibrous healing of the aneurysm until it can be definitely treated and the second is to lyse the subarachnoid clot and reestablish normal CSF circulation around the basal conducting arteries. CSF does not normally contain plasminogen activator activity but gains this following SAH. Obviously, the faster clot lysis is accomplished, the less will be the exposure of the VSMC to the vasoconstrictor agonists.

In the early decades of direct surgery for aneurysms the preservation of the hemostatic plug took precedence over the dissolution of the subarachnoid clot since surgery was generally delayed for a week or two and rebleeding was considered the principal threat. Antifibrinolytic therapy was therefore instituted and widely practiced. The stability of the hemostatic plug is enhanced by naturally occurring inhibitors of the plasminogen tissue activator: plasminogen activator inhibitor-1 and $\alpha_2$-antiplasmin. These naturally occurring compounds are not available for use therapeutically but synthetic lysine analogs fulfill the same function. These are $\epsilon$-aminocaproic acid (Amicar) and tranexamic acid, which are able to bind to the lysine binding sites of plasminogen, inducing a conformational change in the protein. Curiously, these antifibrinolytic drugs activate plasmin (the fibrinolytic molecule) but they prevent the binding of plasmin to fibrin, thereby preventing fibrinolysis. Tranexamic acid is 6–10 times more potent on a molar basis than Amicar. Amicar is given iv 0.1 g/kg over 30 min followed by continuous iv infusion of 0.5–1 g/hr. Eighty percent of the intravenous dose is cleared within 3 hr. The most common side effects are nasal stuffiness, abdominal cramps, nausea, vomiting, diarrhea, and rashes. Rare but more serious side-effects include myonecrosis, hypersensitivity reactions, and possibly thrombosis. Thrombocytopenia may be seen if doses exceed 21/g/day.

In the Cooperative Aneurysm Study on Timing of Aneurysm Surgery the rebleeding rate was 11.7% in 467 patients treated with Amicar and 19.4% in 205 patients not receiving this drug. Concurrently with a decrease in bleeding rates was an increase in focal ischemic events that rose from 22.7 to 32.4% with Amicar treatment. Overall mortality rates were virtually identical at 1 month following SAH (126). In a prospective randomized trial of tranexamic acid, rebleeding rates were 9% with drug treatment versus 24% without, but again infarction rates increased from 15% without treatment to 24% with treatment. Overall mortality rates were virtually identical (120). In another tranexamic acid trial bleeding rates were not reduced with therapy but cerebral infarction was increased (27 vs 11% in 100 patients) (127).

Based on these results and the results of other studies (126,128–132) the routine prophylactic use of antifibrinolytic drugs in the setting of recently ruptured aneurysms has been abandoned. There might still be a role for such therapy in the rare case in which early admission occurs if early clipping or coiling of the ruptured aneurysm is impossible due to some medical or technical consideration and if the initial CT scan indicates a very small amount of subarachnoid blood with a resultant low expectation of DID from VSP (133).

Also, preoperative high-dose $\epsilon$-aminocaproic acid therapy has been evaluated as a means of reducing rebleeding rate in patients having early surgical intervention. This was used in 307 patients and only 1.3% suffered a recurrent hemorrhage. Twenty-three percent of the patients developed symptomatic VSP and 8.1% had an infarction (134).

### F. Thrombin

#### *1. Role in Coagulation*

$\alpha$-Thrombin is a serine protease generated from its circulating zymogen prothrombin as the final clotting event of blood coagulation (Fig. 3.14) (135). It is the coagulation enzyme found in highest concentration and is central to the bioregulation of hemostasis. It functions to activate and transform plasma proteins such as fibrinogen in the coagulation cascade and to stimulate platelets and other cells to initiate mitogenic events, its catalytically inactivated forms possess biological activities with leukocytes and other cells, and it binds to the endothelial cell protein thrombomodulin (136). As a potent activator for a variety of cellular-mediated events, receptors for thrombin can be found on many different cellular types, including platelets, endothelial cells, fibroblasts, leukocytes, and neurons. Most of these cellular events require the proteolytic activity of thrombin (137). It has been known for decades that thrombin can induce endothelial injury to blood vessels (138).

The predecessor of thrombin is prothrombin which circulates in the plasma with a half-life of 2.5 days and has a concentration of 1.4 $\mu M$ as an inactive zymogen of MW 72 kDa (105). Thrombin can be formed by two pathways, one is the contact pathway of coagulation, consisting of plasma proteins activated by negatively

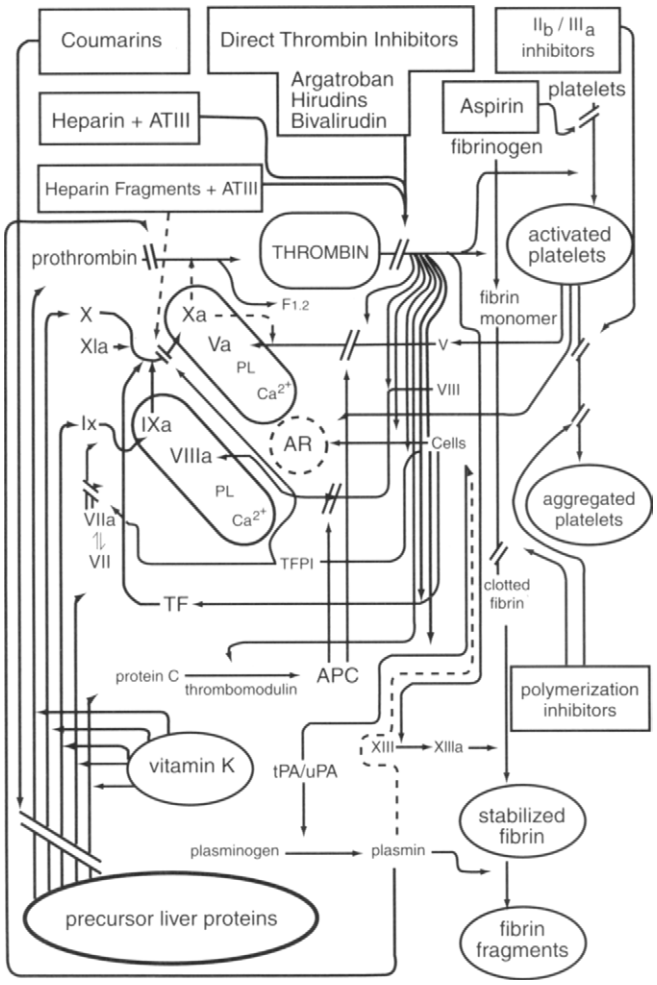


**FIGURE 3.14** Antithrombotic intervention. Distinct modes are shown for coumarins (vitamin K antagonists), heparin and related substances, heparin fragments, direct thrombin inhibitors (e.g., Argatroban, hirudins, and Bilvalirudin), aspirin and related compounds, platelet $II_b/III_a$ inhibitors, and polymerization inhibitors. Coagulation factors are designated by Roman numerals. ATIII, antithrombin III; F1.2, prothrombin fragment 1.2; PL, phospholipids; AR, acitvated receptor(s) on cells; TFPI, tissue factor pathway inhibitor; TF, tissue factor; APC, activated protein C; tPA, tissue plasminogen activator; uPA, urokinase plasminogen activator [reproduced with permission from Fenton, J. W., Ofosu, F. A., Brezniak, D. V., and Hassouna, H. I. (1998). Thrombin and antithrombotics. *Semin. Thrombosis Hemostasis* **24**, 87–156].

charged surfaces, and the other is initiated when calcium and phospholipid-rich extra vascular tissue protein extracts come in contact with plasma. The expression of $\alpha$-thrombin is contributed to by many protein factors. The generation of thrombin is the core reaction of both normal hemostasis and thrombosis. Thrombin can be produced in under 5 min and can activate numerous of its own precursor coagulation proteins. $\alpha$-Thrombin is derived from the carboxy terminal of the prothrombin molecule and consists of two disulphide chains, A and B. The normal inhibition of thrombin in the plasma is due to antithrombin III, a protease inhibitor, and $\alpha_2$-macrogobulin. In circulating plasma there is an adequate concentration of antithrombin III to suppress any thrombin.

### *2. Other Functions*

Thrombin triggers platelet aggregation, secretion, and formation of thromboxane $A_2$ as well as smooth muscle contraction (139). It also causes proliferation of fibroblasts, chemotaxis of monocytes, and mitogenesis of certain other cells. In the circulation it is rapidly bound to high-affinity binding sites on endothelial cells and causes them to release plasminogen activator, platelet-activating factor, ET, prostacyclin, and EDRF. It may produce these effects in a biphasic fashion. Generally at higher concentrations than those used for relaxation, thrombin causes concentration-dependent, slowly developing, sustained contraction and this has been shown in a variety of arteries and veins from different species. The thrombin-induced contractions are not dependent on the presence of endothelium. It is resistant to repeated washouts. The contraction responses to thrombin can be inhibited by both hirudin and synthetic thrombin inhibitors such as $\alpha$-NAPAP. By using various blockers it has been shown that PG, catecholamines, ACh, and histamine are not involved in the thrombin-induced contraction. The thrombin effect, however, does depend on extracellular $Ca^{2+}$ concentration. The thrombin-induced contractions are dependent on the enzyme being catalytically active. Thrombin-induced contractions of several vessels from different species have also been inhibited by the synthetic thrombin inhibitors PACK and MD 805. Noncoagulant forms of thrombin ($\beta$-thrombin, $\gamma$-thrombin, and nitrothrombin) are unable to clot fibrinogen but still produce contractions. Thrombin-stimulated cellular effects are receptor mediated, which requires both receptor occupancy and proteolytic activity of the enzyme. The receptors belong to the G protein-coupled family of receptors. Activation of cell surface thrombin receptors initiates signal transduction which ultimately mobilizes cytosolic $Ca^{2+}$, and protein induces phosphorylation. The receptor-mediated effector coupling involves guanosine triphosphate-binding regulatory proteins and stimulation of phospholipase C. Inositol-1,4,5-triphosphate and 1,2-diacylglycerol are produced.

In human plasma the amount of prothrombin suffices to generate an $\alpha$-thrombin concentration of about 150 U/ml (139). Cellular binding and inactivation by plasma protease inhibitors reduce the actual concentration of thrombin during clotting of whole blood to approximately 10–20 U/ml. During fibrin clotting and thrombosis, active thrombin is incorporated into the

growing clot. It remains active with a long half-life and is protected from inactivation by antithrombin III. Incorporated thrombin in its active form can be released gradually through spontaneous thrombolysis or during the stage of thrombus organization (139).

At the site of vascular injury thrombin is generated and participates in the coagulation cascade and also signals other events related to the development and complications of atherosclerotic plaques. Thrombin-stimulated cells may increase production of matrix metalloproteinases. These substances digest collagen and elastin and may promote cell migration and vascular remodeling (140). The thrombin-generating potential of apoptotic VSMCs was even greater than that of inactivated platelets and similar to that of calcium–ionophore-activated platelets in rat cells. The thrombin-generating capacity is secondary to phosphatidylserine exposure (141).

Recently, specific thrombin receptor blockers have been developed. These show greater promise in being specific inhibitors of thrombin-induced constriction than substances that act at postreceptor sites such as inhibitors of phospholipase C and protein kinase C or calcium antagonists, all of which have nonspecific effects and inhibit a huge variety of receptor-mediated processes (139). However, currently thrombin receptor antagonists lack potency and some are only partial agonists (142).

### *3. Vascular Effects*

Thrombin is central to hemostasis because it activates the cascade and induces VSMC contraction (139). It can stimulate intact endothelium to produce EDRF by the release of NO and prostacyclin. In the absence of endothelium the direct effect on VSMC is sustained contraction. Thrombin activates a unique receptor, which is a member of the rhodopsin superfamily with seven transmembrane domains and a large extracellular amino-terminal extension. In cultured VSMC thrombin receptor activator leads to activation of phospholipase *C*, hydrolysis of phosphatidylinositol 4,5-biphosphate, and the production of two intracellular messengers, $IP_3$ and DAG. $IP_3$ releases sarcoplasmic reticular $Ca^{2+}$ stores and DAG activates PKC.

Thrombin facilitates the induction of ET-1 gene expression to release 5-HT and a platelet-derived cofactor from platelets and the recruitment of inflammatory cells. ET, 5-HT, and platelet-derived growth factor are all potent vasoconstrictors.

Plasminogen activator inhibitor-1 is the principal inhibitor of plasmin formation promoted by tissue plasminogen activators. Thrombin increases plasminogen activator inhibitor-1 antigen, biological activity, and gene expression in cultured baboon aortic smooth muscle cells. Thrombin not only increases plasminogen activator inhibitor-1 transcription but also proteolytically cleaves plasminogen activator inhibitor-1 from the extracellular matrix of VSMCs (143). α-Thrombin impairs the expression of inducible NOS mRNA and protein in VSMC normally induced by IL-1β. Thrombin regulates the expression of inducible NOS at a transcriptional level by proteolytically activating the thrombin receptors in VSMCs (144).

Under the influence of physical changes such as hypoxia or receptor-operated stimuli such as thrombin, endothelial cells produce exclusively ET-1. Most of the ET is released abluminally toward the VSMCs. The main vascular effect of ET-1 is a transient minor vasodilation followed by profound and sustained vasoconstriction as well as proliferation of VSMCs (145).

### *4. Animal Experiments*

Thrombin as well as thrombin receptor activating peptide can produce sustained contraction of endothelium-denuded porcine pulmonary arteries. The vasoconstriction is strongly dependent on extracellular calcium. The PKC inhibitor staurosporine completely inhibits the tonic contraction phase which is presumed due to the activation of PKC (146). Trypsin and other serine proteases elevate $(Ca^{2+})i$ in cultured rat aortic cells, following which the cells become nonresponsive to thrombin $Ca^{2+}$ mobilization. The amount of $Ca^{2+}$ released by thrombin or trypsin seems to depend on the morphology of the cell and the state of the thrombin receptor (147).

Thrombin effects vascular cells by proteolytic activation of G protein-coupled receptors which are rapidly and irreversible desensitized. 5-HT stimulates the expression of thrombin receptors on VSMC by activating its receptors, which subsequently activate PKC and also protein tyrosine kinases. The upregulation of plasma membrane thrombin receptors by 5-HT released from aggregating platelets at sites of vascular injury perhaps potentiates the mitogenic actions of thrombin on the vascular wall (148). Treatment of quiescent rat aortic smooth muscle cell with either α-thrombin or thrombin – receptor-derived agonist peptide causes a pronounced increase in [$^3$H] thymidine incorporation. Both thrombin and the other agonist peptide lead to rapid tyrosine phosphorylation of several proteins (149).

When thrombin is injected into the spinal fluid there is a marked inflammatory response consisting mainly of polymorphonuclear cells. Over the same time period, in dogs the PG $F_{2\alpha}$ and $E_2$ levels both increase significantly. These PGs are known to increase during inflammation. Shortly after these observations were made, purified human and bovine thrombin were noted to induce tonic contractions in isolated canine arteries. Thrombin, as a

spasmogen, had a slower onset of action than 5-HT or PG $F_{2\alpha}$ but was more potent. Its tonic contraction was not terminated by equivalent washing. The contraction was inhibited by prostacyclin (150). The same investigators subsequently showed that nimodipine, a calcium channel blocker, could reduce the contractions induced by thrombin as well as blood 5-HT and PG $F_{2\alpha}$ (151). Canine arterial rings showed dose-dependent contraction to α-thrombin. The mesenteric and renal artery rings did not contract but the coronary and basilar ones did following an initial period of relaxation. The initial basilar artery relaxation to α-thrombin was blocked by removal of the endothelium or heating the α-thrombin (152). Human α-thrombin in physiological concentrations caused rabbit thoracic aorta to slowly contract. The clotting activities could be impaired by chemical manipulation without interfering with the contractile activity. The thrombin-induced contractions were inhibited by D-600 but not by atropine, phentolamine, or indomethacin. The aortic preparations with intact endothelium relaxed in the presence of very low concentrations of α-thrombin prior to contracting in response to higher concentrations.

The contractile responses in canine arteries to thrombin, uridine triphosphate, 5-HT, and KCl were all inhibited by antithrombin III. $\alpha_2$-Macroglobulin also inhibited the contractile response to thrombin, KCl, and 5-HT. The serine protease kallikrein selectively blocked the thrombin-induced contraction (153).

Rabbit aorta was contracted by human plasma exposed to thromboplastin $Ca^{2+}$. The spasm developed slowly and persisted after washout. The agonist produced contraction lasted less than 3 min and its duration paralleled that of thrombin in plasma. Human α-thrombin also caused a similar contraction of this preparation which was not inhibited by phenoxybenzamine, atropine, or angiotension inhibitor but was blocked by hirudin. Heparin caused partial relaxation (154). In canine cerebral arteries thrombin caused slight and transient relaxation followed by dose-dependent, persistent contraction. Treatment with MD 805 (a synthetic inhibitor) attenuated this contractile response in a dose-dependent fashion. The thrombin-induced contraction did not occur in $Ca^{2+}$-free media. The calcium antagonist verapamil attenuated the contractile response of basilar artery to thrombin and KCl (155). The effect of bovine thrombin on rabbit aortic ring preparations was studied, and it was shown that the contractions increased when the endothelium was removed and that the contractions were dependent on $Ca^{2+}$ in the bath. Two specific thrombin inhibitors were found to be potent inhibitors of thrombin-induced contraction but this required higher concentrations than those for these inhibitors to inhibit fibrinogen clotting. The thrombin-induced effect was not mediated by PG, catecholamines, ACh, histamine, or 5-HT (156). Rabbit aortic rings denuded of endothelium were reversibly contracted by both thrombin and trypsin. Such contractions were reduced to 30% of control after removal of extracellular $Ca^{2+}$ and to 70% of control by the $Ca^{2+}$ channel blocker nifedipine. Precontraction by a maximally effective concentration of thrombin prevented a second contraction to subsequent thrombin applications (157). Rabbit arterial rings contracted by 5-HT could be relaxed by the endogenous plasma glycoprotein antithrombin III. Additionally, animals sacrificed on day 3 post-SAH showed less VSP if they had received a 2-hr intracisternal infusion of antithrombin III (158). In guinea pig coronary artery thrombin had a dual action on thromboxane $A_2$-induced contractions—an initial relaxation followed by contraction. In endothelium-denuded tissues thrombin enhanced the thromboxane $A_2$-induced contraction without any preceding relaxation. Studies of the membrane potential of the artery showed that both thrombin and ET consistently depolarized the membrane (159). Bovine carotid arteries were contracted on exposure to thrombin. The contraction induced by thrombin was similar to that produced by ET. In the presence of 1.5 nmol/liter $Ca^{2+}$ there were similar contractions at the $10^{-6}$ and $10^{-7}$ mol/liter doses of both agonists. Median effective dose of both agonists was $8 \times 10^{-8}$ mol/liter. The thrombins induced contractions were completely relaxed with the addition of the adenylate cyclase activator forskolin (10 μmol/liter) or the guanylate cyclase activator sodium nitroprusside (10 μmol/liter). Following thrombin stimulation there was an increase in tyrosine phosphorylation of a 44-kDa protein corresponding to the mitogen-activated protein kinase and this peaked after 1 min of thrombin stimulation. Prothrombin in blood is able to generate thrombin concentrations of 130–160 U/ml (160).

### 5. *Effects on Brain*

Thrombin can produce brain injury by direct brain cell toxicity. Intracerebral injection of thrombin produced focal motor seizures in all animals. Seizures could be prevented by a thrombin inhibitor added to the thrombin. Thrombin injection increased local water content, $Na^+$ and $Cl^-$ ion content, and it reduced $K^+$ content (161). Thrombin has been shown to contribute to the formation of brain edema following ICH using a rat model. Thrombin induces BBB disruption as well as death of parenchymal cells without affecting rCBF and vasoreactivity. These workers previously showed that the degree of conversion of prothrombin to thrombin in a clot correlates with the amount of brain edema around the hematoma. Brain edema following ICH can be attenuated by thrombin-specific inhibitors. Whole blood produces more edema than either plasma or RBCs alone (162).

### 6. *Human Studies*

Prothrombin is converted to thrombin over 2–7 hr depending on the cellular makeup of clot. Thrombin binds to fibrin and is protected from inactivation while in the clot matrix and is released slowly from the hematoma during fibrinolysis. Thrombin is released from intracranial clot in humans over a period of 2 weeks after the hemorrhage (163).

Human basilar arteries obtained within 24 hr of death from non-SAH cases were studied in response to KCl, 5-HT, PG, $D_2$, $PGF_{2\alpha}$, and plasmin. Thrombin reduced the basal tone in these human arterial segments and inhibited the contractions elicited by KCl, $PGF_2\alpha$, and plasmin. The relaxant effect of thrombin was dependent on intact endothelium and showed tachyphylaxis. Antithrombin III also reduced the basal tone in the arteries and inhibited in a concentration-dependent manner the contractile responses to KCl, 5-HT, and the two PGs. Unlike thrombin, there was no tachyphylaxis to this vasorelaxant effect nor was it dependent on intact endothelium. In these vessels, therefore, the contractile response to thrombin was not observed. The doses of thrombin used were 1 and 10 U/ml (164). When thrombin causes the formation of fibrin from fibrinogen, fibrinopeptide A is released. The concentration of this peptide is therefore considered to reflect the activity of thrombin. Levels of fibrinopeptide A were studied in plasma and CSF of 25 patients post-SAH. The levels were extremely high on days 0 and 1 but decreased rapidly by days 2–4 and continued to fall progressively. There were no significant changes in the levels in plasma. This finding demonstrated that the coagulation system in the subarachnoid space was strongly activated immediately following SAH (116).

Thrombin–antithrombin complex was examined sequentially in CSF and blood of 10 patients with severe SAH (diffuse thick SAH on CT scans). Severe diffuse VSP, defined as a >70% reduction in diameter over 2 cm in length, developed in 6 of the 10 cases. Half of these cases showed neurologic deficits as well. The thrombin–antithrombin complexes fell from close to 1000 ng/ml on Days 2–5 post-SAH to extremely low levels over the next 2 weeks. The blood levels showed a similar decrease, but they were at a much lower level than in the CSF (165).

Fibrinopeptide A levels were measured in blood and CSF from patients following SAH. Fibrinopeptide A levels in CSF of SAH patients averaged 81.6 pg/ml compared to 20.3 pg/ml for controls. This was a highly significant difference. Unfortunately, the concentrations at the time period post-SAH were not given. There was a demonstrable association between outcome as measured 3 months post-SAH and the CSF fibrinopeptide A level (166).

Tissue factor occurs in the brain and is the primary initiator of the coagulation cascade which activates thrombin. Using the thrombin–antithrombin III complex as a molecular marker of thrombin formation, tissue factor was studied in 19 patients with SAH and compared to 14 control patients who were undergoing myelography and 5 patients who were having craniotomy for causes other than SAH. The CSF level of tissue factor and that of the thrombin–antithrombin III complex were considerably elevated in SAH patients compared to controls and were most elevated in the first few days following SAH. The levels were higher in poor-grade neurological patients and those having the most blood on the CT scan. Patients subsequently developing cerebral infarction due to VSP had levels greater than those who did not develop cerebral infarction in the first 4 days. Patients with severe disabilities or worse had higher levels than those with only moderate disability or better. The same was true for tissue factor. It was concluded that CSF levels of tissue factor and thrombin–antithrombin III in the early stages after SAH could predict the severity of brain injury and the subsequent likelihood of cerebral infarction from VSP (108).

Fifty patients were studied post-SAH with sampling of CSF (Fig. 3.15). Tissue thrombin–antithrombin II complex was markedly elevated when CSF was collected daily through cisternal catheters for a period of 2 weeks. Patients with the thickest subarachnoid clots had the highest values and there was a tendency for patients with poor outcomes to have higher values. Values were highest in the first 2 days and progressively fell and leveled off by days 9 or 10. The levels in patients who developed VSP were higher, particularly between days 6–8, but these differences did not achieve statistical significance. In the same study, levels of active plasminogen activator inhibitor and the complex of this inhibitor with tissue plasminogen activator tended to increase progressively, peaking between days 6 and 11; the levels were higher in patients with VSP and some of these differences achieved statistical significance. Plasminogen activator inhibitor-1 levels were less than 20 ng/ml (167). Thirty-six patients had CSF fibrinopeptide A levels measured in CSF. Initial CT was done after <12 hr and surgery carried out in <48 hr. The mean fibrinopeptide A level for CT grade 3 patients was 182 ng/ml vs 36 ng/ml for CT grade 2 patients. Patients were ranked by rate of blood clearance on CT. The faster clearance cases had levels of 79 ng/ml, whereas the slow clearers averaged 466 ng/ml. The slow clearers had a significantly higher rate of infarction (168).

### 7. *Cell-Free Plasma Clots*

In experiments using cats (169), long-lasting cell-free plasma clot did not induce chronic VSP. Similarly,

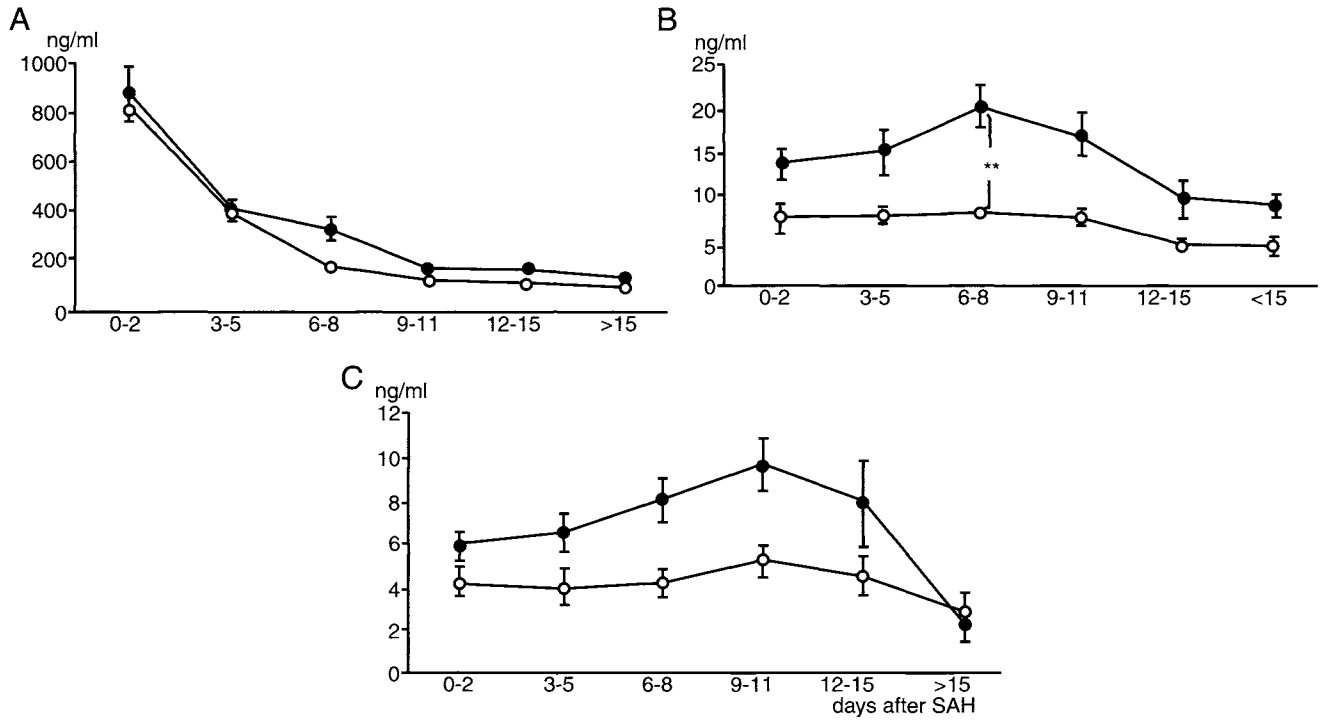


**FIGURE 3.15** Sequential changes of the CSF levels of (A) thrombin–antithrombin III complex, (B) active plasminogen activator inhibitor I, and (C) tissue plasminogen activator–plasminogen activator inhibitor I complex. Closed circles indicate mean values in patients with symptomatic VSP ($n = 17$ on days 0–2, $n = 24$ from days 3–5 to days 12–14, and $n = 12$ after day 15); open circles indicate the mean values in patients without VSP ($n = 21$ on days 0–2, $n = 26$ from days 3–5 to days 12–14, and $n = 14$ after day 15). Vertical bars indicate standard error of the mean; $^{**}$ $p < 0.01$ [reproduced with permission from Ikeda, K., Asakura, H., Futami, K., and Yamashita, J. (1997). Coagulative and fibrinolytic activation in cerebrospinal fluid and plasma after subarachnoid hemorrhage. *Neurosurgery* **41**, 344–350].

platelet-rich plasma provoked no VSP at 72 hr in a dog model (170). Would high concentrations of thrombin have been in contact with the arteries? If so, this would be important negative evidence with respect to thrombin being a significant factor in the etiology of VSP.

Human blood contains enough Hb in RBCs to generate concentrations of up to 2.3 m*M* upon cell lysis. Smaller concentrations of free hemoglobin can prolong clotting times in fresh human plasma (171).

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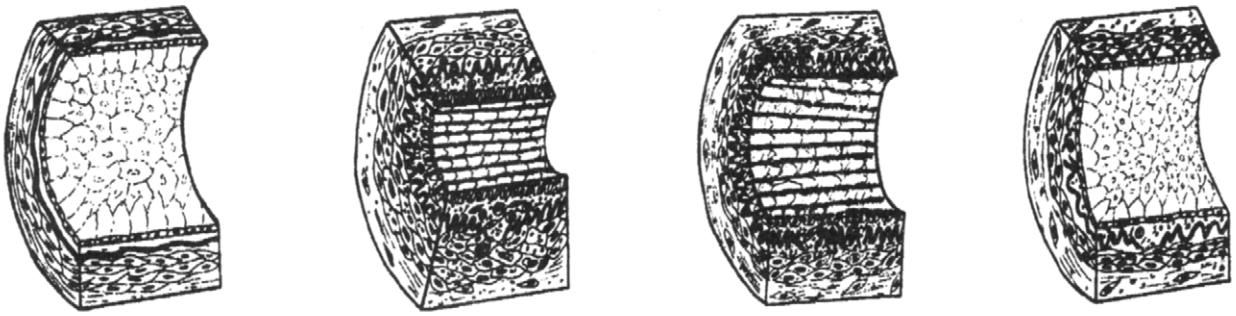
CHAPTER 4

# PATHOLOGY AND PATHOGENESIS



## I. Introduction

Millions of years of evolutionary changes developed to ensure against death of the organism by exsanguination from blood vessel trauma. Extremity or truncal arteries which constrict in response to abluminal blood are performing a homeostatic life-preserving function. Within the relatively closed subarachnoid space, however, prolonged vessel constriction can have catastrophic consequences for the brain, which is more sensitive to ischemia than tissues such as skeletal muscle or the abdominal organs. If sufficient blood is deposited during the active bleeding following aneurysmal rupture it will form actual clot around the basal arteries. A desperate race ensues in which the white cells seek to engulf and remove the red ones before the latter lyse and spill their deadly contents in close proximity to the vascular smooth muscle cells of the conducting arteries. As the level of spasmogens builds over days, the smooth muscle cells progressively constrict to the point that the lumen is dangerously reduced and the cells in the vessel wall are damaged by an unremitting squeeze (Fig. 4.1). Of the hundreds, perhaps thousands, of potential spasmogens produced in the normally bland subarachnoid fluid, the one in overwhelmingly greatest concentration is hemoglobin. The interaction between brain, clot, and damaged vessel wall as well as the process of inflammation and repair can produce many potential spasmogens (Fig. 4.2). The time course of its breakdown and removal, and the nature of its by-products, suggests that it may meet the criteria for the main cause of vasospasm (Table 4.1). The brain is potentially damaged by the acute ischemia resulting from intracranial pressure elevation at the time of rupture, by anoxia due to cardiorespiratory failure, by



| | | | | |
|---|---|---|---|---|
| Intima: | flattened endothelial cells<br>tight junctions | vacuolated endothelium<br>loss of tight junctions, corrugated | near normal caliber<br>necrosis, some fibrosis | fibrotic, thickened |
| IEL: | smooth, not<br>corrugated | fragmented<br>corrugated | corrugated | |
| Media: | spindle shaped<br>smooth muscle cells | smooth muscle cells contracted<br>markedly thickened, vacuoles,<br>some necrosis | adventitial cellular infiltrate | foamy<br>regenerating smooth<br>muscle cells |

**FIGURE 4.1** Graph of the time course of angiographic and symptomatic vasospasm and pathological changes in arteries. Angiographic vasospasm is maximal 7 days after a single SAH, whereas symptoms from vasospasm (symptomatic vasospasm) have their most frequent onset at 8 days. Pathological changes in the cerebral arteries over time are shown at the bottom. Initially, there is contraction of the smooth muscle cell. During the second week after SAH, there is some necrosis of smooth muscle and endothelial cells and possibly fibrosis of the arterial wall and infiltration with inflammatory cells. Weeks after SAH, there is fibrosis in the tunica media and adventitia and varying degrees of endothelial proliferation. *IEL* [reproduced with permission from Weir, B., Macdonald, R. L., and Stoodley, M. (1999). Etiology of vasospasm. *Acta Neurochir.* **72,** 27–46. Copyright © Springer-Verlag GmbH & Co.].

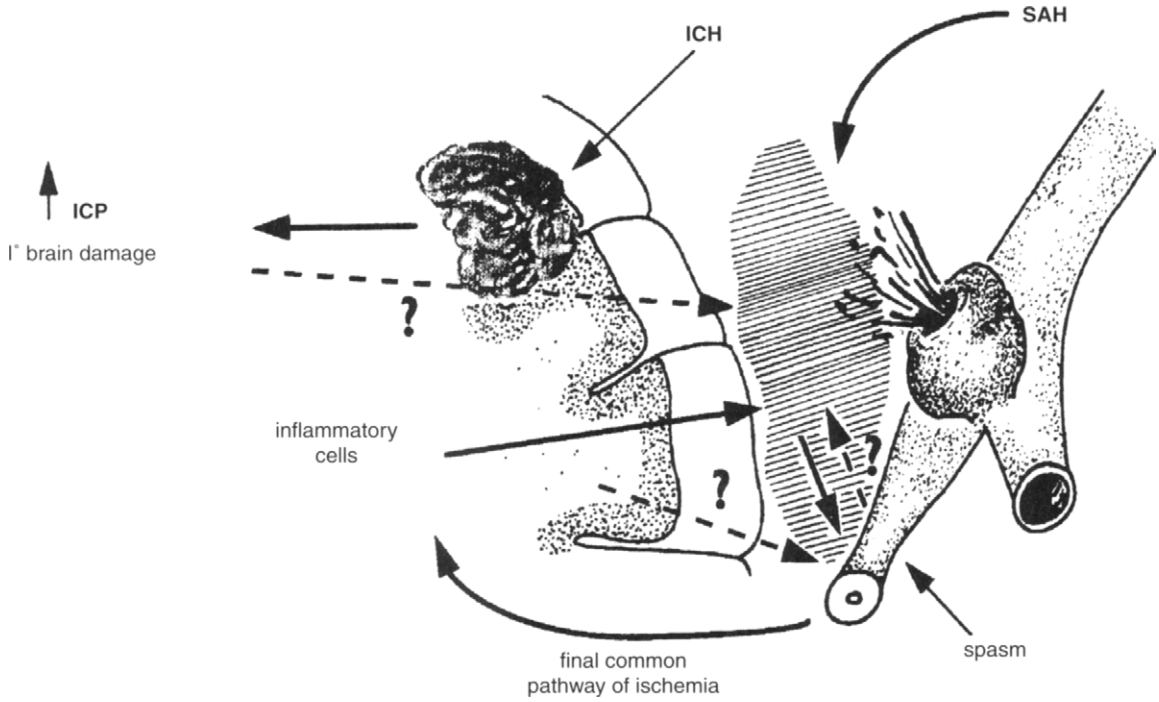



**FIGURE 4.2** Diagram of the possible interactions between subarachnoid blood clot, the arterial wall, and the brain that may be important in the pathogenesis of cerebral vasospasm. Most research has focused on the clot – arterial wall interaction, which is probably the most important. Other interactions have not been investigated, such as the brain and arterial wall affecting the subarachnoid clot so as to promote its breakdown or reactions in it that then cause vasospasm. The effect of the arterial wall on the clot is also not known. *ICH*, intracerebral hemorrhage; *SAH*, subarachnoid hemorrhage; *ICP*, intracranial pressure [reproduced with permission from Weir, B., Macdonald, R. L., and Stoodley, M. (1999). Etiology of vasospasm. *Acta Neurochir.* **72,** 27–46. Copyright © Springer-Verlag GmbH & Co.].

**TABLE 4.1 Criteria for a Spasmogen That Could Cause Vasospasm[a]**

| |
|---|
| Present in blood clot |
| Released in progressively increasing amounts for 5–10 days then in diminishing amounts over 7–14 days |
| Able to penetrate to the vascular smooth muscle layer and possibly endothelium |
| Vasoactive, causes sustained and ≥50% reductions in arterial diameter |
| Present in subarachnoid space or periarterial region in concentrations that are adequate to cause severe contraction |
| Causes smooth muscle necrosis and possibly endothelial cell damage, contractions associated with decreased arterial contractility and compliance after prolonged exposure |
| Contractions not readily reversed by known receptor antagonists |
| Not present in subarachnoid space in other conditions that alter the cerebrospinal fluid such as neoplastic or inflammatory meningitis |
| Vasospasm does not occur if it is removed from the subarachnoid blood clot or its action is blocked prior to vasospasm |

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**TABLE 4.2 Pathological Sequelae of Aneurysmal Rupture**

| |
|---|
| Acute |
| Hemorrhage |
| Subarachnoid |
| Subdural |
| Intracerebral |
| Intraventricular |
| Intra-aneurysmal |
| Secondary brain stem hemorrhages |
| Brain herniation |
| Subfalcine |
| Transtentorial |
| Foramen magnum |
| Acute hydrocephalus |
| Acute brain swelling |
| Aneurysmal/arterial thrombosis and/or embolism |
| Chronic |
| Aneurysmal rebleeding |
| Cerebral edema |
| Cerebral infarction |
| Vasospasm |
| Local pressure from intracerebral hematoma |
| Arterial compressions from cerebral herniations |
| Decreased cerebral perfusion due to systemic hypotension |
| Intracranial hypertension, hypovolemia, hyponatremia, and hypoxia |
| Hemorrhage into ischemic infarct |
| Chronic hydrocephalus |

surgical retraction, venous sacrifice, and temporary vessel clipping, by endovascular vessel damage, by iatrogenic systemic insults such as hypotension—all in addition to the "second" stroke resulting from vasospastic ischemia. As the foreign cells and their debris are finally mopped up after days to weeks, the cerebral arteries gradually and irregularly dilate back to normal dimensions. During the period of vasospasm, however, cerebral infarction is a serious risk and a complex interplay of factors determines whether this process becomes lethal. It can also tip the scales against survival by adding to the burden imposed by the other pathologic sequelae of aneurysmal rupture (Table 4.2).

## II. The Subarachnoid Space, Pia-arachnoid, Arachnoid Villi, and Cerebrospinal Fluid

### A. Subarachnoid Space and Pia-arachnoid

The leptomeninges form a complete investment for the brain and spinal cord. The arachnoid and pia mater are connected by dentate ligaments in the spinal canal and numerous trabeculae in the cranial subarachnoid space. They comprise the leptomeninges and are relatively avascular. The arachnoid membrane in man is formed by two layers of cells (Fig. 4.3). Basement membrane separates the superficial dark cells (electron dense) from the collagen fibers of the inner layer of the arachnoid membrane which occurs among elongated cells similar to fibroblasts. Macrophages are observed within the subarachnoid space (Fig. 4.4). Sometimes the arachnoid and pia mater are in contiguity, in which case the subarachnoid space is not in evidence. In humans the outer arachnoid cells have long interweaving processes containing numerous vacuoles. Desmosomes are frequent between the cells (1). The dural arachnoid junctions are apparently tight collagenous dural areas in continuous contact with the outer border layer of the arachnoid, whose cells show periodic tight junctions occluding the intercellular space (2). As blood vessels enter or leave the brain or spinal cord the pia invaginates into the nervous system to form the outer surface of the perivascular space. The perivascular space of Virchow–Robin extends from the subarachnoid space to a variable depth within the brain. The subarachnoid space is filled with almost protein-free cerebrospinal fluid (CSF). The barrier between blood and CSF is due to special permeability characteristics of brain endothelial cells, the choroid plexus, and the arachnoid membrane. The CSF space protrudes into the dural venous sinuses in the arachnoid villi and granulations (pacchionian bodies) which are outpouchings containing CSF and consisting of

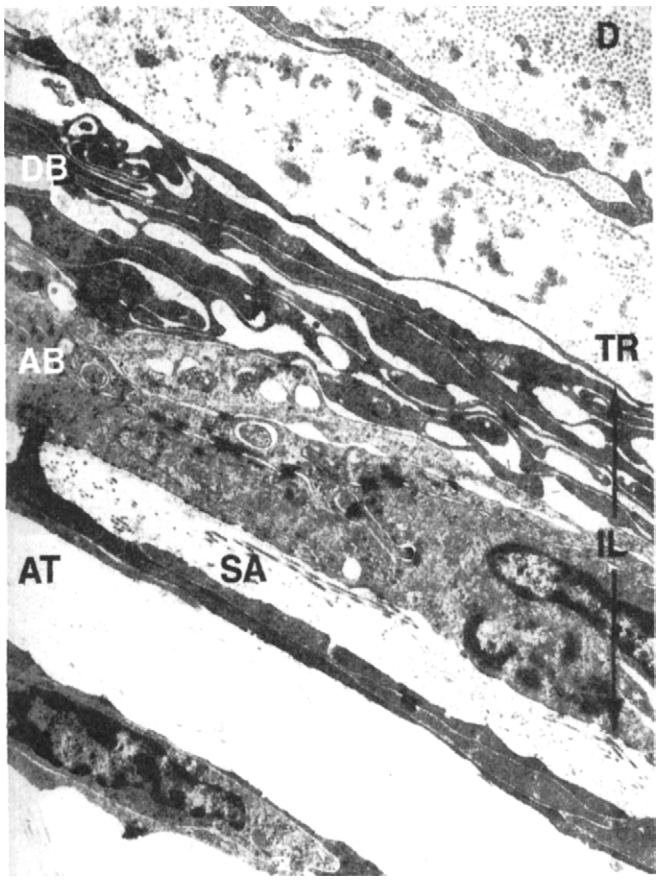



**FIGURE 4.3** Typical organization of the dura-arachnoid interface layer (*IL*). Between the network of arachnoid trabecules (*AT*) traversing the subarachnoid space (SA) and the transitional zone (*TR*) of the dense collagenous tissue of the dura mater (*D*) appear two tightly apposed cell layers, the arachnoid barrier layer (*AB*), and the dural border layer (*DB*). No "subdural space" exists anywhere between dura and arachnoid. Connective tissue fibers are completely absent from the interface layer. The interdigitating tiers of cells of the arachnoid barrier layer are attached by series of desmosomes and intermediate, gap, and tight junctions. The intercellular spaces between the flattened dural border cells are enlarged, and there are fewer cellular attachments [reproduced with permission from Schachenmayr, M. D., and Friede, R. L. (1978). The origin of subdural neomembranes, 1. Fine structure of the dura-arachnoid interface in man. *Am. J. Pathol.* **92,** 53–68].

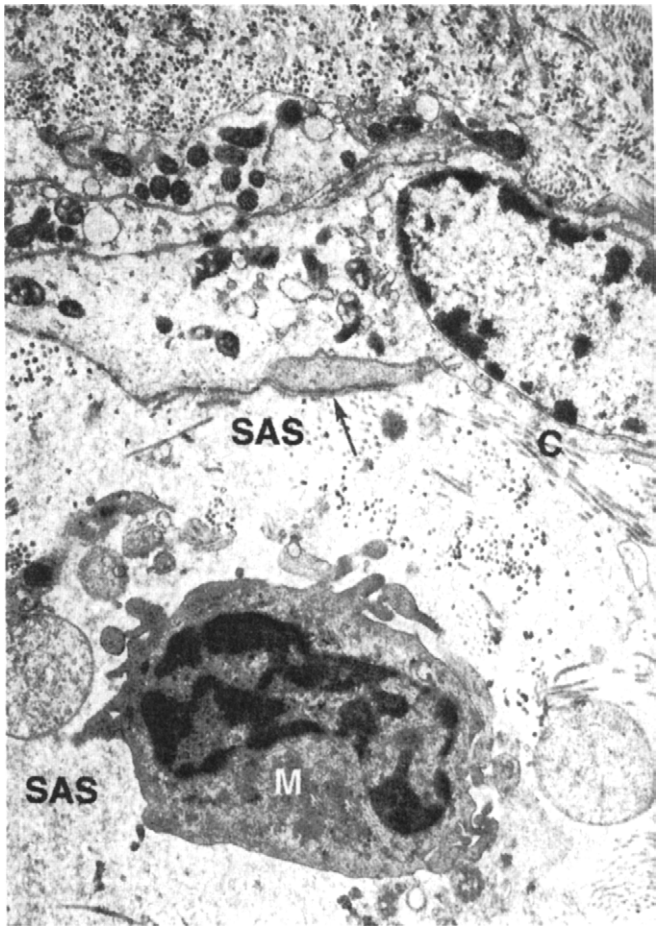



**FIGURE 4.4** The arachnoid cells bordering the subarachnoid space (*SAS*) present an interrupted layer of basement membrane (arrow) on their interior aspect. Macrophages (*M*), collagen fibers (*C*), and granular material lie in the subarachnoid space [reproduced with permission from Lopes, C. A., and Mair, W. G. (1974). Ultrastructure of the arachnoid membrane in man. *Acta Neuropathol.* **28,** 167–173].

specialized arachnoidal cells and the endothelium lining the invaginated venous channels (Fig. 4.5). The arachnoid villus is generally considered to be the major route of CSF absorption into the bloodstream. An arachnoid villus is not grossly visible. Clusters of arachnoid villi are known as arachnoid granulations or pacchionian bodies (Fig. 4.6). These are projections of arachnoidal cells into or through the dura into the venous sinuses or their intradural extensions which are known as lacunae laterales. While pictures exist of apparent open pores between the interior of the villi and the intravascular space, most authorities believe that CSF is mainly transported by a dynamic transendothelial process which involves mobile vacuoles (3). The cells of the arachnoidal villus membrane have multiple vacuoles that permit vesicular transport (Fig. 4.7). Some of these vacuoles have both basal and apical openings and constitute a system of transcellular channels or pores. Intact red blood cells (RBCs) have been seen within some vacuoles, which accounts for the reported transfer of isotopically labeled RBCs from the CSF to the blood in some animals (4). Arachnoid villi are also associated with some intraspinal veins.

The CSF space consists of two systems of hollow cavities which intercommunicate—the subarachnoid space and the ventricular system. The former is lined by mesenchymal tissues—pial cells on the surface of the brain and arachnoid cells abutting the most inner layer

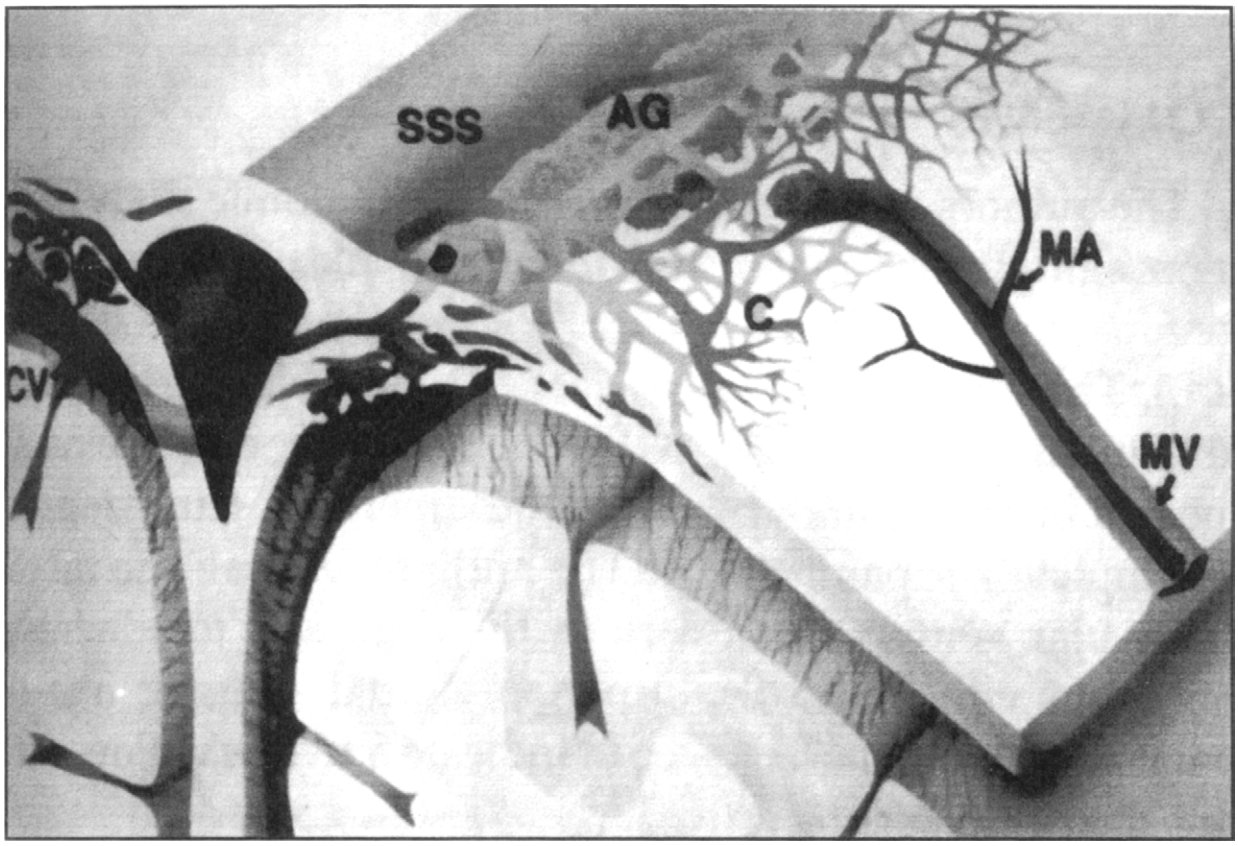



**FIGURE 4.5** SSS, superior sagittal sinus; CV, cortical vein; C, intradural channels; AG, arachnoidal granulations; MA, meningeal artery; MV, meningeal vein [reproduced with permission from Fox, R. J., Walji, A. H., Mielke, B., Petruk, K. C., and Aronyk, K. E. (1996). Anatomic details of intradural channels in the parasagittal dura: A possible pathway for flow of cerebrospinal fluid. *Neurosurgery* **39,** 84–91].

of the dura matter. The ventricular wall is lined by neuroectodermal tissue—ependymal cells and choroidal cells.

The arachnoid was established as a membrane separable from the pia matter in the middle of the seventeenth century by the Dutch anantomist Blaes; the term arachnoid was derived from the Greek word meaning spider's web. In 1829, Saint-Ange injected colored fluids and showed a communication between the subarachnoid space and the ventricles. In 1842, Magendie proved that the CSF was contained in the subarachnoid space, that it circulated, and that it was under a positive pressure (5).

In 1875, a classic text appeared with exquisite illustrations of the subarachnoid space and its structures. Key and Retzius (6) described in detail the subarachnoid cisterns of the brain and named seven major ones (Fig. 4.8).

Another admirable study of the subarachnoid space was achieved by injections performed by Locke and Naffziger. They illustrated the large Sylvian subarachnoid cisterns which are of so much clinical importance in SAH and VSP (Fig. 4.9). In addition, they showed the close contiguity between the cisterna lamina terminalis and the anterior third ventricle as well as the crural cistern and the temporal horn of the lateral ventricles that are locus minoris resisentiae for the rupture of subarachnoid blood into the ventricular system (5). Using pneumography as well as subarachnoid injections, in 1959 Liliequist published a detailed roentgenologic study of the subarachnoid cisterns. He illustrated the sheath of subarachnoid tissue stretching between the temporal lobes and oculomotor nerves which is one boundary of the interpeduncular cistern and which membrane subsequently bears his name (7).

In 1978, Schachenmayr and Friede published a detailed description of the external layer of the arachnoid. They termed this the arachnoid barrier layer, which is characterized by numerous tight junctions that presumably prevent the egress of CSF. This outermost arachnoid layer is intimately bound to the innermost cellular layer of the dura. The outer arachnoid and the inner dural cellular layers are actually more tightly bound to each other than

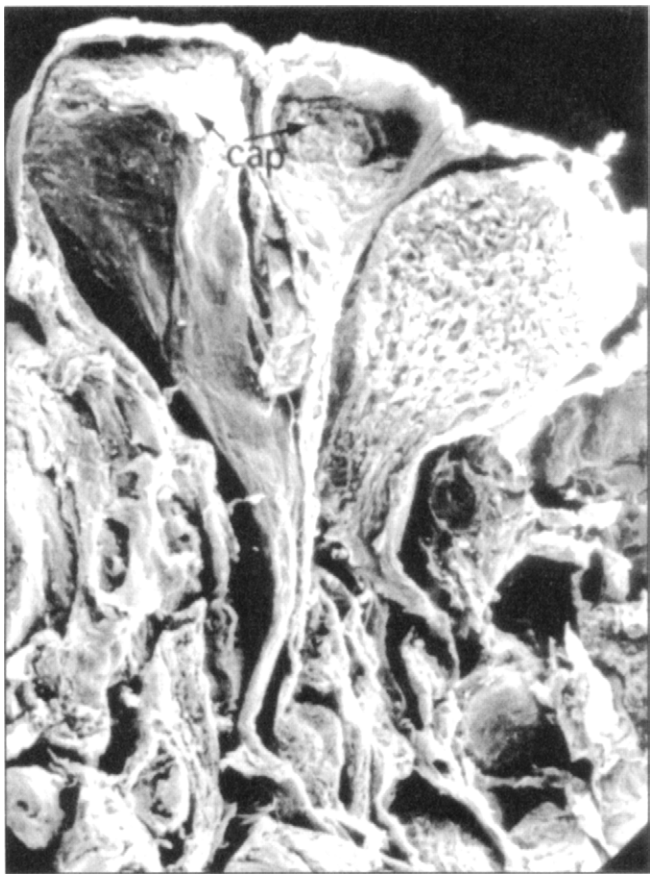



**FIGURE 4.6** Scanning electron micrograph of a vertical section through granulations. The core is complete on the left but there is avulsion of the granulation core in the center. The arachnoid cap of the granulation and its area of attachment (cap) have been exposed. A bisected core on the right shows the internal system of channels. The sagittal sinus is seen above and the dura below [reproduced with permission from Upton, M. L., and Weller, R. O. (1985). The morphology of cerebrospinal fluid drainage pathways in human arachnoid granulations. *J. Neurosurg.* **63,** 867–875].

are the inner dural cellular layer and the collagenous dura. They believed that a true subdural space does not exist and that subdural masses develop within the dural border layer whose cells do not have tight junctions or significant collagenous tissue. This inner dural layer of cells is also characterized by large extracellular cisterns separated by thin cytoplasmic bridges. The arachnoid barrier layer is structurally different from the dural border layer because the arachnoid cells have a system of tight junctions. Both the dural and the arachnoid border layers show a complete absence of connective tissue fibers. The arachnoid barrier cells were usually, but not always, more electron lucent than the darker dural border cells. The trabeculae traversing the subarachnoid space are anchored to the inner side of the arachnoid cell layer by a variety of pedicles that have numerous desmosomal, intermediate, and gap junctions at the opposing cell membranes. Other trabeculae fold button-shaped contact faces between short cytoplasmic protrusions of barrier cells and long-stretched extension of arachnoid cells contacted only by short desmosomal segments. The inner layer of the arachnoid barrier layer is covered by an almost complete basement lamina (8,9).

Animal studies have been consistent with human observations that the subarachnoid space could be accurately categorized as the cleared out portion of a general connective tissue space. Free cells are abundant on all leptomeningeal spaces. These presumably could be the precursors of some of the macrophages responsible for cleaning up the subarachnoid clot. In some animal studies apparent circular fenestrations are evident on the pial side of the arachnoid (10). Free cells in the subarachnoid space have been demonstrated to be macrophages by various authors (11–13). The possibility that various fiber structures or chordae that traverse the subarachnoid space and envelop or attach to arteries of passage could be involve in VSP was hypothesized by Arutiunov (14).

The contemporary concept of the subarachnoid cisterns was to a large extent shaped by the microsurgical observations by Gazi Yaşargil. His fashion of performing intracranial operations by moving methodically from one cistern to another has shaped current practice. He noted that the basal cisterns were only separated from one another by trabeculated porous walls with various sized openings and that there is considerable variation. Apertures between cisterns can be plugged or obliterated after SAH. There are characteristic condensations of the arachnoid over the proximal A1 and M1 arteries between the carotid and the lamina terminalis cisterns and the carotid and Sylvian cisterns, respectively (15).

The cisterns that are fairly characteristic and large enough to be engorged with substantial amounts of clot include the carotid (internal carotid artery), lamina terminalis (anterior cerebral artery and anterior communicating artery), corpus callosum (distal anterior cerebral artery), Sylvian (middle cerebral), crural (anterior choroidal artery), and interpeduncular (basilar artery) (16).

### B. Arachnoid Villi

#### *1. Human Studies*

Human arachnoid villi (microscopic) and arachnoidal granulations (grossly visible) are generally accepted as the principal means by which CSF drains from the subarachnoid space to the interior of the dural venous sinuses. At the base of the villi a thin neck of arachnoidal tissue

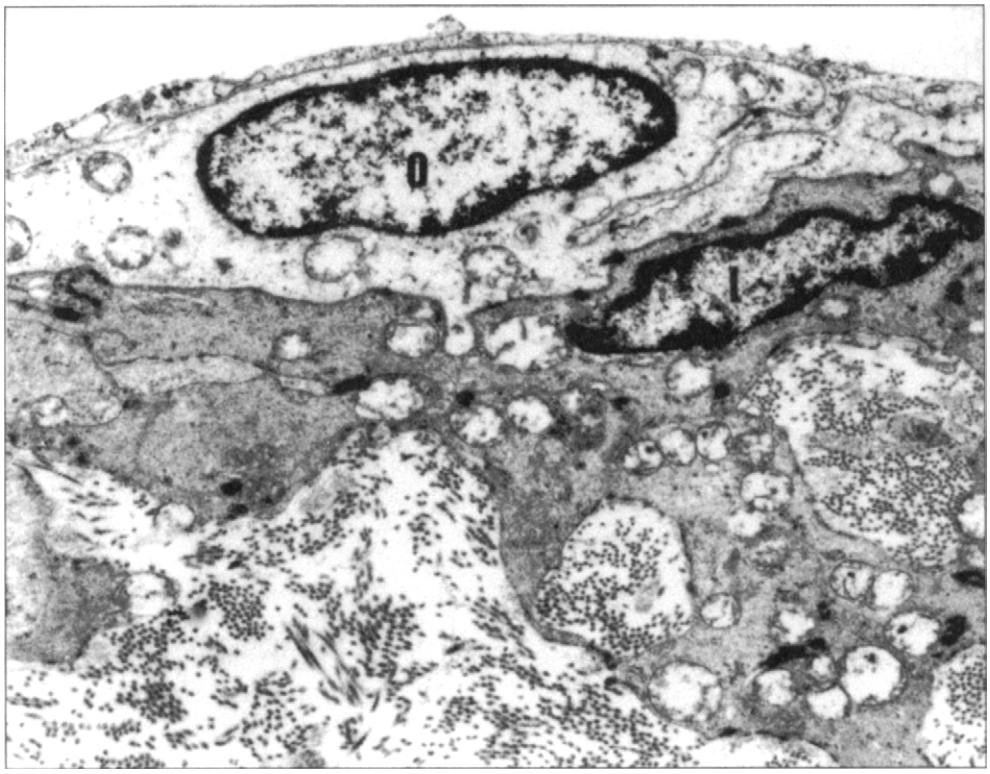



**FIGURE 4.7** Electron micrograph showing the arachnoid cell layer of the apical portion. The arachnoid cell layer consists of an electron-lucent outer zone (O) and an electron-dense inner zone (I) [reproduced with permission from Kida, S., Yamashima, T., Kubota, T., Ito, H., and Yamamoto, S. (1988). A light and electron microscopic and immunohistochemical study of human arachnoid villi. *J. Neurosurg.* **69,** 429–435].

projects through an aperture in the dural lining of the venous sinus and expands to form a core of collagenous trabeculae and interwoven channels. At the apex a cap of arachnoidal cells about 150 μm thick surrounds the collagenous core. Channels within the core extend through the cap to reach the subendothelial region of the granulation. The channels within the granulations are lined by compacted collagen and can contain macrophages. The channels are in continuity with the subarachnoid space. The cap of the granulation is attached to the endothelium over an area 300 μm in diameter; the rest of the granulation core is separated from the endothelium by a subdural space and a fibrous dural copula (17). Whether or not the endothelium of the venous channel is intact is uncertain. Factor VIII stains in one study failed to confirm the complete investment by endothelial cells of the cap of the arachnoidal granulation. Instead, the arachnoid cell was thought to abut directly upon the lumen of the venous sinuses or the lacunae laterales. The arachnoid cell layer was focally thickened to form cap cell clusters. These arachnoid cells stain positively with vimentin (18). There are extensive blunt outpouchings from the dural venous sinuses into the dura that are termed lacunae laterales. Arachnoidal granulations interdigitate with these outpouchings (19). An electron microscopic study of human arachnoid villi obtained at surgery demonstrated micropinocytic vesicles, giant intracellular vacuoles, and tubular-like, endothelium-lined structures (20). Illustration of an apparent gap between endothelial cells was not convincing. In a human study of arachnoidal granulations they were found to be engorged with blood following trauma and SAH from aneurysms. Actual passage of RBCs through the vascular endothelium was not documented. The degree of engorgement of the different lobules of the pacchionian granulations was variable in both time and extent (21).

In the nineteenth century various dyes were injected into the subarachnoid space of cadavers under pressure and were subsequently found in the cores of pacchionian granulations. These bodies were found not to exist in infants or in nonhumans (6). Weed (1914) showed that they were essentially exaggerated forms of the structures seen in the dural sinuses of all animals and infants. They were invasions of the dura by arachnoid but on a macroscopic scale. He noted that the arachnoidal villus is an interlacing cord continuing the outer arachnoid

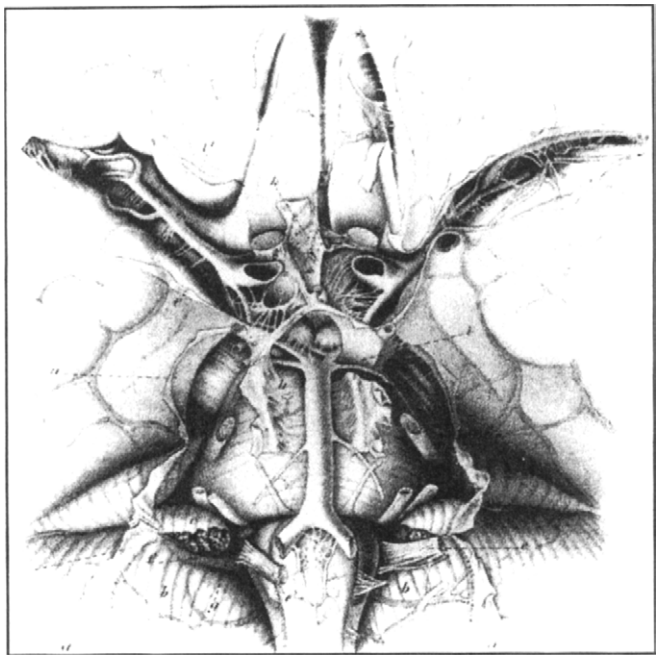


**FIGURE 4.8** Basal cisterns illustrated by Key and Retzuis in 1875 [from Key, A., and Retzius, G. (1875). Studien in der anatomie des nervensystems und des bindegewebes. *P. A. Norstedt & Söner*, p. 220. Stockholm].

membrane into the dura. The villus is surrounded by a web-like sleeve of cerebral vein on its way to the dural channels. The villus is a myxomatous structure that is capped on all sides by a mesothelial cover of arachnoidal cells so that cerebrospinal fluid in the lumen of the villus is separated from the blood in the sinus by this layer of cells (22).

When an arachnoid villus was frozen *in situ* in a living monkey there appeared to be an open tubular structure in the villus. When formalin was injected under high pressure into the subarachnoid space, however, no openings of valves was demonstrated (23). In 1968 and 1971, Shabo and Maxwell considered that these large channels were artifactual. By electron microscopy the endothelial layer of the villus is intact and the interendothelial clefts are sealed by tight junctions that prevent the passage of protein through them (24–26). In 1972, Shabo and Brightman showed that horseradish peroxidase does not usually penetrate interendothelial clefts and there are no large channels that would permit the passage of particulate matter (27).

In 1968, Tripathi found that giant vacuoles would form within the endothelial cells lining the canal through which aqueous fluid drained from the eye (28). In subsequent studies Tripathi and Tripathi performed electron microscopic studies of the meninges in relation to the arachnoid villi and found that at irregular intervals there were transdural openings up to 100μm in diameter leading to transdural channels of a smaller caliber serving to connect the subdural space with the lumen of the sinus or its lacunae (29). It has been suggested that a unique feature of the mesothelial cells lining the arachnoid villi and granulations is the presence of uni- or multilocular giant vacuoles. Electron microscopy showed that mesothelial lining cells of the arachnoid adjacent to the superior sagittal sinus contain many giant vacuoles. Vacuoles are invaginations from the basal aspect of the cell surface which is in direct communication with the subarachnoid space. Some vacuoles also have openings into the subdural space, thus constituting a potential transcellular channel. It was suggested that vacuoles are stages in the formation of a dynamic system of transcellular channels or pores which allow the bulk outflow of CSF across the mesothelial border (30).

In the spinal column the arachnoid invades the dura in relation to the spinal epidural veins similarly as it does in the pacchionian granulations of the superior sagittal sinus (31).

The meninges in nervous tissues are not provided with lymphatic channels in the sense of endothelium-coated tube. Dyes introduced into the subarachnoid space that are subsequently found in lymph nodes must have escaped into the surrounding connective tissue, such as the epidural tissue of the spinal cord, by pathways such as the trunks of the spinal nerves. The subarachnoid space is continuous with the space around the connective tissue sheath of the olfactory, optic, and acoustic nerves. This may be a potential meeting point of the subarachnoid space and the lymphatic system. Colloidal material injected into the subarachnoid space has been found in nasal mucosa and thoracic lymphatics. When dye–plasma protein complex is injected into the subarachnoid space, within 30 min half is found in the cervical lymph and half in the circulation (32).

Human studies of arachnoid villi post-SAH have shown them to be packed with RBCs (Fig. 4.10). Viewed from the inside of the venous sinuses, some granulations are apparently distended with blood (Fig. 4.11), whereas immediately adjacent ones may not be. The appearance is that of an intact surface, not a porous one.

### *2. Animal Studies*

Arachnoidal granulations increase with age in humans and are not present in many animals which have only arachnoidal villi, not gross granulations. Dyes infused into the subarachnoid space localize in the arachnoid villi (33,34). The villi do not have true one-way valves, but the movement through them of fluid and perhaps particles such as RBCs or their fragments probably occurs

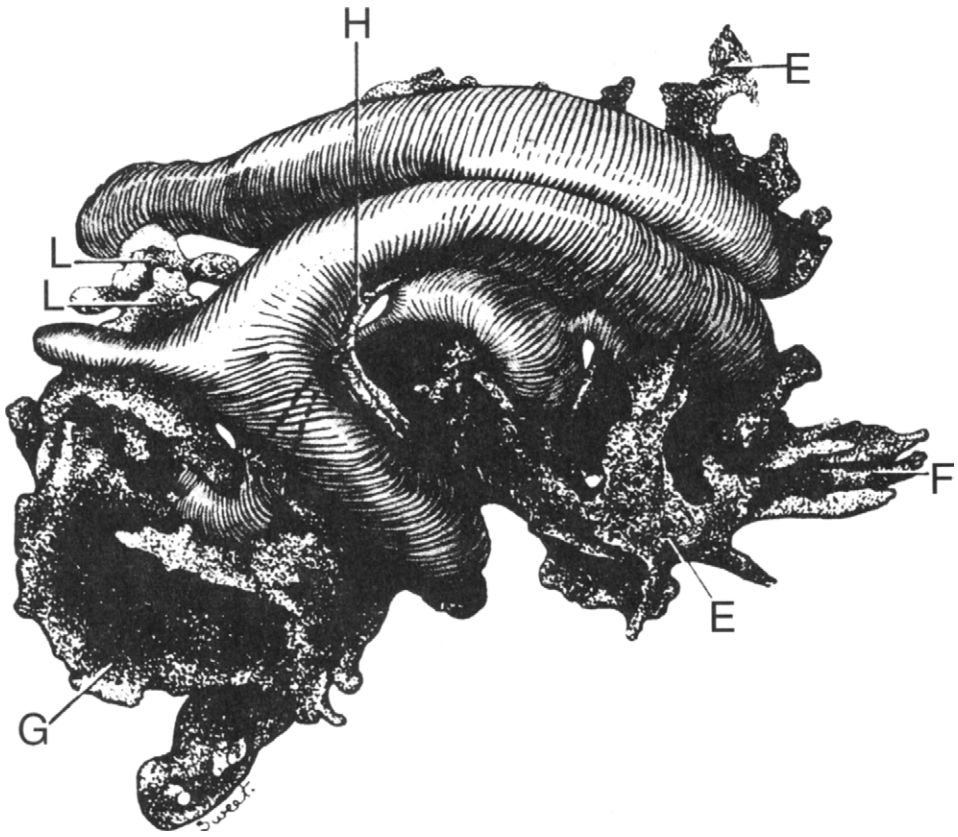



**FIGURE 4.9** Lateral view of cast of the cerebral ventricles and cerebral subarachnoid space. (The cerebrosagittal channel was broken from this cast in the process of cleaning.) The lateral ventricles, foramen of Monro, third ventricle, aqueduct, and fourth ventricle are seen; the lateral cerebrocortical channels are seen at both E's, the occipital subchannels at both L's, and a portion of the right internal channel at H. F, origin of the cerebrosagittal channel; G, lateral cerebellocortical channel.

by pinocytosis or vesicular transport and energy-requiring processes such as phagocytosis and cell degradation. The injection of RBCs or carbon particles into the subarachnoid space in advance of saline injections blocks the flow of saline into the subarachnoid space (4). In most studies there is an intact tight layer of endothelial cells over the surface of the arachnoidal villi that tends to prevent the passage of intact RBCs from the subarachnoid space to the systemic circulation (25). In dogs an injection of RBCs into the cisterna magna without induction of elevated intracranial pressure (ICP) was followed by the demonstration of RBCs within the arachnoid villi. They appeared to be progressively engulfed by phagocytes within the villi, and the villi came to contain fine debris resulting from RBC degeneration. There is no convincing direct evidence that any passageway exists through the endothelium of the villus. Some experiments using tagged RBCs in dogs suggested that there can be immediate passage of labeled cells out of the subarachnoid space. This may have partly been due to extrasubarachnoid injection, passage of RBCs through lymphatics present in animals, or other unknown mechanisms (32,35,36). Tripathi found evidence for the existence of a cyclic process of vascuolization within the cytoplasm of endothelial cells. He interpreted the vacuoles as a system of transcellular channels large enough to allow the passage of proteins and particulate matter across the endothelium (30). In an isolated flux chamber, Welch and Pollay found that intact monkey RBCs (7.5 μm) could pass through the arachnoid villus as did polystyrene microspheres under 6.4 μm (37). In dogs some of the injected RBCs appeared to move out of the subarachnoid space along the olfactory nerve filaments because a large collection of cells were found in the nasal mucosa and in the lymphatic pathways of the nose. It is very unlikely that this system works in humans. In animals the egress of RBCs was increased by the head-down position (38). Simmonds found that 12% of injected RBCs could be recovered in the general circulation after 16 hr. Ligation of cervical lympatics did not significantly affect the rate of absorption. With

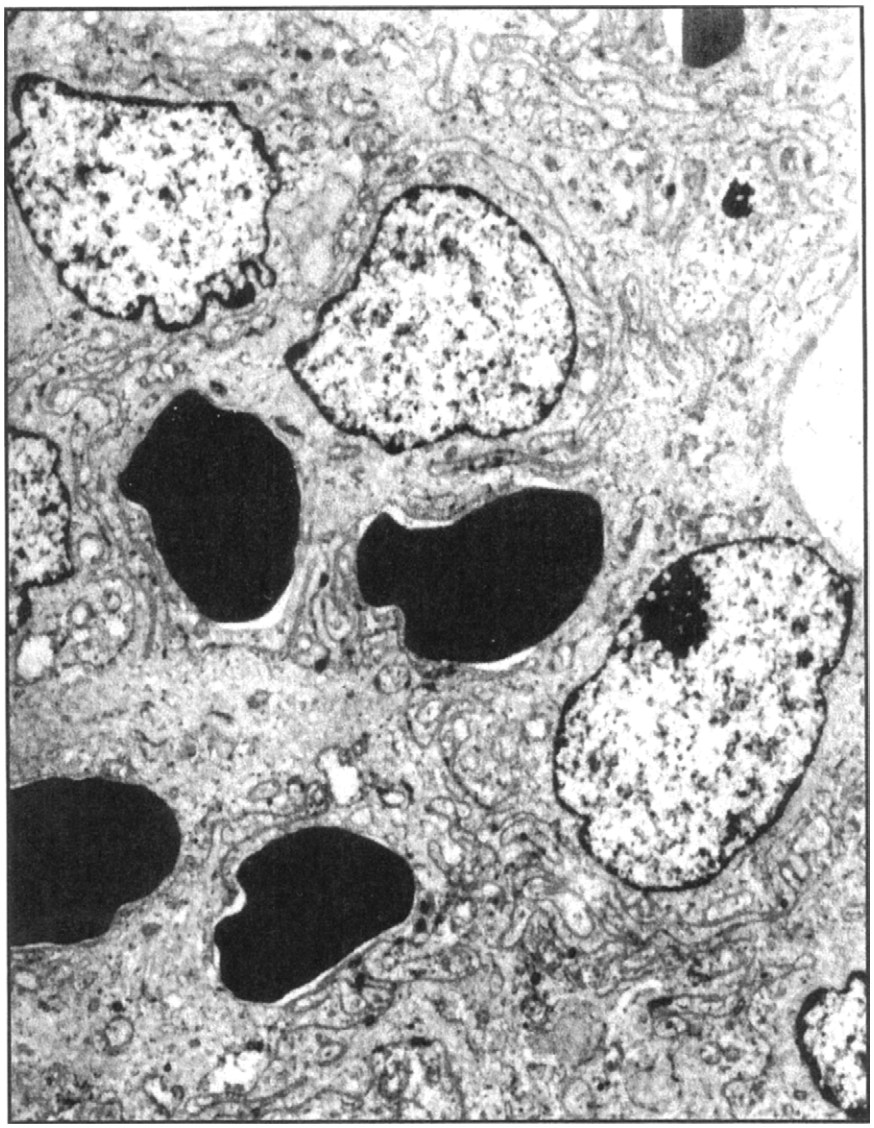


**FIGURE 4.10** Electron micrograph of a specimen with subarachnoid hemorrhage. The extracellular cisterns of the arachnoid cell layer are packed with red blood cells extending from the cranial subarachnoid space. Dark cells are RBCs, and clear cells are nuclei of arachnoid cells [reproduced with permission from Kida, S., Yamashima, T., Kubota, T., Ito, H., and Yamamoto, S. (1988). A light and electron microscopic and immunohistochemical study of human arachnoid villi. *J. Neurosurg.* **69,** 429–435].

breakdown of the blood–brain barrier (BBB) in inflammation there is a tendency for those substances in CSF that are in excess over those in plasma ($Mg^{2+}$ and $Cl^-$) to decrease, whereas the concentrations of those that are less in CSF (phosphorous, $K^+$, and protein) will increase (39).

In an electron microscopic study in dogs the arachnoid villi were found to be distended with the RBCs for a few days after SAH but then began to degenerate. Ten days post-SAH remarkable phagocytosis and micropinocytosis were observed. Thirty days post-SAH, increased cellularity in the arachnoid villi developed and the intracellular spaces became very narrow. Ninety days later the arachnoid villi demonstrated many cytoplasmic filaments and hemidesmosome-like structures in the arachnoid cells as well as narrowed intracellular space and increased cellularity. Microfibrils increased and structures resembling basal lamina were observed in the stroma (40).

Blood injected into the subarachnoid space of dogs disappears in 1 or 2 weeks. The fibrosis and thickening of the arachnoid membrane appears in 1–3 weeks and

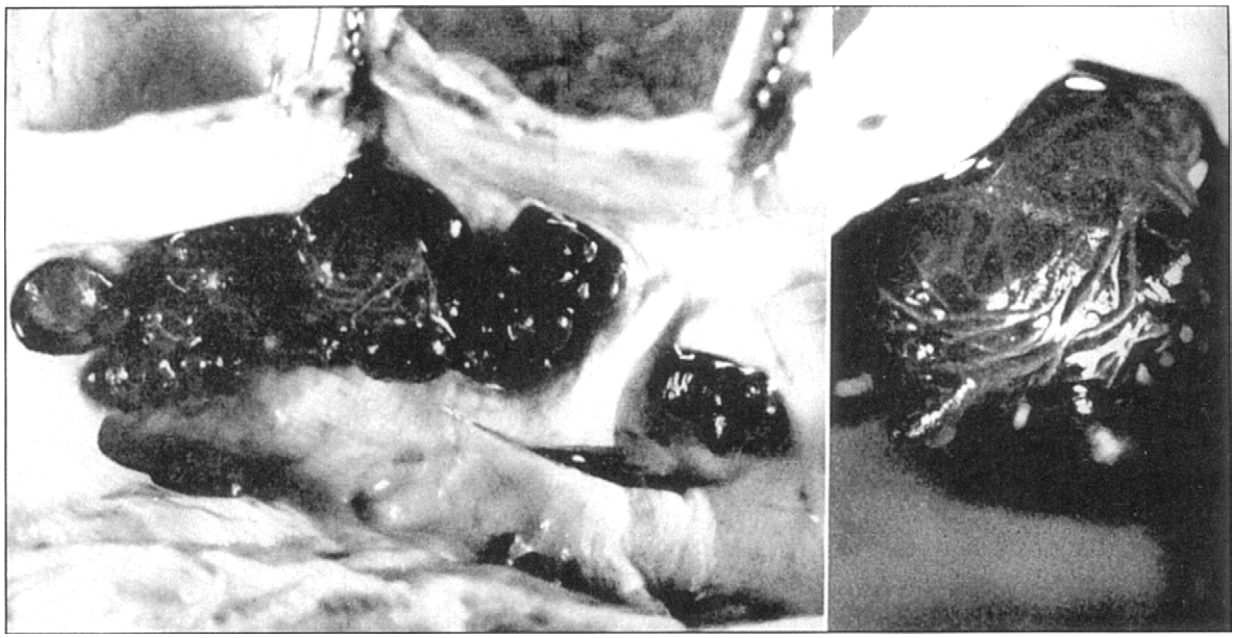


**FIGURE 4.11** (*Left*) Case 1: Internal aspect of superior sagittal sinus showing blood-filled arachnoid villi (black) protruding into the lumen. (*Right*): Closer view of a distended villus. Crossing fibers are of collagenous dural origins. Note tiny diverticulae [reproduced with permission from Ellington, E., and Margolis, G. (1969). Block of arachnoid villus by subarachnoid hemorrhage. *J. Neurosurg.* **30**].

then returns to normal in months in instances of rapid recovery. There are cases in which fibrosis persists for a long time and becomes chronic (41).

### C. Cerebrospinal Fluid

#### *1. Production*

The bulk of CSF is formed within the ventricular system. At least 80% is produced from the choroid plexus. CSF is formed at the rate of about 20 ml/hr or 500 ml/day. CSF is an ultrafiltrate of plasma produced by the passage of fluid through non-tight-junctional, choroidal capillary endothelium by hydrostatic pressure and which is subsequently transformed into a secretion (CSF) by active metabolic processes within the choroidal epithelium (3).

#### *2. Absorption*

CSF absorption depends upon bulk flow, passive diffusion, facilitated diffusion, and active transport of specific solutes. The rate of CSF absorption is pressure dependent and relatively linear in the physiological pressure range. The site of CSF absorption may be via the arachnoid villus, via the lymphatic system, via the brain, or via the choroid plexus.

Weed's concept of the pressures governing absorption of CSF was that the forces tending to draw fluid into the dural sinuses were (i) the difference between the higher pressure in the subarachnoid space and the lower venous pressure and (ii) the colloid osmotic pressure resulting from a higher protein content in the venous blood than in the subarachnoid space (42). Davson *et al.* found that resistance to flow from CSF to blood was not affected by the colloid osmotic pressure of an artificial CSF infused into the ventriculosubarachnoid system. Therefore, they concluded that the drainage channels allow an unrestricted passage of proteins through their pores. Regional increases in resistance could be provoked by introducing whole blood, kaolin, or colloidal graphite particles into the ventricles (43).

Injection of different marker substances into the subarachnoid space led to the idea that such substances can leave the cranium through the lymphatic system. Marker substances have been detected in the mucosa of the perinasal sinuses, cranial nerve sheaths, and cervical lymph nodes following subarachnoid injection. It is not clearly established that extensive lymphatic drainage of CSF occurs in man. It has been proposed that CSF might be absorbed by capillaries of the brain. In certain cases of CSF blockage there is undoubted development of periventricular lucency as seen on imaging studies. This might mean only that the brain is acting as a conduit for CSF to pass from the ventricles to the subarachnoid space rather than acting as a true absorbing site. The possibility that

the choroid plexus might not only act as the producing site for CSF but also act as an absorbing site has also been proposed. There is evidence that this might occur under the pathologic condition of intraventricular hypertension.

There is little evidence that direct absorption through the arachnoid membrane occurs. When the arachnoid is disrupted at high pressures there can be passage of tracers through it. This is not likely to be an important physiological mechanism. Circulating RBCs are a marker of CSF flow. The bulk of RBCs escaping at the time of aneurysmal rupture are in the basal cisterns. Over the course of several days as the density of blood in the basal cistern diminishes there is often an increase in density over the sylvian fissures, and a progressive migration superiorly over the cortex may be seen in sequential CT scans. The most common site of blockage of CSF pathways by SAH is at the tentorial hiatus and basal cisterns where arachnoidal fibrosis can occur. Early studies of post-SAH Hyc by pneumography frequently demonstrated blockage at this site (44). Blood has been known to cause subarachnoid fibrosis ever since the classic studies of Bagley performed in the 1920s (45).

#### *3. Pressure*

Normal CSF can range from 30 to 200 cm $H_2O$ pressure. Merritt and Fremont Smith considered that pressure on lumbar puncture was definitely normal up to 180 cm $H_2O$ (24 mmHg) and possibly normal up to 20 cm $H_2O$ (27 mmHg) (46).

#### *4. Volume*

Early anatomically based estimates of the volume of the adult CSF spaces (lateral ventricular, 25 ml, III and IV ventricular, 5 ml; cranial subarachnoid, 25 ml; and spinal subarachnoid, 75 ml) apparently grossly underestimated the volume of the subarachnoid space (47). The brain occupies 82–97% of the intracranial volume. Using magnetic resonance imaging (MRI)-based computerized segmentation techniques, recent estimates of the mean volume of the subarachnoid space in 27 to 56-year-old controls was 89 ml and in 56 to 80-year-old controls was 142 ml. The corresponding ventricular volumes were 17 and 27 ml. Cases with aqueductal stenosis had ventricular CSF volumes of 253 ml and extraventricular cranial volumes of 172 ml. For those with Alzhemimer's disease the comparable volumes were 55 and 196 ml. The ratio of extraventricular to ventricular CSF volumes is about 6 in control cases (48).

In another MRI study of 64 normals with a mean age of 38 years, total cranial CSF volumes ranged from 57 to 287 ml. Total intracranial CSF volumes increased more steeply with age than did ventricular or posterior fossa CSF volumes. Elevation of $P_aCO_2$ by 17.2 mmHg on average decreased CSF volumes by a mean of 9.2 ml (49).

## III. Cytopathology of Cerebrospinal Fluid and Subarachnoid Hemorrhage

### A. Cellular Responses

Cytological studies should be done as soon as possible after lumbar puncture to avoid autolysis of cells and to preserve their morphology. Specimens should be promptly refrigerated if not immediately examined. Normal CSF contains no more than five lymphocytes or mononuclear cells/$mm^3$. Mononuclear phagocytes, including microglia and perivascular cells, have been demonstrated to originate from the blood using radioactive labels (50). Normal CSF contains less than 44 mg/dl of protein (50). The rapid lysis of RBCs in CSF has not been well explained (35,51,52). The RBCs are not destroyed by osmostic forces. Since CSF does not have the same plasma proteins as blood, this may be a factor in the destabilization of the RBC membrane.

During the repair and reorganization of damaged leptomeningeal tissue, phagocytes remove cell debris, RBCs, fibrin, and collagen. Neutrophils as well as monocytes probably enter the CSF space, attracted by some chemotaxic mechanism. The macrophages persist, whereas the polymorphonuclear cells rapidly disappear. Once the RBC is ingested by a phagocyte the Hb is broken down into coarsely, granulated, crystalline granules known as hemosiderin (Table 4.3). These stain black with the May–Grunwald–Giemsa stain. Some macrophages appear to be completely filled with coarse granules of various sizes and the nucleus may be barely visible as a result (53–62). (Fig. 4.12). Phagocytes containing hematoidin (a yellowish caramel crystalline-appearing granule, which does not give an iron reaction) are found 10–14 days after SAH. Various white blood cells (WBCs) may also be taken up by the macrophages in the subarachnoid space. The presence of polymorphonuclear leukocytes in the CSF is always pathological.

It is not certain what happens to the RBCs following SAH (35,63,64). In experimental situations some appear in the lymphatic drainage of the head (38,65) or are apparently absorbed directly into the blood (4,36,66). It has also been proposed that RBCs remain in the CSF until they are completely destroyed by hemolysis (67) or phagocytosis (68) (Fig. 4.13). Perhaps several of these processes are active following human SAH.

In a recent case of ours in which a lumbar puncture was performed within a few hours of ictus, the RBC/WBC

**TABLE 4.3 Characteristic Histopathological Features of Blood Breakdown Products**[a]

| Substance | Characteristics | Perls' reaction | Iron |
|---|---|---|---|
| Hemoglobin | Orange-red to orange-brown round granules, much variation in size and may form spherules, immunohistochemical stain available | Negative | $Fe^{2+}$ |
| Methemoglobin | Oxidation product of hemoglobin with similar morphologic features | Negative | $Fe^{3+}$ |
| Hematin | Degradation product of methemoglobin after removal of the globin moiety | Negative | $Fe^{3+}$ |
| Biliverdin (green) | Cleavage of the pophyrin ring of heme results in biliverdin: amorphous, green-brown to black granules or sheaves of crystals, Gmelin test positive | Negative | — |
| Bilirubin (yellow) | Amorphous, green-brown to black granules or sheaves of crystals, Gmelin test positive | Negative | — |
| Hematoidin | Formed under hypoxic conditions through aggregation of bilirubin, brown rhombic crystals or amorphous burrs, Gmelin test positive | Negative | — |
| Ferritin | Not identifiable on light microscopy, immunohistochemical stain available (apoferritin and iron) | May be slightly positive | $Fe^{3+}$ |
| Hemosiderin | Intracellular, golden-yellow to brown granular or crystalline pigment, irregular in size and shape (concentrated, polymerized ferritin) | Positive | $Fe^{3+}$ |
| Acid hematin | "Formalin pigment" brown-black fine needle-like crystals, birefringence, Gmelin test positive (artifact of acid and hemoglobin) | Negative | — |

[a] Reprinted from *Surv. Opthalmol.*, Vol. 42, Spraul, C. W., and Grossnitilaus, H. E., Vitreous hemorrhage, 3–30, Copyright 1997, with permission from Elsevier Science.

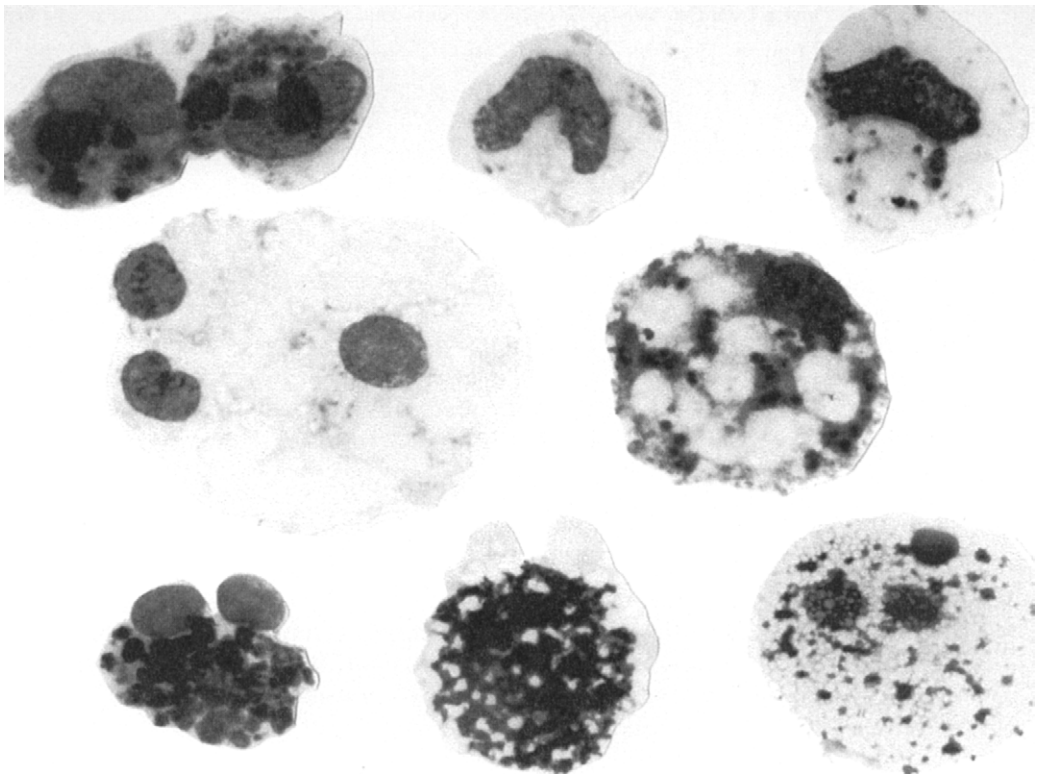


**FIGURE 4.12** Erythrophages and siderophages (macrophages). Illustrated are macrophages with isolated fine and coarse siderin granules, the breakdown products of RBCs within the macrophage [reproduced with permission from Oehmichen, M. (1976). Cerebrospinal fluid cytology. In *An Introduction and Atlas*. Thieme, Stuttgart].

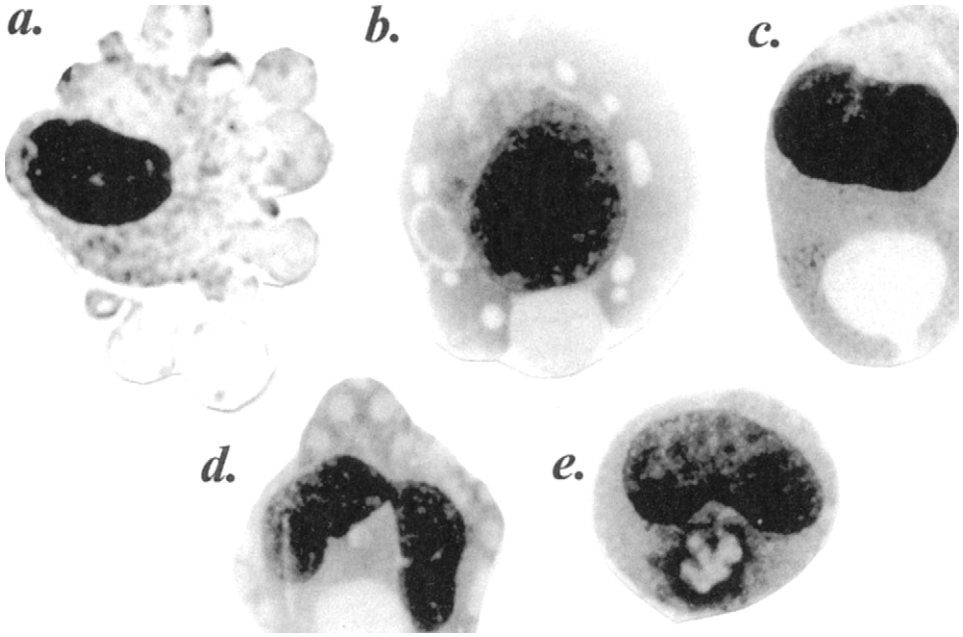



**FIGURE 4.13** Phagocytosis of RBCs by mononuclear phagocytes in various stages. (a) attachment, (b and c) protrusion of cytoplasmic processes and the final covering of the RBCs, (d) ingestion, and (e) beginning digestion [reproduced with permission from Oehmichen, M. (1976). Cerebrospinal fluid cytology. In *An Introduction and Atlas*. Thieme, Stuttgart].

ratio varied between the collection tubes from 633/1 to 295/1. The RBC/WBC ratio in the peripheral blood at the time was 388/1.

The acute phase of cellular response to SAH is characterized by an elevated proportion of neutrophilic WBC (67–72). The polymorphonuclear cells appear within a few hours (73–75), occasionally within a couple of hours (76), and reach a maximum proportion within 24 hr. The numbers usually fall off after about 1 day (77). The WBC level may reach 300 cells/mm$^3$ (53). One-third to two-thirds of WBCs are neutrophils (71,72), with the remainder being monocyte–phagocytes. During the second 24-hr period, neutrophils become less abundant and there is an absolute and relative increase in macrophages. During this time and in the days following, the RBCs begin to adhere to the surface of the phagocytes (65). RBCs within phagocytes may be seen as early as the first couple of hours following SAH (54–57) but are usually seen later in the first 24 hr (58). Lymphocytes and plasma cells may be observed on the second day. These were seen in 16% of 186 CSF specimens examined in one series (54,56). Eosinophilic granulocytes have also been noted (54,56,59,60).

The acute phase after SAH is followed by an increasing outpouring of mononuclear phagocytes (macrophages) and some round cells (53,61,74). Macrophages with contained RBCs are usually seen for 2–4 weeks (52). Such cells have been first observed on the third (78), fourth (57,70,79), fifth day (58,80) after SAH. Sometimes choroid plexus cells are seen with engulfed RBCs (62). The phase of repair can last for weeks to months, and during this period there is a relative increase in the proportion of mononuclear phagocytes with an otherwise normal cell count. Macrophages with contained RBCs have been observed even after several months (58,81–84).

### B. Red Blood Cell Clearance

#### *1. Human Studies*

Aneurysmal rupture results in the outpouring of RBCs and protein-rich blood into the colorless, parvocellular, and low-protein CSF. Depending on the rate and the amount of bleeding, clotting sometimes occurs mainly in the basal subarachnoid system Scanning electron micrographs of clot that has been in the subarachnoid space for days show RBCs trapped in a fibrin network (Fig. 4.14). The fibrin lattice of the clot has the appearance of a honeycomb (Fig. 4.15). Some of the RBCs after a week

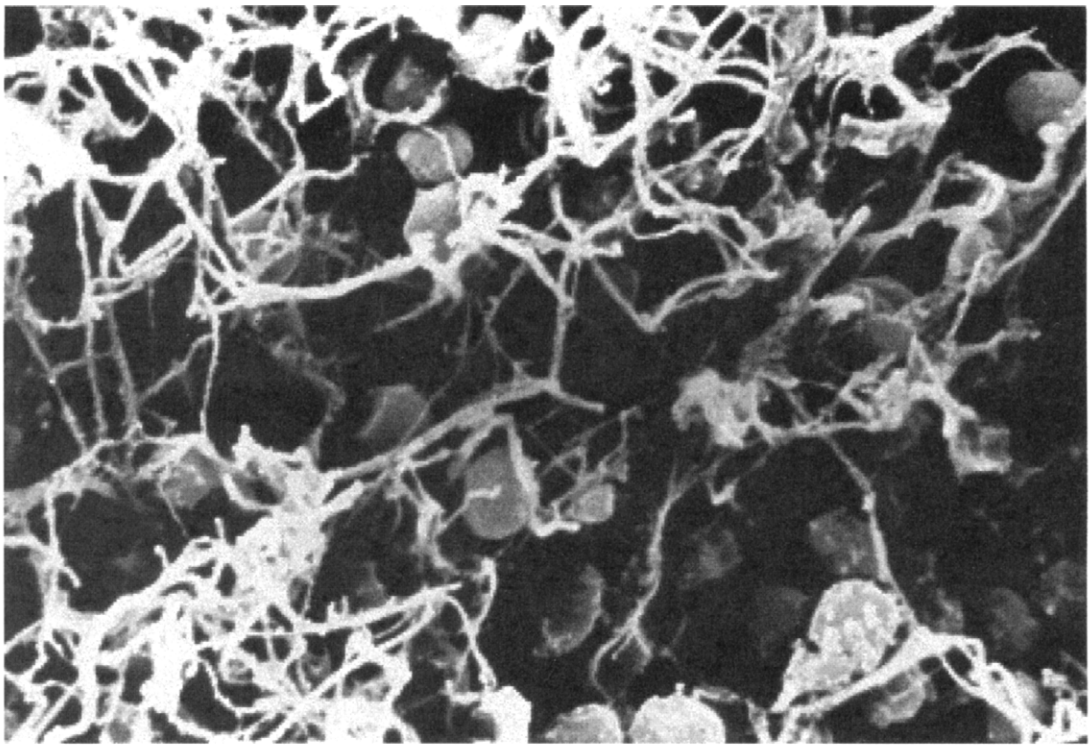


**FIGURE 4.14** Fibrin network with enmeshed RBCs which have lost their normal discoid appearance.

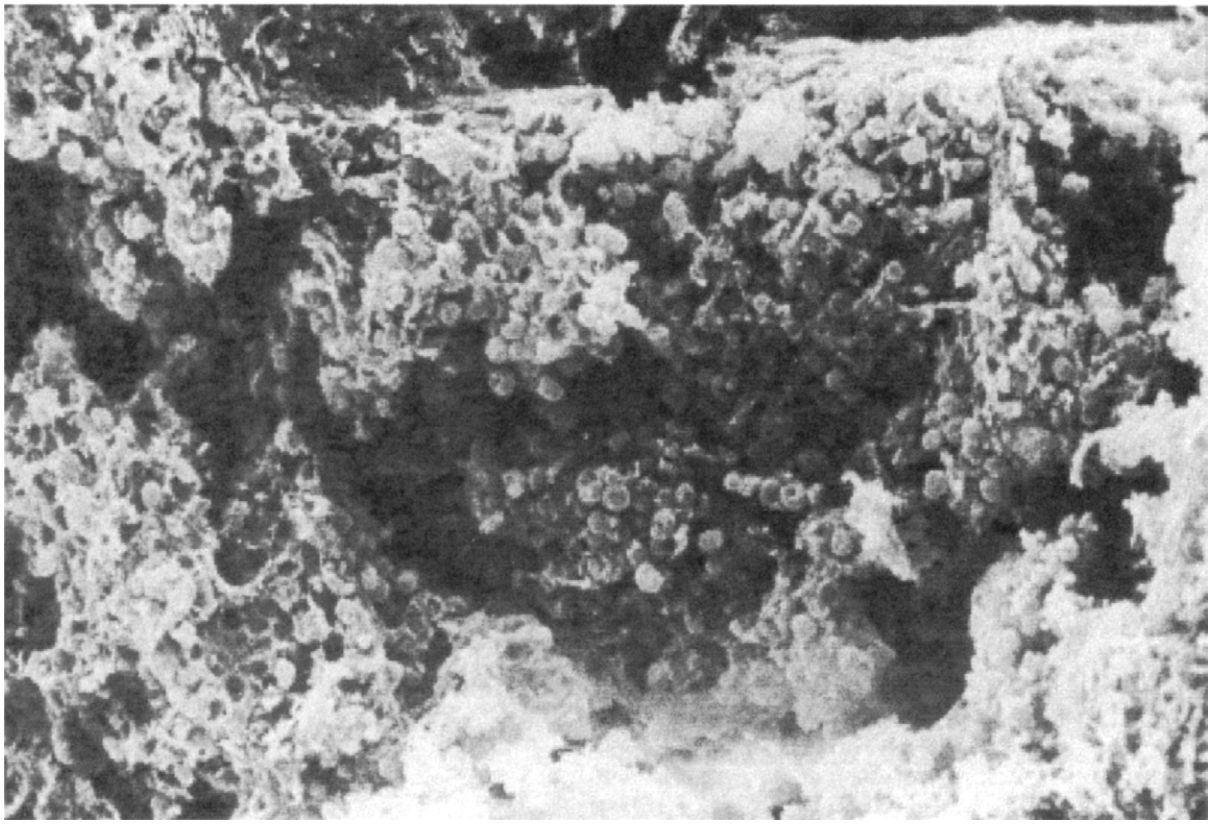


**FIGURE 4.15** Honeycomb-like appearance of fibrin meshwork with RBCs in various stages of disintegration.

remain surrounded by the fibrin, others have presumably disintegrated or been phagocytozed. Within the first instants the pressure within the CSF as well as its chemical composition change. Since the CSF is relatively easily sampled, many studies have been conducted on its changing composition in an effort to understand the pathogenesis of cerebral VSP and ischemic infarction.

The number of RBCs in the lumbar CSF is an imperfect reflection of the volume of blood coagulated in the basal cisterns.The rate of clearing of RBCs from CSF is variable and can range from 6 to 30 days. The clearance rate is reduced in older patients and certain systemic disease states. The enzymes necessary for the degradation of heme compounds such as hemeoxygenase are present in the macrophages of the arachnoid and choroid plexus (85). Hemolysis of RBCs begins within a few hours post-SAH. Early studies suggested that maximal hemolysis occurs on about the fifth day (67).

In a study of clearance rates, the CSF of a 16-year-old patient with an initial lumbar CSF count of 17,000/ml became clear (less than 100 cells/ml) in 6 days. In a 59-year-old patient with an initial cell count of 26,000/ml, it took 30 days for the fluid to become clear. In those patients whose fluid was clear by about 9 days, the xanthochromia index (total absorption properties of CSF from 400 to 615 μm area under the absorption curve), Hb, bilirubin, CSF protein, and WBCs were all highest on days 2 or 3. For patients clearing more slowly (by 19–22 days) the same indexes tend to be maximal around days 7–9 (51). In an *in vitro* study in which human RBCs were mixed with human CSF and incubated *in vivo*, the RBCs that initially were about 15,000 cells/ml had disappeared by 2.5 days after incubation. Crenation of the RBCs was evident within the first hour. The number of ghost cells progressively increased from 10 hr onward. Xanthochromia was present within 4 hr after the onset of mixing. Development of color progressed slowly at first but increased markedly after 12–24 hr incubation and was completed within 2–2.5 days. The color was due to Hb, and bilirubin was not present.

In five patients studied with daily lumbar puncture, initial RBC counts ranged from 22,000 to 680,000/ml. The fluid became clear between 5 and 6 days after the ictus. Evaluations of the blood actually removed by lumbar puncture compared to the estimates based on initial counts of RBCs demonstrated the futility of lumbar puncture as a means of removing a substantial amount of subarachnoid blood. In dogs complete drainage of the entire volume of CSF 1 hr postcisternal injection of 2 cc's of homogenous blood resulted in recovery of only 30% of the injected RBCs (86).

Forty-seven CSF samples were analyzed for cells with iron content. Such cells, first detected at 1 week post-SAH, increased to 8.5% of total nucleated cells at 4–6 weeks and subsequently decreased to 1% by 15–17 weeks. All 27 samples obtained 2–9 weeks post-SAH showed iron positivity. Of 37 samples obtained within 17 weeks, the false-negative rate was 8.1% (87).

Lumbar puncture from 66 patients with hemorrhagic infarction and 16 with lobar hematoma demonstrated a transient increase in WBCs in the CSF in 70% of patients, and the peak occurred 3 or 4 days after onset. Those patients showed a WBC count greater than 10/ml. Half the patients had a pleocytosis greater than 100/ml. In contradistinction, in pale infarcts the maximum response was usually only 10–20 WBC/ml between days 4 and 14 (88).

Hemolysis can result from the intracellular release of lysosomal enzymes by macrophages or it can be caused by autohemolysis. If glucose and oxygen fall below minimal concentrations *in vitro*, RBC autohemolysis occurs. The dissolution of RBCs usually is an intracellular process secondary to engulfment by macrophages. RBCs need to be covered by opsonins in order to be ingested by macrophages (89). Denatured Hb bonds to the internal membrane of the disintegrating RBCs to form a Heinz body. This is believed to be composed of precipitated globin, porphyrins, ferritin, and nucleic acid (90). Ferric iron ($Fe^{3+}$) is liberated during the catabolism of Hb. This occurs within the macrophage, and it is stored as ferritin or hemosiderin. Extracellular iron binds to proteins such as lactoferrin and transferrin (91). No correlation was found between the number of RBCs in CSF collected by lumbar puncture and the amount or extent of blood detected by CT (92). Erythrophages containing iron are found 4 days after spontaneous or traumatic subarachnoid hemorrhage and can persist for up to 120 days. These findings were based on samples obtained from 105 patients (93).

### 2. *Animal Studies*

Macrophages were demonstrated to derive from the lining cells in the subarachnoid cavity in response to stimulus of particulate matter in an early classic study (74). Sprong's earlier demonstration of the inefficacy of repeated lumbar punctures as a means of removing RBCs was confirmed by Meredith (94).

Various fluids were injected into the cisternal magna of dogs and the lethal doses for 50% of the animals were calculated to be the following: plasma, 12 ml/kg; heparinized whole blood, 5.75 ml/kg, unaltered whole blood, 1.9 ml/kg, washed RBCs 5.75 ml/kg, and reconstituted Hb, 3.0 ml/kg body weight. Radioactive labeled proteins appeared within the systemic circulation within minutes of injection, and labeled RBCs also appeared within minutes. In the first 8 hr, 12% of the radioactivity was

estimated to be in the systemic circulation. It is unlikely that this mechanism is operative in humans (95).

## IV. Arterial Changes in Vasospasm

### A. Systemic Arterial Response to Injury

The most common injury to human blood vessels is caused by arterial hypertension (Table 4.4). This results in a stereotypic response consisting of thickening of the arterial media due to smooth muscle cell proliferation (96,97). Similar changes occur in animals (98, 99). Acute severe hypertension can induce edema in the vessel wall, with resultant fragmentation of the elastica and myonecrosis. The latter is a signal for subsequent proliferation (96,98,99). The endothelial layer may also be damaged, resulting in permeability changes or actual sloughing of the endothelium (99,100).

Arterial injury resulting in platelet deposition may cause the diffusion of platelet-derived growth factor into the vessel wall to stimulate smooth muscle cell division (101,102). Other substances released from aggregated platelets include adenine nucleotides, 5-HT and $Ca^{2+}$ (103). The etiological link between endothelial damage and smooth muscle proliferation in systemic arteries has been demonstrated in a variety of animal arterial injury models (104–107). In the acute phase of endothelial injury arteries may show intense vasoconstriction (106,108). The proliferative response in the media can occur within several days (104,106). Increased protein synthesis is demonstrable in the arterial media for several months (104,107). The new smooth muscle cells can migrate through the elastica into the subintimal region and narrow the vessel lumen (104,106). Exposure of the subendothelium for days may be required for the initiation of cell proliferation.

Smooth muscle cells can exist in a contractile mode or in a synthetic state (109). A phenotypic change may occur in tissue culture in which contractile smooth muscle cells lose their myofilaments, increase their collagen production, and alter the actin content (102,110). Systemic arteries can mount a vigorous repair response to a variety of injuries. These responses can be associated with chronic vasoconstriction. The vasoconstriction can result from the inhibition of normal regulatory tone mechanisms. This presumably occurs in atherosclerosis and hypertension (111).

### B. Morphometry of Vasospasm

Controversy has surrounded the question of whether the reduction of lumen seen in angiographic VSP is due to

**TABLE 4.4 Changes in Arterial Morphology Related to Various Pathologic Conditions[a]**

| | SAH (human autopsy) | SAH (animal) | Hypertension | Arteriosclerosis | Endothelial damage |
|---|---|---|---|---|---|
| Intima | | | | | |
| Thickening/edema | + | + | + (acute) | + | + |
| Endothelial morphology | + | + | – | – | + |
| "Myointimal" cells | + | + | – | + | + |
| Corrugation | + | + | + | – | + |
| Altered permeability | – | + | + | + | + |
| Media | | | | | |
| Thickening/edema | + | + | + | + | + |
| Necrosis | + | + | – | – | – |
| Vacuoles | ? | + | – | – | – |
| Proliferation | ? | + | + | + | + |
| Fibrosis (late) | + | + | + | + | + |
| Adventitia | | | | | |
| Inflammation | + | + | – | ? | – |
| Axonal changes | + | + | – | – | – |

[a] Reproduced with permission from Mayberg, M. R., Okada, T., and Bark, D.H. (1990). Morphologic changes in cerebral arteries after subarachnoid hemorrhage. *Neurosurg. Clin. North. Am.* **1**, 417–432.

simple vasoconstriction of the medial smooth muscle or whether some form of arterial injury results in edema of the vessel wall or a proliferation of elements within the wall which by virtue of increased mass would reduce the lumen. Most studies have concluded that there is no significant increase in area of the vessel wall, at least in the first week or so following the onset of VSP, which could account for the observed reduction in lumen. There is no doubt that structural changes do occur (myonecrosis in the media and subendothelial cellular infiltration on the luminal side of the elastica) (112). An early study of the morphology of vasoconstriction used mesenteric arteries of dogs prepared by an instant freezing method. In contrast to formalin-fixed specimens, the walls were thin in relation to the vessel lumen. Endothelial cells were flat and the intima was not convoluted. Smooth muscle cells were long and thin, with nuclei 10 times longer than their width. Pharmacologically induced constriction caused a significant reduction in external diameter and lumen with an increase in the ratio of wall thickness diameter from 1:30 to 1:3. After constriction the intima became a densely packed series of irregular convolutions and the flat endothelial cells became progressively columnar. Nuclei appeared to perch on the top of convolutions. The largest increase in wall thickness occurred in the media. Deformation of the shortened smooth muscle cells was least adjacent to the internal elastic lamina (113).

Porcine middle cerebral artery (MCA) exposed to blood for 10 days showed a 56% reduction in luminal cross-sectional area and an increase in radial wall thickness of 75%, with only a minimal increase in cross-sectional area of 13%. Stereological analysis revealed that the volume density of individual components of the arterial wall were unchanged following the exposure to blood. There was a 44% reduction in smooth muscle cell immunoreactive actin, with a concurrent increase in collagen in the extracellular matrix (112).

In rabbits, induced SAH caused a significant biphasic constriction of the basilar artery without any changes in the cross-sectional area of the media. The relative amount of smooth muscle cell decreased significantly in the late stage of hemorrhage (114). There was a highly linear correlation between morphometric and angiographic determinations of the degree of VSP. The morphometric approach documented far greater relative constriction in the major cerebral arteries than in smaller arteries under chronic hypertension.

### C. Pathology of Arteries in Vasospasm

#### *1. Human Studies*

Postmortem angiograms revealed that vessels showing spasm remained constricted for at least 36 hr following death, although relaxation would occur 2 days following (115). John Hunter observed that placental arteries would constrict for a couple of days after separation from the body. Since histological examination of these spastic vessels did not show any obvious change except for a possible instance of fragmentation of elastica, it was assumed that spasm was a functional constriction and not a wall infiltration or swelling (116). Crompton described morphological changes at autopsy in human cerebral arteries following fatal SAH. Changes included edema, medial necrosis and fibrosis, and adventitial inflammatory infiltrates. These autopsies were performed 2–20 weeks after SAH and the illustrations were of small arteries sometimes showing complete autolysis.

Histological changes in the intradural arteries of 12 patients dying between 1 day and 15 months post-SAH were documented. In all patients surviving more than 4 weeks (with one exception surviving 4 days) the lumens of the intracranial arteries were narrowed by subendothelial granulation tissue which thickened the intima. In 58% of cases vessels near the site of rupture were involved. Less frequently, vessels remote from the site of hemorrhage were also involved. Changes were restricted to vessels in the subarachnoid space. Intimal thickening was proportional to the amount of subarachnoid blood. Only 2 of 3 cases showing diffuse VSP between days 6 and 9 post-SAH had subendothelial proliferation shown at autopsy performed between 2 weeks and 4 months. The case without these changes was autopsied earliest—at 2 weeks. One case had no angiographic VSP at 1 month but had intimal cellular proliferation at 4 months. Another case was said to show severe angiographic narrowing at 9 months with appropriate pathologic changes at 12 months. This was obviously not the usual VSP post-SAH. Another case showed no angiographic narrowing on day 1 post-SAH, but intimal changes were observed at autopsy on day 4. It is surprising that such a reaction could develop in only a few days. Hemosiderin was demonstrated in a perivascular distribution in 86% of vessels showing intimal proliferation but only 57% of those vessels not showing this change. The intimal thickening was greatest at vessels supplying infarcted areas. There was no apparent involvement of other layers of the vessel wall, including the media and the intact internal elastic membrane. There was considerable variation even between vessels in the same vicinity. Because 3 of the 4 patients with the most marked subendothelial proliferation survived more than 3 months, it was suggested that the changes might be progressive and permanent (117).

The arteries of 6 patients with angiographically demonstrated VSP were studied histologically. In patients dying within a day of onset from VSP, contraction of the medial smooth muscle was the main cause of lumenal narrowing.

In those dying between 1 and 2 weeks of onset, the arterial wall demonstrated reduction in luminal size with medial thickening, corrugation of the internal elastic lamina, intimal edema due to endothelial injury, and thrombus formation. In patients dying more than 2 weeks after the onset of VSP, there were necrosis and a reduction in numbers of smooth muscle cells. One case showed progressive angiographic narrowing for more than 2 weeks; the arterial wall showed lumenal stenosis with cellulofibrous thickening of the intima and organization of thrombus (118). Seventeen cases with radiological and pathological evidence and 3 with only pathological evidence of VSP were studied postmortem. In 12 dying within 3 weeks of SAH, the most significant change was necrosis of the tunica media (Fig. 4.16). In 8 cases dying later, there was marked concentric intimal thickening by subendothelial fibrosis (Figs. 4.17 and 4.18). Focal and asymmetrical intimal changes can be observed in control material as well as post-SAH. These changes have been associated with degenerative arterial disease. Concentric arterial thickening may a specific sequela to SAH but it can resemble Heubner's arteritis (119).

Based on a series of 44 patients known to have angiographic VSP, the following observations were made. In patients dying between 4 and 12 days, marked irregularity of the elastica, cloudy swelling of muscle cells, and medial thickening were present. In those dying between 10 and 25 days, myonecrosis, macrophagic infiltration in the media, and deposition of acid mucopolysaccharides were described; plasma cells, lymphocytes, and hemosiderin deposits were seen in the adventitia. In those dying between 20 and 57 days, subendothelial thickening, atrophy of the elastica, atrophy and fibrosis of the media, and a slight increase in collagen fibers in the adventitia were seen. After at least 49 days, remission of thickening in the intima and occasional muscular regeneration in the media were observed (120).

In a series of 28 cases, pathologic changes and angiographic VSP were considered to have occurred concurrently in 64% of SAH patients undergoing angiography. The principal pathologic change in the vessels was subintimal cellular proliferation, made up predominantly of interposition of smooth muscle cells and fibroblasts between the internal elastic membrane and the endothelial layer. These changes were apparently limited to the involved major cerebral vessels and seldom extended beyond primary branches. Intramural vascular hemorrhage and subsequent hemosiderin pigmentation were also noted in the vessels with severe subintimal proliferation. However, intimal proliferation without medial necrosis was suggested to be a nonspecific feature of vascular injury produced by a wide variety of factors. In comparison, angiographic constriction seen following the second week of SAH was almost always related to morphological thickening of the arterial wall. Medial necrosis and diffuse rather than focal constriction seemed to be more frequently associated with severe vessel pathology. These arterial changes appear to be greatest in those cases having survived the longest post-SAH (121). Sixteen patients dying of SAH who had angiographic VSP showed signs of cerebral arterial intimal thickening,

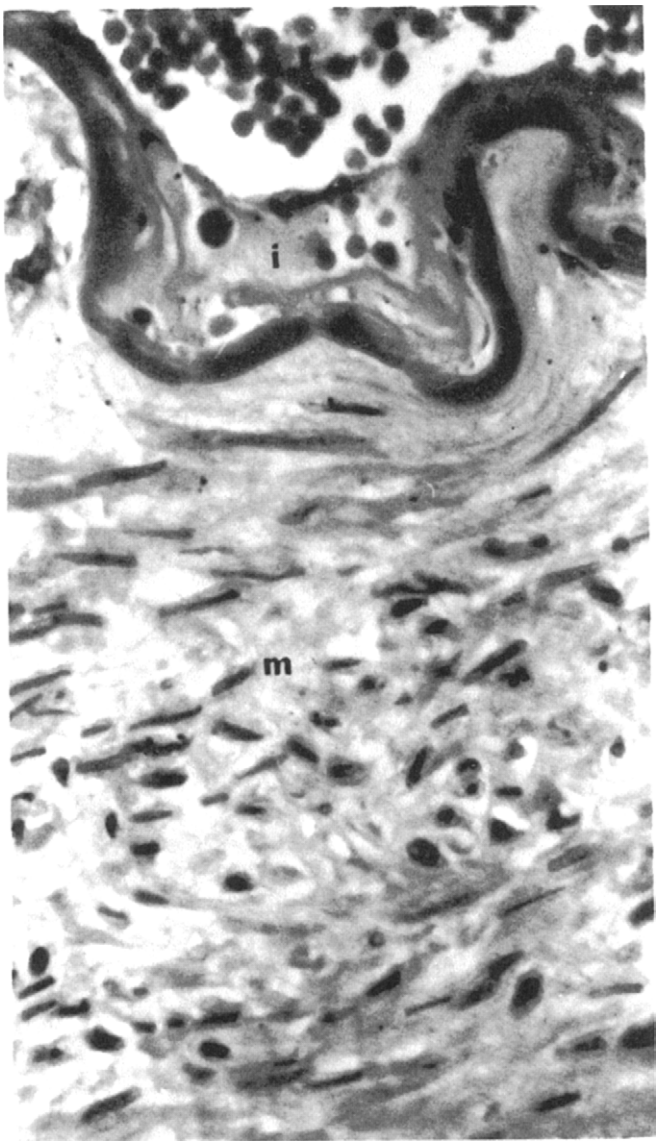



**FIGURE 4.16** Photomicrograph of transverse section of the right middle cerebral artery. The lumen of the artery is above. The tunica intima (i) is swollen and the tunica elastica irregular and probably abnormal. The tunica media (m) is disorganized, with necrosis of smooth muscle cells and the presence of "plump" cells [reproduced with permission from Hughes, J. T., and Schianchi, P. M. (1978). Cerebral artery spasm: A histological study at necropsy of the blood vessels in cases of subarachnoid hemorrhage. *J. Neurosurg.* **48,** 515–525].

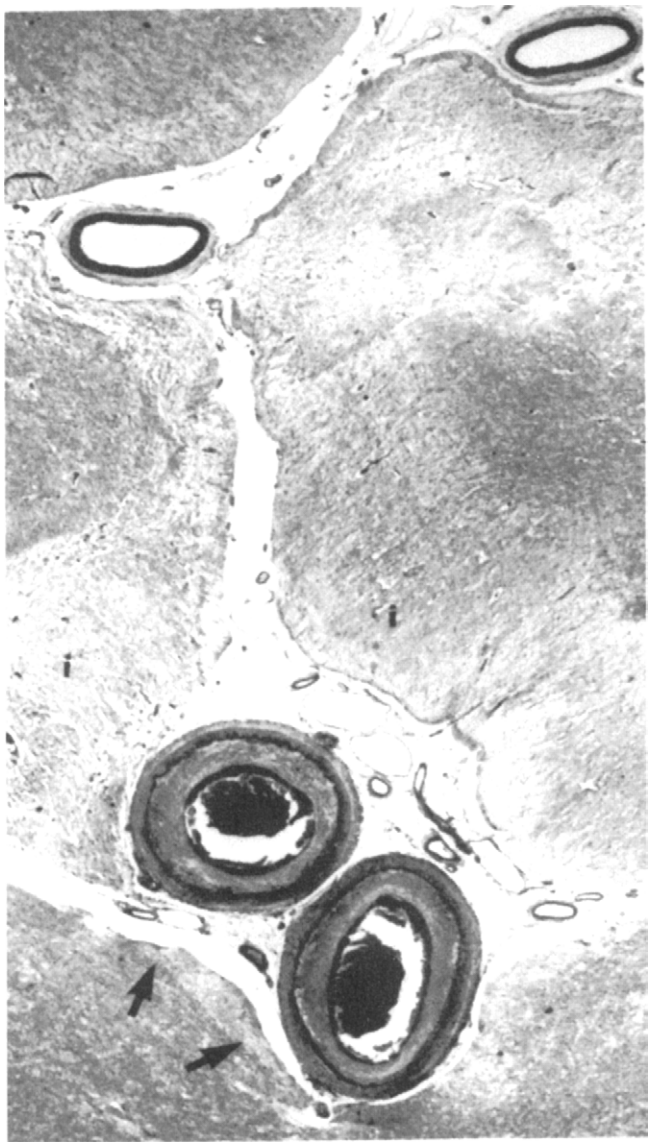


**FIGURE 4.17** Both anterior cerebral arteries (arrows) show thinning and fibrosis of the media but marked enlargement of the intima by a subintimal proliferation of connective tissue. Note the bilateral infarction (i) of the cingulate gyri [reproduced with permission from Hughes, T. J., and Schianchi, P. M. (1978). Cerebral arterial spasm. A histological study at necroscopy of the blood vessels in cases of subarachnoid hemorrhage. *J. Neurosurg.* **48,** 515–525].

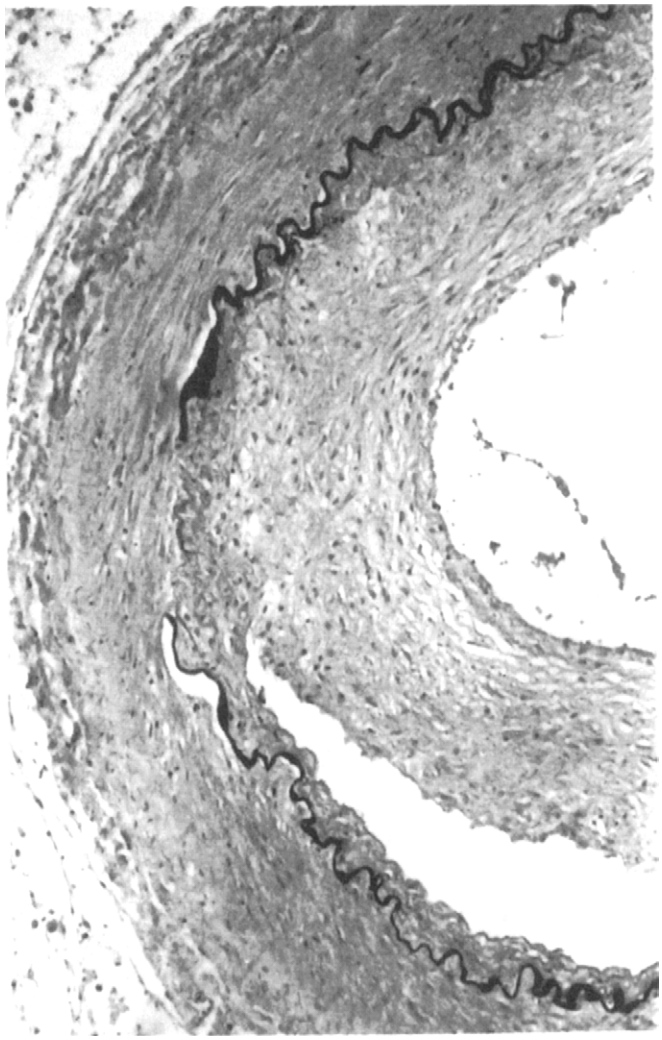


**FIGURE 4.18** Photomicrograph of a transverse section of the anterior cerebral artery. The tunica media is atrophied and fibrotic. The tunica elastica is irregular and in one place broken. The tunica intima is elevated above a thick concentric layer of fibrosis [reproduced with permission from Hughes, T. J., and Schianchi, P. M. (1978). Cerebral arterial spasm. A histological study at necroscopy of the blood vessels in cases of subarachnoid hemorrhage. *J. Neurosurg.* **48,** 515–525].

necrosis of the media, and leukocytic infiltration of the adventitia. Glycosaminoglycan histochemical evaluation was performed but the SAH arteries did not seem to differ from those obtained from presumed normal controls (122). Scanning electron microscopy of the vascular luminal surface of narrowed human arteries in SAH cases showed the occasional absence of endothelial cells and crisscrossing fibrin nets. These specimens had been removed postmortem and had been irrigated with saline prior to fixation; as a result, and because of the fact that control series also showed some fibrils and loss of endothelial cells, the observations are open to question (123). Mast cells in the arteries of 7 patients with aneurysmal SAH were found. Using the same techniques, they were not observed in arteries of patients dying from trauma (124). Some human cases have shown medial thinning with necrosis of smooth muscle cells associated with scattered eosinophilic cellular debris in the outer media (125).

Autopsies were performed on 45 cases dying from ruptured aneurysms. All showed SAH, 33% showed

intraventricular hemorrhage (IVH), 20% showed intracranial hemorrhage (ICH), and 13% showed IVH and ICH. Infarction (encephalomalacia), the primary cause of death, was present in 80%; as a contributing cause it was present in the other 20%. The time of death post-SAH was as follows: days 2–10, 62%; days 11–24, 27%; and days 25–50, 11%. Reduction in the diameter of arteries was present in all the patients dying in the early phase, but was of varying degrees ranging from moderate narrowing to apparent complete obliteration of the lumen and contiguity of the intima. On some parts of the surface of the interior wall, hemorrhages were noted between the internal elastica and the media. Smooth muscle cells tended to show twisted nuclei, indistinguishable cell borders, and condensation of the cytoplasm. Clots were noted around the arteries. By days 11–24 post-SAH, the periarterial clots were beginning to lyse. The smooth muscle cells of the media showed dystrophic changes including perinuclear halos and edema. Patients dying in the late phase showed sclerosis of the media (126).

Using Gomori's reticulin stain, a dense network of fibers surrounding smooth muscle cells was uniformly distributed in the media of "normal" patients as opposed to 35 patients who died of aneurysmal rupture, whose arteries showed fewer reticular fibers in the inner media (127).

The arteries of four patients who died from VSP were compared with those of two who died without VSP. The former patients showed many white, fibrinous microthrombi in ischemic and infarcted areas served by angiographically spastic arteries. These regions corresponded to low-density areas observed on CT scans. The cases dying without VSP showed only negligible thrombi. The distribution of microthrombi was significantly greater in regions clinically identified as having been ischemic (128).

### 2. *Animal Studies*

The model that we and our colleagues at the University of Alberta developed of clot application directly to surgically exposed basal arteries has provided interesting views of spastic arteries. The decrease in lumen with apparent wall thickening is dramatic (Fig. 4.19–4.21). The flat endothelial surface of normal vessels becomes corrugated and the longitudinal folds, parallel to the direction of blood flow, contained blood elements despite perfusion fixation (Fig. 4.22). In cross section, the endothelial folds frequently showed progressive damage to the endothelial cells and apparent regions of edema under the apices of the folds (Fig. 4.23). The smooth muscle cells undergo a variety of changes, including that of shape and development of lytic changes. All degrees of smooth muscle damage were documented (Fig. 4.24). Such changes in the dog have been recorded by others (Fig. 4.25).

When viewed by scanning electron microscope, preparations of vessel wall in which intercellular material has been removed demonstrate apparent connections between vascular smooth muscle cells (Fig. 4.26). The normally smooth, elongate vascular smooth muscle cells (Fig. 4.27), when contracted by various vasoconstrictors including hemolysate, have an accordian-like spiral of ridges in their surfaces, at right angles to the long axis of the cells (Fig. 4.28).

It has been hypothesized that blockage of potential drainage or nourishing networks in the vessel wall by RBC debris might be a factor in the genesis of VSP. Scanning electron micrographs of adventitial surface of normal vessels are consistent with such apertures (Figs. 4.29 and 4.30).

In a porcine model, MCA exposed to blood or heme-containing blood fractions for 10 days showed apparent thickening of the vessel walls (112,129). Endothelial cells can show a progression of changes, including swelling, intracellular vacuoles and subendothelial blisters, proliferating myointimal cells migrating through the disrupted elastica into the subintima, and frank denudation of endothelium (112,130–141).

Brain parenchymal vessels were studied in cats subjected to SAH. Cortical tissues were frozen *in situ* and morphometric analysis demonstrated that SAH induced parenchymal vasoconstriction (142). VSP of the basilar artery occurred following the direct injection of vasopressin. Microcorrosion vascular casts of polyester clearly demonstrated numerous longitudinal foldings and endothelial cellular impressions (143). Stereological morphometry on the intraparenchymal capillary network and microgravimetry were performed in a rabbit SAH model. The volume and surface densities of capillaries were significantly reduced; the maximum and the minimum intercapillary distances were significantly increased on days 1 or 2 after SAH. Concurrently, the specific gravity was significantly decreased. It was suggested that the formation of brain edema in the period 1 or 2 days following SAH might be associated with impaired capillary perfusion and reduced capillary blood volume (144). The sulcal arterioles of the cervical spinal cord of rats subjected to trauma showed evidence of VSP up to 24 hr postinjury. Arteriolar morphology showed a large decrease in smooth muscle cell length and increases in width. Arteriolar cross-sectional area decreased concurrently. The endothelium of the sulcal arteries developed microvilli (145). Perfusion-fixed specimens of intraparenchymal arterioles in dogs subjected to SAH were investigated by arterial injections of polyester resin. These corrosion casts showed tapered narrowing with endothelial folding after SAH. The width of the arteriole significantly decreased 3–7 days post-SAH. The significant decrease of internal diameter of

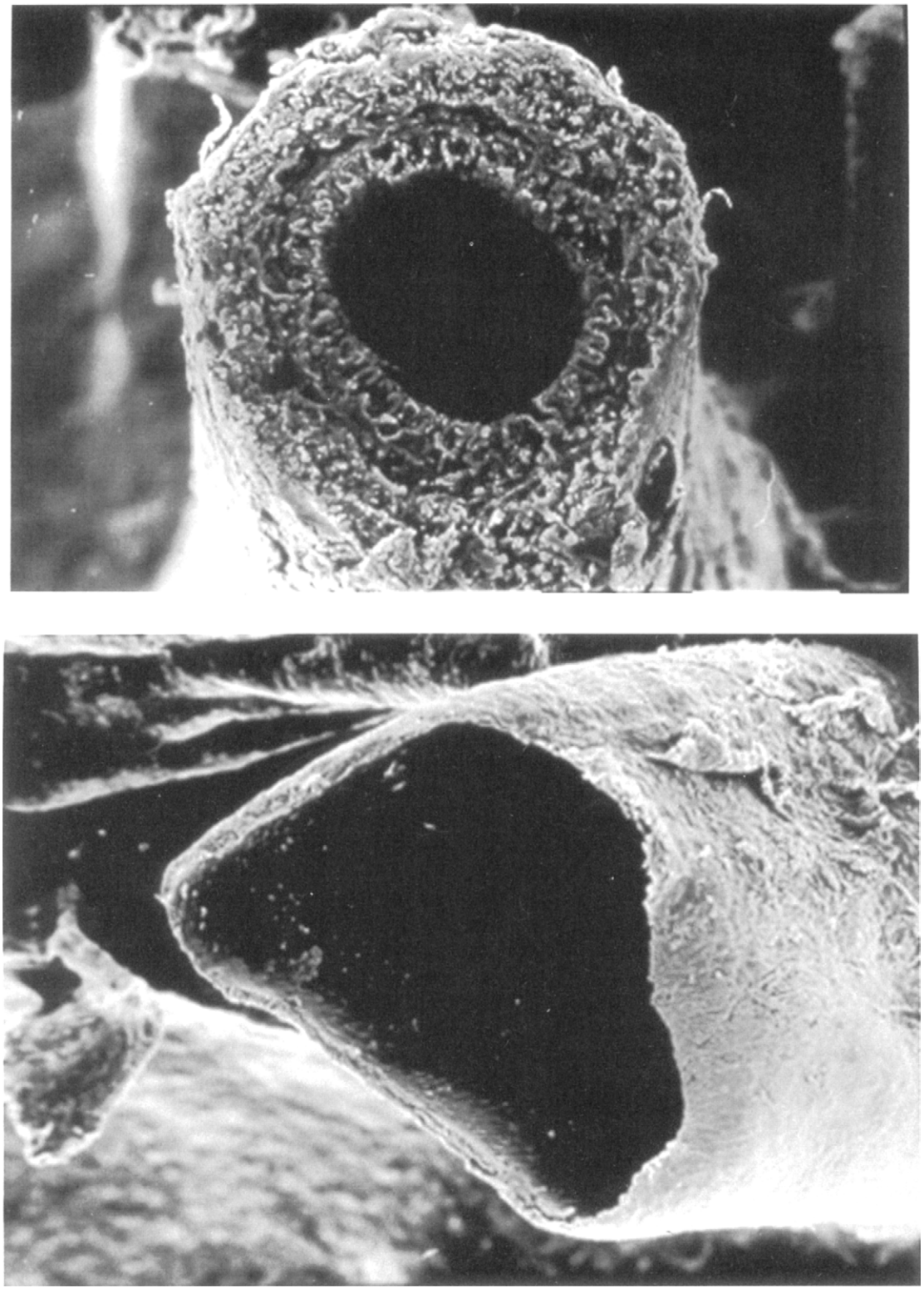


**FIGURE 4.19** Scanning electron micrograph of vessel in spasm (top) which was encased in clot for 7 days and contralateral MCA (bottom) which was not.

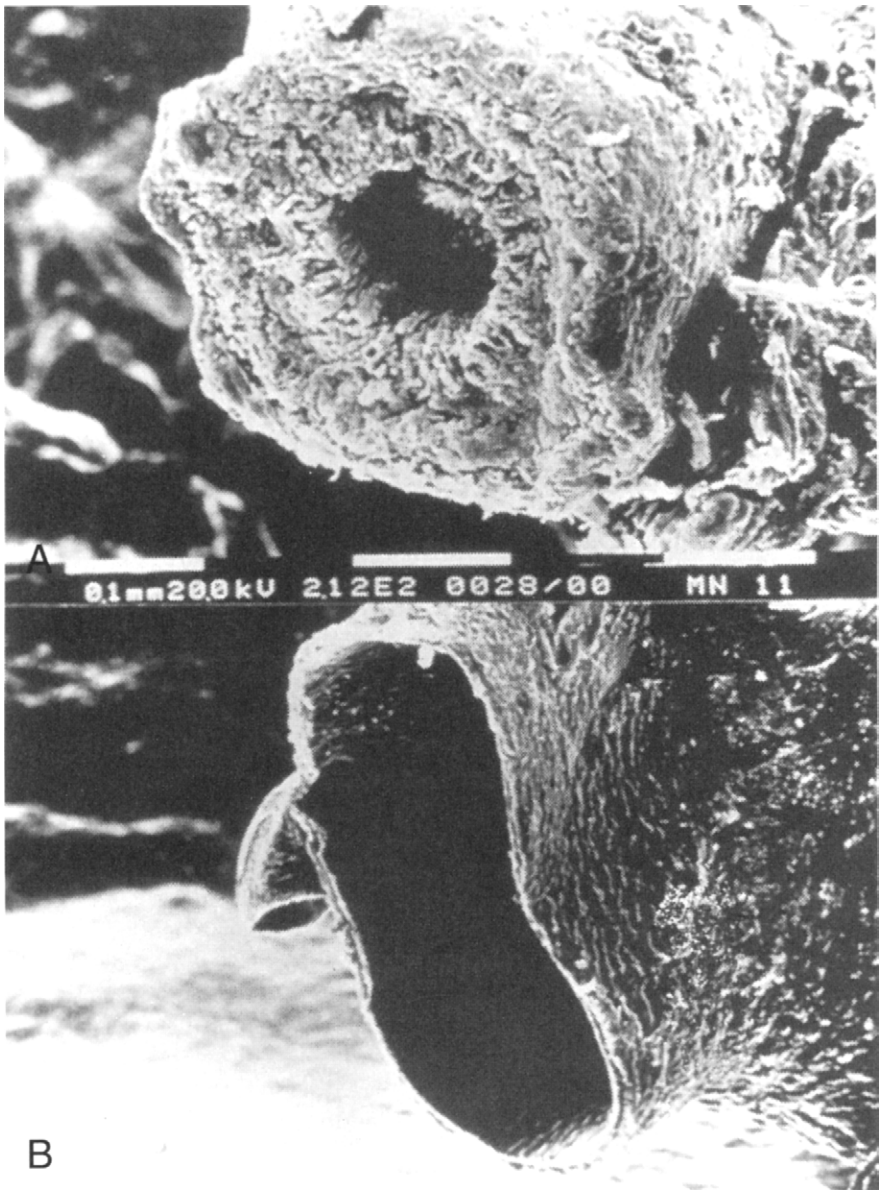



**FIGURE 4.20** Representative scanning electron micrographs of the MCA of a monkey treated with nimodipine (6 mg/kg po 8 hr). (A) MCA clot side. (B) Nonclot side [reproduced with permission from Nosko, M., Weir, B., Krueger, C., Cook, D., Norris, S., Overton, T., and Boisvert, D. (1985). Nimodipine and chronic vasospasm in monkeys: Part 1. *Neurosurgery* **16,** 129–135].

arterioles was associated with a significant increase in wall thickness, at any depth, from the brain surface 3 and 7 days post-SAH (146).

A corrosion cast technique was used to evaluate hemolysate-induced VSP in rats using three-dimensional analysis. The acute (10 min post-SAH) phase was studied. The basilar artery showed apparent VSP over its entire length and corrugations were observed on the inner surface. Endothelial cell nuclei were distorted. The inner basilar artery diameter was 38% contracted in a hemolysate-injected group compared to that in a saline-injected control group (147).

### D. Changes in Arterial Innervation

There is a rich plexus of adrenergic, cholinergic, and peptidergic axons originating from the superior cervical ganglion which exist around the cerebral arteries.

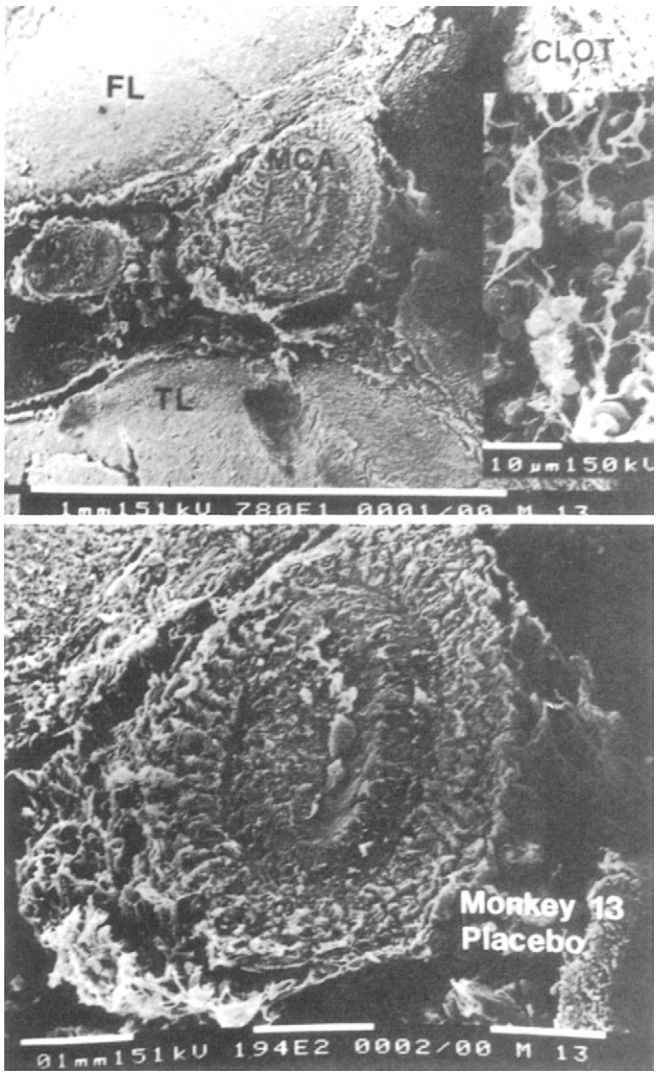



**FIGURE 4.21** Scanning electron micrographs of the right MCA (clot side) from a monkey not receiving intrathecal rt-PA. Severe VSP with intraluminal thrombosis is present. (Top) The subarachnoid clot, which has separated from the vessel adventitia during fixation. FL, frontal lobe; TL, tempolobe [reproduced with permission from Findlay M., Weir, B., Gordon, P., Grace, M., and Baugham, R. (1989). Safety and efficacy of intrathecal thrombolytic therapy in a primate model of cerebral vasospasm. *Neurosurgery* **24,** 491–498].

Following SAH, the catecholamine-induced fluorescence is usually reduced for at least a month (148,149). Ultrastructural changes are evident in the perivascular axons after SAH (150). All types of nerves are probably equally damaged since there is a marked reduction in catecholamines, ACh, vasoactive intestinal polypeptide (VIP), and substance P (SP) (149,151). Axonal degeneration occurs after exposure to whole blood, RBCs, or Hb (112,129). After SAH there may be a transient increase in $\alpha_2$ and $\beta_2$ adrenergic receptor sites (148,152). It has been speculated that a type of denervation supersensitivity might exist after VSP, but there is no direct convincing evidence. NE (131,138,153) and 5–HT (153–155) have been implicated in morphologic changes after SAH. The bulk of recent evidence, however, has indicated that vessels in VSP become progressively refractory to both vasoconstrictor and vasodilator agonists (156). Destruction of the posterior hypothalamus of dogs decreases the perivascular adrenergic nerve plexus fluorescence. It takes up to 7 days for immunohistochemical recovery. The amount of acetylcholinesterase was not diminished by this lesion (157). In another study in hypothalamic lesions in dogs, degeneration of both adrenergic and cholinergic nerves was observed (158).

### E. Arterial Wall Barrier Disruptions

#### *1. Animal Studies*

In a rabbit SAH model the presence of perivascular blood disrupted the normal blood arterial wall barrier at 48 hr. This was demonstrated by the leakage of horseradish peroxidase into the subendothelial space (159). Other workers have shown that the endothelial integrity was maintained at 10 days post-SAH and at that point there was no passage of tracers (112). Also, in the rabbit disruption of the arterial wall barrier was observed after experimental SAH. There was a markedly increased transfer of staining through the vessels embedded in cisternal clots. This staining extended toward the core of the brain stem on the first day after SAH (144).

Horseradish peroxidase injected iv in dogs does not normally penetrate the arterial endothelium to stain the vessel wall; however, this occurs following SAH or increased ICP from subarachnoid saline injection. The mechanism may be the opening of interendothelial junctions. This could account for abnormal post-contrast enhancement on CT scans (159).

In the primate clot application model when chronic VSP was reliably produced, the injection of horseradish peroxidase by the arterial route did not stain the arterial wall. When it was injected into the subarachnoid space it permeated the vessel wall from the adventitial side, apparently passing through adventitial pores (160).

### F. The Functional Significance of Morphologic Changes

#### *1. Human Studies*

Mayberg concluded that the hypothesis that luminal narrowing is caused by an increased mass of the vessel wall (due to myointimal proliferation) is probably not valid. He considered that histologic changes in the arteries

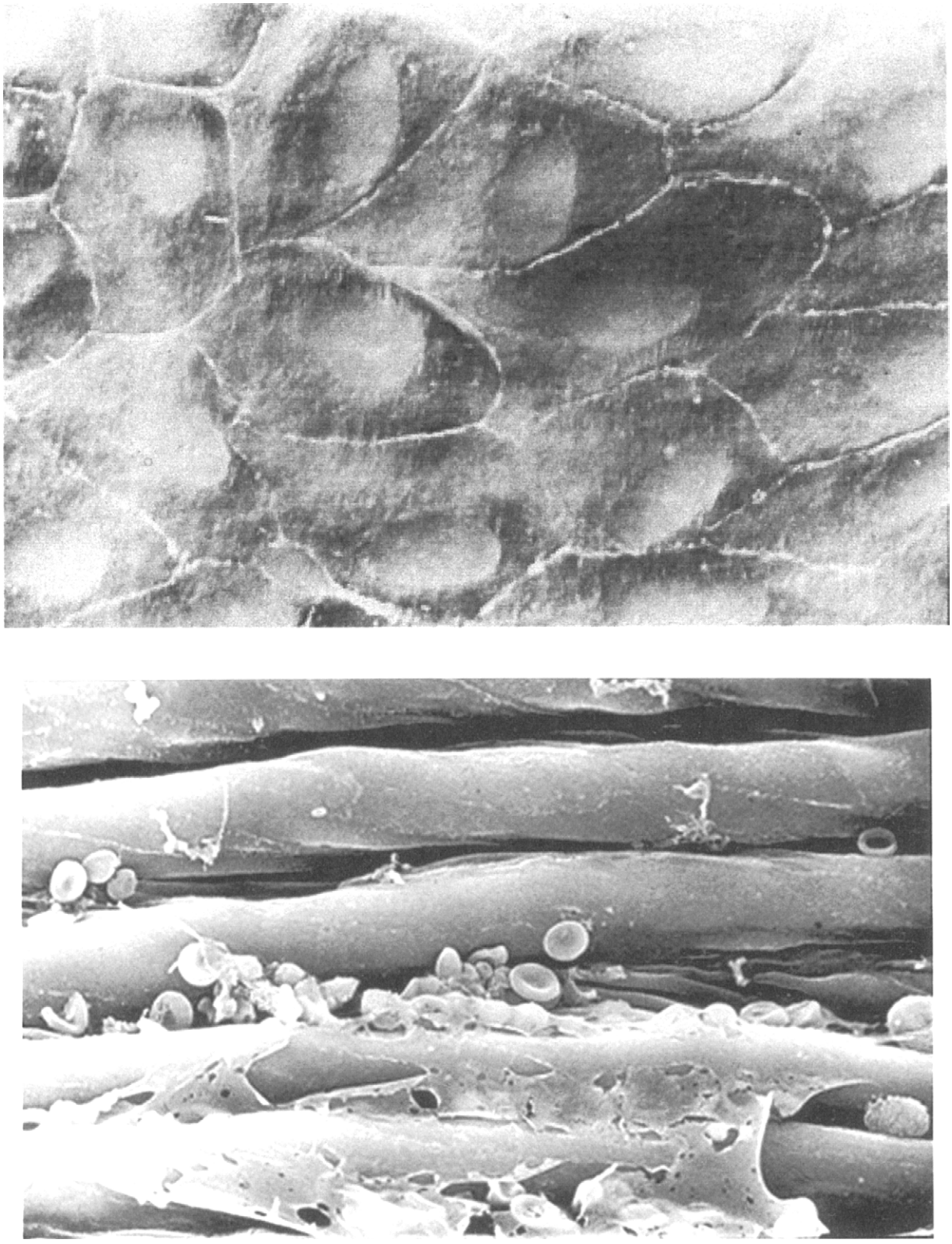


**FIGURE 4.22** (Top) Electron micrograph of the luminal surface of a monkey MCA which is not in spasm. The nuclei do not line up in any particular axis. The surface is flat. (Bottom) Despite perfusion fixation, this MCA, which was surrounded by clot for 7 days, has RBCs and leukocytes trapped in the interstices of longitudinal folds caused by VSP that are parallel to the long axis of blood flow.

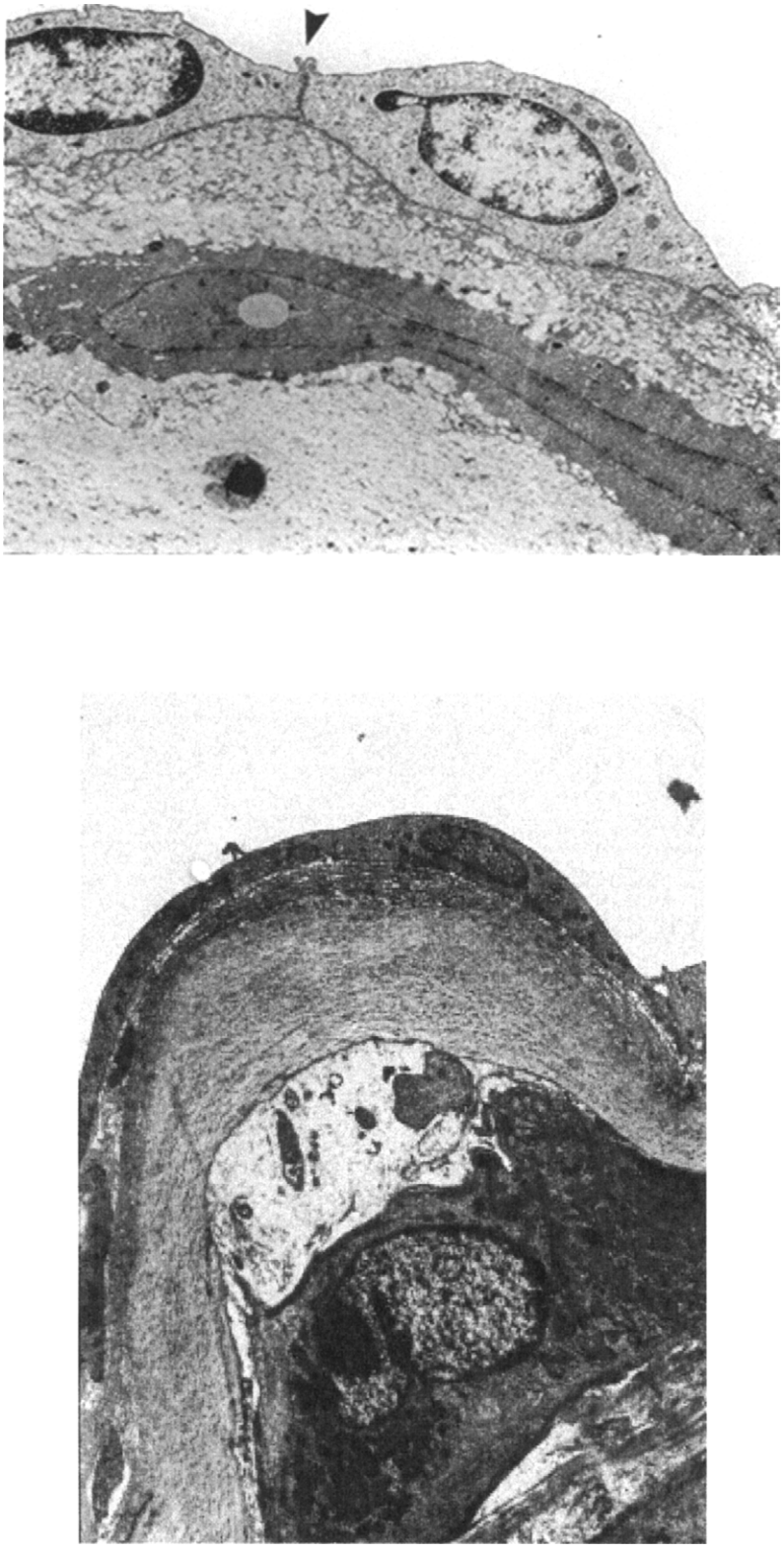
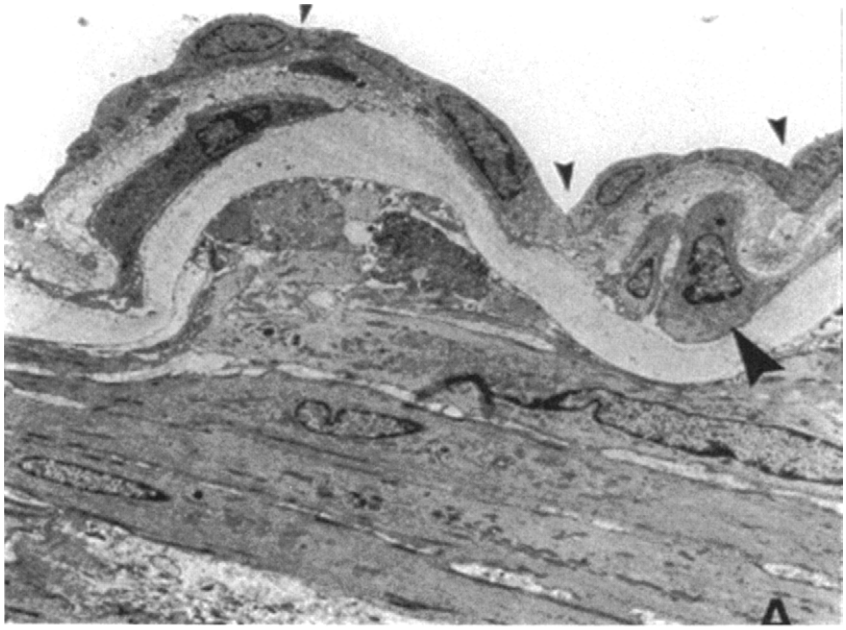


**FIGURE 4.23** Transmission electron micrographs of cross sections of the lumenal surface of monkey MCA. (Top left) Normal endothelial cells and internal elastica. Note the tight junction. (Bottom left) With more marked folding, edematous regions appear under the apices as though the muscle layer is pulling off the abluminal side of the elastica.



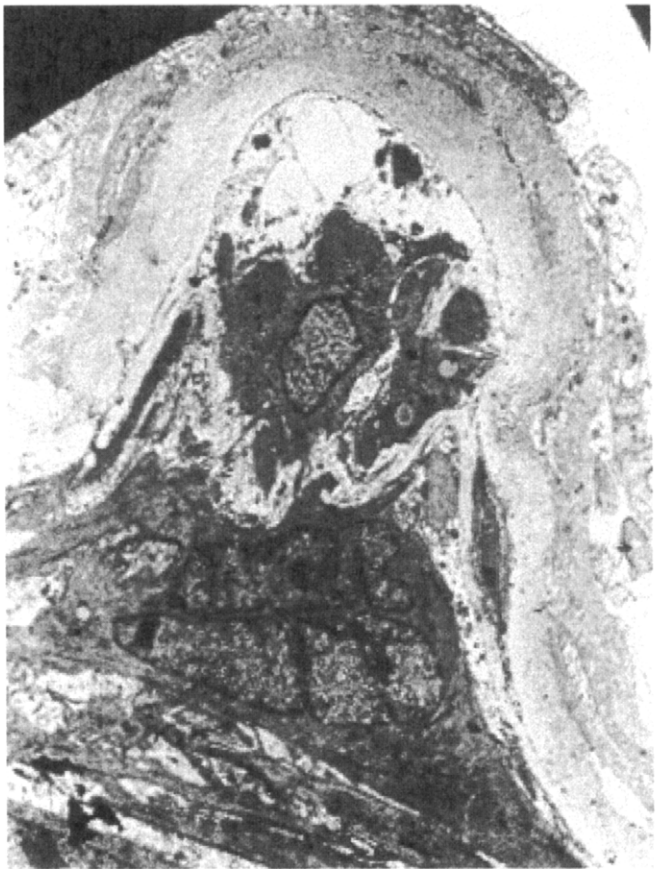


**FIGURE 4.23** (*Continued*)
(Top right) MCA exposed to clot shows some corregation of elastica and multiple cell types in the endothelial layer. (Bottom right) Same with degenerating smooth muscle cells.

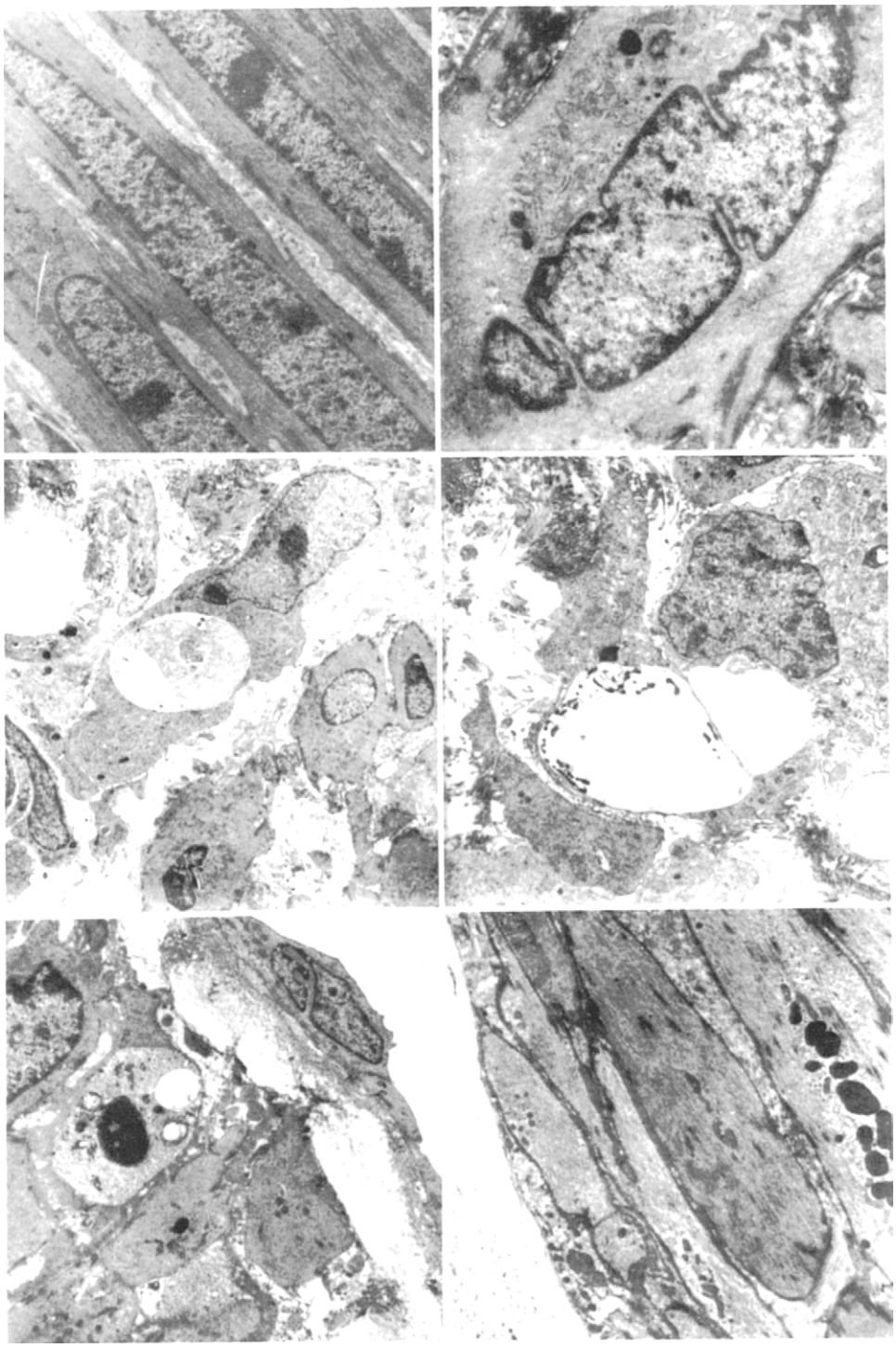


**FIGURE 4.24** Transmission electron microscopy of smooth muscle cells. (Upper left) Normal smooth muscle, (Upper right) contracted nucleus of media muscle cells, (Center left) vacuolization and marked fibrosis of lamina muscularis, (Center right) vacuolization and fibrosis of the media, (Lower left) smooth muscle cell with pyknotic nucleus and intracytoplasmic vacuoles (Lower right) smooth muscle cell with condensed lysosomes [reproduced with permission from Espinosa, F., Weir, B., Shnitka, T., Overton, T., and Boisvert, D. (1984). A randomized placebo-controlled double-blind trial of nimodipine after SAH in monkeys. Part 2: Pathological findings. *J. Neurosurg.* **60,** 1176–1185].

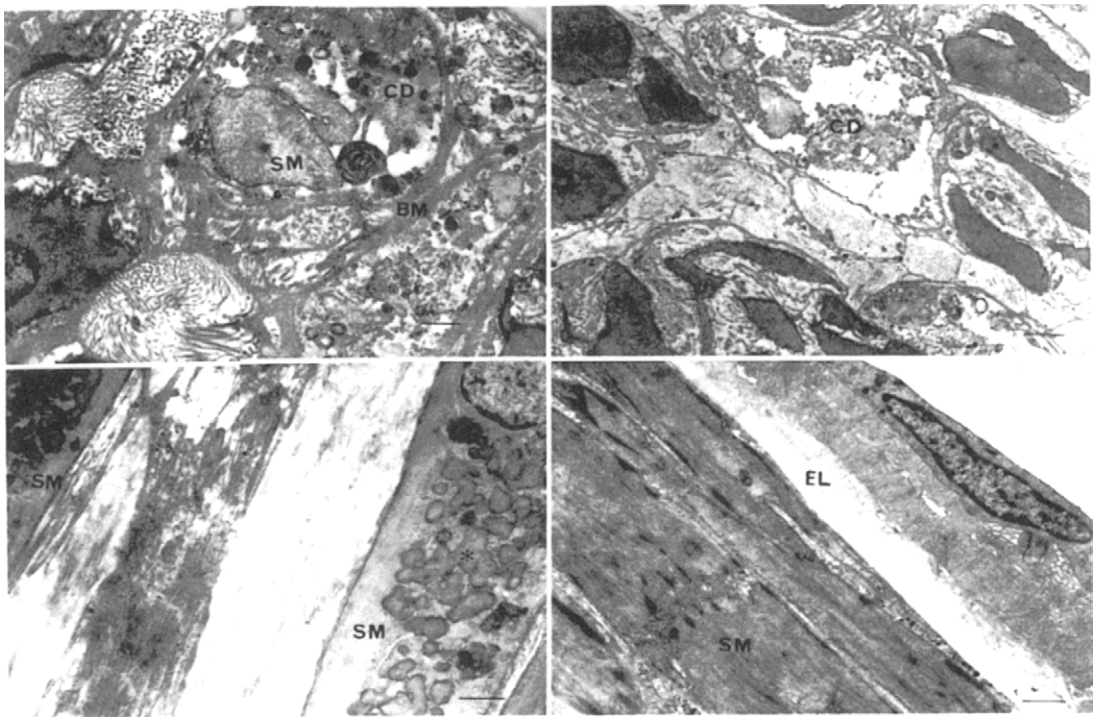



**FIGURE 4.25** Electron microscopy. (Upper left) Basilar artery 1 month after SAH. An abnormal smooth muscle cell (SM) with low-density cytoplasm and a clear membrane is seen. In its vicinity various kinds of dense or amorphous materials (CD), which are considered to be cell debris, are seen. A basement membrane (BM) is seen to surround them all. In the interstitial space there are abundant dense particles. (Upper right) Basilar artery 4 months after SAH. There is considerable cell debris (CD) surrounded by basement membranes, and the smooth muscle cell population in the media is reduced. (Lower left) Basilar artery 7 months after SAH. Smooth muscle cells (SM) containing numerous lysosome-like dense bodies (asterik) are seen. (Lower right) Basilar artery, 24 months after SAH, has normal elastic lamina (EL) and smooth-muscle cell (SM). No abnormal finding is observed [reproduced with permission from Tanabe, Y., Sakata, K., Yamada, H., Ito, T., and Takada, M. (1978). Cerebral vasospasm and ultrastructural changes in cerebral arterial wall. An experimental study. *J. Neurosurg.* **49,** 229–238].

after chronic exposure to blood might reflect pathologic processes that act to maintain arterial narrowing. The endothelial barrier might be disrupted with exposure of the arterial smooth muscle to serum constituents instead of the usual ultrafiltrate of plasma. The complex metabolic interaction between endothelium and smooth muscle contraction might also be disrupted with impairment of usual secretion of potent vasodilators such as prostacyclin and adenosine (112).

Contractile responsiveness of small vessels to NE and $K^+$ was found in vessels removed from patients within 48 hr of SAH. These segments showed spontaneous rhythmic increases in tone. Such changes, however, were also found in arteries removed from non-SAH cases. The heightened agonist responsiveness was thought to result from exposure to agents that damage the blood vessel wall, resulting in partial depolarization of endothelial and smooth muscle cells. It should be noted that these were very small vessels and that this was not the time during which chronic VSP is present (161).

### 2. *Animal Studies*

Hemorrhage caused by puncture of the ICA in monkeys resulted in large, prolonged, and spontaneous increases in muscle tone when the vessels were studied *in vitro*. There was also a marked reduction in the ability of the vessel wall to contract, a reduction in constrictor and dilator nerve influences on vascular tone, and some increased sensitivity to 5-HT (156).

In a rabbit model of chronic VSP in which the basilar arteries were studied between 1 and 9 days, the initial phase of VSP was reversible by intraarterial papaverine. The second phase exhibited an increasing component of narrowing that was papaverine insensitive. Increasing basilar artery stiffness occurred over 9 days as the contractility decreased (162). Penetrating arterial

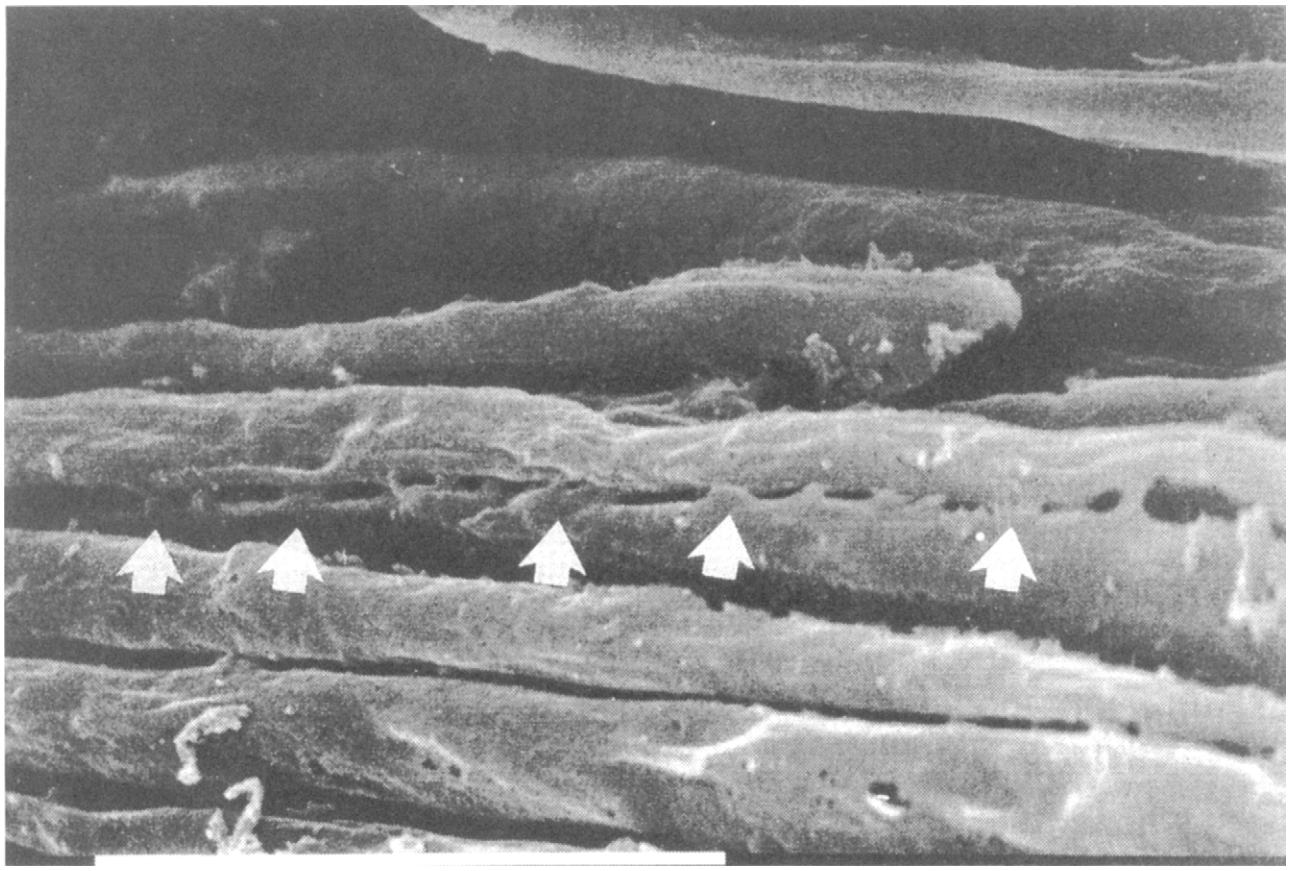


**FIGURE 4.26** Scanning electron photomicrograph of anterior cerebral artery from a monkey given intrathecal injections of oxyhemoglobin. There are multiple intercellular contacts visible between smooth muscle cells (arrows). Mild folding of the cell membrane is seen (scale bar = 10 μm) [reproduced with permission from Macdonald, R. L., Weir, B., Chen, M. and Grace, M. (1991). Scanning electron microscopy of normal and vasospastic monkey cerebrovascular smooth muscle cells. *Neurosurgery* **29,** 544–550].

microvessels from rabbits 3 days after SAH showed no significant differences in spontaneous tone or reactivity to a number of vasoactive stimuli, including $Ca^{2+}$, serotonin, and ACh compared to controls. In contrast, the basilar artery after SAH showed an attenuation of the relaxing effect of ACh following 5–HT-induced contraction and of ATP after KCl-induced basilar artery contraction (163). The basilar artery also showed increased contractions to the vasoconstrictor agonists.

### G. Blood-Brain Barrier

The concept of a BBB grew from the fact that certain dyes that stain systemic organs if given iv fail to stain the brain. It was also noted that certain dyes if injected intracisternally would stain the brain even though they failed to do so after iv administration. The barriers to transport are specific to given molecules. The barrier depends on morphological constraints, biochemical characteristics of the solute, and transport systems within choroidal epithelium and cerebrocapillary endothelium. The structural basis of the BBB is the modified type of endothelium of the blood vessels. The capillary endothelial cells have tight junctions and a very low permeability to hydrophilic nonelectrolytes on the end of specific membrane carrier molecules (50).

The physical basis of the BBB is the closely apposed margins of vascular endothelial cells. The BBB is not a single hurdle to all substances but varies with the size and nature of the compound. There is also a blood ventricular (CSF) barrier around the choroid plexus vessels that is formed by tight junctions of the choroidal epithelium. On the brain subarachnoid space surface, the interface between brain tissue and CSF is a fine avascular membrane overlying tightly packed layers of cytoplasmic processes of astrocytes. Early studies in which dyes were introduced under pressure into the CSF showed penetration of the leptomeninges and ependyma with staining of

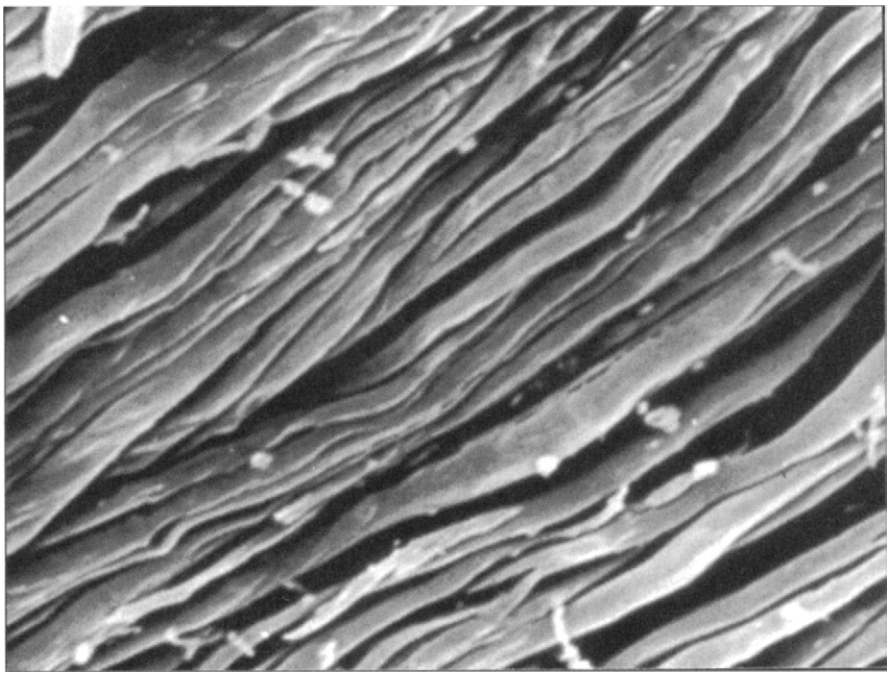


**FIGURE 4.27** Scanning electron micrographs of uncontracted monkey vascular smooth muscle cells after removal of adventitial connective tissue by hydrolysis with HCl.

the brain to a depth of several millimeters: These findings were probably spurious and resulted from toxic damage to the brain subarachnoid space barrier.

Studies of the BBB following hypoxia or ischemia have indicated that it becomes more permeable to large molecules such as radiographic contrast material and Evans blue dye (164). There may be a selective increase in the transport of small molecules, such as horseradish peroxidases, after short periods of ischemia that antedate the later general increase in permeability which indicates only advanced necrotic changes in blood vessels.

Substrates can penetrate the brain by dialysis, ultrafiltration, osmosis, Donnan's equilibrium, electric charges, lipid solubility, special tissue affinity, or metabolic activity. The movement of a substance into a brain cell ultimately requires penetration of the plasma membrane, which is a bimolecular lipid layer with an inner and outer absorbed protein layer. The equilibration of the brain with labeled $Na^+$, $K^+$, or $Cl^-$ injected into the plasma is slow. The movement of $CO_2$ across the BBB has a profound and complex effect on the acid-based balance since it moves much more rapidly than both $H^+$ and $HCO_3^-$. Any change in $P_aCO_2$ is rapidly reflected in the brain and CSF by a corresponding change in pH, whereas metabolic alterations in blood acid–base components will be reflected only slowly in the brain. Ventilatory responses to acid–base disturbances are controlled in part by the concentrations of $H^+$ and $HCO_3^-$ in the CSF which do not directly reflect levels in the blood.

### *1. Human Studies*

In a study of augmented CT scans in 80 patients after SAH, abnormal enhancement was seen in 26 cases in regions bordering the subarachnoid spaces. Such enhancement was associated with a deteriorated clinical condition, VSP, and poor outcome. It was suggested that the abnormal enhancement was parenchymal and resulted from a breakdown of the BBB (165). This can occur early after SAH. The pathological contrast is most obvious in the cortex and near the subarachnoid spaces but probably occurred throughout the brain (165–167).

Twenty-six patients with SAH were investigated with 68–Ga-EDTA and position emission tomography (PET) to evaluate the presence of a BBB disturbance. Only 1 patient showed such a disruption. A patient with abnormal PET examinations showed an accumulation of the 68-Ga-EDTA dye at the site of the aneurysm. The only patient with an apparent disruption of the BBB showed an increased accumulation in the parietal region supplied by one of the MCAs. No abnormal accumulation of tracer occurred in or close to the subarachnoid space in these SAH patients using this technique (168).

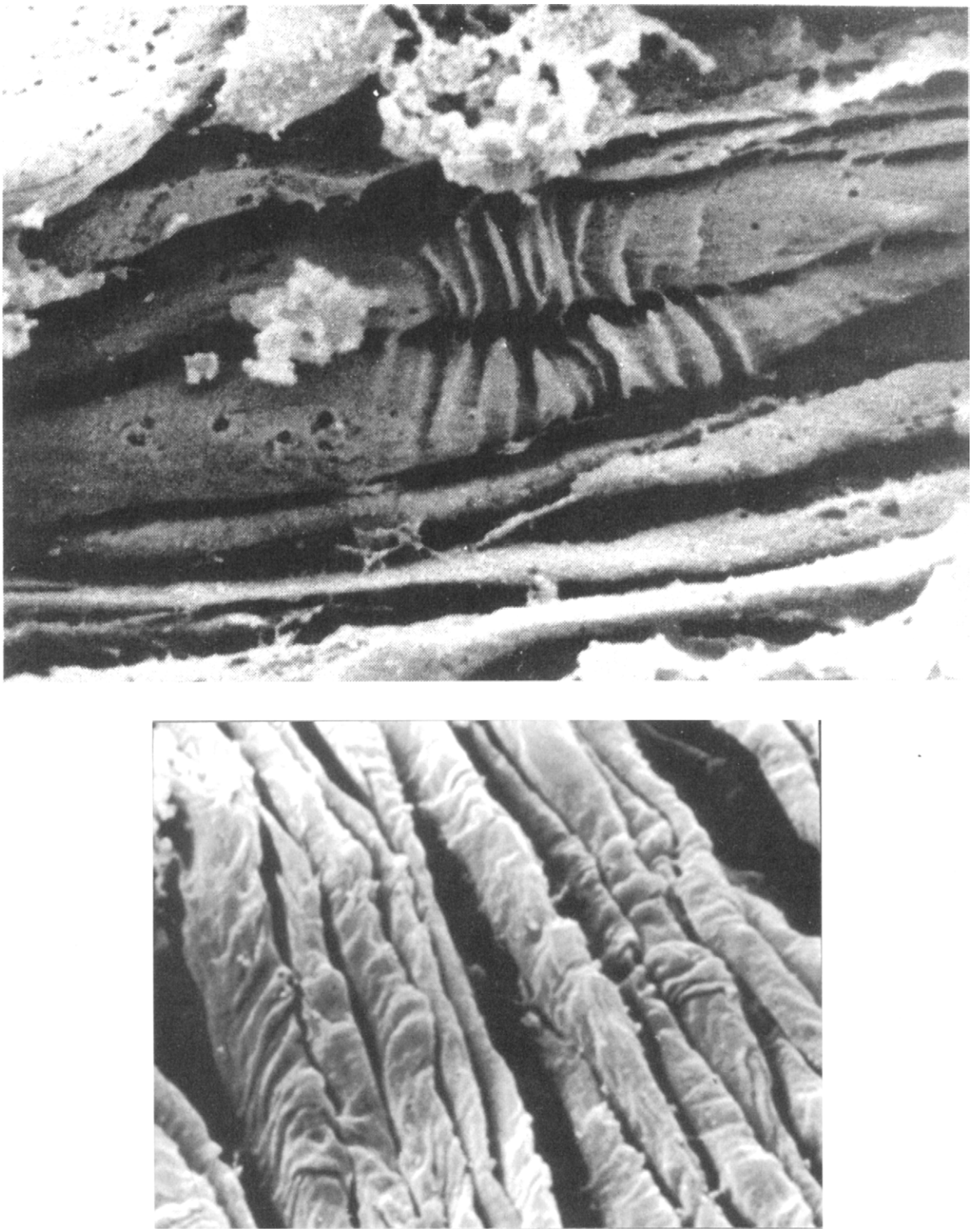


**FIGURE 4.28** (Top) Scanning electron micrograph of monkey basilar artery contracted *in vitro* with prostaglandin $F_{2\alpha}$. Note marked folding of the cell membranes. (Bottom) Following exposure to oxyhemoglobin cells become shorter and the membrane surfaces develop ridges or convolutions [reproduced with permission from Macdonald, R. L., Weir, B., Chen, M., and Grace, M. (1991). Scanning electron microscopy of normal and vasospastic monkey cerebrovascular smooth muscle cells. *Neurosurgery* **29,** 544–550].

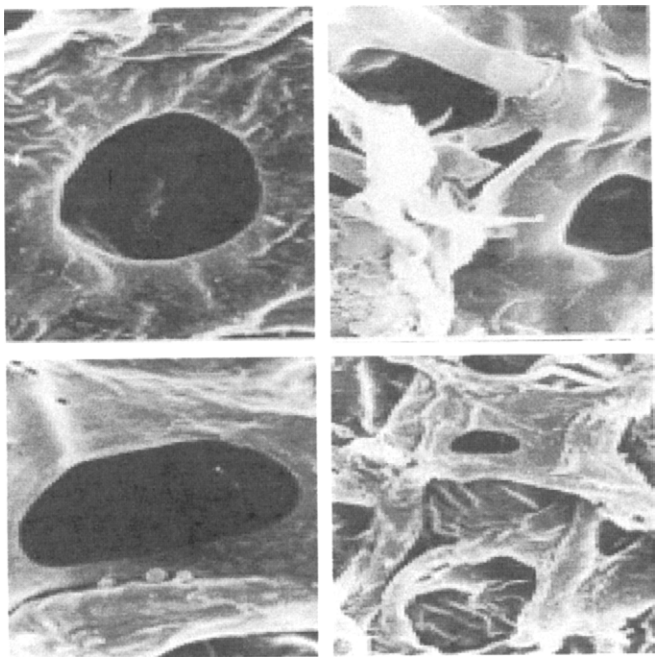


**FIGURE 4.29** Scanning electron micrograph shows apparent stomas on the surface of the adventitia.

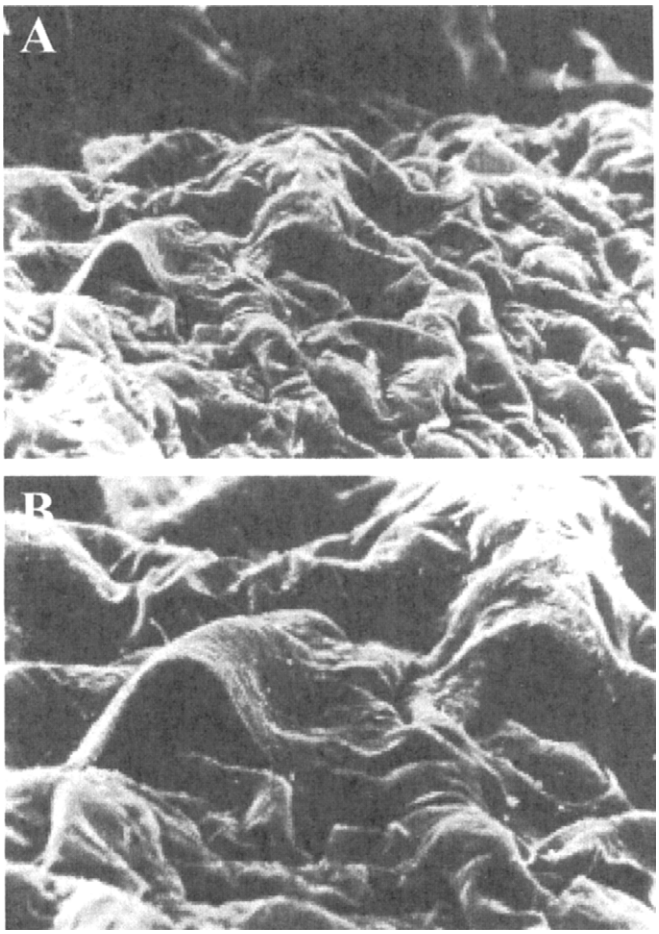



**FIGURE 4.30** Scanning electron micrographs at different magnifications showing paired stomas that have the appearance of tunnel-like structures on the surface of the adventitia [reproduced with permission from Espinosa, F., Weir, B., and Shnitka, T. (1986). Electron microscopy of simian cerebral arteries after subarachnoid hemorrhage and after the injection of horseradish peroxidase. *Neurosurgery* **19,** 935–945].

### 2. *Animal Studies*

SAH was induced in rats by injecting autologous blood into the cortical subarachnoid spaces. SAH alone caused significant extravasation of dye, whereas sham operation did not. Spontaneously hypertensive strains of rats showed a greater increase in Evans blue extravasation than did normal animals (169). Following cortical subarachnoid space blood injections, brain edema developed in the acute stage (170). In 20 rats having intracisternal injection of blood and 10 control animals it was found that intraarterial injection of dehydrocholate resulted in BBB disruption in both groups. The extent of disruption, however, was significantly greater in the control group than in the SAH group. The animals with the lowest pre-SAH mean arterial blood pressure (MABP) demonstrated the greatest resistance to experimental BBB disruption (171). SAH caused a six fold increase in Evans blue extravasation in a rat model. The capillary permeability to this tracer was normalized by pretreatment with a 21–aminosteroid (172). In a model, the novel antiischemic compound Bimoclomol, which is used against peripheral vascular complications of diabetes mellitus, was found to reduce the extravasation of Evans blue dye into the cerebral tissue of rats with SAH (173). The BBB changes were evaluated by means of the quantitative [$^{14}$C]α-aminoisobutyric technique. The animals were studied on the second day post-SAH. There was an increased passage of the tracer across the BBB into the brain (174).

In a series of cats subjected to SAH, Evans blue dye was injected intravenously. One hour post-SAH there were discrete spots of fluorescence relating to individual parenchymal vessels, appearing bilaterally in the cerebral cortex and to a lesser extent in the white matter. Four hours later the extravasations and the tissue staining became more widespread and confluent. Changes were predominant on the side of the punctured vessels, presumably the site of the most blood. In a different feline series of SAH, the permeability of the BBB to Evans blue protein appeared to be maintained by rendering the animals hypertensive by the intracarotid injection of mercuric chloride (175).

## V. Changes in Composition of Cerebrospinal Fluid, Blood, and Adjacent Tissues

### A. Cerebrospinal Fluid

The composition of cerebrospinal fluid is given in Table 4.5.

#### *1. Hemoglobin*

Hb is usually evident in the supernatant CSF within the first 4–10 hr after SAH. The pink color is usually maximal within the first 24–48 hr and then gradually fades. Bilirubin is detected later than Hb, first appearing 9–15 hr after bleeding, and it imparts a yellowish color. It is present for up to 2 weeks following SAH (50). Spectrophotometric investigations of CSF show that oxyHb is situated in the absorption curve with a distinct maximum at 415 nm, methHb has a corresponding peak at 406 nm, and the bilirubin curve shows a single broad absorption band with a maximum at 455 nm when the solution contains albumin (176).

Total Hb was determined in 85 CSF samples from aneurysm patients. No significant correlation could be observed between VSP and the amount of Hb. VSP was seen in 39% of those containing oxyHb and 64% of those containing oxyHb and methHb. The relatively high frequency of VSP in the presence of methHb was attributed to the larger amounts of Hb and blood clots in the subarachnoid space (177). In a similar study in 15 patients post-SAH the median Hb concentration was 19.5 mg%. Increased Hb concentrations could be found up to 26 days after the initial SAH. Two patients reached normal Hb values rapidly, 1 within 3 days and the other within 6 days (178). Eighty-one specimens from 31 cases with SAH and intraventricular hemorrhage (IVH) showed Hb in the CSF beginning about 2 hr post-SAH and starting to decrease in the second week. Methb was not

**TABLE 4.5 Composition of Cerebrospinal Fluid and Serum**[a]

| | Cerebrospinal fluid | Serum |
|---|---|---|
| Osmolarity (mOsm/liter) | 295 | 295 |
| Water content (%) | 99 | 93 |
| Sodium (mEq/liter) | 138.0 | 138.0 |
| Potassium (mEq/liter) | 2.8 | 4.5 |
| Calcium (mEq/liter) | 2.1 | 4.8 |
| Magnesium (mEq/liter) | 2.3 | 1.7 |
| Chloride (mEq/liter) | 119.0 | 102.0 |
| Bicarbonate (mEq/liter) | 22.0 | 24.0[b] |
| $CO_2$ tension (mmHg) | 47.0 | 41.0[b] |
| pH | 7.33 | 7.41[b] |
| Oxygen (mmHg) | 43.0 | 104.0[b] |
| Glucose (mg/dl) | 60.0 | 90.0 |
| Lactate (mEq/liter) | 1.6 | 1.0[b] |
| Pyruvate (mEq/liter) | 0.08 | 0.11[b] |
| Lactate:pyruvate ratio | 26.0 | 17.6[b] |
| Fructose (mg/dl) | 4.0 | 2.0 |
| Polylols (nmol/dl) | 3.4 | 1.5 |
| Myoinositol (mg/dl) | 2.6 | 1.0 |
| Total protein (mg/dl) | 35.0 | 7.0 |
| Prealbumin (%) | 4 | Trace |
| Albumin (%) | 65 | 60 |
| $Alpha_1$, globulin (%) | 4 | 5 |
| $Alpha_2$, globulin (%) | 8 | 9 |
| Beta globulin ($beta_1$+ tau) (%) | 12 | 12 |
| Gamma globulin (%) | 7 | 14 |
| IgG (mg/dl) | 1.2 | 987 |
| IgA (mg/dl) | 0.2 | 175 |
| IgM (mg/dl) | 0.06 | 70 |
| Kappa:lambda ratio | 1.0 | 1.0 |
| Beta-trace protein (mg/dl) | 2.0 | >0.4 |
| Fibronectin | 3.0 μg/ml | 300 g/ml |
| Total free amino acids (mol/dl) | 80.9 | 228.0 |
| Ammonia glutamine (g/dl) | 24.0 | 37.0[b] |
| Urea (mmol/dl) | 4.7 | 5.4 |
| Creatinine (mg/dl) | 1.2 | 1.8 |
| Uric acid (mg/dl) | 0.25 | 5.50 |
| Putrescine (pmol/ml) | 184.0 | |
| Spermidine (pmol/ml) | 150.0 | |
| Total lipids (mg/dl) | 1.5 | 750.0 |
| Free cholesterol (mg/dl) | 0.4 | 180.0 |
| Cholesterol esters (mg/dl) | 0.3 | 126 |
| cAMP (nmol/liter) | 20.0 | |
| cGMP (nmol/liter) | 0.68 | |
| Homovanillic acid (g/ml) | 60.0 | |
| 5-Hydroxyindoleacetid acid (g/ml) | 0.04 | |
| Norepinephrine (pg/ml) | 200 | 350.0 |
| 3-Methoxy-4-hydroxyphenylglycol (mg/ml) | 15.0 | |
| Acetylcholine (mg/dl) | 1.8 | |
| Choline (nmol/ml) | 2.5 | |
| $PGF_{2\alpha}$ (pg/ml) | 92.0 | |
| Insulin (mol/ml) | 3.7 | 36.0 |
| Gastrin (pmol/liter) | 3.4 | |
| Cholecystokinin (pmol/liter) | 14.0 | |
| Beta endorphin (pmol/liter) | 145.0 | 10.0 |
| Phosphorus (mg/dl) | 1.6 | 4.0 |
| Iron | 1.5 g/dl | 15.0 mg/dl |

[a] Major brain metabolite of norepinephrine. Reproduced with permission from Fishman, R. A. (1992). In *Cerebrospinal Fluid in Diseases of the Nervous System*, 2nd ed., Table 8–1, Saunders, Philadelphia.

[b] Arterial blood.

found after SAH but was found in subdural hematoma fluid (179).

Within 1 week post-SAH, images from T1-weighted magnetic resonance imaging (MRI) start to show blood products. It has been suggested that metHb formation with T1 signal shortening partly accounts for the increasing intensity of the CSF (180).

### 2. *Pigments*

Xanthochromia is a descriptive concept and refers to a yellowish or pinkish discoloration of the spinal fluid which can be present in (i) jaundice in which total bilirubin levels exceed 10–15 mg/dl, (ii) protein concentrations in CSF greater than 150 mg/dl, (iii) hypercarotenemia, and (iv) rifampin intake (181). It is present within 4 hr after onset of SAH and increases for a few days. It begins to decrease after 6–10 days (46,182). The Van den Berg reaction is negative in fluids made xanthochromic *in vitro*, whereas it is positive in the CSF of patients. The fact that bilirubin is produced from hemolyzed RBCs is strong evidence that the destruction of the RBCs takes place within the lining of the subarachnoid space or within clot. CSF is normally as clear as water. At least 1 ml of CSF is required to be spun down to make a judgment that the supernatant is clear. Supernatant fluid may remain clear for up to 12 hr after SAH despite the presence of RBCs in the CSF (183).

Serial studies of xanthochromia were performed on CSF from 15 patients post-SAH without evidence of rebleeding. The index rose in some patients up until the $17^{th}$ day. It was concluded that because the proportion of oxyHb or the absolute concentration of Hb fluctuated, the diagnosis of rebleeding based on CSF examination could only be made by observing an increase in xanthochromia but not Hb, if previous samples had shown a decrease. Five of 6 patients with CT-demonstrated rebleeding showed increased xanthochromia as evidence of rebleeding (184). The conversion of reduced Hb to methHb is possible *in vitro* and *in vivo*. However, the conversion of heme compounds to bile pigments requires specific intracellular conditions of temperature, concentration, and pH and the presence of both $O_2$ and an appropriate reducing system (179). Bilirubin begins to appear 3 or 4 days post-SAH and increases in the second week. In *in vitro* experiments the xanthochromia which is due to Hb and not bilirubin begins about 4 hr postincubation (64).

### 3. *Proteins, Peptides, and Amino Acids*

The CSF contains some proteins but concentration is very much less than that in plasma; CSF can be regarded as essentially a protein-free fluid in physiological circumstances. Various authorities give a range for an upper limit of lumbar human CSF protein of between 25 and 59 mg/100 ml. Protein concentration in the lumbar CSF is higher than cisternal or ventricular CSF concentration (185). The proportion of prealbumin decreases despite the fact that the total concentration of protein rises on going from the ventricles to the lumbar sac. The normal CSF proteins include orosomomucoid, $\alpha_1$-antichymotrypsin, haptoglobin, ceruloplasmin, $\alpha_1$-antitrypsin, $\beta$-trace protein, $\alpha_2$-macroglobulin, prealbumin, albumin, $\gamma$-globulin, IgA, transferrin, hemopexin, C′3-complement, IgG, and $\gamma$-trace protein (186). Protein concentrations in CSF tend to increase with age. In seven series the percentages of protein fractions ranged as follows: total protein, 21–40 mg/100 ml; prealbumins, 4.3–6.8 %; albumin, 54.5–63.7%; $\alpha_1$-globulin, 3.4–6.1%; $\alpha_2$-globulin, 4.9–6.7%; $\beta$-globulin, 8.5–9.7%; $\tau$-globulin, 5.3–6.1%; and $\gamma$-globulin, 8.3–11.6% (186). In one group of 10 patients with SAH, prealbumin was significantly lower than that in other conditions or controls (187). In another study of SAH, the $\beta_2$-microglobulin level was elevated (188). Oligoclonal Ig bands were found in the serum and CSF of 16% of 83 patients with SAH. These were more common in SAH than cerebral ischemia. In serum, the bands were less common than in the CSF of these SAH patients (189).

The concentrations of amino acids in CSF of normal subjects from four series showed that those present in greatest concentrations are threonine, alanine, serine, and arginine. In contrast, in plasma the most common acids are alanine, glycine, valine, proline, and lysine (186). The only amino acid with a CSF concentration approaching or exceeding that of its plasma concentration is glutamine (186).

In 247 CSF samples from cases with cerebral hemorrhage of all types, only 34 had less than 45 mg/dl of protein, and 140 had greater than 100 mg/dl. The average was 270 mg/dl. Normal concentrations of proteins in CSF (mg/liter) were as follows: albumin, 155; prealbumin, 17.3; transferrin, 14.4; ceruloplasmin, 12.3; $\alpha_2$-macroglobulin, 2; IgA, 1.3; and IgG, fibrinogen, IgM, and $\beta$-lipoprotein, $< 1$ (190). In 254 patients diagnosed with intracranial bleeding, the CSF total protein was associated with death if concentrations exceeded 3000 mg/100 ml (191).

The levels of the two calcium-binding proteins, S-100 and calmodulin, which are highly concentrated in the CSF, were elevated in the earliest postoperative CSF samples in SAH patients. Patients in poorer grades showed higher levels of S-100. The prognosis correlated with the S-100 protein levels. There was a lack of correlation between the calmodulin levels and the preoperative grade or outcome. S-100 is considered to be a nervous system-specific protein, localized in the glia and Schwann cells, and is utilized as an immunohistochemical marker for brain tumors. Calmodulin is an activator of cyclic

nucleotide phophodiesterase. It appears to be a chief mediator of $Ca^{2+}$ effects on various cellular processes (192).

Astroprotein is immunologically identical to glial fibrillary acidic protein. Levels in CSF are markedly increased in acute cases of ICH and slightly to moderately increased in acute cases of SAH and cerebral infarction. Levels relate to the general neurological state of patients (193).

The intracellular neuronal protein MAP-tau was measured in the CSF of 12 SAH patients. Levels were elevated about 10,000-fold compared to controls (mean = 56 ng/ml). The patient's current clinical condition was highly correlated with levels in 82 study patients. Patients without VSP showed a steady decline in this protein during hospitalization. VSP patients showed initial levels of 66 ng/ml, during VSP they showed levels of 198 ng/ml, and post-VSP they showed levels of 24 ng/ml (194).

CSF and plasma levels of the 52-amino acid peptide adrenomedullin were measured in 13 SAH patients. Levels significantly increased in the CSF of 6 patients with transient ischemia symptoms and in 3 cases with permanent ischemic deficits. Peak values for all of the permanent deficit group were >100 fmol/ml. Patients with ischemic symptoms had lower plasma levels than did patients without (195).

#### *4. Complement and Inflammatory Factors*

The complement pathway is a nonspecific immune defense mechanism. Anaphylatoxins $C3_a$ and $C4_a$ increase vascular permeability and exacerbate inflammation. CSF from 29 SAH patients was examined. The $C3_a$ normal level in CSF was 165 ng/ml. In severe VSP cases the level was 2500 ng/ml on day 1 dropping to 750 ng/ml by day 7. For cases with mild VSP the day 1 level was 1000 ng/ml, again falling to about 700 ng/ml by day 7. CSF $C4_a$ normal level was 166 ng/ml. In severe VSP, it was 1400 ng/ml on day 1, falling to 500 ng/ml on day 7. For milder cases it was 1100 ng/ml on day 1 and 490 ng/ml on day 7. The CSF SC5b-9 (membrane attack complex) was found to have a normal value of 0.19 ng/ml. The day 1 level in severe VSP cases was 3700 ng/ml, decreasing to 500 ng/ml by day 7. For milder cases it was 1100 ng/ml on day 1 and 300 ng/ml on day 7. It was hypothesized that the plasma protein cascades initiated by SAH might include complement activation (196). In another study, CSF C3a and C4a levels in the first 2 days post-SAH were significantly higher in patients with delayed ischemic deficit (DID) than in those who did not subsequently develop this complication of VSP (197).

A mixture of CSF and blood was incubated at body temperature for 7 days. $C1_q$-binding immunoglobulin was detected. This protein is considered to activate the complement system with the production of such vasoconstrictors as $C3_a$ and $C5_a$ (198). The central nervous system (CNS) is relatively isolated from circulating immune factors such as complement. In 15 patients following SAH, the terminal complement complex (TCC) concentration on days 0–2 was higher in CSF (210 ng/ml) than in plasma (63 ng/ml), but none was present in the CSF of controls or patients with ischemic strokes. By the 7 to 10-day interval TCC was absent from the CSF. Complement activation may initiate inflammation and aggravate brain damage (199).

#### *5. Platelet-Activating Factor*

Platelet-activating factor is a lipid autocoid that was originally recognized as a chemical mediator released from the antigen-stimulated basophils. It is produced by a variety of inflammatory cells, platelets, and vascular endothelium following chemical or immunological stimulation. Platelet-activating factor has a wide spectrum of activities, including stimulating platelets and WBCs, enhancing vascular permeability and causing vasoconstriction. Twenty-one patients post-SAH had serial samples of CSF obtained from continuous ventricular or systemic drainage. Six patients had cerebral infarction and 15 did not. The platelet-activating factor concentrations between days 5 and 9 were greater in patients with cerebral infarction from VSP than in those without. The concentration is regulated by platelet-activating factor acetylhydrolase, an enzyme normally present in serum. The activity of this enzyme gradually decreased following SAH and then increased as a mirror image to the platelet-activating factor concentration. The enzymatic activity was less in the patients with cerebral infarction than in those without. The activity of this enzyme increased in a temperature-dependent manner (200).

#### *6. Neopterin*

Neopterin is a substance produced by macrophages in response to the synthesis by T cells of γ-interferon. CSF samples from 14 patients post-SAH showed large variations in levels. The mean control CSF neopterin level was 1.3 n*M*. In the SAH group the new CSF neopterin values were higher in patients with clinical symptoms of DID compared to patients without such deficits (21.9 vs 9.3 n*M*). No strong correlation was found between levels and clinical outcome, ischemia, or detectable blood on the CT scan (201).

#### *7. Bradykinin*

Bradykinin CSF concentrations in 21 patients post-SAH showed values of 135 pg/ml on day 0, 38 pg/ml on day 1, 23 pg/ml on day 2,17 pg/ml on day 3, 16 pg/ml on

day 4, and 15 pg/ml on day 5. The control CSF showed concentrations averaging 8 pg/ml. Patients with IVH did not show high levels of bradykinin (BK). It is thought that activation of the Hageman factor would require contact with collagen bundles in the trabeculae of the subarachnoid space, which would be the case in SAH but not IVH (202). In 25 patients, BK levels were examined within 3 days post-SAH and the concentrations on the days 0–1, days 2–4, days 5–7 periods and later were 122.7, 38.6, 22.7, and 17.1 pg/ml. Plasma BK levels post-SAH were higher than in the control groups but showed no change over time (203).

### *8. Interleukin*

Interleukins (IL) are immunomodulators that regulate cellular events within the immune system. Localized brain immune responses lead to increased levels of the immunomodulator "cytokines." Serum and CSF samples of 12 patients were analyzed for IL-6, soluble IL-2R, and soluble CD8 levels to establish whether immunactivation occurred following SAH. The levels of IL-6 dramatically increased and soluble IL-R moderately increased in the CSF in 11 of 12 patients. Slightly elevated levels of soluble CD8 were seen in 6 patients. The IL-6 levels peaked at day 6. The increases in IL-6, soluble IL-2R, and soluble CD8 levels in CSF were not paralleled by increased values in serum, so it was assumed that they were being synthesized in the subarachnoid space. Also, transfer of IL-6 via the BBB was unlikely since there was a negative correlation between serum and CSF levels. These findings were interpreted as reflecting a severe inflammatory effect on the CNS (204).

Cisteinyl-leukotrienes were elevated in CSF shortly after ictus. Levels were significantly higher in patients who subsequently developed VSP assessed by transcranial Doppler ultrasonography (TCD). Normalization of Doppler values was accompanied by decreasing levels of CSF cisteinyl-leukotrienes (205).

IL-IRa mean levels were significantly higher in poor-grade SAH patients on admission than in good-grade ones (318 vs 82 pg/ml). Levels increased during episodes of DID and postoperatively. Levels of this interleukin and TNF-α increased significantly on days 4–10 in patients who ultimately had a poor outcome. Both of these compounds are known to induce fever, malaise, leukocytosis, and NO synthesis and to mediate ischemic brain injury (206).

### *9. Adhesion Molecules*

Adhesion molecules are involved in WBC adherence to the endothelium and subsequent diapedesis and migration into SAH clot. CSF from 17 patients post-SAH was compared to that of 16 controls. Concentrations of soluble adhesion molecules which facilitate the passage of WBCs across the vascular endothelium were assessed. Levels of soluble forms of E-selectin, ICAM-1, and VCAM-1 were all elevated in CSF of patients post-SAH compared to controls. After the initial ictus, levels fell over time. The levels of E-selectin were highest in patients who later developed VSP (207).

### *10. Enzymes*

#### *Creatine Phosphokinase*

Creatine phosphokinase (CPK) in CSF from 30 patients post-SAH showed increases but no diagnostic specificity. The increases seemed to be proportional to the degree of cerebral destruction (infarction, ICH, and IVH). VSP without infarction did not increase the levels of CPK (208). A large number of samples of CSF from patients with neurologic disease showed lactose dehydrogenase levels of 8.4 IU/liter. There was no relationship between enzyme levels in CSF and its protein or cell content. CPK levels in normal CSF were 0–2.1 ng/ml, whereas in patients with acute cerebrovascular accidents the levels were 6.5–14.0 ng/ml. The presence of CPK in the CSF was not specific for any disease. In general, there were raised levels after any kind of acute stroke. In bacterial meningitis lactate and glutathione levels are increased. Enzyme levels in the CSF of controls were as follows: enolase, 0.72; aldolase, 0.37; pyruvate kinase, 1.47; LDH, 7.90; and CPK, 1.9 IU/liter (186). CPK levels were measured in 35 CSF specimens from 30 patients post-SAH. Levels of the enzyme were increased where there had been brain destruction such as Hyc, infarction, or parenchymal hemorrhage. VSP without infarction did not raise CSF CPK levels (208). Levels of LDH, CPK, and of CPK isoenzymes were examined in 148 CSF samples. There was a tendency for increased enzyme levels in patients with ICH. There was no correlation between total CPK activity in the serum and the CSF (209).

#### *Phospholipase*

Phosphoinositide-specific phospholipase activities in CSF post-SAH were significantly higher than control CSF. The preoperative clinical grade correlated with the PLC activity in the CSF on day 3. Phospholipase (PLC) activity was closely correlated with the activity of neuron-specific enolase, which reflected the degree of brain damage. PLC enzymes were subclassified as PLC-β, PLC-δ, and PLC-ϐ. It was hypothesized that the enzymes were released into the CSF by brain tissue damaged at the ictus and that the degree of activity reflected the extent of brain damage (210).

*Superoxide Dismutase*

In the cisterna magna levels of superoxide dismutase (SOD) were obtained in aneurysm cases. Control levels were 12.99 U/ml, which were higher than that in 26 patients operated days 1 and 3 post-SAH (4.44 U/ml) and that in 40 patients treated by delayed surgery (7.64 U/ml). In 13 patients presenting with DID from VSP, SOD levels were 12.23 compared to 5.43 U/ml in 27 cases without VSP. The SOD levels are thought to decrease post-SAH because of impaired synthesis. Subsequent elevated SOD levels might reflect cerebral ischemia (211).

*Catalase and Glutathione*

Lipid peroxides increased in CSF during the first 4 days post-SAH in 25 patients treated by surgery within 72 hr post-SAH. Those with symptomatic VSP had a greater increase in peroxides than those that did not have it. Symptomatic VSP was associated with a marked decrease in SOD activities on days 3 and 4 followed by a gradual increase, whereas patients without VSP showed little change. Catalase, on the other hand, increased on days 3 and 4 in patients who developed symptomatic VSP and gradually increased thereafter. The gluthathione peroxidase levels did not change (212).

*Heme Oxygenase*

In 23 cases of ruptured aneurysms the CSF levels of heme oxygenase in the CSF increased (213).

### *11. Glucose*

There is normally a gradient between higher ventricular glucose levels and lumbar sac CSF glucose levels (116 to 88) (186). SAH is sometimes associated with a decrease in CSF glucose presumably since it is metabolized by RBCs. Merritt and Fremont-Smith found that 7 of 199 cases of SAH had CSF glucose levels of under 30 mg/dl (46). The lowest glucose levels usually occur about 4–8 days post-SAH (214). The lumbar CSF glucose concentration is normally 60–70% of blood glucose concentration. Of 33 patients with nontraumatic SAH, 70% developed CSF glucose levels of less than 40 mg/100 ml and a CSF–blood glucose ratio of less than 0.5. The patients with the lowest glucose levels and lowest CSF–blood glucose ratio (27 mg/100 ml and 0.21, respectively) had their initial lumbar punctures preformed on average 5.4 days post-SAH when the RBCs were 182,000/ml. Patients with normal initial findings (85 mg/100 ml and 0.61) had their initial lumbar punctures preformed 1.2 days post-SAH when the RBCs were 480,000/ml. In the literature reviewed by Vincent, 11 of 71 patients had CSF glucose levels less than 40 mg/100 ml and findings that suggested a nadir in glucose about 1 week post-SAH (215).

### *12. Electrolytes*

*Sodium*

Concentrations of various solutes in plasma and (CSF) in normals are as follows: $K^+$, 4.63 (2.86); Mg, 1.61 (2.23); $Ca^{2+}$, 4.70 (2.28); $HCO_3$, 26.8 (23.3); osmolality, 289 (289); and $pCO_2$ (mmHg), 41.1 (50.5) (186). In primates the $K^+$ concentration is considerably higher in plasma than in lumbar CSF, which in turn is higher than the cortical concentration. $Ca^{2+}$ also has a higher plasma concentration than CSF, which is just slightly less than the cortical concentration. $Mg^{2+}$, on the other hand, has a slightly lower plasma concentration than CSF and the cortical concentration is higher than CSF (186). CSF and serum have identical osmolarities and sodium content in under normal circumstances, (Table 4.1). In 24 patients with SAH, $Na^+$ levels in CSF averaged 142 mEq/liter compared to plasma at 138 mEq/liter (190).

*Potassium*

In a series of 24 patients having repeated CSF electrolyte sampling, the most consistent change was a decrease in $K^+$ concentration over the first 5–10 days post-SAH. The lowest values ranged from 2.6 to 1.8 mEq/liter which represented an almost 40% decrease in the concentration of $K^+$. Patients who were drowsy or confused had lower $K^+$ levels than patients who were fully alert. No patient was fully conscious or alert following SAH with a $K^+$ value less than 2.4 mEq/liter. In instances of ICH or IVH, the relationship between $K^+$ and consciousness was absent. The decrease in CSF $K^+$ could be due to (i) impairment of the blood–CSF barrier in the presence of a decreasing plasma $K^+$ level, (ii) an increase in blood pH or its decrease in CSF, (iii) impairment in the mechanism for controlling CSF $K^+$ resulting from pial or ependymal damage, or (iv) an increase in sugar metabolism by RBCs in the subarachnoid space. CSF $Na^+$ and $Cl^-$ changes only occurred in association with variations in plasma concentrations and did not occur independently of them; however, the relative stability of CSF $Na^+$ and $Cl^-$ was reduced after SAH although not after ischemic stroke (190).

Presumably there is an efficient removal mechanism for $K^+$ since, despite the fact that the interior of RBCs contains high concentrations, the CSF level post-SAH has been observed to decrease from 2.7–3.9 to 2.1 mEq/liter at 5–10 days. If cerebral infarction occurs, the CSF $K^+$ can increase to 4 or 5 mEq/liter and this increases rapidly following death (190).

*Magnesium*

In 14 cases post-SAH the serum $Mg^{2+}$ was observed to decrease and the CSF $Ca^{2+}$ level was found to increase

from days 3 to 9 (216). In 15 SAH patients, 8 developed VSP. They had a slight but significant decrease in CSF $Mg^{2+}$ between days 6 and 8, but no changes were noted in serum $Mg^{2+}$ or $Ca^{2+}$ (217).

### *13. Gases*

#### $pO_2$

The $pO_2$ of the CSF is less than that of blood. Lumbar CSF $pO_2$ averaged $43 \pm 10.8$ mmHg compared to normal arterial $pO_2$. Jugular venous blood oxygen tension was usually greater than CSF oxygen tension. There were no significant differences between lumbar and cisternal fluid $pO_2$, although it has been reported that cisternal $pO_2$ is 6 mmHg greater than lumbar CSF $pO_2$ (218). In ventilated rabbits subject to hypoxia the CSF $pO_2$ was usually higher than blood $P_aCO_2$. This difference was attributed to the Bohr effect that increases the $pO_2$ in the blood of the choroid plexus capillaries as a result of its acidification (219). Measurements of $P_aO_2$ and CSF $pO_2$ during operation and for several days thereafter showed a consistently lower CSF $pO_2$ than $P_aCO_2$. The difference was in the 50–100 mmHg range (220). In patients with a decreased level of consciousness, VSP, and markedly bloody CSF, the CSF $pO_2$ was significantly decreased and did not return to normal even with normalization of the $P_aO_2$. CSF $pO_2$ varied between 44.1 mmHg in alert patients to 32.5 mmHg in comatose ones following aneurysmal rupture (221). CSF $pO_2/P_aO_2$ was 0.50 in controls, 0.50 in SAH cases without VSP, and 0.27 in cases with VSP (222). Normal means of brain cerebral interstitial gas values in humans measured at operation and postoperatively were as follows; $pO_2$, 33 mmHg; $pCO_2$, 48 mmHg; pH, 7.19 (223).

#### *pH, $pCO_2$, and Lactate*

Normal values for pH in plasma range from 7.422 to 7.397, CSF 7.311–7.439; $pCO_2$ plasma 35.4–41.1 mmHg, CSF 45.0–50.5 mmHg; $HCO_3$ plasma 22.6–26.3 mmHg, CSF 22.5–24.8 mmHg (186). Values for arterial blood, cisternal CSF, and lumbar CSF are as follows: pH, 7.397, 7.346, and 7.325; $P_aCO_2$, 40.5, 46.5, and 49.1 mmHg; and $HCO_3$ 24.3, 24.7, and 24.9 mmHg. CSF has a higher $pCO_2$ and a lower pH than blood serum. Lumbar CSF is more acidic and has a higher $P_aCO_2$ and a slightly lower $HCO_3$ than either arterial blood or cisternal CSF (224). The CSF $pO_2$ changes quickly in response to hypoxemia. Reducing cerebral perfusion pressure reduces both brain tissue and CSF $pO_2$, but in the reperfusion state after complete ischemia the CSF $pO_2$ can be restored to normal while the brain tissue $pO_2$ may remain low or progressively decrease (225). When brain surface pH was directly measured in dogs subjected to respiratory arrest, the changes in cisternal CSF pH grossly underestimated the surface acidosis. CSF analysis may therefore provide unreliable information about the severity of brain–acid base changes. During respiratory arrest the changes in the venous blood acid–base variables are a better indicator of the severity of the metabolic aberrations than the cisternal CSF (226).

In a case of traumatic SAH, arterial blood was at a consistently higher pH than CSF. Simultaneously, the $pCO_2$ of arterial blood was lower than the CSF $pCO_2$. $HCO_3^-$ was higher in blood than in CSF. The patient was not on a respirator and was consistently hyperventilating. The hydrogen ion ($H^+$) concentration of CSF is increased and the bicarbonate concentration is reduced in the presence of SAH. The decrease in pH is associated with increased lactate concentration. This is presumed to derive from glycolysis in the shed RBCs. The mean values for normal and SAH patients, respectively, were as follows: CSF pH; 7.326 and 7.239; arterial pH, 7.409 and 7.469; CSF bicarbonate, 25.1 and 16.7 mEq/liter; arterial bicarbonate, 24.8 and 24.1 mEq/liter. The $HCO_3^-$ concentration, pH, and $pCO_2$ of hemorrhagic CSF were all slightly reduced. The lactate concentration was increased almost eightfold. Acid–base imbalance in CSF persisted for almost 6 days after a single SAH. Lactic acid generation is considered the cause of the CSF acidosis, glucose is almost quantitatively converted to lactic acid by the RBC enzyme systems.

Nineteen patients studied within the first 24 hr after SAH infarction showed a higher lactate level in the SAH group compared to ischemic strokes (4.88 vs 2.78 μmol/ml) (227). Seven patients with ruptured aneurysms showed both a blood and CSF acidosis, whereas in unconscious patients pH was in the 7.2 range, but with neurologic recovery plasma pH increased to 7.4 and CSF pH to 7.3 (220).

One hundred and two patients with acute ischemic strokes were also noted to have increased lactate and pyruvate concentrations in CSF for the first 12 days. Corresponding decreased $HCO_3^-$ levels occurred in CSF. Respiratory alkalosis and hypoxemia were common. Jugular venous $O_2$ levels were frequently above normal (228). In an effort to treat CSF acidosis six patients post-SAH had intracarotid injection of 7% sodium bicarbonate solution. The treatment was believed to result in improvement in level of consciousness. In two patients angiograms performed on patients with known VSP showed no dilation 15–30 min after the intracarotid $HCO_3^-$ injection (229).

Twenty-four patients post-SAH were divided according to the pH of their hemorrhagic CSF. Of those whose lowest pH was greater than 7.30, 5 of 10 remained fully conscious. Of those with pH level below 7.30, only 1 of 14 remained conscious. The group with higher CSF pH had

lower lactic acid (3.97 vs 5.47 mmol/liter) than the more acidotic, poor-grade patients. Glucose was higher in the good-grade patients with more normal CSF pH (57 vs 50.2 mg%). No correlation was found between single lumbar CSF hematocrit levels and CSF pH. CSF pH was considered to be a useful indicator of the neurologic condition: The more acidic it was the more likely the patient was to be comatose. $HCO_3^-$ less than 20 mEq/liter, or CSF lactic acid concentration greater than 5 mmol/liter during the first 48 hr post-SAH, was associated with an adverse prognosis particularly if the CSF pH was less than 7.30 at any time post-SAH. Falling CSF pH was attributed to (i) change in blood acid–base values, (ii) release of acid metabolites from infarcted cerebral tissue, and (iii) an increase in CSF lactic acid concentration. Increased lactic acid in CSF was attributed to increased production from glucose metabolism of the RBCs in the CSF and systemic hyperventilation. Increased CSF lactic acid concentrations were not always accompanied by significant increases in ventilation. Mortality was 79% in those patients with a pH less than 7.30 compared with only 20% in those who did not have a decrease in CSF (190).

In 52 patients with ruptured aneurysms the CSF lactate levels were as follows: clear CSF, 2.32 mmol/liter; xanthochromic CSF, 2.66 mmol/liter; and hemorrhagic CSF, 2.77 mmol/liter. In 16 patients with no VSP and an average ICP of 11.8 mmHg, the lactate acid level was 2.23 mmol/liter. In 19 patients with VSP and a higher ICP (24.55 mmHg) the lactate levels were increased to 2.85 mmol/liter. Patients with lactate levels greater than 3.5 mmol/liter were considered to have a poor prognosis (230).

Monitoring of CSF lactate concentrations and lactate:pyruvate ratios was performed daily in 20 patients from days 1 to 12 post-SAH. All patients tended to have a high CSF lactate concentration on day 1. Patients who never developed symptomatic VSP had a progressive decrease in lactate concentration and a normalization of the lactate:-pyruvate ratio. Patients who developed symptomatic VSP showed an initial decrease in lactate but a secondary increase on days 5–7 which correlated well with the onset of VSP. The delayed increase in CSF lactate of this group was accompanied by an increase in the CSF pyruvate level and the CSF lactate:pyruvate ratio (231).

### *14. Biogenic Amines*

#### *5-HT*

Several neurotransmitters are known as biogenic amines. These are derived from amino acids by several modifications. The aromatic amino acid tyrosine is converted in the hypothalamus and the adrenal medulla to dopamine, in peripheral nerve endings to NE, and in brain to melanin and pigment. Tryptophan is converted in the brain to 5–HT and in the pineal gland to melatonin. The major catecholamines are dopamine, NE, and Epi, which are derived from tyrosine synthesized by chromafin cells in the CNS and the adrenal. Tyrosine hydroxylase converts tyrosine to DOPA, DOPA decarboxylase converts DOPA to dopamine, and dopamine hydroxylase converts dopamine to NE. Catecholamines have half-lives between 15 and 30 sec and are rapidly metabolized. Catechol-*O*-methyltransferase, a cytosolic enzyme and monoamine oxidase, a mitochondrial enzyme, catalyze the oxidative deamination of monoamines. The end product of dopamine degradation is homovanilic acid (HVA), whereas that of NE and Epi degradation is vanillylmandelic acid. 5-HT is found in brain cells, platelets, and elsewhere. It is sythesized and stored in the brain. 5-HT synthesis involves the hydroxylation of tryptophan followed by decarboxylation. In addition to being a regulator of sleep, temperature, and blood pressure, 5-HT is also a powerful constrictor of vascular smooth muscle. Levels of HVA in normals in the ventricular CSF are 0.466 μg/ml; in cisternal CSF, 0.185 μg/ml; and in lumbar CSF, 0.053 μg/ml (186).

5-HT levels in ventricular CSF collected between days 2 and 15 post-SAH ranged between <2 and 5 nmol/liter. There was no difference between these levels and those found in controls. 5-HT concentrations did not correlate with the severity of angiographic VSP or with CSF pressure or grade. Cisternal CSF collected during operation on two patients who developed severe postoperative VSP, which was contaminated by fresh blood, showed 5-HT concentrations >25 nmol/liter. Voldby and colleagues suggested that 5-HT did not play a major role in sustained delayed VSP but that fresh platelets might be a source of sufficient 5-HT to initiate VSP (232).

Blood and tissue from the region of ruptured aneurysms were obtained at surgery. Assays for 5-HT showed values ranging between 0 and 88 ng/g in four patients who had VSP but none was detected in six other patients with ruptured aneurysms. One patient showing no 5-HT was operated 12 days post-SAH, whereas the other three patients with VSP and 5-HT were operated on day 3 post-SAH (233).

#### *Catecholamines*

The amine levels in plasma levels of 35 cases post-SAH were studied. Twenty-two cases showed no VSP and had CSF NE levels of 0.154 μg/liter and Epi levels of 0.045 μ/liter. In 7 cases with focal VSP NE levels were increased to 0.341 μg/liter. Six cases showed intense VSP and the NE in the CSF was very significantly raised above normal (0.764 μg/liter). The CSF levels of NE

patients were independent of plasma levels. Epi and 5-HIAA levels were not significantly increased in these patients (234). During general surgical operations both Epi and NE are increased in the plasma and attain much higher levels than the corresponding level in the CSF (235).

Postsurgery levels of Epi and NE were increased in the plasma of SAH patients compared to normals. Patients with focal ischemic deficits had significantly higher levels of Epi in the cisternal and ventricular CSF at the time of surgery than patients who did not have these deficits. In focal ischemic deficit cases this Epi level was 0.85 nm/liter in cisternal CSF and NE was 1.66 nm/liter. In patients with no ischemic deficits the corresponding levels were 0.12 and 1.50 nm/liter. The CSF levels of Epi tended to fall progressively from the day of SAH. For days 0–4, the level was 0.46 nm/liter, for days 5–14 it was 0.36 nm/liter, and for days 15–19 it was 0.18 nm/liter. NE levels at the same time intervals were 1.06, 1.16, and 0.66 nm/liter (236).

Twelve patients were operated within 3 days post-SAH prior to the development of VSP and had continuous cisternal or ventricular sampling for NE. Its concentration increased in all CSF samples concurrently with the appearance of VSP. The cisternal CSF of patients with VSP contained significantly higher NE (0.246 ng/ml) compared to those without VSP (0.075 ng/ml). This was a highly significant difference. The concentration was still considered too low to produce significant local vasoconstriction and it was concluded that the elevated catecholamine level might be due to the release of NE into the CSF from various brain sources (237). Intravenous therapy with NE to raise the blood pressure was found to be associated with extremely elevated concentrations of 4-methoxy-hydroxyphenyglycol (MHGP), the primary metabolite of NE, in the CSF of patients with SAH. The concentration of MHPG in CSF correlated significantly with MHPG in plasma and NE in plasma. The concentrations of NE, Epi, and dopamine in CSF did not correlate with their respective concentrations in plasma. It was suggested that exogenously infused NE might be quickly transformed into MHPG, which could rapidly penetrate the BBB and accumulate in CSF in excessively high concentrations that might have vasoconstrictor effects. The highest MHPG level recorded in CSF was 70.5 ng/ml (238). Dopamine-β-hydroxylase activity in CSF showed no relationship to VSP (239).

### *15. Prostaglandins*

One of the first studies of $PGF_{2\alpha}$ concentrations measured by radioimmunoassay failed to show a correlation between CSF values and the appearance of VSP. CSF $PGF_{2\alpha}$ values were as follows: normal control, 37.7 pg/ml; non-SAH neurological disease controls, 195.9 pg/ml; nonaneurysmal SAH, 406.3 pg/ml; and aneurysmal SAH, 655.5 pg/ml. Although a correlation between $PGF_{2\alpha}$ and VSP was not evident, very few samples were obtained days 1–12 post-SAH (240). In one case with VSP, the $PGF_{2\alpha}$ level was 720 ng/ml in the CSF (241). The level of prostaglandin was elevated in most SAH patients at some time in the course of their illness in one series. The elevation of $PGF_{2\alpha}$ in lumbar CSF is considered to reflect an impairment of transport out of the CSF compartment due to the SAH. Evidence for this was considered to be the early appearance of $PGF_{2\alpha}$ in the jugular vein after it was injected into the cisterna magna of dogs. Control $PGF_{2\alpha}$ in lumbar CSF samples was 73 pg/ml in 5 cases. The highest levels in 6 patients ranged between 1257 and 7583 pg/ml; all these patients had neurological deficits. One SAH case with minimal deficit had a level of only 116 pg/ml. Prostaglandins, by causing hypertension, fever, cerebral edema, and VSP, may contribute generally to the symptoms of SAH. These vasoactive lipids are synthesized in platelets, brain, and cerebral vessels. The synthesis of prostaglandins is increased by thrombin, catecholamines, 5-HT, and BK, all of which are released at the time of SAH. Serial studies have shown that prostaglandin levels can vary from day to day in the same patient (242). Fifty-four controls had $PGF_{2\alpha}$ of 67 pg/ml (range, 25–150 pg/ml). Significant increases occurred in different CNS diseases with extremely high values being found in patients with stroke and SAH when the samples were collected shortly after the attack began. Patients with transient ischemic attacks had average levels of only 170 pg/ml (range, 35–355 pg/ml) (243).

Prostaglandin production by RBCs incubated in human CSF at body temperature increased over 48 hr and then began to decrease. Levels were higher in bloody specimens (approximately 300,000 RBCs/ml CSF) compared to clear CSF. Mean values of PG in lumbar CSF collected from 5 patients post-SAH were as follows: $PGF_{2\alpha}$, 1548 pmol/liter; $PGE_2$, 1651 pmol/liter; 6-keto-$PGF_{1\alpha}$, 1924 pmol/liter; and $TXB_2$, 103 pmol/liter. The biological significance of changes in prostaglandin levels was questioned since these would be dependent on the method of dissection of the arteries, different *in vitro* conditions of incubation, and different analytical procedures. Different-sized vessels or the presence of VSP might also affect PG production (244). In 32 cases of SAH, CSF was assayed by bioassay for $PGE_2$ levels and correlation with VSP was sought but not found; the highest level was from a patient who died. Two cases post-SAH were monitored by serial lumbar puncture for levels of $PGD_2$ and 6-keto-$PGF_{1\alpha}$, the stable metabolite of prostacyclin $PGI_2$. In one case with VSP, $PGD_2$ had a concentration with a characteristic peak apparently

related to VSP. The synthesis of 6-keto-$PGF_{1\alpha}$, on the other hand, appeared to be inhibited after the hemorrhage. The patients without radiologic evidence of VSP showed a steady state for these PG metabolites. Cisternal $PGD_2$ in the presence of VSP was twice the highest lumbar concentration, whereas the 6-keto-$PGF_{1\alpha}$ was very low (245). $TXB_2$ and 6-keto-$PGF_{1\alpha}$ were monitored in CSF in patients post-SAH. The mean $TXB_2$ levels in cases with VSP was 610 pg/ml and in cases without was 78 pg/ml. The mean value of CSF 6-keto-$PGF_{1\alpha}$ in cases with VSP was 1152 pg/ml and in cases without 65 pg/ml. Sequential measurements in 5 cases with VSP and 5 cases without were performed. The values of these prostaglandins in the CSF were elevated in the early stage of SAH in cases with subsequent VSP and the levels decreased gradually. In cases without VSP the levels in CSF were consistently low. In one case with VSP, the cisternal CSF had higher $TXB_2$ and 6-keto-$PGF_{1\alpha}$ than ventricular CSF (246).

In 12 patients elevated levels of $TXB_2$ and 6-keto-$PGF_{1\alpha}$ were found in CSF post-SAH. The initial $TXB_2$ level was thought to correlate with the amount of SAH on the CT scan. Further increases were thought to correlate with ischemic deterioration. The CSF $TXB_2$ levels ranged between 26 and 9832 pg/ml (247).

In five patients post-SAH, indwelling lumbar spinal catheters were used to sample CSF daily. $PGF_{2\alpha}$ was highly correlated with the development of and fluctuations with clinical VSP, with angiographic findings, with neurologic grade on admission, and with outcome. The same study found that tryptophan content increased marginally in association with clinical and angiographic VSP. Unlike these substances, the 5-HT level was highest early in the hospital course and not later during VSP (248). Raised PG levels in CSF are of uncertain significance. Raised levels could be due to delayed clearance associated with Hyc or abnormalities of the BBB. The more likely explanation of raised levels of prostanoids is increased synthesis. In 39 patients with SAH, CSF levels were examined. CSF levels (pg/ml) from SAH and controls, respectively, were as follows: $PGE_2$, 39 and 13; $TXB_2$, 72 and 11; 6-keto-$PGF_{1\alpha}$, 61 and 16. These were all significant differences. This large study used contemporary techniques and performed a statistical analysis of a large body of clinical and radiologic data. In contrast to the foregoing, no significant correlation was found between the level of each prostaglandin measured and the following variables: clinical grade on admission, amount of SAH on CT scan, occurrence of ischemic deterioration, low-density changes on CT scan, the presence of VSP on angiography or clinical outcome, and the incidence of ischemia as a cause of death or disability at 3 months post-SAH (249).

### *16. Leukotrienes*

Leukotrienes are products of arachidonic acid metabolism via the lipoxygenase pathway. There are synthesized in gray matter and blood vessels. Their production is enhanced in ischemic conditions and experimental SAH. Lumbar and cisternal CSF levels of leukotriene C4 were assayed in 48 patients post-SAH. Twelve patients had symptomatic and radiologic VSP. CSF levels were significantly higher in SAH than control cases. Cisternal CSF levels were higher than lumbar levels (250).

### *17. Acetylcholine*

Fifty-eight CSF samples were obtained from 23 patients for 21 days post-SAH. CSF ACh levels in normal controls averaged 35.8 pmol/ml and were significantly higher than those in SAH patients (9.9 pmol/ml). Low levels of ACh have been associated with impairment in learning and memory. ACh is destroyed by the enzyme butyrylcholinesterase, which is 5–200 times higher in concentration in plasma than CSF. Contamination of CSF by this enzyme might contribute to the reduction in acetylcholine levels in CSF. However, a measurement of CSF butyrylcholinesterase showed a return to normal control levels within 1 week post-SAH, whereas the low ACh levels post-SAH persisted for at least 3 weeks (251).

### *18. Phospholipids*

Phospholipids are found in normal CSF and include lysolecithin, sphingomyelin, lecithin, and phosphotidylethanolamine. In the CSF the saturated fatty acids dominate (186).

### *19. Neuropeptides*

Cerebral blood vessels are supplied by vasodilatory perivascular nerves and parasympathetic and sensory nerves. In the parasympathetic nerves VIP coexists with ACh. These fibers have origin in the sphenopalatine and otic ganglia and in small nerve cell clusters in the base of the brain. VIP may be an important vasodilator. The sensory fibers store at least three peptides: tachykinins (SP and neurokinin A) and calcitonin gene-related peptide (CGRP). These fibers mainly originate in the trigeminal ganglion.

CGRP concentrations were measured in patients following strokes. The CSF levels with ischemic vascular diseases averaged 152 pg/ml, which is not significantly different from the control level of 45 pg/ml. In hemorrhagic cerebrovascular diseases the level was significantly higher (3965 pg/ml) (252). CGRP, SP, VIP, and neuropeptide Y (NPY) were analyzed in CSF in the postoperative course in 14 patients who had SAH. The CSF VIP-like

immunoreactivity (LI) was lower in SAH than in controls. The CGRP-LI level was measurable in CSF post-SAH but not in control CSF; in individual patients with marked vasoconstriction increased levels of CGRP-LI (up to 14 pmol/liter) and NPY-LI (up to 232 pmol/liter) were observed (253).

The "trigeminocerebrovascular" system can be activated by perivascular administration of various vasoconstrictors. Both NE and NPY are strong vasoconstrictors. Lumbar CSF VIP levels were low in patients undergoing aneurysm surgery. The same authors observed a circadian pattern of CSF VIP concentration variations (254). In 14 patients post-SAH concentrations of CGRP and pituitary polypeptide 7B2 were not significantly different from those of controls. ANP was significantly lower. Serial determinations showed an increase in NPY concentrations 6–11 days post-SAH, although the mean levels were not higher than those in controls. The secondary rise in NPY might indicate that this vasoconstrictor had a role in VSP (255). The NPY concentration in human CSF from a variety of hemorrhagic strokes was 4148 ± 397 pg/ml, which was significantly higher than the control level of 1083 ± 245 pg/ml. The NPY concentration in CSF in patients following ischemic strokes was not significantly different from the level in controls (256).

Hemorphins are peptides with opioid activity that are enzymatically released from Hb. Following hemorrhagic stroke the peptide LVV-hemorphin-7 was recovered in high amounts (115–300 pmol/ml) but was not present in control CSF (257).

### *20. Free Radicals and Lipid Peroxides*

Free radicals may be detected by electron spin resonance spectroscopy and chemiluminescence or by reacting them with spin traps to form stable hydroxylation products. Other methods detect the products of free radical reactions such as lipid peroxide, malondialdehyde, conjugated dienes, and hydrocarbons or on the consumption of scavengers. Indirect methods such as detection of malondialdehyde by thiobarbituric acid reactions are fraught with imprecision.

Electron spin resonance was used to observe the phasic changes of oxyHb during the course of autooxidation. The characteristic changes of electron spin resonance signals of ferric protein compound from high to low spin corresponded to the changes from oxyHb to superoxide metHb, metHb, and hemichrome during the incubation of blood. These changes were observed in CSF from patients who suffered from VSP post-SAH (258). OxyHb in its autooxidation to metHb produces active species of oxygen ($O_2$-•, $H_2O_2$, $OH^•$, $^1O_2$). These free radicals in turn may generate toxic reactions such as peroxidation of polyunsaturated fatty acids in the biomembrane of the adjacent cells. The peroxide content of lumbar CSF was assessed in 25 patients post-SAH; the values in patients with VSP were higher than those without. A statistically significant difference between VSP and non-VSP groups was evident in the time periods days 1–3 and days 7–9 post-SAH (259). Normal controls showed no evidence of hydroperoxy eicosatetraenoic acids and hydroxy eicosatetraenoic acids in CSF. In SAH patients such peaks were recognized during the occurrence of VSP. In 10 SAH patients, semiquantitative analysis of 5-hydroxy eicosatetraenoic acid correlated closely with the occurrence of VSP (260). CSF was sampled from 10 patients who had VSP; one of the compounds appearing on day 7 post-SAH was identified as 5-hydroxy eicosatetraenoic acid. It was concluded that the peroxides of arachidonic acid are present in the CSF following SAH and that they correlate with the occurrence of VSP (261). Twenty-five patients treated with early surgery post-SAH had CSF examined for lipid peroxide concentrations and the activities of SOD, catalase, and glutathione peroxidase (GSH-α). The concentration of lipid peroxides increased during the first 4 days post-SAH in patients who developed symptomatic VSP. Patients with VSP had a marked decrease in SOD activities on days 3 and 4 followed by a more gradual decrease, whereas patients without VSP showed little change. There was a significant difference in catalase activity which was the reverse of the SOD acitivity. GSH-α showed no change. There was a close correlation between the increased lipid peroxide concentrations and the decrease in SOD activity in the CSF. The determination of lipid peroxide concentration was estimated using malondialdehyde (262). Many diseases involve increased free radical production. Probably all tissue damage results in lipid peroxidation, so it is difficult to determine if an increase in peroxidation is the cause or the result of tissue damage. Free radicals damage not only lipids but also nucleic acids and proteins.

In four studies involving 90 patients, CSF was assayed for thiobarbituric-reactive substances and higher levels were found in those patients developing VSP (212,259, 263–266). Most investigations reported little thiobarbituric acid-reactive substance in the first few days after SAH, although there was one report of the highest values being found on days 1–3 (259,264–266). It has been suggested that there is an inverse relationship between polyunsaturated fatty acid content of CSF and the thiobarbiturate substance concentration (265). Lipid peroxidation products were studied in 16 patients post-SAH. Their levels were significantly elevated in SAH. The lipid peroxidation products were twice as high in patients with an unfavorable outcome as those with a favorable course. It was claimed that administration of antioxidants such as α-tocopherol and ascorbic acid inhibited the elevated lipid

peroxidation products as manifest by a decrease in the content of CSF malonyl dialdehyde (267).

### *21. Growth Factors*

Nerve growth factor was found in some brain-injured patients soon after injury. It was also detected post-SAH (268). In a patient post-SAH, nerve growth factor levels in CSF were below the level of detection. Nerve growth factor was found to increase in CSF transiently following craniotomies. This may reflect neurotrophic activities involved in the regeneration of neural networks (269).

Transforming growth factor (TGF-β1) is a multifunctional polypeptide controlling the production of extracellular matrix protein. In 24 patients post-SAH its level rapidly decreased from the onset of SAH. The level in 13 patients showing ventricular dilatation with periventricular low density on CT scan was 1.07 ng/ml on days 12–14; this was significantly higher than the 0.52 ng/ml in patients without ventricular dilatation, which suggested that it might play a role in generating communicating Hyc (270).

No significant increase occurred in the CSF of dogs in platelet-derived growth factor after experimental SAH (271).

### *22. Antidiuretic and Natriuretic Factors*

Antidiuretic hormone (ADH) was measured in CSF of 42 patients post-SAH. Increased concentrations were present in 10 patients, 8 of whom had bled from an anterior communicating artery (A Com A) aneurysm. In 3 patients high blood levels were associated with hyponatremia. Five patients had increased ADH concentrations in the presence of normal plasma values and all showed severe disturbances of consciousness (272).

Twenty-six patients post-SAH had plasma and CSF natriuretic factors/peptides (ANF/ANPs) measured. The ANF/ANP concentration in human CSF is one or two orders lower than that in the plasma and there was no significant correlation between the levels in the CSF and the plasma levels. After SAH in patients with raised ICP there was an increase in concentration in the CSF but the plasma concentration did not change (273). Four patients with moderate to severe SAH had higher mean CSF ANF values (17.7 pg/ml) compared to 5 patients with minimal SAH (0.6 pg/ml) or 9 control subjects (3.7 pg/ml) (274).

ANF factors were highest in patients with ICH followed by those with obstructive Hyc and SAH (19, 13, and 8 pg/ml, respectively). Concentration was less than 4 pg/ml in controls. Levels were higher in patients on fluid restriction. Since the CSF concentration did not correlate with ICP, CSF $Na^+$ or osmolality, serum $Na^+$ or hemodynamics, it was concluded the CSF ANF concentration is a nonspecific indicator of brain injury (275).

### *23. cAMP*

The normal levels of cAMP in CSF are 15–30 mmol/liter. Blood levels increase in ischemia and decrease in coma (50). The degree of elevation of CSF cAMP appeared to correlate with the degree of hypertension in the patients and the size and time of destruction of cerebral cells in ischemia (276).

### *24. Acids*

#### δ-*Aminobutyric Acid*

CSF levels of GABA increase in ischemia. The normal level is about 150 pmol/ml (277).

#### *Cholic Acid*

A type of cholic acid (7-α-hydroxy-3-oxo-4-cholestenoic acid) first observed to be elevated in chronic subdural hematomas was subsequently shown to be significantly higher in the CSF in aneurysmal SAH patients than other patients undergoing craniotomy. Since plasma levels showed no postoperative increase it was assumed that extra hepatic intracranial production of this cholic acid occurred after SAH (278).

### *25. Hemolysate Vasoconstrictors*

Using rabbit aortic preparations the CSF from 43 patients with intracranial VSP and 175 control patients were studied. Sixty-seven percent of the plasma samples caused significant contraction of the aortic strip. The responses of the sample of patients with SAH and VSP were the same as the responses of the control samples. Only 3% of clear and colorless CSF samples showed significant activity compared to 33% of xanthochromic specimens (278b).

CSF from 3 patients post-SAH obtained on days 2, 5, and 7 produced dose-dependent contractions of dog basilar artery ring segments. The CSF was xanthochromic. The contraction in one case was blocked by phenoxybenzamine. Using an indirect method it was considered that 5-HT was present in these samples. CSF samples from 2 patients with VSP, in which the CSF was xanthochromic, caused dose-dependent contractions in both human and canine cerebral artery segments (279). Lumbar CSF from 34 patients post-SAH was used to contract human basilar artery segments. The vasoconstriction was not antagonized by 2-bromolysergic acid diethylamide, methysergide (5-HT antagonist), phentolamine (α-adrenergic blocker), mepyramine (antihistamine), and atropine (anti-cholinergic). CSF caused a slow-developing vasoconstriction as opposed to a rapid constriction

resulting from exogenous prostaglandins. CSF from patients augmented the contractile response to 5-HT, prostaglandins, and to a lesser extent catecholamines. The CSF from patients also relaxed a few specimens (280).

CSF removed up to 6 weeks post-SAH from 19 patients was assayed biologically. This pattern was not significantly influenced by surgery. Serial angiography of the patients showed progressive arterial dilatation as the vasoconstrictor activity of the CSF was progressively reduced and the clinical improvement occurred (281). Serial samples of CSF were obtained from 10 patients who underwent aneurysm clipping within 0–2 days post-SAH. No relationship could be demonstrated between vasconstrictor activity and postoperative CSF samples, the patients clinical condition, or angiographic VSP. Using appropriate antagonists it was considered that the prime vasoconstrictor agent in CSF was not 5-HT, histamine, NE, Epi, ACh, or angiotension II. There was a correlation between the CSF $K^+$ and the vasoactive substance in CSF, but $K^+$ did not account for the vasoconstrictor activity of CSF (282). CSF from 4 patients post-SAH caused consistent vasoconstrictor response in exposed cat cortical pial arterioles. The effect was not attributable to pH or $K^+$. The vasoconstriction was more marked in smaller arterioles. Nifedipine applied topically could reverse the vasoconstriction (283).

Human pial arterioles were obtained at tumor craniotomies. The vessels were exposed to CSF from six patients 6–12 days post-SAH. All the CSF samples induced constriction in vessels from one or several donors. Twelve vessels did not react to any CSF. Although there was a conspicuous inconsistency in response, the pattern of individual vessel responses to different CSF specimens was more consistent. The vasoconstrictor response from the CSF of two patients who had a normal level of consciousness was just as pronounced as the responses from comatose patients (284). When small human cerebral arteries were preincubated with indomethacin, CSF from SAH patients caused a markedly increased contraction. Also, contractions induced by NE but not 5-HT were augmented. Prostacyclin and its metabolite 6-keto-$PGF_1$ reversed the contractions induced by CSF as well as NE, 5-HT, and $PGF_{2\alpha}$. It was suggested that a reduction in these dilator arachidonic acid (AA) metabolites might result in an imbalance between contractile and relaxant forces acting on the arterial wall leading to VSP (285).

Samples of SAH CSF, subdural hematoma fluid, and tumor cyst fluid were tested for constriction with canine arteries and rat stomach fundus. All the samples containing blood produced contractions. Neither methysergide (5-HT antagonist) nor indomethacin (inhibitor of PG synthesis) significantly diminished the contraction due to blood containing CSF, although the calcium antagonist D600 successfully antagonized the response in all groups. The antagonist effect of D600 on the CSF-induced vasoconstriction was more effective in the cerebral artery preparations than on stomach fundus. CSF from patients with angiographic VSP was significantly more active than CSF obtained from patients without VSP (286).

Vasoactive substances were assayed using isolated human cerebral arteries obtained postmortem and isolated rat stomach fundus. Thirteen patients provided CSF which was collected an average of 16 days post-SAH (range, 7–32 days). All of the patients had good or excellent clinical outcomes. These preparations produced dose-related contractions in response to 5-HT. The CSF produced vasoconstriction equivalent to 40 nmol/liter of 5-HT. There was a 76% inhibition of the arterial contractile response resulting from use of the 5-HT antagonist ketanserin. The fact that most of this CSF was obtained after the risk of VSP had passed is noteworthy (287). An analysis of the ghost-free hemolysate of RBCs on exposed basilar artery of cats was performed. The vasoconstrictor substance was heat labile, had a MW greater than 20 kDa, and in gel filtration came out in the same fraction as oxyHb did. In dog basilar artery strip preparations *in vitro* the constrictor activity of the hemolysate was decreased by aspirin (antiprostaglandin) and by polyphloretin phosphate (an antiprostaglandin constrictor) (288).

Partially purified hemolysate protein was injected into the cisterna magna of dogs. This produced basilar artery VSP which relaxed 4–10 days postinjection. The specimen contained Hb as well as other compounds (289). Hemolysates of Hb caused contractions of guinea pig basilar artery *in vitro*. One response was inhibited by indomethecin and a second was resistant. Thromboxane $A_2$ did not affect the constriction. Removal of the endothelium modified the vasoconstriction (290). RBC hemolysate caused significant vasoconstriction in dog basilar artery but only slight constriction in mesenteric ones. The vasoconstriction was reduced by polyphloretin and aspirin. Cylcooxygenase and Hb stimulate the conversion of AA to prostaglandin endoperoxides (291).

Bloody human CSF was found to cause *in vitro* isometric contraction of canine basilar artery strips. The disulfide bond-reducing agents dithiothreitol ($10^{-4}M$) and dithioerythritol ($10^{-4}M$) suppressed the contraction due to bloody CSF by an average of 40 and 61%, respectively. These substances did not alter KCl-induced contraction. The sulfhydryl group oxidizing agent 5,5′-dithiobis-(2 nitrobenzoic acid) ($10^{-4}M$), reversed the inhibitory effect of dithioerythritol on the contractile response of bloody CSF. No significant suppression of any response resulted from the use of the standard antagonists methysergide ($10^{-7}M$), mepyramine ($10^{-7}M$), phenoxy-

benzamine ($10^{-5}M$), propranolol ($10^{-6}M$), or atropine ($10^{-6}M$). It seemed therefore that 5-HT, histamine, NE, and ACh were probably not involved in the vasconstriction resulting from bloody CSF, whereas PG, Hb, and lipid hydroperoxides might be involved (292).

CSF was obtained from 32 patients post-SAH at a mean time of 11 days. The CSF was tested on the rat stomach muscle preparation. The mean constrictor activity in the 7 cases with severe VSP was 67 nmol/liter $PGE_2$ equivalents, which was 10 times greater than that in the 19 patients in whom VSP was not demonstrated (6.7 nmol/liter $PGE_2$ equivalent). The patients who died had the highest concentration of vasoconstrictor material—73.8 nmol/liter $PGE_2$ equivalent compared to 6.8 nmol/liter for those with good outcomes. Serial lumbar CSF samples from 1 patient were tested against both rat fundus and isolated human basilar artery and there was good agreement between the values for smooth muscle constrictor activity between the two preparations (293). CSF was obtained from 11 patients: 4 had VSP preoperatively and 2 had it postoperatively. In only 1 patient was the VSP judged to be severe. The vasoconstrictor responses of canine basilar artery were tested. The magnitude of the responses did not correlate with the patient's VSP history. The maximal response of basilar arteries to xanthochromic CSF varied between 4 and 123% of the $K^+$ response. The concentration of oxyHb in the CSF ranged from $2.76 \times 10^{-5}$ (0.18%) to $53.7 \times 10^{-5}M$ (3.46%). MetHb varied from $0.04 \times 10^{-5}$ to $7.18 \times 10^{-5}M$, and deoxyHb varied from $0.003 \times 10^{-5}M$ to $5.41 \times 10^{-5}M$. Total Hb varied from $3.33 \times 10^{-5}$ to $66.79 \times 10^{-5}M$. The average oxyHb concentration was $23.22 \times 10^{-5}M$, whereas the average total Hb was $31.19 \times 10^{-5}M$. The oxyHb content averaged 74% of the total. No correlation between total Hb and the contractile response was evident. The Hb content was not related to the interval between the bleed and the collection of the CSF sample. Only 3 of the 11 CSF samples produced a sustained contractile response. The responses elicited by xanthochromic CSF were variable in comparison to the consistent contractions resulting from high $K^+$ or 5-HT. The vasodilator protein antithrombin III (1 U/ml) inhibited the sustained contraction produced by xanthochromic CSF (294). Pretreatment of canine arterial ring with human CSF post-SAH resulted in a dose-dependent inhibition of relaxation induced by A23187. Hb produced a similar inhibition of the relaxation. Normal CSF from patients without SAH did not affect endothelium-dependent relaxation (295).

CSF from 7 patients with and 5 without VSP post-SAH were obtained between days 2 and 16. Using cultured vascular smooth muscle cells, an elevation of cytosolic-free $Ca^{2+}$ occurred which was greater than the response to control CSF (296). CSF collected on days 7–10 post-SAH was treated by heating, ultrafiltration, or salting out with ammonium sulfate. The resulting solutions were tested for their effect on cytosolic-free $Ca^{2+}$ in cultured vascular smooth muscle cells. Heated CSF and ultrafiltrated solutions containing substances with MW of less than 10 kDa caused no significant elevation of cytosolic-free $Ca^{2+}$. The factor responsible for any increasing $Ca^{2+}$ was considered to be a protein with a MW of greater than 10 kDa. The substance also caused a rapid accumulation of inositol-1,4,5-triphosphate in vascular smooth muscle cells, so the active factor presumably stimulates receptor-mediated phosphoinositide breakdown (297).

CSF from patients on day 2 post-SAH induced a transient elevation of cytosolic-free $Ca^{2+}$ greater than that from control patients. In cultured porcine cerebral arterial smooth muscle cells the SAH CSF promoted levels of [$^2$H]-thymidine incorporation (DNA synthesis) more than 2.5-fold higher than that promoted by CSF from control patients (298). A $[Ca^{2+}]i$ elevating factor from CSF post-SAH was purified to homogeneity by ammonium sulfate precipitation and a combination of Mono Q, Superose 12, and Mono S columns using liquid chromatography. Fifteen micrograms of purified protein was obtained from 340 mg of CSF protein. The molecular mass was estimated to be 81 kDa by electrophoresis. The purified protein was cross reactive with anti-human transferrin antibody. It was suggested that transferrin may be involved with VSP post-SAH (299).

Small human arteries obtained at craniotomy were exposed to hemorrhagic CSF. Prostacyclin ($PGI_2$) and its metabolite (6-keto-$PGF_1$) reversed vasoconstriction from hemorrhagic CSF as well as from 5-HT, $PGF_{2\alpha}$, and NE (285).

### *26. Clotting and Fibrinolytic Factors*

Coagulation and fibrinolytic studies were performed in 30 patients post-SAH and the results were compared to those of 30 control patients. Recalcification time, fibrinogen, partial thromboplastin time, K value of thromboelastogram, and euglobulin lysis time were all measured. There were significant differences between patients and controls in fibrinogen, PTT, and K values of the thromboelastogram. The abnormalities were considered to reflect tissue damage and/or meningeal reaction to SAH. Similar abnormalities have been noted following brain injury and some nonhemorrhagic strokes. SAH patients may exhibit simultaneous increased coagulability and activation of fibrinolysis. Fibrinogen levels >400 mg% were associated with a mortality rate of 87% compared to a mortality rate of 36% in those with lower values (300). The fibrinolytic activity of CSF in 63 patients with various neurological diseases was assessed by a modified fibrin plate method. In 26 cases of SAH the fibrinolytic activity

was markedly increased in the 2 weeks following the hemorrhagic ictus (301).

Fibrin degradation products (FDPs) were assayed in CSF of 252 patients with a variety of neurological diseases. These were found in 23% of cases. Proteins of similar MW such as plasminogen and factor IX were also present. Nonspecific vascular damage such as that in some systemic degenerations and tumors can also cause protein extravasations into spaces where they are not normally found. Ten of the 13 patients whose CSF was examined in the first 4 days post-SAH showed one or two low MW coagulation proteins in CSF. These included FDP (fragments B and E), plasminogen, and/or factor IX. Three patients studied on days 4–16 showed only one low MW coagulation protein (302).

Forty-one post-SAH patients had serial assays of fibrin/FDP and plasminogen activator activity on fibrin plates of blood and CSF. Plasminogen activator activity slightly increased post-SAH in the CSF compared to control patients at 1 week post-SAH. FDPs were also elevated in CSF around 1 week post-SAH and in patients treated with antifibrinolytic agents. These values tended to decrease more than the levels seen in untreated patients (303). CSF FDP levels were measured in the first 3 days post-SAH in 50 grade 1 and 2 patients. The patients who subsequently showed clinical evidence of DID had initial FDP levels ranging from 80 to 320 mg/ml compared to levels of 20–80 mg/ml in the 30 patients who did not show evidence of delayed ischemia (304). CSF levels on days 3–5 post-SAH for fibrinopeptide A and fibrinopeptide B beta were elevated compared to levels in other time periods. These elevations occurred 2–4 days prior to the appearance of symptomatic VSP (305). Forty-eight patients had CSF analysis performed 9–15 days post-SAH. Twenty-two received tranexamic acid (antifibrinolysis). No differences in FDP levels were found between the patients treated with tranexamic acid and those who were not. Twenty patients had FDP in the CSF (4–22 mg/liter range and mean 11 mg/liter). SAH patients had plasminogen values ranging from 7 to 60 mU compared to 14–16 mU in controls. Patients with FDP tended to have higher CSF total protein. Eleven of 20 patients with FDP had CSF total protein greater than 1 g/liter compared to only 2 of 28 who did not have detectible FDP. FDP in the CSF could reflect both a damaged blood–CSF barrier and local fibrinolysis (306).

Platelet-secreted proteins and fibrinopeptide A were cleared from the CSF within 3 days post-SAH. Increased thrombotic activity of the CSF as reflected by higher levels of fibrinopeptide A and platelet-secreted protein seem to be associated with the occurrence of neurological deficit (203). Fibrinopeptide A levels were studied in CSF and plasma in 25 patients post-SAH using radioimmunoassay. CSF levels were extremely high days 0–1, 1253 ng/ml; days 2–4, 11.3 ng/ml; days 5–7, 10.7 ng/ml; and days 8–14, 6.3 ng/ml. In the controls fribrinopeptide A concentrations were 1.2 ng/ml. The plasma levels showed no statistically significant changes with time. The elevated fibrinopeptide A was considered to be an indicator of thrombin activation in the early stages of SAH. The formation of elevated bradykinins was thought to be due to contact activation of the Hageman factor (intrinsic factor) within the subarachnoid space (307).

The D dimer assay of 40 CSF samples post-SAH appeared to be a means of accurately differentiating true SAH from traumatic lumbar puncture (LP). The D dimer assay was positive in all 6 patients with SAH and negative in a control group of 14 patients with traumatic LPs and in 20 patients with normal CSF. The D dimer assay was a better test than xanthochromia or the decline in RBC count in sequential tubes in differentiating SAH from traumatic LP (308). Fibrinopeptide A level is a quantitative index of thrombin generation when measured in blood and CSF post-SAH. The levels of fibrinopeptide A in both blood and CSF were significantly raised compared to controls. The levels correlated with the amount of SAH on the initial CT scan. The CSF fibrinopeptide A levels had a statistically significant correlation with outcome at 3 months postictus. No statistically significant correlation was found between blood and CSF fibrinopeptide A levels (309).

FDPs were measured in 29 patients in the first and second weeks after SAH. Values above 40 μg/ml were found in 8 of 9 patients showing signs of cerebral ischemia, whereas only 7 of 15 patients without VSP showed similar elevations (310). Using an enzyme immunoassay for thrombin activity, thrombin – antithrombin complex (TAT), the CSF, and blood levels of 10 patients with severe SAH, who were operated in 48 hr post-SAH, were measured serially. Angiography was performed on days 7–9 and the degree of VSP defined as a >70% reduction in diameter over at least 2 cm in length was observed in 6 of 10 patients. TAT levels in the CSF of the VSP group averaged 938 mg/ml compared to a mean levels of 183 mg/ml in the non-VSP group. These initial levels decreased sequentially (311). Nineteen patients undergoing surgery within 48 hr post-SAH who had ventricular and cisternal drains had the CSF analyzed for membrane-bound tissue factor and thrombin–antithrombin III complex and myelin basic protein. They were studied on days 0–4 and 5–9 after the ictus. In the early time period the tissue factor and thrombin–antithrombin correlated with clinical condition and the degree of SAH on CT scan. There was also a correlation with subsequent

cerebral infarction from VSP. Only tissue factor correlated significantly with clinical outcome in this early time period. The three factors assayed all correlated significantly with each other. From days 5 to 9 only tissue factor correlated with cerebral infarction, infarction volume, MBP levels, and outcome. Unlike tissue factor, the thrombin–antithrombin level did not correlate with VSP during the interval when it most commonly occurs, which does not support a role for thrombin in the pathogenesis of VSP. Tissue factor value in control craniotomy subjects was 145 pg/ml compared to 602 pg/ml during days 0–4 and 197 pg/ml during days 5–9 post-SAH. Comparable thrombin–antithrombin values (mg/ml) were 40, 2110, and 316. Both tissue factors and thrombin–antithrombin levels were much higher in patients with ICH and IVH as well as SAH than in the SAH group alone. The levels were also much higher in patients who developed cerebral infarction due to VSP, particularly in the early time period (312). CSF levels of thrombin–antithrombin, PAI-1, and tPA-PAI-1 activities in plasma and CSF collected from cisternal drainage catheters were assessed in 50 patients post-SAH. The CSF levels of all these parameters and plasma PAI-1 levels were significantly higher in patients with severe SAH than in those with mild SAH. The CSF level of tPA-PAI-1 and the initial neurologic status were related. The CSF PAI-1 level increased to >20 ng/ml near the time of VSP development, whereas it remained below 20 ng/ml in patients without VSP. The CSF tPA-PAI levels showed the highest peak near the time of VSP remission. Patients with good outcomes had significantly lower levels of CSF PAI-1 and tPA-PAI-1 activity. It appears that both the coagulative and the fibrinolytic systems are activated in CSF and plasma post-SAH and that the degree of such activation correlates with the amount of SAH clot. The author suggested that the intrathecal administration of fibrinolytic agents should be started early after surgery before CSF PAI-1 levels increase (313).

The CSF and blood from 38 patients were studied serially after SAH. Thrombin–antithrombin III complex and prothrombin fragment F1+2 levels were significantly higher in the CSF but not the blood compared to controls. Levels were also higher on days 7–9 in those with VSP compared to those without. Tissue factor level was higher in CSF than in blood in patients. Tissue factor inhibitor could not be detected in CSF. One hundred to 200 ml of CSF was drained per day. Since the thrombin–antithrombin complex and prothrombin fragments are considered to be markers for thrombin, the latter was considered to be involved in VSP. The markers did not correlate with grade at outset or outcome. Relatively few patients (6 of 38) had evidence of VSP (314).

### *27. Endothelin*

Endothelins (ETs) are peptides originally isolated from endothelial cells which have extremely potent and long-lasting vasoconstricting effects on cerebral vessels both *in vitro* and *in vivo*. Astrocytes also produce these peptides and the production can be stimulated by thrombin or potentiated via an autocrine mechanism.

Suzuki *et al.* (315) reported a considerable increase in ET immunoreacitvity in the CSF from patients with SAH and found levels of 0.4 pmol/liter on days 0–1 increasing to 2.2 pmol/liter on day 6 and thereafter decreasing gradually. CSF samples from 17 control patients found detectable endothelin with a preponderance of big ET-1 relative to ET-1 and ET-3. The mean concentrations of ET-1 and ET-3 in simultaneously collected plasma were significantly higher than those in CSF, although the levels for big ET-1 in plasma were similar to those in CSF (144). CSF of patients post-SAH ranged from 0.3 to 4.5 pmol/liter. No ET immunoreactivity was observed by the same investigators in CSF from controls or from patients with cerebral infarction, subdural hematoma, and tumors (315). ET was present in both plasma and CSF in SAH patients but the levels were not found to correlate with VSP. Phosphoramidon, an agent that inhibits the conversion of big ET to ET, did not ameliorate the development of VSP (316). ET-3 levels were significantly elevated in patients post-SAH (317). Plasma levels of ET were measured at 4.92 pmol/liter in 14 patients post-SAH. ET was found in all the normal body fluids examined (318).

Peaks of both ET-1 and ET-3 were observed in the CSF of 5 patients post-SAH who developed VSP but not in 2 other patients. The CSF levels of ET-1 and ET-3 displayed a striking parallelism. Plasma ET-1 levels remained in the normal range and ET-3 levels were not detected in plasma. Patients with high ET levels in CSF showed a simultaneous peak in urinary ET secretion. It was therefore suggested that ET might be an etiologic factor in VSP rather than a marker (319). ET-1 immunoreactivity was assessed in 27 patients for 2 weeks post-SAH. Plasma levels were highly elevated during the whole study. Levels of CSF of the same patients were not stable. Plasma levels were higher in patients with larger SAH judged by CT scan. Plasma ET-1 levels in 12 patients with VSP were higher in those without VSP during the first week than in those who did not develop VSP. ET-1 levels were in the normal range on days 0–3 in the CSF but became elevated during days 5–14. CSF ET-1 levels in patients without VSP were within the normal range during the entire period of study. The time course of VSP and the increase in CSF ET-1 coincided precisely (320). Eleven patients studied post-SAH had big ET levels determined by

radioimmunoassay. There were no elevations in big ET in 59 plasma samples and the 17 simultaneously estimated CSF samples. Differences between plasma and CSF did not reach significant levels. Big ET values between patients with and without VSP showed no significant differences (321). ET-like immunoreactivity (ET-LI) was studied in plasma and CSF from patients post-SAH. Normal ET-I levels in plasma and CSF were 12.4 and 9.1 pg/ml, respectively. Levels increased with surgery. The plasma and CSF ET-LI levels in patients who developed symptomatic VSP became concomitantly elevated again (322). Big ET, ET-1, and ET-3 were measured serially for 2 weeks post-SAH in 22 patients. Big ET-1 was the predominant peptide in the CSF of SAH patients. The CSF concentrations of big ET-1, ET-1, and ET-3 were significantly higher in older than in younger patients. In SAH patients without VSP (diagnosed by clinical signs and TCD) the concentrations of ET in CSF decreased with time. In those patients who developed VSP, this coincided with an increase in the concentration of big ET-1 and ET-1. The volume of hematoma in the basal cisterns on the initial CT scan was predictive of the subsequent concentrations of ET in the CSF. The plasma concentrations, on the other hand, did not correlate with VSP (323).

#### *28. Nitric Oxide, Nitrite, and Nitrate*

Thirty-one patients having aneurysm clippings within 3 days post-SAH had concentrations of nitrite and nitrate measured in their CSF. Control CSF level was 2.6 μmol/liter in non-SAH craniotomies. The concentration of NO metabolites was greater in patients with larger SAH shown on CT scan. Concentration of nitrate was greater than that of nitrite suggesting that NO in the subarachnoid space is mainly absorbed by Hb and degraded to nitrate. No differences were seen between patients on high-dose methylprednisolone or the usual dose of steroids. Steroids may prevent the formation of inducible NO synthase mediated by inflammatory cytokines. It was concluded that nitrate is the dominant NO metabolite in CSF post-SAH and that NO metabolism is stimulated by SAH. NO normally has a vasodilator action on the cerebral blood vessels and is released from both nervous and vascular tissues. It has an extremely high affinity for Hb, especially oxyHb, and becomes nitrosyl Hb after being absorbed. Its dissociation from Hb is slow, with a half-life of approximately 3 hr (324).

Nitrite and nitrate were measured in the CSF of 22 control patients, 23 with SAH, and 6 with SAH and VSP. Levels in SAH patients were higher than normal, but those with VSP had significantly lower levels on days 7–9 than those without VSP (325).

#### *29. Lattice Molecules*

CSF samples from patients with recent SAH accelerate the contraction of the collagen lattice formed by rat tail collagen and cultured human dermal fibroblasts (326). The rate of acceleration was significantly related to the clinical grade of VSP. Moderate and high concentrations of PKC peptide inhibitor impair the lattice compaction with or without acceleration by post-SAH CSF. Adenyl cyclase activators also inhibited compaction. Compaction was not inhibited by HA-1077, which is an antagonist of several protein kinases.

### B. Changes in Blood Serum and Plasma

#### *1. White Blood Cells*

A retrospective analysis of 173 aneurysmal SAH cases was performed. An admission WBC count >15,000/μl was associated with a 55% mortality as opposed to a 25% mortality with a lower WBC count. The mortality rate with a temperature >37.5°C on day 0 was 60% compared to 35% for those with a lower temperature. A WBC count of >15.000/μl on day 0 was associated with a VSP rate of 40%; a lower WBC count was associated with a VSP rate of 30%. day 0 temperatures >37.5°C were associated with a VSP rate of 30%. By day 6 patients with temperature >37.5°C had a VSP rate of 60%, double that of the VSP rate of those with temperatures <37.5°C. The WBC count was apparently more closely linked to the chance of dying than the chance of developing VSP. Development of fever a few days after SAH is related to both an increased mortality and an increased chance of developing VSP (327).

Serum soluble E-selectin levels were measured in 54 SAH patients. This is an adhesion molecule for WBCs. Levels were significantly higher for poor-grade patients on day 10 post-SAH (70 vs 37 ng/ml) and on day 12 (64 vs 43 ng/ml) (328).

#### *2. Platelets*

The platelet count in 110 SAH patients was analyzed. A minimum platelet count was similar in patients with or without symptomatic VSP, but the minimum occurred later in symptomatic VSP patients regardless of neurologic grade or timing of surgery (329).

#### *3. Blood Chemistry*

Buckell *et al.* studied 134 cases with spontaneous SAH, of whom 67 had documented aneurysms. Blood urea was above 47 mg/dl in 23% of alert patients and 56% of comatose ones. In comatose patients, the mean urea concentration rose from 30 to 50 mg/100 dl between the second and the sixth decades. Forty-five percent of comatose patients

had osmolalities outside the normal range of 280–300 mOs/liter compared to 12% of alert patients. The longer the patient was comatose, the higher the osmolality tended to become. Twenty-seven percent of comatose patients and 7% of alert ones had plasma proteins >7.5 g/100 ml. No alert patient had a raised Hb concentration, as did 22% of comatose patients. No patient had a plasma $Na^+$ concentration <133 mEq/liter. Seven patients had $K^+$ <3.5 mEq/liter. Glucose levels >110 mg/100 ml occurred in 45% of comatose patients and only 15% of alert ones. Six aneurysm patients had raised serum glutamic–oxalacetic transaminase levels (>40 U/ml). The erythrocyte sedimentation rate tended to increase progressively with the passage of time from the ictus. It was increased in 26% of patients on day 0 and increased steadily to 63% of patients on day 4, 43% on day 5, and 80% on day 6 (330).

#### *4. Antibodies*

Thirty-two patients were operated on within 72 hr of SAH. Anti-phospholipid antibodies such as lupus anticoagulants, anti-cardiolipin IgG, and anti-cardiolipin IgM were measured repeatedly after admission. Thirty-four percent tested positively. Although anti-phospholipid antibodies did not predict symptomatic VSP once it occurred, patients with them frequently demonstrated cerebral infarction and their outcome was worse. Anti-phospholipid antibodies were associated with poorer initial clinical and SAH grades on CT scan. They were detected between days 1 and 7. Sixty-seven percent with these antibodies became negative between days 7 and 13. A reduction in platelet count, increased platelet aggregability, and an increased plasma platelet factor 4 concentration were also observed in anti-phospholipid antibodies-positive patients with symptomatic VSP (331).

#### *5. Complement*

Serum complement levels (C3 and C4) decreased severely in patients with severe VSP (332). Serum complement levels were monitored in 42 patients for a 2- or 3-week period post-SAH. Kawano and Yonekawa had previously found that decreased serum C4 levels 5 days post-SAH were likely to be associated with symptomatic VSP, but in this group of patients a subset of 8 treated with nizofenone (a presumed antivasospastic agent) showed decreased serum C4 levels 5 days post-SAH but only 2 of them showed symptomatic VSP (333).

#### *6. Enzymes*

Plasmin renin activity was considered to be one of the markers of post-SAH autonomic disorder. The plasmin renin activities seemed to be linearly correlated with the amount of blood on the initial CT scan (334). Serum immumocomplexes were measured in 54 patients post-SAH. In 37 with VSP the changes in immunocomplex content during the first week after SAH correlated well with the clinical course. The high immunocomplex content preceded the onset of VSP and low content preceded clinical improvement. The C3-containing immunocomplex content in serum was approximately 2.8 μg/ml 5 days before the onset of VSP and 0.5 μg/ml 5 days after (335).

Seventy percent of 13 patients with postoperative increases in lactate dehydrogenase isoenzymes in their serum developed neurologic symptoms compared to 21% of patients with normal enzyme activity. It was considered that the enzyme elevation might be in response to operative brain damage. Hydroxybutyric dehydrogenase and lactate dehydrogenase were studied in serum of 44 patients operated on for aneurysms. Seven patients had a postoperative increase in serum enzyme activity above the normal range. All these patients showed neurological symptoms compared to only 14% of those who had normal postoperative enzyme levels (336). The myocardial isoenzyme of creatine kinase was demonstrated in the serum of 7 of 16 patients with SAH. All these patients developed VSP on angiography or a focal reversible neurological deficit. The release of this myocardial creatine kinase isoenzyme was considered to be the result of sympathetic nervous system activity related to intracranial VSP. Patients with cerebral hemorrhage (controls) showed levels of creatine phosphokinase of 11 (4.7), lactate dehydrogenase of 245 (188), and serum glutamic oxaloacetic transaminase of 20.7 (15.7). The enzyme levels were expressed in okinaka units. No brain creatine kinase isoenzyme was detected in any patient. Patients with positive serum levels of creatine kinase-MB had more evidence of acute myocardial ischemia on ECG. The slow and progressive increase in myocardial isoenzyme was considered to be an indication of acute myocardial dysfunction as a consequence of an acute cerebrovascular lesion (337).

Neuron-specific enolase was measured in 29 patients post-SAH. Serum levels were significantly higher in patients in poor neurologic condition and in those with greater amounts of SAH. Patients with good outcome had a low serum neuron-specific enolase level throughout their course. The levels increased with the development of DID and particularly in poor-outcome patients (338). Neuron-specific enolase is localized in neurons and axonal processes and escapes into blood and CSF at the time of neural injury. In a group of 20 SAH patients, 7 developed angiographic VSP and showed concurrent elevated serum neuron-specific enolase levels from days 5 to 15 (339). Patients with no delayed neurologic deficit have a monophasic peak associated with aneurysmal rupture and surgery, whereas patients with DID from VSP tend to have a

second peak at 5–10 days post-SAH. Other than neuron-specific enolase, the following are sometimes used as markers of neural injury: myelin basic protein, which accounts for 30% of myelin protein in the CNS; glial fibrillary acidic protein; and cytokines such as IL-6, tumor necrosis factor-α, and IL-1β. IL-6 is one of the cytokines that can be measured in human serum. Its elevation may lead to inflammation. Transforming growth factor-β is believed to downregulate the inflammatory response and antagonize the effect of proinflammatory cytokines such as tumor necrosis factor-α. Intracellular adhesion molecule 1 may participate in the ischemic processes through the recruitment of blood-borne WBCs (340).

Dopamine-β-hydroxylase was measured in peripheral venous blood in 24 patients post-SAH. The levels increased markedly in the first 48 hr and thereafter declined over 2 weeks. There was no significant difference in the plasma levels on admission between the patients with and without subsequent VSP revealed by angiograhy (239). Plasma renin activity was estimated in 23 patients during the first week post-SAH and compared to that of 6 control postlaminectomy patients. Levels were higher in patients who were comatose or had neurologic deficits. Patients with high renin levels (>2.5 pmol/ml/hr) had significantly higher morbidity and mortality than those with low renin levels. There were also higher levels of urinary catecholamines in the high renin group of patients. A combination of impaired consciousness, high levels of urinary catecholamines (particularly Epi), and high plasma renin activity in the first week post-SAH was associated with a poor prognosis (341).

Dopamine-β-hydroxylase serum activity is proportional to the release of NE from sympathetic nerve endings. Levels were increased post-SAH and postoperatively. No relationship was found to VSP (239).

### *7. Electrolytes*

Fourteen patients with VSP, had a $Mg^{2+}$ level of 1.87 ± 0.16 mEq/dl in serum compared to a level of 1.95 mEq/dl in 54 patients without VSP. Patients with favorable results had a level of 2.00 mEq/dl compared to an unfavorable result case level of 1.74 mEq/dl (342). In 173 operated aneurysm patients post-SAH, high osmolality shortly after admission was related to mortality but not VSP. Changes in $Na^+$ and $K^+$ had no obvious relationship to either mortality or VSP (343). Ionized $Ca^{2+}$ concentrations in whole blood from 22 SAH patients were compared to those in 14 normal volunteers. The average value of control patients was 1.23 mmol/liter compared to lower levels in patients with poor neurologic condition or severe SAH. In patients with VSP the values were significantly decreased, particularly between days 8 and 14 post-SAH (344).

### *8. Biogenic Amines*

Plasma Epi and NE levels were measured in 21 post-SAH patients and compared to those of 13 controls. NE levels were significantly increased in patients compared to controls. Plasma Epi concentrations in patients with a poor outcome were significantly higher at the time of admission compared to those of patients who subsequently had a good result. The differences became even more significant in the 2 or 3 days post-SAH (345).

Serial urine catecholamine and plasma cortisol levels were obtained for 37 patients. Fourteen days post-SAH mean levels were consistently above the normal range of apparently well patients or those of other hospitalized patients. Patients showing radiologic VSP showed significantly higher levels of urine catecholamines (346). Increased concentrations of Epi and NE were found post-SAH. Reassessment at variable intervals showed that patients whose concentrations showed a decline often had a good result. The plasma dopamine-β-hydroxylase levels remained in the normal range (347). Plasma levels of physiological amine metabolites are difficult to measure. This study was done using high-performance liquid chromotography in 14 SAH patients. In the seriously ill group the concentrations were as follows: NE, 550 pg/ml; Epi, 672 pg/ml; dopamine, 119 pg/ml; 3-methoxy-4–hydroxyphenyl glycol, 17.3 ng/ml; hydroxyphenyl acetic acid, 33 ng/ml; and 5-hydroxy-indole-3-acetic acid, 11.2 ng/ml. The only significant difference between the two groups was in plasma 3-methoxy-4-hydroyphenyl glycol, which is a major metabolite of brain NE, highly concentrated in the hypothalamus. The elevation in the hydroxyphenyl acetic acid level tended to occur in the plasma coincident with deterioration in consciousness level (348). Plasma NE concentrations in 40 patients post-SAH were measured serially. Initial abnormally elevated NE was associated with cardiac abnormalities for longer than a week. Sinus tachycardia and negative T waves tended to be positively associated with NE concentrations, but other EKG abnormalities were not (349).

A three-fold increase in total body NE spillover into the plasma occurred within 48 hr post-SAH. This sympathetic activation persisted throughout the days 7–10 period but normalized at 6 months post-SAH follow-up (350).

### *9. Neuropeptides*

Neuropeptide Y is a vasoconstrictor neurotransmitter mainly colocalized with NE in sympathetic fibers innervating cerebral blood vessels. Most of these fibers originate in the superior cervical ganglion. Serial measurements in external jugular venous blood were performed. NPY

immunoreactivity was 166 pmol/liter in 14 controls. Post-SAH increased levels up to 253 pmol/liter were correlated with increased TCD velocities. When MCA mean velocities exceeded 120 cm/sec, levels of NPY were 129 pmol/liter compared to 113 pmol/liter for those with lower velocities (351). The same group investigated ANP-LI in jugular venous plasma. Healthy volunteer patients showed no significant differences compared to the control group. One patient had very high ANP-LI levels associated with a high mean plasma $Na^+$ and high urinary $Na^+$ excretion (352).

### *10. Hormones*

Plasma levels of ANP and ADH were measured serially in 23 patients post-SAH. Eighty-five percent of 23 patients developed angiographic VSP, which was symptomatic in 65% . Low-density areas in CT scan developed in 41% and hyponatremia in 35%. Symptomatic VSP developed in 88% of patients with hyponatremia and low-density areas on CT. Plasma ANP levels were 77 pg/ml and ADH levels were 2.2 pg/ml in patients with symptomatic VSP compared to 38 and 2.4 pg/ml, respectively, in cases without symptomatic VSP. Plasma ANP levels also became significantly raised in patients with symptomatic VSP. Levels were also higher in patients who developed low densities on CT compared to those without (353).

### *11. Prostaglandins*

Plasma thromboxane levels were estimated using 11-dehydro-$TXB_2$ as a measuring index. This compound is the major long-lived metabolite of $TXB_2$. Plasma 11-dehydro-$TXB_2$ levels tended to be higher in the early stages post-SAH but decreased thereafter to normal or low normal levels. The stable metabolite of prostacyclin, 6-keto-$PGF_{1\alpha}$, tended to decrease mildly during the time of VSP predilection (354). Two of 5 patients with symptomatic VSP showed markedly raised concentrations of $TXB_2$ after day 8 (355). ADP-induced platelet aggregation and the associated release of $TXB_2$ were studied in 49 SAH patients. Patients with diffuse angiographic VSP had significantly higher levels of $TXB_2$ release than other patients, even after adjustment for clinical grade on admission and before surgery and other factors (356).

### *12. Clotting and Fibrinolytic Factors*

Patients with aneurysmal SAH showed higher levels of blood fibrinogen levels in VSP than in non-vasospastic states, and the severity of VSP was associated with the degree of elevation of the fibrinogen. Patients with marked spasm averaged about 750 mg/dl and patients with no spasm averaged about 425 mg/dl. There was also a tendency toward a decreased level of antithrombin III and a shortened PTT time in patients with marked VSP. The number of activated platelets in the systemic circulation increased over the first 3 days following SAH and leveled off on about day 6, after which it decreased. A similar tendency was seen in platelet aggregation. Fibrinogen levels also increased between days 0 and 3 post-SAH (357,358).

Blood samples from internal jugular and peripheral veins in 13 SAH patients showed high platelet aggregability during the early stages of VSP. The concentrations of β-thromboglobulin increased several days after the onset of VSP, reaching 80 ng/ml or more in patients with a poor prognosis.

Of 69 studied SAH patients, 16 developed DID. None of the wide variety of coagulation and hemorrheological variables or cardiolipin antibodies were significantly different between patients with and without later development of DID. Elevation of fibrin fragment D-dimer was found in 8 patients with ischemia on admission and 49% of all patients, but it was not associated with delayed ischemic deficit. Fibrin D-dimer levels rose significantly postoperatively and after the onset of delayed ischemia (from 4.71 to 5.84 ng/ml) (359).

Thrombin–antithrombin III complex and plasmin-$\alpha_2$ plasmin inhibitor complex levels in peripheral venous blood from 51 patients with SAH correlated with neurological grade at outset and outcome. Patients whose thrombin–antithrombin III complex levels were $\geq$25 ng/ml and inhibitor complex levels were >3.0 μg/ml had only a 25% chance of a fair or good outcome. Patients with lower levels had an 83% chance. There were no significant differences in the levels of these compounds between patients who experienced VSP and those who did not (360).

One hundred and sixty-seven patients admitted within 24 hr of the onset of SAH showed raised levels of the thrombin–antithrombin complex (elevation indicating the activation of the blood coagulation system), plasmin–antiplasmin complex, and D-dimer (elevation indicating the activation of the fibrinolytic system) which were significantly associated with increases in the neurological deficit severity, amount of subarachnoid clot, and the poor clinical outcome. Levels of the thrombin–antithrombin complexes and plasmin–antiplasmin complexes were significantly higher in patients with ICH or IVH than in patients without those hematomas. Also, for almost all the neurological and CT grades, the levels of the thrombin–antithrombin complexes were significantly higher in the patients with poor outcomes (361).

Several instances of disseminated intravascular coagulation as a complication of aneurysmal rupture have been reported, but such cases do not seem to be related to the occurrence of symptomatic VSP (362).

Plasma fibronectin concentrations are significantly lower on days 3–9 post-SAH in patients with poor outcomes compared to those with good ones. Patients with VSP had lower fibronectin concentrations during the 4 weeks following SAH (363).

### C. Changes in Vessel Wall, Leptomeningeal Cells, Brain, and Clot

#### *1. Human Studies*

*Vessel Walls*

Morphological examination of human blood vessels using fluorescence antibodies and a ferritin antibody demonstrated that Hb was distributed in the adventitia in the smooth muscle layer of the media (364).

In five cases of fatal SAH, vessel wall calcium reactive products were measured. On days 8 and 9 the volume percentage was 3.04 ± 0.36, compared to levels of 2.63 ± 0.14 on days 10–13 and 2.51 ± 0.30 on day 15 (365).

Isolated human cerebral arteries were contracted with KCl. Desensitization and tachyphylaxis greatly reduced or abolished the contractile responses to NE, 5-HT, angiotension II, arginine vasopressin, SP, NPY, neurotensin, thrombin, uridine triphosphate, linoleic acid, melittin, and cathepsin D. Some arteries failed to respond to some of these agonists. ACh or BK did not produce any contractions. Sustained contractions were produced by $PGE_2$, $-D_2$, and $-F_{2\alpha}$ as well as plasmin. $PGE_2$ and plasmin produced contractions comparable to those of KCl at concentrations of $10^{-7}M$ or less. The antiproteases leupeptin and pepstatin did not inhibit the contractile responses to KCl, whereas antithrombin III in concentrations of $6 \times 10^{-8}M$ did inhibit the contractions (366). Human cerebral vessels were found to contain CGRP-LI. The levels were significantly lower in arteries removed from patients who had fatal SAH (367). NE content of human cerebral arteries following fatal SAH was about 5% of the control group. SAH was shown to produce sympathetic denervation and subsequent alterations in $\alpha_2$-adrenergic receptors (368). Vasoconstriction of human cerebral arteries was produced by ET. The dose range was similar to that seen with other vasoconstrictor substances such as 5-HT and $PGF_{2\alpha}$. The response was resistant to antagonist of NE, isoproterenol, histamine, ACh, and angiotensin II. The ET response on these human arteries was inhibited by SNP, verapamil, and a disulfide bond reducing agent (dithiothreitol). The physiologic properties of ET were considered to be similar to those of a vasoconstrictor protein found in human CSF (369).

*Leptomeningeal Cells*

Human leptomeningeal cell proliferation was studied *in vitro*. Proliferation of these cells was stimulated by thrombin, transforming growth factor-β, epidermal growth factor, α-acidic fibroblast growth factor, and platelet-derived growth factor (370).

*Brain Changes*

Intracerebral microdialysis was performed between days 2.3 and 8.3 in 4 patients, 1 of whom had severe SAH. Concentrations of energy-related metabolites such as lactate, pyruvate, and hypoxanthine as well as multiple amino acids were measured. There was a 25-fold increase in extracellular fluid glutamate, aspartate, and taurine under conditions of energy perturbation as indicated by high levels of the lactate:pyruvate ratio, lactate level, and hypoxanthine (371). Extracellular fluid from the gyrus rectus and subarachnoid fluid were sampled by microdialysis probe following clipping of aneurysms in 11 patients. The concentrations of amino acids and nucleotides were monitored in 60-min samples collected over 2–4 days. Markedly elevated concentrations of the excitatory amino acid glutamate were observed in the extracellular fluid of only 1 patient who underwent surgery within 8 hr post-SAH. Moderate glutamate elevations were seen in 2 other patients and elevations of aspartate occurred in another. Five patients showed elevations of taurine on occasion. It was concluded that the level of consciousness in the postoperative period was inversely related to the total amino acid concentrations in the extracellular fluid. Excitatory amino acid levels were not shown to be appreciably elevated following aneurysm clipping (372).

Perilesional samples of brain tissue were obtained from 5 patients with SAH and production of PG was performed "*ex vivo*" and correlation sought with the amount of edema on the CT scan. The capacity to synthesize leukotriene C4 was significantly elevated after SAH (13.91 ± 2.6 mg/ml of incubation medium) compared with control patients ( 5.56 ± 0.91 mg/ml) (373). Brain samples of patients operated on for ACom A aneurysms were compared to those of control cases of unruptured aneurysms. Comparisons were made between tissue obtained days 1–4 post-SAH and days 10–14 post-SAH. The *ex vivo* release of $PGD_2$, $-E_2$, 6-keto-$PGF_{1\alpha}$, and leukotriene C4 was measured. There was a greater release of $PGE_2$ in patients operated acutely compared to patients with unruptured aneurysms. Patients operated acutely also showed a higher release of leukotriene C4. The release of 6-keto-$PGF_{1\alpha}$ was higher in patients operated in a delayed fashion (stable metabolite of the vasodilator prostacyclin) (374).

*Clot*

Hypertensive ICH material collected during surgery was analyzed for $K^+$ concentration. The $K^+$ level reached its peak within days 3 or 4 posthemorrhage and gradually decreased to normal over 3 weeks (375).

### 2. *Animal Studies*

*Hemoglobin*

*In vitro* microdialysis sampling of spinal fluid around subarachnoid clot in a primate model showed that perivascular concentrations of oxyHb, deoxyHb, and metHb peaked on day 2 post-SAH in control monkeys and could not be detected on days 5–12. In distinction, perivascular concentrations of oxyHb and deoxyHb peaked on day 7 after in the SAH, at which time the concentrations in the dialysate were 100 times higher than those in any sample obtained from control animals. MetHb levels increased only slightly, peaking between days 7 and 12, at which time the concentration in the dialystate was 10-fold higher than that in control animals. At the time the concentrations of oxyHb and deoxyHb were increased angiographic VSP was demonstrated. This was strong evidence that oxyHb is involved in the pathogenesis of delayed cerebral VSP and also implicated deoxyHb (376).

The trypsinogen IV genes have been detected in human brain. In a rat SAH model the level of trypsinogen activation peptide was 152 m*M* pre-SAH and decreased to 44 post-SAH (377).

*Electrolytes*

Experimental SAH was induced in dogs. Angiography demonstrated early spasm lasting a few hours and delayed vasoconstriction occurring more than 24 hr later. During both stages of vasoconstriction pH and bicarbonate ion concentration in the CSF were reduced by 20% below normal controls (378). In the dog double-SAH model, CSF showed a 3.5-fold elevation of uric acid. Parenteral administration of allopurinol (a specific blocker of xanthine oxidase) every 6 hr blocked the elevation of uric acid levels. However, angiographic VSP on day 7 post-SAH continued to show the morphologic changes of VSP by electron microscopy and CSF PG levels continued to be elevated. Although the enzyme xanthine oxidase is a potential source of oxygen free radicals in ischemia in some organs, it was not considered to play a major role in the pathogenesis of VSP (379).

*Neuropeptides*

In the primate model, sampling of blood and CSF was performed on days 0, 7, 12, and 28 postclot placement. SAH did not invoke changes in CSF or plasma levels of the vasoconstrictor transmitter NPY. NPY levels were significantly higher in CSF than in arterial plasma (380). In a rabbit SAH model, the concentration of NPY was measured in CSF 3 days post-blood injection and was found to be 5971 pg/ml, whereas in control animals the levels was 992 pg/ml. Porcine NPY caused a threefold potentiation of NE-induced contraction of rabbit cerebral arteries (381). The time course of changes in CSF immunoreactivity for the vasodilator peptide SP and CGRP contained in the trigeminal vascular system were studied following induced SAH in a rabbit model. CSF at intervals up to 3 days following a single injection of blood showed a large increase in CSF SP-like immunoreactivity and CGRP-like immunoreactivity 30 min after SAH. Arterial and hemorrhagic CSF levels differed suggesting that increased CSF levels did not result from the blood contamination alone. Immunoreactivities continued to be elevated on day 1 but returned to baseline by day 3 (382).

*Endothelin*

Serial ET levels were measured from the perivascular CSF space using the microdialysis technique and simultaneous plasma levels were measured in the primate model of SAH. There was no apparent correlation between perivascular levels of ET-1 and the development of VSP or its resolution. CSF fluid and plasma levels of ET-1 were not affected by VSP. CSF ET-1 levels averaged 9.3 pg/ml and ET-1 plasma levels 1.2 pg/ml before SAH and remained unchanged when VSP developed. In this study transient MCA occlusion was also performed. This ischemia evoked an increase in ET-1 levels in CSF (1 pg/ml at onset of occlusion vs 3.1 pg/ml after 4 hr of reperfusion). Endothelial cells and astrocytes in culture showed inhibition of ET-1 production 6 hr after exposure to hemoglobins. Hypoxia inhibited ET-1 release by endothelial cells, but with astrocytes hypoxia induced an increase in ET-1 production. It was concluded that ET-1 is released from astrocytes but not endothelial cells during hypoxia induced by transient ischemia. There is no relationship between ET-1 and VSP *in vivo* or between ET-1 and oxyHb *in vitro*. The increase in ET-1 levels in CSF after SAH is probably a result of cerebral ischemia rather than the cause of cerebral VSP (383).

The conversion of big ET-1 to ET-1 is specifically inhibited by the metalloproteinase inhibitor phosphoramidon. In the canine double-hemorrhage model on day 7 post-SAH basilar artery diameter decreased to 55% of control values at which time CSF levels of ET had significantly increased. Intracisternal pretreatment with phosphoramidon potently supressed the decrease in diameter of the basilar artery post-SAH. It decreased only 20% compared to 55% in the control group (384).

*Prostaglandins*

Eicosanoid concentrations in cortical periarachnoid fluid were studied using the cortical window technique in newborn pigs. SAH results in a 20–30% decrease in average diameter after exposure to blood for 48–96 hr. No changes in 6-keto-$PGF_{1\alpha}$ and $PGE_2$ were detected. These are dilator prostanoids. Concentrations of vasoconstrictor prostanoids in cortical CSF increased. $TXB_2$ increased to 430 pg/ml and $PGF_{2\alpha}$ increased to 1370 pg/ml compared to 250 and 860 pg/ml, respectively, in the control groups. The concentrations of peptidoleukotrienes increased to 400–600 pg/ml 4 days post-SAH, whereas the level in the control group was <80 pg/ml. The ratio of vasoconstrictor to vasodilator prostanoids therefore increased (385). *In vivo* microdialysis was performed after experimetal SAH in rats. The extracellular content of 3,4,-dihydroxy-phenylacetic acid abruptly increased after SAH, peaking at 20–40 min and then decreasing over 120 min. The contents of HVA and 5-hydroxyindoleacetic acid changed as well. These increases were attributed to a nonspecific brain stem ischemia response because similar changes were seen after cisternal saline injection (386).

In a rat SAH model CSF levels of $PGE_2$, $PGF_{2\alpha}$, and $TXB_2$ were studied 6,12, and 36 hr after cisternal injection of blood. Angiographic VSP was verified in parallel groups. CSF levels of all three eicosanoids were significantly higher in the SAH groups compared to those of both noninjected and mock-CSF-injected control rats. These increases in eicosanoid concentrations were accompanied by a decrease in mean vascular diameter to 78–82% of controls on day 2 following SAH (387).

In a canine SAH model day 8 angiography was performed. The degree of vessel constriction was linearly related to the volume of the blood injected. A volume-dependent significant increase in all eicosanoids measured was also demonstrated. Despite highly elevated CSF levels of the vasodilating eicosanoid prostacyclin, severe angiographic constriction was still present, perhaps related to the high concentrations of $PGE_2$ and $TXA_2$ (388).

*Animal Tissue Experiments*

**i. Clot** Clots were applied to the supraclinoid subarachnoid arteries of dogs. Seven days later the concentration of hemin in the clot was 390$\mu M$. Dissociated VSMC exposed to hemin (50 $\mu M$) shortened from 51.6 to 37.3 $\mu$m on average. The mean percentage permeation of $^{45}Ca^{2+}$ into vascular smooth muscle cells (VSMCs) exposed to hemin was 201% (control 103%). On a basis of hemin buildup in subarachnoid hematoma, its constrictor effect on VSMCs, and the increase in cytosolic $Ca^{2+}$, it was considered that hemin is a potential etiologic agent for cerebral VSP (389).

Xanthochromia can be observed as early as 12 hr post-SAH. The yellow color of CSF is attributable to its bilirubin content. Bilirubin formation is catalyzed by a mixed function oxidase, heme oxygenase (HO), which converts heme to equimolar amounts of bilirubin and carbon monoxide; HO has an obligatory requirement for NADH and $O_2$. HO is stimulated by its substrate, heme. HO activity in both arachnoid and choroid plexus tissue of rats was strongly stimulated by the subarachnoid injection of methemalbumin, which contains 2.5 $\mu M$ of heme per milliliter. The experimental injection of heme, heparinized blood, or Hb solution induced a four fold increase in HO activity in arachnoid membrane and choroid plexus. HO is composed of at least two isozymes, HO-1 and HO-2. HO-1 is the heat shock protein HSP32 and is inducible by many factors, including heme and heat shock. Following SAH in rats large increases in HO-1 immunoreactivity were seen throughout the brain. This followed injections of lysed blood, whole blood, and oxyHb but not saline. HO-1 immunoreactivity was greatest in the regions adjacent to the basal subarachnoid cisterns where blood and oxyHb concentrations were highest. HO-1 was mainly in microglia but also occurred in some astrocytes (390).

The subarachnoid clot present in dogs 8 days after SAH injection contained significant amounts of 12-HETE (1.8 nmol/g wet weight). The activities of 12- and 5-lipoxygenases were significantly increased in the subarachnoid clot and the basilar artery after SAH (263).

**ii. Blood Vessels**

*Enzymes.* In canine basilar artery undergoing chronic VSP the turnover of phospholipids was increased on day 7. The cytosolic PKC activity was downregulated on days 4 and 7, whereas membrane PKC activity remained unchanged (391). In a rabbit basilar artery 2 hemorrhage model a significant decrease in protein phosphatase 1 activity occurred on days 2 and 4 in spastic arteries. A similar significant reduction in protein phosphatase 2A occurred in the spastic vessels at the same time. Vasoconstriction induced by local application of PKC or 5-HT failed to produce similar reductions. Protein phosphatase 1 in a smooth muscle extract catalyzes the dephosphorylation of myosin light chain and calponin, whereas protein phosphatase 2A in cytosolic extract catalyzes the dephosphorylation of calponin and caldesmon. The phosphorylation of calponin and caldesmon results in their inability to inhibit smooth muscle contraction. The decreased activity of these phosphatases might contribute to VSP since they are unable to inhibit the continued phosphorylation of not only MLC but also calponin and caldesmon

(392). The actin–tropomyosin-binding protein calponin has an inhibitory effect on smooth muscle actomyosin activity. In a canine double-hemorrhage model there was a reduced expression of calponin in basilar artery extract on days 2 and 7 post-SAH. The proportions of calponin to actin–tropomyosin on days 0, 2, and 7 were 13, 6, and 4%, respectively (393).

*Potassium.* In a rat SAH model basilar arteries were removed at various time intervals and the electrolyte concentrations of the vascular smooth muscle cytoplasm were measured. Extracellular $K^+$ in the vessel wall increased up to eight times baseline 24 hr post-SAH. The cytoplasmic concentrations of $Ca^{2+}$, $Na^+$, and $Cl^-$ also increased. The $K^+$ concentration in extravasated RBCs decreased over 24 hr (375).

*Nucleotides.* In rabbits following SAH the mean ATP content decreased from 0.38 to 0.17 μmol/g 2 days post-SAH. Hypoxia significantly decreased ATP content in control arteries. The same degree of hypoxia did not decrease the ATP in the basilar artery post-SAH. The release of L-lactate was significantly higher from arteries post-SAH than from control arteries under both aerobic and hypoxic conditions (394). Spastic canine arteries post-SAH showed markedly reduced levels of ATP, guanosine triphosphate, and creatine phosphate. The ratios of ATP to ADP, guanosine triphosphate to guanosine diphosphate, and creatine phosphate to total creatine were all significantly decreased as well. Results demonstrated a serious perturbation in energy metabolism during chronic VSP (395). The time course of reductions in these metabolites was studied in the same model. The nadir for guanosine triphosphate was day 3 and that for ATP and creatine phosphate day 7. Total adenylate (ATP + ADP + AMP) and total creatine (creatine + creatine phosphate) content diminished markedly over the 14 days of this study. Some angiographic recovery was observed in the absence of metabolic recovery (396). Purified Hb solution or autologous whole blood was injected into the cisterna magna of pigs. Intra but not extracerebral vasoconstriction resulted, which persisted for up to 7 days. cGMP was measured in involved arteries to quantify the inhibition of vascular endothelial-derived relaxant factor by Hb. The depression of cGMP levels by Hb was reversible and equivalent to the effect of endothelial denudation or incubation with NG-nitro-L-arginine methyl ester. The effect of Hb was attributed to a specific action on endothelial-derived relaxant factor rather than an interaction with NO-like substances produced by vascular smooth muscle or adventitial nerves. After 7 days of *in vivo* exposure to whole blood cGMP levels were not further reduced by intraluminal perfusion with 1 μ*M* Hb. Exposure for shorter times did not prevent reduction by this maneuver. Adventitially applied Hb inhibits basal endothelial-derived relaxant factor (EDRF) activity and reduces basal cGMP levels (397). The cGMP content in rabbit arteries 2 days post-SAH was significantly lower than control artery content (31.5 vs 57.3 pmol/g tissue). In preparations incubated with SP the cGMP increased to 440% in controls but only 97% in arteries post-SAH. This indicates an impairment of vasodilator activity (398). Mild VSP in a single SAH canine model was associated with a slight decrease in arterial cGMP level on day 4 that returned to baseline on day 7 post-SAH. PKC activity was slightly enhanced throughout this time. In a 2-hemorrhage model that produced severe VSP, a significant decrease in cGMP was observed on day 5 that persisted until day 7. There was a remarkable enhancement of PKC activity from day 5 until day 7. It was hypothesized that SAH disturbs the feedback control exerted by NO on PKC activation leading to a PKC-dependent VSP (399).

*Neuropeptides.* Following SAH injection in rats the density of 5-HT-containing and NPY-containing perivascular nerve fibers per unit area of vessels was measured for up to 5 days post-SAH. In addition, high-performance liquid chromatography studies were performed. There was a major increase in sympathetic nerve content of 5-HT arising by uptake from clot within the first 3 hr post-SAH. NPY content decreased from 3 up to 48 hr post-SAH. The majority of clot was absorbed by 3 days and the sympathetic nerve content of both NPY and 5-HT had returned to normal (400). Rat femoral arteries exposed to periarterial blood were examined for up to 14 days following clot application. The expression of procollagen types I and III messenger RNA increased threefold at day 7 post-clot application. The expression of transforming growth factor-β, an important regulator of collagen synthesis, was markedly increased by 3 days after clot application and then gradually declined. Cultured VSMC treated with higher concentrations of serum increased the expression of procollagen types I and III and transforming growth factor-β but exposure to oxyHb did not (401).

*Leukotrienes.* Leukotriene production from incubated basilar artery was studied in a canine SAH model. The increased production of leukotriene C4, D4, and E4 varied by time post-SAH and tissue examined. In the blood clot the biosynthetic capacity of these leukotrienes increased continually after the first injection of blood (402). Leukotriene formation in isolated basilar arteries of dogs was examined. The calcium ionophore (A 23187) resulted in production of a significant amount of leukotriene B4 and C4, in addition to 5-(*S*)-hydroxy-

6,8,11,14-eicosatetraenoic acid. SAH prominently activates 5-lipoxygenase, which is not normally detectable in basilar artery. This resulted in the production of 2.1 nmol 5-HETE/5 min/mg of protein (408).

*Cytoskeletal Matrix Proteins.* In the primate model spastic arteries were examined on days 7, 14, and 28. Immunohistochemical staining for collagens, desmin, myosin, laminin, or α-actin was utilized. Fibronectin immunoreactivity increased 14 days after SAH. Seven days post-SAH occasional areas of tunica media showed immunoreactivity to fibrinogen. Intimal thickening on day 28 demonstrated immunoreactivity to α-actin, myosin, vimentin, desmin, fibronectin, laminin, and each type of collagen. No significant increases in the number of intimal cells showing immunoreactivity to α-actin were seen and no significant change in hydroxyproline content of cerebral arteries was seen at any time post-SAH. The results were interpreted to show that the rigidity in luminal narrowing of VSP was not due to increased arterial collagen, although other proteins in the wall might relate to these changes. There was no indication that smooth muscle contractile proteins changed during VSP or that an increase in the number of α-actin-containing myointimal cells contributed to VSP. The occurrence of intimal thickening and the medial increases in fibronectin suggested that VSP damages smooth muscle (403).

#### iii. Brain

*Nitric Oxide Synthase.* In a single hemorrhage porcine SAH model, nitric oxide synthase (NOS) immunoreactivity was assessed semiquantitatively. Endothelial NOS nitrotyrosine rose rapidly, peaking at 12 hr and then subsiding to rise again 4–7 days post-SAH. Neuronal NOS rose rapidly and then fell permanently. Inducible NOS was progressively expressed by macrophages peaking at 7 days and returning to baseline by 14 days (404).

*Free Fatty Acids.* Following SAH in rats, brain lactate levels rose significantly, as did the free fatty acid levels; the largest component was stearic acid. These measurements done in the first hour post-SAH showed that significant degradation of cell membranes occurs in the first minutes after SAH (405).

*Free Radicals.* Following SAH induction in rats, various brain areas were assayed for up to 48 hr. After the hemorrhage the Cu–Zn and Mn SOD were all significantly reduced (406). In this model, lipid peroxidation (quantified as thiobarbiturate reactive substance content) did not show significant changes. There was a concurrent enhancement of the release of leukotriene C4. Glutathione peroxidase was only significantly reduced 48 hr post-SAH, unlike the earlier reductions in the SOD (407).

*Gene Expression.* The expression of c-fos protein was examined by immunohistochemistry in brain stem sections following mock CSF or SAH injections into rats. Blood in the subarachnoid space was an effective stimulus to activate c-fos expression within subpopulations of brain stem neurons. The c-fos expression within the nucleus of the solitary tract and area postrema may reflect a direct blood response leading to activation of the autonomic system (409).

## VI. Cerebral Infarction from Vasospasm

### A. Physiology of Aneurysmal Rupture and Vasospasm

The brain normally receives about 15% of the cardiac output (410). Middle cerebral flow has been measured at 97 ml/min and proximal anterior cerebral arterial flow at 65 ml/min. The brain normally has a high and constant blood flow. In response to regional changes in metabolism, however, the regional cerebral blood flow (rCBF) can vary greatly (411). The resistance of the cerebral vasculature changes in relation to the CPP and the metabolic needs of the brain. This varies in such a way as to produce an adequate and relatively constant flow over a wide range of pressures in both physiologic and pathologic conditions. Complete cessation of blood flow for 6–15 sec can result in loss of consciousness and if extended for 3–15 min cerebral infarction results.

The major extrinsic physiologic factor governing CBF is the CPP, which is the MABP minus the ICP and the arterial blood gas tensions. Hypercapnia causes dilation and hypocapnia causes constriction of cerebral blood vessels. In the normal range of arterial pressure of $P_aCO_2$, CBF changes 5% for each 1 mmHg change in $P_aCO_2$. $P_aCO_2$ effects override the $P_aO_2$ changes until levels of extreme hypoxia (± 50 mmHg) are reached. CBF varies directly with $P_aCO_2$ and inversely with $P_aO_2$. An increase in $P_aCO_2$ of 1 mmHg is associated with an increase of CBF of 2 ml/100 mg/min and increase in cerebral blood volume (CBV) of 0.04 ml/100 g in the physiologic range. Failure of cerebral $O_2$ transport leads to increased lactic acid production (412). Hydrogen ions accumulate and hyperemia results. If CBF is excessive for a particular level of cerebral metabolism, venous blood is relatively hyperoxygenated. Cerebral acidosis and venous hyperoxemia may be associated with generalized hypoxia, ischemic infarction, and SAH. Cerebral edema may be

aggravated by the loss of normal vasomotor control over resistance vessels. The constancy of brain tissue pH is safeguarded by local changes in CBF, changes in pulmonary ventilation, the buffering capacity of the brain, and metabolically induced $HCO_3^-$ changes in the cerebral tissue. The ratio between arterial $P_aCO_2$ and CSF $HCO_3^-$ is of importance in determining how the tone of VSMC will vary. The pH of CSF is normally lower than that of the blood (7.30 vs plasma pH 7.40) (413). In patients chronically hypercarbic, acute reduction in the $P_aCO_2$ can result in ischemic cerebral dysfunction because the CSF $HCO_3^-$ levels have undergone compensatory changes. Passive hyperventilation in the presence of high CSF $HCO_3^-$ concentrations can produce extreme cerebral vasoconstriction. Since severely brain-damaged patients are usually already hyperventilating and optimally hypercarbic, additional passive hyperventilation may not induce desired reductions in ICP.

Ischemic brain hypoxic signs begin when $P_aCO_2$ tensions are reduced to around 20 mmHg in normal subjects. The cerebral vasculature in injured areas of the brain, however, may be refractory to hypocapnia or vasoparalyzed. In a primate model of SAH there was reduced $CO_2$ reactivity. A large increase in CBF was demonstrated only when $P_aCO_2$ was raised to extreme levels (60–65 mmHg) acutely post-SAH. $CO_2$ regulation was present in 81% of grade I and II patients; however, 79% of grade III and 70% of grade IV patients showed impairment of chemical regulation (414).

Extracranial and intracranial blood vessels are innervated by both adrenergic and cholinergic nerves. Most of these originate from the extracranial and parasympathetic autonomic nervous systems. There is no evidence for tonic neurogenic control of CBF. Continued maximal stimulation of the superior cervical ganglion produces a decrease in CBF of 5–10%. Although many drugs produce dramatic changes in arteriolar tone when injected into the lumen of systemic arterioles, they often do not affect CBF. For instance, intracarotid injection of NE does not change CBF and apparently does not cross intact cerebral vascular endothelium (415). We are unaware of the effects on ischemic areas where the arterial blood vessel barrier may become variably permeable. Adrenergic innervation of extraparenchymal vessels is more plentiful than that around intraparenchymal arteries and arterioles. There is evidence for a central noradrenergic system directly innervating cerebral blood vessels. A parasympathetic supply to the pial arterioles is probably carried by cranial nerve VII. There is a growing list of substances carried in the perivascular system which includes VIP, SP, neurotensin, 5-HT, gastrin-releasing peptide, cholecystokinin, NPY, somatostatin, and noradrenergic fibers. The autonomic nerve fibers are located in the loose connective tissue abutting the adventitia of arteries. There is close cytoplasmic contact between the smooth muscle cells and nerves (416).

### B. Impairment of Autoregulation

The brain possesses an intrinsic mechanism by which its vascular supply can be varied locally to respond to local variations in functional activity. Vascular smooth muscle normally has tone in response to normal blood pressure. Lowering that pressure can be followed by a relaxation of vascular smooth muscle. This is known as the Bayliss effect. This reactive tendency enables the arteries to maintain a constant blood flow through the tissues regardless of the height of the systemic blood pressure. This is an intrinsic reflex in the wall of the cerebral arteries and results in constriction in response to hypertension and dilation in response to hypotension. Myogenic autoregulation results in the maintenance of relatively stable CBF in the face of a changing CPP. Normally autoregulation is operative between about 60 and 160 mmHg (417). Above this level, MABP hypertensive breakthrough occurs, the BBB is damaged, and there is forced vasodilation and focal plasma leakage. In chronically hypertensive patients the autoregulatory curve is moved to the right so that the CBF may fall at relatively higher levels of MABP than in normotensive patients. This may result from the fact that there is a structural vascular adaptation to hypertension with wall thickening and luminal narrowing. Cerebral ischemia seldom results from antihypertensive treatment because pressure can be lowered about 25% before the lower limit of autoregulation is reached in hypertensives and by about 50% before symptoms of ischemia are produced. Although blood pressure may fall below the lower limit of autoregulation, the brain may still be able to compensate by increasing the $O_2$ extracted from the blood. Typically, the $O_2$ content is approximately 60% in internal jugular venous blood, which indicates that there is still a comfortable margin of $O_2$ supply that could be extracted by the brain. This is higher than the $O_2$ content in the coronary sinus of the heart, which is only 20–30%.

SAH is a common cause of loss of autoregulation. Since impairment of autoregulation is highly variable in different brain regions, a reduction in CPP may cause extreme ischemia in some areas but not others. The focal regions of edema can impair autoregulation so that hypotension may produce a dangerous fall in CBF despite the fact that overall ICP and CCP are normal. Because venous pressure is only slightly greater than ICP, the difference between MABP and ICP is a reasonable approximation of CCP.

In 34 patients having CBF responses to hypotension and hypocapnia studied between days 3 and 13 post-SAH, a good correlation was found between poor neurological grade and a greater degree of impairment of pressure autoregulation. The reduction of CBF with hyperventilation was preserved but was less than normal. Values for 11 patients without VSP and 11 patients with severe VSP were respectively as follows: ICP, 12 mmHg without and 28 mmHg with; rCBF (ml/100 g/min) before and after a reduction in MABP of 13%, 44/43 without, and of 12%, 34/30 with; rCBF before and after a decrease in $P_aCO_2$ of 9 mmHg, 43/32 without, and 7 mmHg, 32/25 with. The use of hyperventilation in poor-grade patients therefore produces complex and unpredictable effects. Injurious effects of declining CBF may or may not be countered by a decrease in ICP.

### C. Cerebral Edema

Edema is defined as an increase in $H_2O$ and $Na^+$ content of the brain (418). Normal whole brain is 78% $H_2O$. Cerebral edema may be classified as as vasogenic, cytotoxic, or interstitial. The most common form is vasogenic, which results from an increased permeability of damaged brain capillary endothelial cells. The increased permeability of various markers is inversely related to their molecular weight. Edema fluid is composed of plasma filtrate including plasma proteins. In cytotoxic edema all the cellular elements of the brain swell and there is a resultant reduction in brain extracellular fluid space. Interstitial edema is an increase in the $H_2O$ and $Na^+$ content of the periventricular white matter due to the movement of CSF across the ventricular walls. Obstructive Hyc is an example (419). The extracellular space of the brain is generally considered to be approximately 14%. Progressive swelling of the brain leads to various types of herniation.

### D. Cerebral Volume Changes

When patients die within hours or a few days after SAH, the brain usually shows significant swelling. This can be attributed to increased CBV and/or cerebral edema.

Intracranial blood volume is mainly in the venous sinuses and the veins of the pial circulation (420). The CBV is approximately 2–4 ml/100 g brain tissue. The major controlling factors for CBV are $P_aCO_2$ and local metabolic needs of the brain tissue. CBF changes are associated in a linear fashion with CBV. If autoregulation is abolished, CBF and CBV respond passively through changes in MABP. In normal brain at a $P_aCO_2$ of between 20 and 80 mmHg, CBV increases 0.04 ml/100 g for every increase in $P_aCO_2$ of 1 mmHg (421,422). In pathologic states such increases can affect ICP adversely. Autoregulation is abolished in ischemic areas that become acidotic and edematous. At this point, flow passively follows CPP. A small decrease in blood pressure can cause marked ischemia and elevations of CBF may induce increases in CBV and ICP, resulting in enhancement of severe edema formation.

PET studies conducted on patients post-SAH found an average CBV in normals of 3.6 ml/100 g, in grades III and IV post-SAH it was 3.9 ml/100 g, and in grades III and IV with VSP it reached 5.7 ml/100 mg. CBF in normals was 54 ml/100 g/min, which decreased in grades III and IV post-SAH patients to 35 ml, reaching 33 ml if VSP was present. $CMRO_2$ was normally 3.6 ml/100 g/min, falling to 3 ml/100 g/min in grades III and IV aneurysm patients without VSP and 2.5 ml/100 g/min in such patients with VSP (423).

### E. Cerebrospinal Fluid and Intracranial Pressure

CSF is formed at the rate of 0.35 ml/min; 80% is produced by the choroid plexus and 10–20% may be produced by the brain interstitium (420). Unless it is extremely high, the ICP does not determine CSF formation rate. CSF is not a simple plasma filtrate but is actually produced by secretion and osmosis (424). Protein concentration averages 25 mg/100 ml of CSF compared with the plasma concentration of 6500–7500 mg/100 ml. Most of the CSF is absorbed superiorly via the arachnoid villi lying in close proximity to the major venous sinuses. The villi function as if they were "valves" that open at 5 mmHg and the rate of passage of CSF through them increases with increasing ICP (23,37,425). The valves permit fluid to pass only in the direction of CSF to blood. A lesser amount of CSF is absorbed around spinal nerve roots. Many of the events that follow aneurysmal rupture can disturb CSF circulation, including, brain shift, obliteration of subarachnoid spaces by clot, and obstruction of the absorption pathways. The one-way flow of CSF is termed bulk flow since all constituents of CSF leave with the fluid.

The contents of the intracranial space consist of brain tissue water, CSF, and intravascular blood with the compartments being contiguous and having interchangeable volumes but the overall content being essentially incompressible. The readily displaceable elements of the intracranial fluids are blood and CSF. CSF volume may be reduced by an increase in CSF absorption rate, a decrease in CSF production rate, or both, as well as by displacement of fluid from the intracranial to the intraspinal space (426). With rupture of an aneurysm it is likely that the immediate buffering is by the latter mechanism.

Normally, the ICP trace is of low amplitude with fluctuations. As compliance decreases the height of the baseline and the amplitude of the pulse waves of ICP both increase. The ICP waveform consists of miniature arterial waves superimposed on sinusoidal respiratory waves. With an elevation of ICP the arterial waves increase in amplitude and the respiratory waves decrease. Increases in ICP below the level of MABP have no effect on pulse rate, respiratory rate, or systemic blood pressure (427). Variations in systemic blood pressure, pulse, respiratory rate, and the level of consciousness, either alone or in combination, do not allow one to reliably infer the level of ICP (428). There are no absolutely pathognomonic signs or symptoms of increased or decreased ICP. Headache, papilledema, unilateral pupillary dilation, oculomotor or abducens nerve paresis, and irregular respirations raise the suspicion that such a state exists (428). The foundations of therapy for acute intracranial hypertension are the provisions of a patent airway, adequate oxygenation, and modest hyperventilation. In the presence of severe VSP and a developing infarction, ICP increases and a brain shift usually develops. The absolute level of ICP may be of less importance in determining the ultimate extent of neuronal damage than the development of brain shifts and distortions.

Surgery can exacerbate the deleterious effects of ischemia due to VSP by subjecting regions of the brain to retractor pressure. In animals subjected to a brain retractor pressure of more than 40 mmHg for 1 hr, somatosensory cortical envoked responses were absent at 24 hr, there was extravasation of Evans blue dye, and infarction was seen on histologic sections 3 days following surgery. Lesser degrees of pressure were associated with variable loss of evoked potentials and a lesser degrees of breakdown of the BBB to dye. Edema, but not infarction, was noted pathologically. Since the brain is semisolid, stresses and strains applied locally can exceed regional blood pressure and induce discrete cerebral ischemia. It is clear that extensive surgical dissection and retraction performed in the period of maximal vasospastic ischemia is likely to add to the surgical morbidity. The key question is always whether or not this is more than compensated for by the reduction in morbidity and mortality from rebleeding, the intraoperative removal of clot, or the postclipping opportunity to be more aggressive in the treatment of VSP.

Intracranial contents consist of solid brain and intracellular water (80–85%), blood (3–6%), and CSF (5–16%) (412). A change in volume of one of these components must result in a reciprocal and opposite change in the volume of one or both or the others, owing to the incompressibility of the contents. The primary means of compensating for increased volume intracranially is by the increased translocation of CSF from intracranial to extracranial storage sites and into venous blood by increased reabsorption. Normally, CSF can move freely into the expansile spinal subarachnoid space and has access to the arachnoid villi reabsorptive sites mainly along the superior sagittal sinus. To some extent, increased CSF absorption is pressure dependent to an upper limit of 30 mmHg (429). ICP increases when CSF isobaric spatial compensation is exhausted. Additional spatial compensation may be obtained by reducing the intracranial CBV. The low-pressure venous or capillary beds may be collapsed, leading to cerebral ischemia and increased cerebrovascular resistance. When bridging veins are compressed there is an additional transmitted increase in back pressure to the cerebral capillary bed, which further elevates the ICP and increases CBV by causing stasis in the vascular bed. This in turn can accelerate the formation of cerebral edema fluid. The intracranial volume of an abnormal mass can increase in a given patient for some time before ICP begins to rise. The rate of increase in pressure is initially slow and then becomes progressively more rapid. The compliance curve is the slope of the change in pressure for changes in intracranial volume. The shape of this curve is related to factors such as lesion size and location, rate of expansion, plasticity of the skull and the presence of fontanelles or surgical openings, the occurrence of brain herniations, the level of blood pressure, and $P_aCO_2$ (430).

The brain is relatively incompressible to change in volume. As long as there is displaceable blood or CSF within the cranium, an increase in intracranial volume causes only a slight transient elevation of ICP. Once all of the CSF and blood that can be moved have been displaced further increments in foreign masses may cause a large increase in ICP and the cranium is said to be tight. It is important during the period of maximum risk of infarction that repeated objective measures of the patients' neurologic status be performed by the nursing staff. Repeated CT scans should be performed in the event of significant clinical change. Continuous monitoring of the ICP should also be performed in patients at high risk of infarction.

While the adult human brain is relatively incompressible, it may show plastic creep with time (420). The compliance of the brain will determine pressure changes resulting from a given volume increase of a space-occupying lesion such as a vasospastically induced infarct (410). Sustained elevations of ICP can result from increased dural sinus pressure, an increased rate of CSF formation, or an increased resistance to CSF absorption such as occurs after SAH (428). Compliance refers to the first derivative of the pressure–volume curve ($dV/dP$) which varies inversely with the pressure. Compliance is a

measure of the stiffness or rigidity of the craniospinal compartment, which is derived principally from the elasticity of the craniospinal venous bed. The viscoelastic properties of the brain and the spinal cord also contribute to cerebrospinal compliance. SAH has prompt effects on the CSF system because the resistance to its absorption is increased since blood cells and plasma proteins increase CSF viscosity and plug arachnoid villi. The rapid addition of subarachnoid blood to the intracranial contents leads to a rise in CSF pressure, which is one determinant of craniospinal compliance. Compliance is equal to an increase in volume divided by an increase in pressure and is the reciprocal of elastance. In low-compliance states, small increases in volume may produce fatal elevations in ICP. As ICP increases, compliance decreases and the brain is relatively tighter (428). The sudden addition of volume to the subarachnoid space such as occurs with SAH is probably different from sudden bleeding into the brain parenchyma. In an experimental model, volume loading of the epidural compartment produced a much larger increase in CSF pressure than loading in the CSF space (431). The fatal volumes of blood are greater for subarachnoid space injection than for brain injection in several animal studies.

Various radioactively labeled blood products were injected into dogs via cisterna magna and lumbar subarachnoid (SA) catheters. The dosage that was lethal in half the dogs was as follows: plasma, 12 ml/kg; anticoagulated blood, 5.75 ml/kg; and unaltered whole blood, 1.9 ml/kg. A plasma rhinorrhea was commonly observed when the injected volumes were above 8–10 ml/kg. At least three factors were involved in the initial fate of blood released into the SA space: volume, rate of injection, and the increase in SA fluid pressure. Albumin molecules reached the systemic system faster than RBCs (432). In another canine study, blood was introduced by an extracorporeal femorointrathecal shunt or by injection into five different sites. RBCs were the component that induced an increase in the outflow resistance by clogging the pathway of CSF. The lethal volumes were specific for each site: for brain parencyhma, 8.1 ml; lateral ventricle, 16.2 ml; chiasmatic cistern, 17.1 ml; cisterna magna, 30 ml; and spinal subarachnoid space, 55 ml. Intrathecal infusions of saline showed that spatial decompensation rather than cumulative ischemic effects caused death (433). In a similar canine autologous shunt model of SAH, the duration and volume of hemorrhage into the suprasellar cistern were measure in relationship to variable controlled flow rates. At high rates of bleeding (18.7 ml/min) the duration of hemorrhage was 191 sec and hemorrhage volume was 15.1 ml. At low flow rates (4.4 ml/min) the duration of hemorrhage was 394 sec and the volume was 10.9 ml. CPP decreased at all hemorrhage rates but never decreased to 0 mmHg (perfusion arrest). The initial flow rate of SAH had a positive linear correlation with the volume of hemorrhage. Presumably in humans the size of the initial rent in the aneurysm and the blood and CSF pressure at the time would have a primary influence on the volume of the SAH (434).

By analogy, if we assume a human intracranial SA space volume of 100 ml and if the volume injected into the basal cistern can be twice the volume that can be acutely tolerated in the brain parenchyma (in humans this can range between 80 and 120 ml), it is possible that in severe bleeds virtually the entire volume of cranial subarachnoid CSF could be displaced. Obviously, there must be a point at which the concentration of blood in the blood CSF mixture would be sufficient to induce clotting rather than simple staining of the still liquid CSF.

The viscoelastic properties of the brain can be adversely changed by hypoosmotic brain edema, drug-induced hypotension, hypercapnia, hypertension, hypercapnic anoxia, and cerebral vasodilation even at a time when ICP is normal. In the presence of space-occupying lesions such as regions of cerebral edema, hematomas, or dilated ventricles, the capacity of the intracranial volume to compensate for an increase in volume declines.

When aneurysms rupture in humans the ICP may rapidly rise to the level of diastolic blood pressure and end-diastolic flow arrest may occur (435). These very high pressures may last for only seconds or minutes, and during these episodes there is ischemic–anoxic global brain damage that accounts for the deaths from the initial episode in the subsequent days. This mechanism is entirely independent of VSP but the adverse effects of both processes can obviously be additive.

Induced arterial injuries in primates cause rapid rises in ICP and significant reductions in CPP to an average of 37% of normal in one study (436). Even after short periods of extreme ischemia significant increases in $H_2O$ content of all areas of the brain have been demonstrated (437). Hyc is an abnormally increased volume of CSF in the craniospinal axis and is always associated with lateral ventricular enlargement (418). Obstructive Hyc results from a blockage at some point in the CSF pathway between the lateral ventricles and the outflow foramina of the fourth ventricle. The second major type is communicating , in which CSF can reach the cisterna magna and communicate with the spinal subarachnoid space but in which an obstruction blocks free upward passage around the brain and over the vertex. Following SAH, adhesions in the tentorial hiatus or the engorgement of the arachnoid villi by RBCs and fibrin can cause communicating Hyc.

The brain swelling that occurs acutely after SAH can mask the dilation of the lateral ventricles in the face of

CSF outflow blockage. Vasospastic infarction tends to occur toward the end of the first week post-SAH, earlier than ventricular enlargement from obstructive Hyc post-SAH. For a week or two, however, these processes can overlap. In one series of SAH/aneurysm patients, ICP preoperatively averaged 51 mmHg and postoperatively 30 mmHg. Of 30 patients, in only 1 were plateau or A waves observed (A waves, 5–20/min, 50–100 mmHg). B waves were commonly seen (B waves, 0.2–2/min, <50 mmHg) (438). Higher ICP correlates with poorer clinical grades (439). Since both the occurrence of clinically apparent VSP and post-SAH Hyc are indicative of larger volume hemorrhages, patients with VSP have a higher incidence of shunt requirement and vice versa. In one series, 36% of 73 patients with VSP required a shunt compared to only 16% of 68 patients without VSP (440). It has been suggested that if patients had large volumes of CSF removed by drainage, there may be an increased necessity of subsequent shunting. Fifty-nine percent of patients having such large volumes drained were shunted compared to 22% of those in which lesser amounts were drained. In this group of patients the enlargement of the ventricles tended to occur after the third week post-SAH (440).

In the early phases of diffuse VSP, the ICP tends to be low but it then rises as infarction progresses and serious neurological deterioration occurs. Most patients with diffuse VSP who show a late increase in ICP also develop late enlargement of the ventricular system. Widespread low-density areas occurred on CT scan in 69% of one series. In the absence of late increases in ICP the development of low-density areas was reduced to 25%. CBF in this series was 48 ml/100 g/min if no VSP developed. Diffuse VSP patients had a CBF of 35 ml/100 g/min that decreased to 25 ml/100 g/min during the phase of high ICP (441).

Some patients with full-blown normal-pressure Hyc do not have raised ICP in the chronic phase despite the fact that CBF may be markedly reduced. The CBF reduction may reflect reduced cerebral metabolism (442). Drainage of CSF tends to improve the condition of patients unless they have established severe VSP or structural damage from the initial episode or rebleeding. In 52 patients, ICP averaged 10 mmHg in grades I–II patients, 18 mmHg in grades II and III patients, and 29 mmHg in grades III–V patients. Patients with no VSP showed a mean ICP of 16 mmHg compared to patients with severe VSP who had a mean ICP of 27 mmHg. None of the patients who were ultimately disabled had an ICP lower than 10 mmHg. Plateau waves were not observed. Development of VSP was associated with an increase in ICP, with the pressure peak usually culminating between 4 and 8 days post-SAH. In this series, VSP and acute Hyc did not develop independently but did develop in the same time period. Consciousness was impaired and adverse neurologic signs developed above the 20–30 mmHg range. The failure to obtain a beneficial effect in some patients with DID and poor neurologic grades from ventricular drainage was attributed to the fact that the severely constricted arteries supplied locally damaged and swollen brain regions in which local tissue pressure remained high despite the fact that ventricular pressure was lowered by fluid removal (443).

The following are disadvantages of ventricular catheterization to record CSF pressures: (i) the brain trauma created by the catheter, (ii) the possibility of introducing intracerebral or intraventricular hemorrhage, (iii) the establishment of a pathway that can permit infection to enter the ventricles, and (iv) the inadvertent excessive removal of CSF which may induce subdural clots or brain herniations. Notwithstanding these risks, it is probably a good idea to normalize ventricular CSF pressure in a patient who is in the process of developing infarction from vasospastic ischemia. Although this does not attack the root cause of infarction, it eliminates the possibility of an additive adverse factor. Various authorities recommend CSF drainage at different levels ranging between 15 and 25 mmHg. We tend to drain CSF at the lower value. The first line of defense against elevated ICP is the provision of a good airway, hypothermia, paralysis, and controlled ventilation. Ventricular drainage is usually instituted in poor-grade aneurysm patients, in the acute phase of SAH, who have normal or enlarged ventricles. Mannitol is the mainstay of medical therapy. Barbiturates or propofol anesthesia are used if other measures have failed or in the rare circumstance of acute brain swelling (as opposed to cerebral edema). In extreme circumstances, surgical decompression is employed.

At a certain point the risk of infection becomes greater than the benefit of normalizing CSF pressure post-SAH. We begin to assess the effect of clamping the drain and simply recording the pressure beginning about 10 days post-SAH in patients at high risk of VSP.

### F. Cerebral Blood Flow

Blood flow is directly related to CPP and the fourth power of the radius of resistance vessels and is inversely related to whole blood viscosity. Blood has different viscosities at different shear rates, so the classic Poiseuille equation is not directly applicable. Rouleaux of RBCs break up in small vessels and shear rates increase with a decrease in viscosity. This is abruptly reversed in the capillaries when the diameter of the RBCs is slightly larger than the capillary and RBCs must deform to pass through them. When vessel diameter is reduced, the velocity of blood flow through the stenotic region can increase and

maintain constant flow until the cross-sectional area is reduced by about 70% (444).

When ICP rises and reaches the level of MABP, CBF slows and stops. After CBF has been stopped for a while, it may be impossible to restore flow. Local failure of reperfusion is called "no-reflow" phenomenon. If flow is restored to a previously ischemic area, the flow may be much higher than normal (by a factor of 2 or 3) because vasoactive metabolites accumulate or there is local vasoparalysis, which is called reactive hyperemia. Following SAH in animals, the maximum rate of catalysis for different aerobic and anaerobic enzymes did not significantly change. Mitochondrial respiration was affected particularly in the presence of glutamate or malate, which produce NADH (445,446). Long-term microdialysis sampling of brain extracellular fluid has been performed in a case of SAH. Glutamate, aspartate, and taurine were considerably increased under conditions of energy perturbations as indicated by high lactate: pyruate ratios (371, 447) (Figs. 4.31 and 4.32).

In experimental animals following blood injection into the CSF there is an increased permeability to horseradish peroxidase in the brain stem adjacent to cisternal clot. Sudden increases in ICP can increase the ability of tracer substances to penetrate brain artery walls. It has been suggested that this pressure change, rather than actual contact with SAH, may be an important factor in the breakdown of the blood vessel wall barrier (448). Following SAH horseradish peroxidase particles can enter the arterial wall from either the luminal or the abluminal side. Such studies suggest that spasmogens may also penetrate the wall from either side (449).

Loss of consciousness can be anticipated when MABP falls below 40 mmHg or $P_aO_2$ falls below 50 mmHg in patients without chronic lung disease. Complete cessation of CBF for 5 or 6 min results in irreversible loss of useful

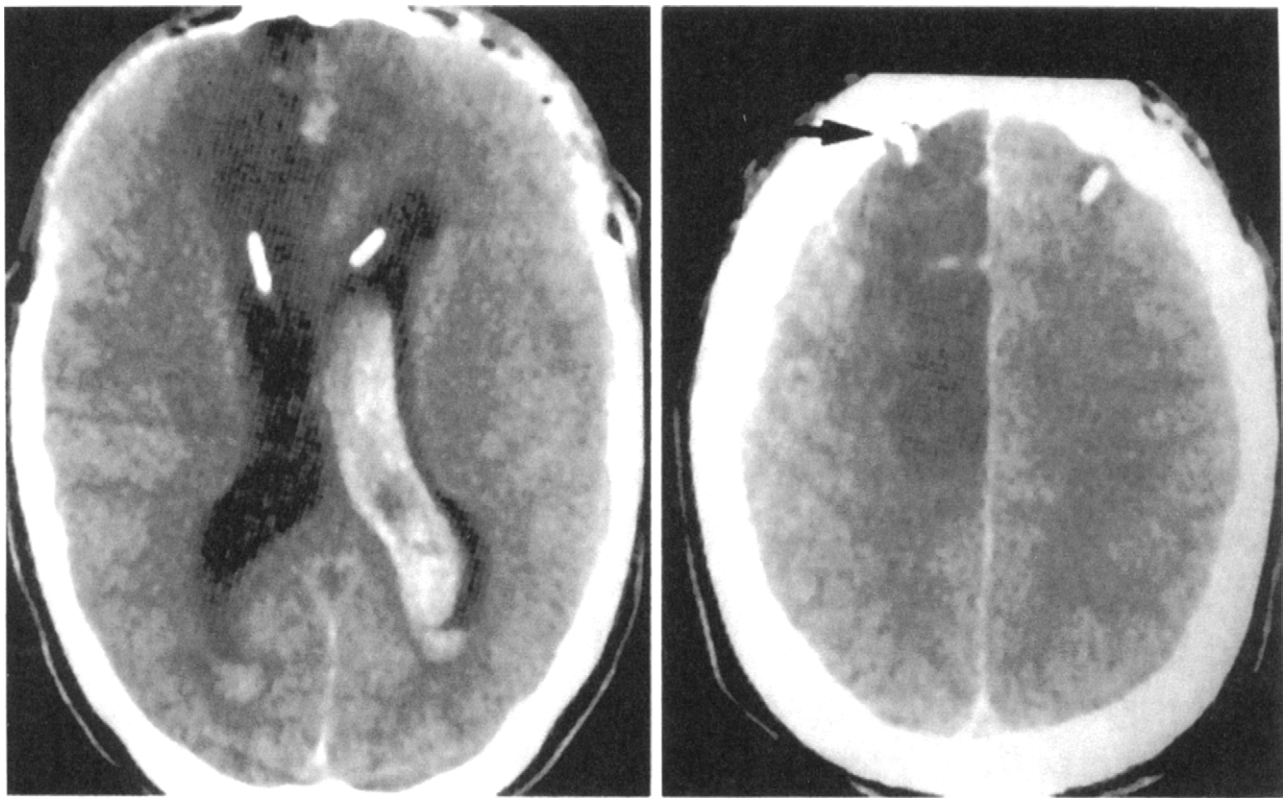


**FIGURE 4.31** (Left) Computerized tomography scans showing the microdialysis probe in conjunction with the intraventricular catheter (*arrow*) located in the hypodense lesion of the right frontal lobe (Case 10). (Right) Case 10 charts showing dialysate levels of the lactate/pyruvate ratio, glutamate, lactate, glucose, and hypoxanthine in microdialysis. Horizontal bar denotes the time of aneurysm surgery. Timescale is hours after subarachnoid hemorrhage [reproduced with permission from Persson, L., Valtysson, J., Enbald, P., Wärme, P., Cesarini, K., Lewén, A., and Hillered, L. (1996). *J. Neurosurg.* **84,** 606–616]. (*Continues*)

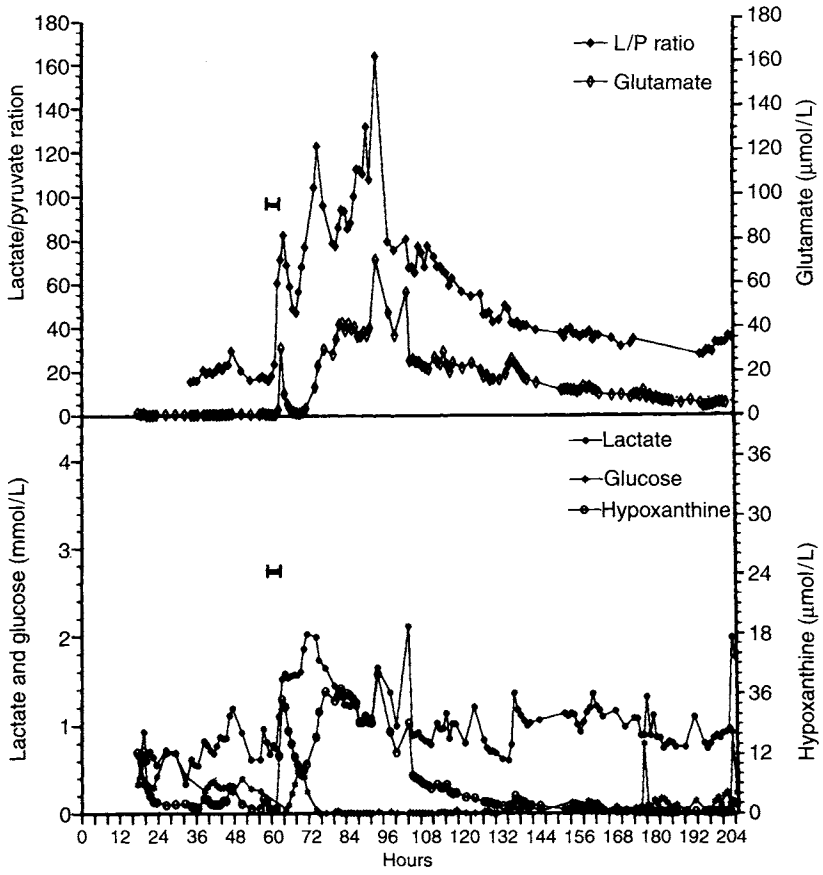



**FIGURE 4.31** (*Continued*)

brain function. Consciousness usually persists until CBF declines below 20 ml/100 g/min. MCA occlusion usually results in a reduction of rCBF below 20 ml/100 g/min and contralateral hemiparesis or hemiplegia. In primate MCA occlusion experiments after CBF reductions to 8 ml/100 g/min for 90 min, reperfusion did not prevent infarction. The CBF threshold for infarction was 12 ml/100 g/min if the period of ischemia was extended to 180 min. On the other hand, periods of ischemia of less than 1 hr duration were well tolerated in terms of functional recovery and histologic evidence of infarction. There is a critical relationship between both the depth and the duration of cerebral ischemia. A patient who develops hemiplegia and unconsciousness from VSP and who remains in that state for several hours is unlikely to be benefitted by any form of therapy because irreversible neuronal destruction has probably occurred (450,451).

The causal relationship between VSP, reduction in CBF and subsequent infarction, and DID is no longer in doubt. The severity of the process, the collateral circulation, and a host of other pathophysiological factors will determine the outcome for a given patient.

Of 18 studies of CBF done prior to 1984, 89% found a decrease in flow following SAH (452–468). In 11 of these reports, in good grade (I and II) post SAH the average was 85% of normal and in poor grade (III–V) it was 69%. Those with impairment of consciousness had an additional reduction in flow averaging 19% compared to alert patients. The more severe and diffuse the VSP, the more likely it is that CBF will decrease to levels that produce symptoms. It is common to find a general reduction of CBF in the presence of impaired consciousness and the radiologic appearance of narrowed cerebral arteries. The reduction of flow is frequently bilateral but is usually greater on the side of the ruptured aneurysm, larger clot, and operative approach. In earlier studies, when CBF was reduced patients tended to be hyperventilating and hypertensive. In more than two-thirds of these investigations, the presence of angiographic VSP correlated with reduction in CBF and failure to demonstrate this relationship was probably due to technical factors or differences in timing of the two types of investigation.

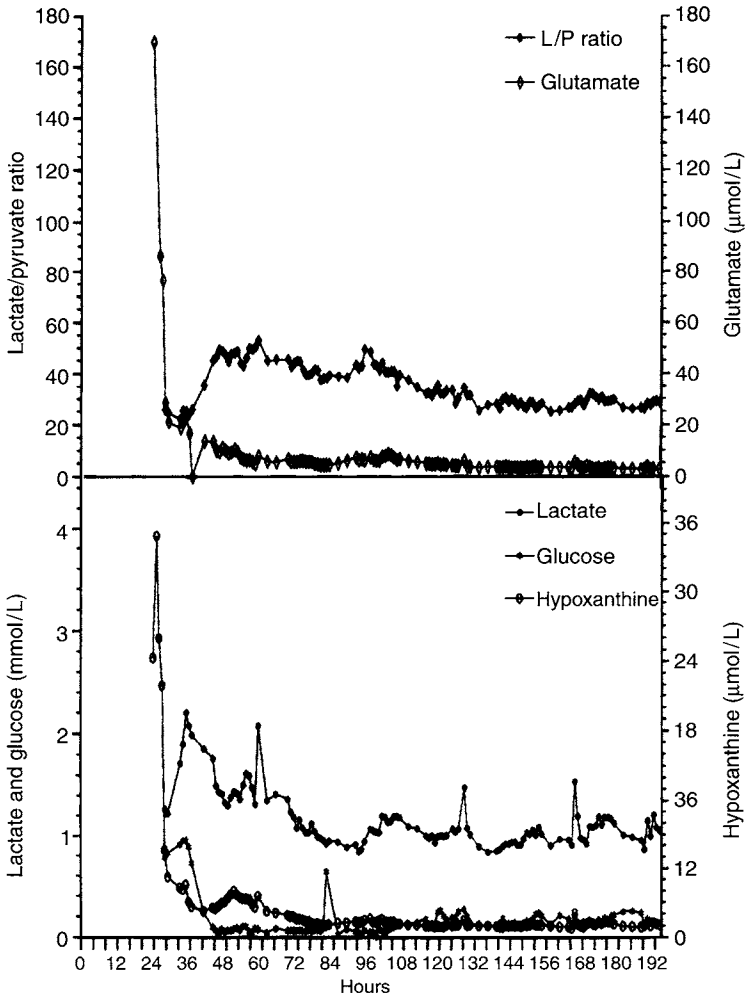



**FIGURE 4.32** Charts show dialysate levels of the lactate/pyruvate ratio, glutamate, lactate, glucose, and hypoxanthine. Time scales are hours after subarachnoid hemorrhage. Microdialysates from a patient with an uneventful clinical course and a good recovery according to the Glasgow Outcome Scale [reproduced with permission from Persson, L., Valtysson, J., Enbald, P., Wärme, P., Cesarini, K., Lewén, A., and Hillered, L. (1996). *J. Neurosurg.* **84,** 606–616].

In 41 patients rCBF reached its nadir in the second week post-SAH. CBF correlated well with the severity of angiographic VSP and the development of DID. Fifteen patients with symptomatic VSP had a mean rCBF of 27 ml/100 g/min compared to 41 ml/100 g/min in 11 patients with asymptomatic VSP. Seventeen patients had MCA aneurysms and VSP. The average rCBF in patients with no deficits at follow-up was 42 ml/100 g/min. Values for rCBF averaged 36 ml/100 g/min in patients with transient hemiparesis and 31 ml/100 g/min in those with permanent hemiparesis (465).

Of 49 patients post-SAH, all those with poor grades and diffuse VSP showed reductions in CBF to less than half their control values with focal areas of decreased flow to less than 30 ml/100 g/min in addition to a reduction in mean values. Serial studies demonstrated that the disappearance of VSP was associated with an increase in rCBF in the ischemic focus and mean CBF (462). The degree of subarachnoid clot within the first 3 days following SAH correlated with subsequent severe diffuse VSP. In a study of 84 patients, those with no VSP had a mean CBF of 42 ml/100 g/min and those with severe diffuse VSP had a mean flow of 30 ml/100 g/min. None of the patients without severe diffuse VSP showed focal ischemia or impaired $CO_2$ response. Ninety percent with severe diffuse VSP showed focal ischemia and 100% showed impairment of $CO_2$ response. Sixty percent of patients with VSP developed low-density areas on the CT scan (469). Severe

diffuse VSP was associated with global ischemia (average flow 21 ml/100 g/min) and was always followed by cerebral infarction. The $CMRO_2$ was even more reduced than CBF. The $CMRO_2$ was linearly related to the initial CBF; at CBF of approximately 25 ml/100 g/min it was about 1 ml/100 g/min. It was concluded that the acute increase in ICP of SAH and the subsequent generalized effect of blood in the SA space depresses oxidative metabolism, decreases the arteriovenous $O_2$ difference, and depresses the $CMRO_2$. At vasoconstriction >50%, CBF was thought to decrease below 20 ml/100 g/min with resulting cerebral infarction. Beyond the angiographically observable spastic arteries there may be a massive dilation of small vessels responding to the lactic acidosis in the brain parenchyma (470). VSP may cause or result from the reduction in the brains $CMRO_2$ and both may reflect the severity of the SAH (471).

Most studies suggest that the poorer the neurological grade post-SAH, the lower will be the CBF, but there is a wide range of flows within each neurological grade. There is a tendency for flow to decline progressively within the first few days after SAH. Ninety-one CBF measurements on 57 patients were less than 30 ml/100 g/min in 31% of patients with a good clinical result and in 73% with a poor one. The CBF declined progressively with increasing ventricular size. Patients with extremely low CBF but normal ventricular size usually had VSP or an ICH (457). A progressive decline in mean CBF values with poor neurological grades was observed in 50 patients. Those under 50 years of age had initial CBF values of 42 ml/100 g/min and those older than 50 years had an initial CBF of 36 ml/100 g/min. The CBF averaged 41 ml/100 g/min in patients who recovered, 37 ml/100 g/min in patients who became disabled, and 33 ml/100 g/min in those who died. The presence of oligemic foci was noted in 26% of patients who recovered, 62% who were disabled, and 67% of those who died. Patients with depression of consciousness and motor deficit showed a mean decrease in CBF of 30% compared with the others. Bergvall *et al.* [457] emphasized that patients in good neurological grades could have abnormally low CBF and vice versa. Overall, however, high CBF in the first 2 weeks following SAH was a favorable diagnostic factor (468). Frequent studies of CBF were performed on 116 patients for the first 3 weeks post-SAH. The values were lower in older patients. The mean CBF of patients whose DID from VSP was 37 ml/100 g/min compared to 52 ml/100 g/min for patients who did not develop such deficits. CBF was most 'reduced the first week after surgery and tended to rise during the second week. It was also more reduced in the hemisphere ispsilateral to surgery. A severe VSP was associated with a flow reduction in contrast to mild VSP. Two-thirds of patients with severe angiographic VSP developed DID; the mean CBF value of this group was 38 ml/100 g/min compared to 52 ml/100 g/min for those who did not develop such deficits. Poor clinical grades correlated with reduced CBF (472). Individuals with flow values less than 20 ml/100 g/min have consistently been grades IV or V and have subsequently progressed to poor outcomes. Flows in the range from 20 to 30 ml/100 g/min are usually accompanied by a decrease in the level in consciousness and mild deficits, whereas values less than this are accompanied by more severe and usually irreversible deficits. rCBF < 12 ml/100 g/min has consistently been accompanied by irreversible ischemia (473). Usually, operation is associated with reduction in rCBF in the operated regions.

A postoperative increase in CBF was associated with the alert state in 83% of cases, whereas patients without a postoperative increase were alert in only 25% of cases (464). In a study of 37 operated patients, mean hemispheric CBF ipsilateral to the operation was lower than that of the contralateral hemisphere (466). Postoperative results correlated with preoperative CBF as follows: excellent, 42 ml/100 g/min; good, 40 ml/100 g/min; fair, 31 ml/100 g/min; and poor, 25 ml/100 g/min (462). In the first week post-SAH 13 good-grade patients had a mean CBF of < 40 ml/100 g/min, 54% developed DID, and 43% died. Thirty-three similar patients had a CBF greater than this and only 24% showed evidence of ischemia, but 38% still died (474). Within a week of surgery CBF averaged 31 ml/100 g/min in comparison to preoperative flows of 36 ml/100 g/min. Control values for normals averaged 45 ml/100 g/min. In one-third of the operated cases there was a poor correlation between neurologic grade and mean CBF (475). Grade I patients could tolerate, without ill effect, intraoperative reductions in blood pressure and resulting CBF reductions as low as 20 ml/100 g/min for up to 35–40 min during halothane-induced hypotension. Patients with impaired consciousness, chronic hypertension, or VSP were considered vulnerable to the ischemic consequences of intraoperative hypotension. Generally, the safest course is to avoid intraoperative hypotension if possible and to use it for as short a time as absolutely necessary and preferably locally rather than generally (476). Earlier studies employed $Xe^{133}$ radioactive gas and linear collimation. This technology had drawbacks, including a dependence on normative partition/coefficient values, poor resolution, insensitivity to low flow regions because of see-through, and no direct anatomical correlation. In addition, it exposed patients to radioactivity. The technology was not easy to apply serially. Single measurements failed to correlate with a dynamic physiologic process. Elevated flows were sometimes misleading because they could represent reactive hypernemia in previously ischemic and infarcted areas (477–479).

The stable Xe-enhanced CT technique has significant advantages over radioactive Xe techniques. It provides high resolution as well as quantitative information in normal and diseased states because the partition coefficient is calculated for each flow value. These studies can be repeated within 20–30 min because of rapid cerebral washout of Xe. Flow values can be coupled to the CT anatomical depiction (480). The Xe/CT is capable of showing patterns of irregular high and low flow values throughout one or more vascular territories. Yonas reported 3 grade I and II patients with this pattern of flow who subsequently progressed to absence of flow in the abnormal regions. The location of ischemia associated with VSP most often involved the distribution of arteries immediately distal to the site of SAH. Severe remote ischemia can also evolve, as can global ischemia. The first evidence of ischemia from VSP was often accompanied by one or more regions of flow near 0. Sequential Xe/CT measurements can be used to assess the value of raising the blood pressure (481). In series of 47 patients studied by Xe/CT, only 7 of 14 patients identified as having symptomatic VSP had flow values < 15 ml/100 g/min in two or more contiguous cortical regions. These all developed infarction. All grade I patients in the series had flow values > 50 ml/100 g/min, whereas only grade V patients had global values < 20 ml/100 g/min (473).

PET has demonstrated that in brain regions served by extremely spastic arteries there is a decrease in blood flow, an increase in blood volume, and a decrease in oxygen metabolism. Cortical CBF values remain above 50 ml/100 g/min and decline only when the primary and secondary collateral capacity to supply blood is impaired beyond the ability of the intrinsic arterial supply to dilate further. Mean values for CBF and $CMRO_2$ decreased progressively with poor neurological grades and in each grade when VSP was present. The reverse relationship is found for CBV (423). In one case studied by PET scanning a hemiplegia was associated with contralateral focal VSP. The control and vasospastic values, respectively, were as follows: CBF, 55 and 30 ml/100 g/min; $CMRO_2$, 3.5 and 2.5 ml/100 g/min; oxygen extraction ratio (OER), 0.42 and 0.53 ml/100 g/min; and CBV, 3.5 and 5.5 ml/100 g/min. There were significant differences in values of CBV and CBF between vasospastic and nonvasospastic hemispheres (482).

Early PET studies suggested that as flow falls to a given region there is dilation of the microvascular bed. Subsequent studies have cast doubt on this finding; indeed, intraparenchymal constriction may occur in VSP. In a further effort to compensate for the reduced flow, relatively more $O_2$ is extracted by an ischemic region. If ischemia is so severe that infarction develops, the metabolic rate of tissue will decrease, although nonneuronal repair mechanisms may give spuriously high values in the chronic stage of repair. There is no entirely satisfactory way to assess these changes when present in the seriously ill patient. The time, expense, and complexity of PET scanning methods limit its widespread availability (483).

Four hemiparetic patients with VSP were studied by PET. The two patients who recovered had minimal contralateral hemispheric rCBF of 15 and 16.2 ml/100 g/min; for the two that did not recover, the flows were 12 and 11.7 ml/100 g/min. The comparable values for the $CMRO_2$ were 1.34 and 2.60 vs 0.72 and 1.66 ml/100 g/min. Measurement of OER and CBV showed no consistent pattern. All patients showed higher CBF before than after VSP (483).

Numerous animal experiments have demonstrated a decrease in CBF in association with VSP (436,484–486).

### G. Cerebral Metabolism

CNS metabolism is almost exclusively aerobic, which necessitates a constant supply of glucose and $O_2$ since there are almost no stores of energy-providing substrates in the brain. The brain, which is 2% of total body weight, receives 15 or 16% of resting cardiac output (418). $O_2$ availability is a function of CBF, $O_2$ saturation, and Hb concentration. A reduction of CBF is known as ischemia, a reduction in $O_2$ saturation is hypoxic hypoxia, and a reduction in hemoglobin concentration is anemic hypoxia (487). The brain normally consumes approximately 45 ml/min of $O_2$. This is about 20% of the basal $O_2$ consumption for the whole body. $CMRO_2$ is normally 3 or more ml/100 g/min and levels below 1 ml/100 g/min generally indicate irreversible brain damage. Cerebral $O_2$ uptake is normally stable in usual activities. Like CBF, however, the global constancy does not reflect regional changes that take place on a moment-to-moment basis. In health, rCBF changes rapidly to couple flow to metabolism.

Energy used by the brain maintains neuronal membrane potential, synaptic transmission, and the structure of the neuropil. Unlike other tissues, the brain cannot use complex substrates for energy production. The net result of the oxidative metabolism of 1 molecule of glucose is the formation of 38 ATP molecules with their high-energy phosphate bonds. Mechanisms of adaptation of the cerebral circulation and pressure regulation are ultimately designed to safeguard the oxidative metabolism of glucose by the brain. When the brain is damaged certain biogenic amines and free radicals that spread cerebral edema may be produced by disruption of fatty acid chains. These substances may be important players in the deleterious cascade initiated by vasospastic ischemia. Ischemia is a level of blood flow insufficient to supply enough $O_2$ to meet local metabolic demand. Flow may be very low and

yet increased $O_2$ extraction may keep $O_2$ supply adequate to the tissues needs (488). Ischemia may be considered to have three thresholds. The clinical symptom threshold is about 30 ml/100 g/min, the electric failure threshold is 20 ml/100 g/min, and the metabolic failure threshold is 10 ml/100 g/min. Below the last threshold, $K^+$ pours out of cells and levels of phosphocreatine and ATP decline. Ischemic brain damage can result from a decrease in MABP, an increase in ICP, loss of autoregulation, VSP, cerebral edema, pathological brain shifts, a decrease in $P_aCO_2$ or hematocrit, and epilepsy. Although CBF begins to decline at a MABP around 45 mmHg, no change in EEG is noted at this point. The $CMRO_2$ does not decline until CBF is reduced to 60% of normal. When substrates are limited, the metabolic rate decreases to preserve normal levels of high-energy phosphates. Anaerobic metabolism increases once CBF has fallen to below 40% of normal and the EEG becomes flattened. Cerebral levels of ATP then decrease precipitously (489). When arterial $P_aO_2$ is below 50 mmHg, CBF increases, and at $P_aO_2$ of 30 mmHg it is almost doubled. Breathing 100% $O_2$ reduces global CBF by about 10%. Flow falls to about 75–80% of normal at a $P_aO_2$ of 800–1200 mmHg (hyperbaric conditions).

Hypoxemic content of the blood (a reduction in the $O_2$) differs from ischemia, which is a state of impaired tissue perfusion (410). When available $O_2$ supply is too low to support normal tissue respiration, metabolism changes to a predominantly incomplete anaerobic form in which the partial oxidization of glucose to lactic acid results in the production of only two molecules of ATP from one molecule of glucose. During brain hypoxia, levels of ATP and other high-energy phosphates decrease. Preceding this is a shift in the redox state of the coenzymes of the electron transport system that normally link hydrogen release with $O_2$. The ratio of the reduced form of NADH increases proportionately to the oxidized form $NAD^+$. When $P_aO_2$ decreases to 35 mmHg there is continued maintenance of high-energy phosphate levels, including glycolysis and lactate production, but decreased neurotransmitter release with minor functional and EEG disorders. When $P_aO_2$ decreases to 10–20 mmHg, ATP and energy charge are decreased. There is an increase in the NADH: $NAD^+$ ratio. At this point consciousness is lost, and EEG slows and becomes isoelectric. Levels of $P_aO_2$ of 0–5 mmHg result in permanent neuronal damage and death from cardiovascular failure (410). Some recovery of energy state is possible even after 1 hr of total ischemia if circulation is supported (490). This is short-term biochemical and EEG recovery, not functional neurological recovery. After only 20 sec of ischemia, $CMRO_2$ decreases and the NADH: $NAD^+$ ratio increases with accompanying EEG slowing and loss of consciousness. Ischemia lasting between 1 and 5 min depletes ATP and the energy charge while increasing lactate, $H^+$ and $[K^+]_e$.$Na^+$ enters cells. All brain reflexes are lost. Ischemia persisting for 5–15 min results in decreased protein synthesis and increases in cAMP, γ-aminobutyric acid, some amino acids, and free fatty acids, which are associated with irreversible damage to some cells. Beyond 60 min metabolism ceases and protein metabolism stops. Although energy failure is invariably associated with ischemia, it is not the only cause of irreversible cell damage, which can also result from seizures or hypoglycemia (164). During a seizure $CMRO_2$ may increase by 300%. The $O_2$ supply to the tissue is maintained by an increase in blood flow (410).

Following any type of brain injury such as SAH, there ensues a complex series of adverse events associated with the impairment of circulation and $O_2$ delivery to the tissue as well as impairment of normal metabolism and neuropil disruption. Hypoxemia may occur from brain damage alone. Following SAH, patients may have bradycardia or asystole and become apneic. Spontaneous respiration may resume. Atelectasis may follow the initial respiratory arrest. The loss of consciousness may impair protective respiratory reflexes. Gastric aspiration may occur or errors in respirator settings may be made. Hypocapnia is probably the most common disorder observed acutely following severe hemorrhage. Arterial pH rises and there is respiratory alkalosis. This arterial pH change shifts the $O_2$ Hb dissociation curve to the left; although the $P_aO_2$ is low, the $O_2$ saturation of Hb remains relatively high so less $O_2$ is available to the tissue (418).

Ischemia causes an increase in tissue lactate concentration with a resultant decrease in pH. If the lactate levels remain above 20–25 mmol/kg, tissues will fail to recover to a normal energy state after an ischemic insult. In primates MCA occlusion of 4 hr duration resulted in a decrease of ATP to 20% of normal and an increase in lactate to 10 times normal. In this model ischemia was largely reversible up to 2 or 3 hr but irreversible after 4 hr. Following 4 hr of complete occlusion the cause of death was edema, which progressed even with restoration of flow (491).

The consequences of an episode of ischemia or hypoxia depend on the state of the brain prior to the onset of the insult as well as its duration and severity. The functional reserve of the cerebral circulation or the coexistence of structural abnormalities are also important. Hypotensive ischemia produces boundary zone infarcts, whereas hypoxia selectively destroys cerebellar Purkinje cells and hippocampal cells. In some conditions of cerebral ischemia due to hypotension, reperfusion of certain areas may not occur even after blood pressure is restored to normal levels. This is called the "no-reflow" phenomenon. In hypoxic neuronal damage, pyramidal cells in layers 3

and 5 or 6 of the cerebral cortex, pyramidal cells in the areas $h_1$ and $h_{3-5}$ of the hippocampus, and the Purkinje cells of the cerebellum are selectively involved (492). The restoration of an adequate CPP at the termination of an ischemic episode may cause CBF to initially overshoot the preischemic values which is termed reactive hyperemia. However, CBF may subsequently decrease and ultimately again reach dangerously low subnormal values in what is known as delayed hypoperfusion. The metabolic effects of focal ischemia of 3-hr duration have been considered to be approximately equivalent to the effects of global ischemia of 15-min duration. During the early phases of focal ischemia, the regional metabolic responses vary considerably depending on the degree of reduction of CBF. Under chronic ischemic conditions sharp metabolic and hemodynamic demarcation develops between ischemic and nonischemic regions (493). During the initial few hours of focal ischemia, an increase in $H_2O$ and $Na^+$ content is almost exclusively confined to the gray matter. Between 12 and 48 hr later, $H_2O$ retention and any $Na^+$ increases become progressively more pronounced in the white matter. In cerebral ischemia there is ultimately a loss of intracellular $Ca^{2+}$ homeostasis that predisposes the cell to a massive influx of $Ca^{2+}$ during reperfusion following partial ischemia. Disturbed $Ca^{2+}$ homeostasis within the cell appears to be the final determinant of cell death. An increase in cytosolic-free $Ca^{2+}$ may activate endogenous phospholipases that degrade the mitochondrial, endoplasmic, and plasma membranes (494).

It used to be customary to routinely reduce MABP during aneurysm surgery. The majority of such patients did well if they were operated on at a time of intact autoregulation. In one series, CBF remained unchanged during arterial hypotension but $CMRO_2$ decreased. The parameters returned to normal when hypotension was discontinued. In two patients, however, there were reductions of 25–30% from their initial higher values of CBF and there was a drastic associated reduction of $CMRO_2$. While these parameters improved during discontinuation of hypotension, both patients exhibited prolonged postoperative coma (495).

Astrup, Symon, and coworkers introduced the concept of ischemic penumbra as being a region of nonfunctioning but potentially viable tissue that might recover its function if restoration of flow is brought about promptly. $CMRO_2$ can be reduced by abolishing synaptic transmission with drugs such as anesthetic gases or barbiturates. These can reduce the $CMRO_2$ only to a certain level, at which point there is isoelectricity on the EEG. Additional doses will not further depress the $CMRO_2$. Different drugs such as lidocaine which interfere with $Na^+$–$K^+$ fluxes can further reduce the $CMRO_2$ but their effect is also limited. Reducing the temperature can further reduce $CMRO_2$ beyond the drug-induced point in a graded and progressive fashion. At 18°C, $O_2$ consumption is reduced to approximately 20% of the anesthetized control level. Some membrane failure occurs at CBF below 8 ml/100 g/min, corresponding to a total $O_2$ supply of 1.4 ml/100 g/min. This is the minimum amount of $O_2$ required to keep the $Na^+$–$K^+$ transport system balanced to prevent a permanent leakage of these ions in either direction.

Patients with severe neurologic deficits or more severe degrees of VSP have the most marked depression of CBF and $CMRO_2$ on PET studies (423). A good correlation exists between the occurrence of diffuse VSP and DID. VSP showed a correlation with $CMRO_2$ as follows: diffuse mild and diffuse severe VSP at 1.42 ml/100 g/min and mild or no VSP at 2.27 ml/100 g/min. The correlation with the cerebral metabolic rate for glucose was as follows: diffuse mild and diffuse severe VSP at 2.44 mg/100 g/min and mild or no VSP at 3.26 mg/100 g/min. Fifty-three percent of the patients with diffuse VSP developed cerebral infarction. Forty percent of patients with diffuse severe VSP showed low-density areas on CT scan, while none of the patients without VSP showed such low-density areas (496).

There is a tendency for OER to increase with age (497), which compensates for a decrease in CBF. The net effect is to maintain $CMRO_2$ at higher levels than would be expected if the $CMRO_2$ and CBF decreased in parallel. Even in older patients, the OER of 0.5 indicates that the venous blood contains 50% unextracted $O_2$ that would still be available to the tissues. Acute ischemia results in a decline in CBF and a compensatory increase in OER. If the reduction in flow is sufficiently great that the $O_2$ carrying reserve is exhausted, cerebral metabolism becomes flow dependent in a linear fashion and true ischemia supervenes. Irreversible structural disintegration appears to start at $CMRO_2$ levels of 1–1.5 ml $O_2$/100 ml/min. The period of grossly elevated OER rarely lasts beyond 24 hr in humans. Cortex may show a higher OER than white matter or subcortical gray matter. Within the first 24 hr, as infarction becomes established there is a low CBF and a high OER. Once infarction is complete there is a low OER and a low $CMRO_2$ compared with normal. This suggests that reestablishment of the blood flow is not influencing the fact that the perfused tissue is incapable of increasing its $O_2$ consumption. The low OER in an infarct represents relative hyperperfusion regardless of the absolute level of CBF. High levels of CBV indicate vasodilation in response to lowered CBF. Persumably there is a point at which maximal vasodilation can no longer compensate for the reduction in CBF and OER begins to increase (497). The OER is mathematically the arteriovenous difference in $O_2$ concentration divided by the arterial concentration of $O_2$. Generally,

when the OER is maximally depressed the tissue is not viable. In the territory distal to arteries in VSP, PET studies have shown initial increases in CBV followed by a decrease in CBF and finally a decrease in $CMRO_2$.

Charbel and colleagues used intracellular electrodes for 7–10 days of postoperative monitoring following clipping or coiling of ruptured aneurysms. Patients who did not develop VSP had brain tissue $pO_2$ >20 mmHg, $pCO_2$ stablized at 40 mmHg, and pH remained between 7.1 and 7.2. Three patients who developed VSP did not show a significant decrease in brain tissue $pO_2$ but did show an increase in $pCO_2$ and the pH decreased to 6.7. Angioplasty reversed the acidosis and hypercapnia within minutes. The brain tissue $pO_2$ increased from about 10 to 15 mmHg in about 10 min (498).

### H. Histopathology

Infarction involves the death of both neurons and glia. Neuronal necrosis follows cell swelling, mitochondrial damage and dissolution, and generalized disruption of internal homeostasis. Membranes lyse and intracellular constituents are released extracellularly, which can invoke a local inflammatory reaction. This is characterized by cellular infiltration, edema, and vascular damage. Ischemically induced extreme energy failure in mitochondria is the presumed essential cause of necrosis.

A recently recognized mechanism of cell death is apoptosis, which is an active process of cellular destruction resulting in chromatin shrinkage and aggregation without initial loss of cell membrane or mitochondrial integrity. Extensive genomic fragmentation is a histologic feature. Phagocytes can sequester the antigenically modified apoptotic cells before their contents spill into the surrounding space where they would elicit an inflammatory response and local damage. The apoptotic cell presumably commits suicide to increase the survival chances of its neighbors. In ischemic infarction the core may die rapidly by necrosis, whereas delayed apoptotic death may occur in the penumbra (499).

Apoptosis is a mechanism to eliminate extraneous, superfluous, or dangerous cells. The enforcer of this controlled cellular deconstruction is provided by the caspase family of cysteine proteases which cleave target cells. The caspases normally exist in cells as inactive proenzymes. This may be the mechanism by which some cells that might otherwise recover from ischemia may die because the injury triggers their suicide programs. Neurons in an ischemic penumbra may be susceptible to this. In apoptosis the cell disassembles its DNA and breaks up its content into membrane-wrapped packets that can be removed without causing inflammation. One of the neuronal signs of apoptosis is the breakage of DNA and an overall granular appearance caused by condensation of the chromosomes in the decomposing nuclei. Two of the cardinal histologic signs of apoptosis are caspase-3 production and cut up DNA coexisting in neurons. There are at least 13 caspases currently known. Caspase-1 cleaves and activates IL-1β, which signals the immune system to trigger inflammation. IL-1 receptor antagonist can reduce the area of ischemic brain damage in stroke models. Caspase-3 gene expression is increased in rodents with global ischemia (500).

Inflammation may contribute to the extent of neuronal damage in ischemia. Capillaries may become plugged by polymorphonuclear leukocytes to create a mechanical obstruction and they may also serve as a source of oxygen free radicals upon release of their intracytoplasmic granules. RBC circulation may also be prevented by structural changes involving the endothelium and the surrounding astrocytes which may swell during ischemia and collapse the lumen of microvessels.

Focal cerebral ischemia produces infarction characterized by microvacuolation, ischemic cell change with incrustation, and homogenizing cell change. Ischemia may result not just in neuronal death through these rapid mechanisms. In experimental models, neuronal loss and atrophy have been observed in the ipsilateral thalamus and the ipsilateral substantia nigra a few weeks after ipsilateral middle cerebral artery occlusion. Neuronal cell damage can therefore occur not only from direct ischemia but also from axonal degeneration with resultant retrograde neuronal degeneration and transsynaptic mechanisms of death. It is possible that a decrease in the level of γ-aminobutyric acid, a potent inhibitor of neuronal discharge, can render substantia nigra neurons liable to excessive firing due to neurotransmitter-mediated disinhibition (501).

Under normal conditions it is highly likely that all capillaries are perfused, although there may well be large flow differences between single capillaries in the brain. Increased CBF is probably accompanied by a more homogeneous pattern of capillary plasma perfusion. In the immediate phase following flow reduction consequent to MCA occlusion there is not a dramatic increase in the amount of nonperfused capillaries, although actual capillary blockade may start 90–120 min after permanent occlusion (502).

A series of molecular and cellular processes underlie the transition from ischemia to inflammation and ultimate tissue dissolution. These involve polymorphonuclear leukocyte activation and chemotaxis, endothelial cell receptor biology, cytokine synthesis and release, leukocyte transmigration, and tissue invasion and microvascular thrombosis. Focal cerebral ischemia leads to inflammatory change in the cerebral vasculature by

hypoxic injury, cytokine release, thrombin generation, and cellular responses. In the endothelium of the downstream microvasculature ischemia converts xanthine dehydrogenase to xanthine oxidase. Upon reperfusion, oxygen free radicals and $H_2O_2$ are generated from hypoxanthine and $O_2$ by the endothelium and by WBCs. In some experimental models following MCA occlusion the cytokines TNF-α and IL-1β are synthesized through transcriptional control and released within hours of flow obstruction. These factors are generated by perivascular astrocytes and other nonvascular cells. The number of intramicrovascular and perivascular polymorphonuclear leukocytes reaches a maximum 24 hr after complete MCA occlusion. Subsequently, monocytes and macrophages transmigrate and contribute to progressive tissue destruction. P-selectin is released to the endothelial surface from Weibel–Palade bodies within 2 hr of MCA occlusion and is continuously expressed in the ischemic territory. Postreperfusion obstruction by leukocytes is one contributor to the no-reflow phenomenon. In certain experimental models, blockade of the interaction of the leukocyte β-integrin CD18 with its counterreceptor ICAM-1 produces a significant increase in microvascular patency (503).

WBCs upon activation may induce vasoconstriction of larger cerebral arteries by reduction of EDRF or increased $TXA_2$ release. Clinically activated polymorphonuclear leukocytes appear in the peripheral circulation as evidenced by CD18 expression in the early days following thromboembolic stroke (503).

Ischemia from VSP can be aggravated by coexistent vascular occlusive disease and other factors, such as advanced age, hypertension, diabetes mellitus, smoking, coronary artery disease, and elevated lipids. Systemic factors compromising the cerebral and blood pressure include rhythm abnormalities, cardiac disease, hypotension, and anemia. Carotid flow can also be affected by conditions such as fibromuscular dysplasia and cervical atherosclerosis. Since many patients with SAH are elderly, they may have a small vessel disease as well that impairs potentially beneficial collateral flow. Microatheromas can occur in the penetrating arteries and lipohyalinosis can affect arterioles. In this small vessel atherosclerosis there is concentric lamellar laying down of collagen and deposition of fibrohyaline substances in the adventitia but generally a limited presence of lipid and little new cellular invasion. Carotid kinking may also be an impediment to flow (504).

Systemic hypotension can aggravate focal cerebral ischemia. Superimposed diffuse cerebral hypoperfusion results in a sequence of clinical symptoms ranging from syncope to focal effects of watershed infarction and ultimately generalized symptoms from diffuse hypoxic ischemic neuronal damage. Syncope can result from impaired baroreceptor reflexes in the elderly, and it can occur in conjunction with fever and adverse medication responses. Hypotension may also result from sepsis, vomiting, and iatrogenic causes (505).

### I. Clinical Studies of Infarction

Robertson studied 27 fatal cases of ruptured aneurysm. He hypothesized that the ischemic changes were due to temporary spasm of the supplying vessels. This was a key insight into the relationship between chronic cerebral VSP and cerebral infarction. In a different analysis of 86 patients dying from ruptured aneurysms, he noted infarction in 30% of cases whom he personally examined (506). Within a few years other workers were able to buttress his observations. In a series of 143 autopsies on ruptured aneurysm cases, focal hemorrhagic or anemic infarction involving both cortical and deep structure were observed in 45% of cases. The site of the lesion could be correlated with the location of the aneurysm (507). The frequency with which infarctions were found probably reflected the assiduousness of the search and the experience of the neuropathologist. In a study of 32 brains, small patchy foci of cortical necrosis were noted. Infarcts were present in 78%, and in 40% they were characterized as being massive (508). The aneurysm-bearing vessels were examined in eight patients who died of ruptured AComA aneurysms; they were thought to be excessively contracted and supplying territories showing softening. Infarction was most extensive in the areas supplied by the parent vessels of the aneurysms, and the septal areas were destroyed in 88% of cases, bilaterally in 50% (509).

In a series of 159 patients who had survived at least 24 hr after admission to hospital with SAH, 75% showed cerebral infarction. Interestingly, 21% of these had stenotic atheromatous involvement of the cerebral arteries. This compared to an incidence of only 15% in 40 patients dying without infarcts. Thirty-six percent of the patients with infarcts had recurrent hemorrhage, as did 48% of the patients without infarcts. This resulted from the larger proportion of patients without cerebral infarction dying from ICH or IVH, which is strongly associated with recurrent episodes of bleeding. Subarachnoid hematomas were present in 50% of all patients. They were more than 1 cm in thickness in 86% of patients with infarction and in 72% without. Intrafrontal subarachnoid hematomas tended to rupture into the brain with greater frequency than Sylvian hematomas. Relatively more Sylvian hematomas were associated with infarction. The high incidence of infarction associated with ruptured MCA aneurysms was due to the presence of Sylvian hematomas. In smaller arteries embedded in subarachnoid clot, subendothelial permeation of polymorphonuclear cells was noted. Con-

centric rings of fibrin were seen to radiate outward from small necrotic vessels in such hematomas. Fifty-nine percent of the 105 patients with infarction had sustained a diastolic blood pressure below 60 mmHg at some time in the clinical course. Among the 35 patients without cerebral infarction, only 17% had experienced a similar documented level of hypotension. Angiographic VSP had been documented in 37% of cases with infarction and 12% without. Of the 40 cases with documented VSP and infarction, in 98% the infarction was present somewhere within the territory of supply of one or more of the spastic arteries, and in 60% of these it accurately occupied the territory of the artery involved. Relationships existed between infarction and atheroma of the cerebral arteries, bilateral hypoplasia or narrowness of the anteroposterior connections between the carotid and vertebral arteries, bleeding in the perivascular sheaths of perforating ganglionic arteries, the presence of larger subarachnoid hematomas (particularly in the Sylvian fissure), vascular hypotension, VSP, direct surgical attack on the aneurysm, and carotid ligation. Crompton reviewed nine pathological series published between 1949 and 1963, and of the 644 cases 42% were said to have shown cerebral infarction (510,511).

In a later series of 53 patients the incidence of infarction in patients who had shown VSP at angiography was 82%. However, 70% of the patients showed infarcts; in these patients there had been no vasospasm demonstrated at angiography. This was probably due to the timing of the angiogram. Infarction almost always occurred in territories supplied by the spastic vessel. Narrowing of the lumen by more than 60% and diffuseness of the VSP appeared to be casually related to the subsequent development of infarction (512).

In a series of 75 patients in which massive infarction occurred in 40%, infarction was noted to be more frequent in older patients. There were no instances of emboli arising from intraaneurysmal thrombus. No differences in infarction rate were demonstrable between patients who had thrombus in the aneurysm sack and those who did not. Any association between infarction and systemic hypotension, pulmonary complications, or the use of specific medications was not evident in this series. SAH was observed near several infarcts and it was suggested that the disruption of small vessels by hematomas might result in necrosis of adjacent brain, but no torn vessels and distended sulci were demonstrated (513,514). The localized infarction was considered to be due to partial or complete arterial obstruction by VSP, thrombus, or embolism, all of which could be superimposed on atherosclerotic changes. The tendency could be increased by hypotension or anoxemia. In none of the 30 cases of extensive infarction was a thrombosed major artery found. Also, there was no evidence of multiple thrombi in smaller vessels. Of 10 patients with advance atherosclerosis of the basal arteries, only 3 had associated massive infarcts. In 1 case with massive bilateral infarction the postoperative angiogram had demonstrated patency of all major arteries. In 53% of the cases with massive infarction there were massive hematomas still present at the time of death. Only 20% of autopsies post-SAH showed no evidence of ischemia, but more than half of these patients had died within a few hours of SAH. The youngest patient with infarction in this series was 30 years (513,514).

In a series of more than 1300 aneurysm patients, 3.1% were 19 years of age or younger, and 58% were neurologically grades I or II on admission. At discharge, one-third of the patients had died but only 2% were severely disabled. Nine percent of the children had previous known hypertension. Only 23% were operated on within the first 3 days post-SAH. VSP occurred in 53% of patients undergoing angiography between days 4 and 16 post-SAH. VSP was not associated with an increased morbidity or mortality in these young patients. No patient had cerebral infarction demonstrated at autopsy. Nineteen cases had preoperative angiograms between days 3 and 17 post-SAH. Twenty-seven percent showed slight VSP and 27% showed severe VSP (515).

Infarction may be recognized within the first 24 hr after ictus on the CT scans. Liquefaction necrosis and the production of an encephalomalacic cavity usually take 4–8 weeks to appear. Contrast enhancement is often demonstrable within 1–3 weeks at the site of an infarction. Significant amounts of blood in the subarachnoid space correlated well with the subsequent development of cerebral infarction in a clinical series of 32 patients (516).

In 135 patients admitted within 2 weeks of the last SAH, who had neither rebleeding nor ICH, 68% had angiographic VSP. Infarction attributable to VSP developed in 21% of all patients. Low-density ischemic lesion showed on the CT scan about 9 days after SAH. Contrast enhancement became positive after 17 days on average in 6 patients and the low-density areas turned into high-density ones, indicating hemorrhagic infarction, at a mean of 26 days after SAH in some cases. The most common pattern was infarction in the territory of the major arterial trunk. Infarction from VSP was most obvious in the cortex and adjacent white matter and relatively unusual in the basal ganglia. Hemorrhagic transformation occurred in 21% of these vasospastic infarcts (517).

In another series of 206 fatal aneurysm cases, 46% showed ischemic sequelae; of these cases, 43% had ischemic infarction at autopsy, 28% showed hemorrhagic infarction, and 9% were combined types (518). In a

different series of 219 patients who had been admitted within 14 days of SAH without intracerebral hemorrhage or operative difficulties, angiographic VSP was evident in 70%. DID attributed to this VSP occurred in 26%; 20% went on to develop infarction. Of these patients, 32% showed hemorrhagic infarction confirmed by CT scan. The CT detected the hemorrhagic transformation between 13 and 40 days after SAH (mean, 24 days). It was considered that hemorrhagic transformation was a manifestation of the angiographic relaxation of VSP and resumption of normal flow into damaged area. Interestingly, only 14% of the patients with hemorrhagic infarction demonstrated clinical worsening coincident with it (519). In an autopsy series of 29 patients, managed without operation, a significant relationship was shown between the occurrence and degree of VSP and the resulting ischemic brain damage. Some degree of VSP was present in 75% of the patients with ischemic brain damage in arterial territories, 16% of patients with boundary zone ischemic changes, and 18% of patients without ischemic brain damage. Of patients showing ischemic brain damage in a given arterial territory, 54% showed arterial constriction of more than one-third of the presumed normal diameter and 60% showed a midline shift of 6 mm or more. Of patients without ischemic brain damage, only 9% showed severe VSP and only 25% had a similar midline shift. VSP corresponded to the territory of infarction in 75% of hemispheres. VSP and ICH were combined in 35% of the hemispheres showing cortical infarction as opposed to only 5% of the hemispheres with no ischemic damage. The presence of both lesions therefore significantly increased the likelihood of infarction (520).

Multiple microthrombi might play a role in the induction of cerebral ischemic symptoms or cerebral infarction. Microthrombi composed of platelet aggregates with polymorphonuclear neutrophils have been found in the arterioles of the parenchyma at autopsy (521). Four patients dying from symptomatic VSP showed many white and fibrinous microthrombi together with infarction in the territories of spastic arteries which correspond to low-density areas seen by CT scan. Two aneurysm patients dying without cerebral VSP showed only negligible microthrombi. The microthrombi were significantly greater in regions of infarction (128) (Fig. 4.33).

Symptomatic VSP developed in 45% and cerebral infarction in 17% of 221 patients who had their first CT scan within 2 weeks post-SAH. Hemorrhagic infarction was noted in 6%. The hemorrhagic infarction occurred between days 20 and 30 post-SAH and corresponded to the remission stage of the VSP in this study from Japan. A massive hemorrhage with mass effect occurred in only 2 cases. These data suggest that carrying induced hypertensive therapy into the third week post-SAH might aggravate the tendency toward bleeding (522).

The very late development of spontaneous ICH in patients having had moderate to severe VSP 31–111 months prior to the second bleeding episode was documented in 11 patients. These intracerebral hemorrhages were somewhat different from the usual hypertensive intracerebral hemorrhages in that they had various

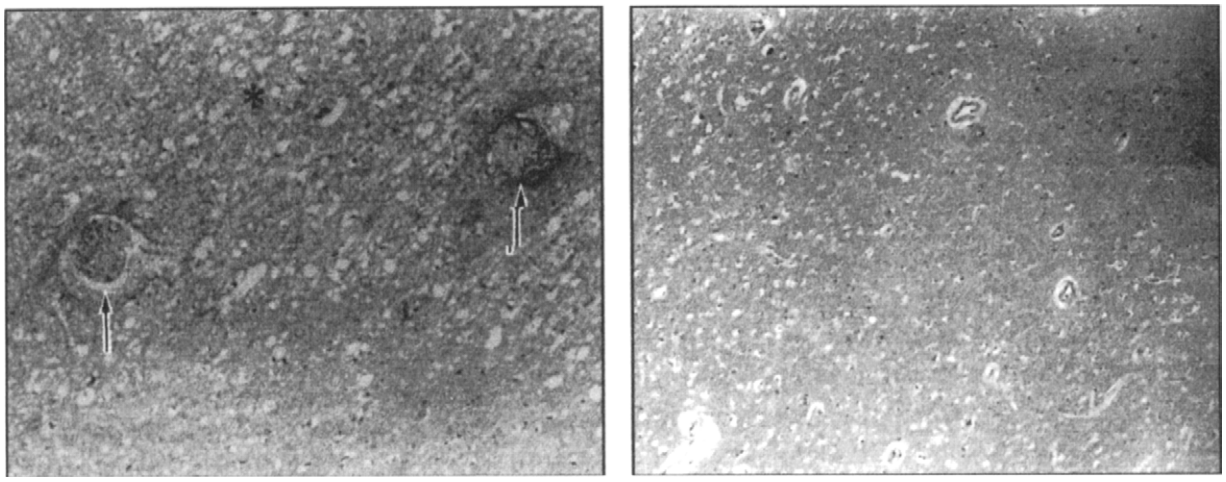


**FIGURE 4.33** Microthrombosis in case of cerebral vasospasm. (Left) Multiple microthrombi (arrows) and ischemic changes (asterisk) are seen in the area of the right MCA. (Right) Neither thrombus nor ischemic change is seen in a specimen taken from symmetrically contralateral region [reproduced with permission from Suzuki, S., Kimura, M., Souma, M., Ohkima, H., Shimizu, T., and Iwabuchi, T. (1990). Cerebral microthrombosis in symptomatic cerebral vasospasm. A quantitative histological study in autopsy cases. *Neurol. Med. Chir. (Tokyo)* **30,** 309–316].

degenerative changes in the elastic lamina and media of the perforating arteries even though most patients were young and normotensive. Severe VSP was considered to be a potential prognostic factor for the subsequent development of ICH in Japanese patients (523).

In a prospective clinical series of 265 patients who had an initial CT scan within 3 days post-SAH and a final CT study 1–3 years (mean, 1.4 years) after surgery, a logistic regression analysis revealed (in order of importance) the following prognostic factors for cerebral infarction: the amount of blood on the CT scan, postoperative angiographic VSP, the timing of operation (increased with earlier surgery), and a history of hypertension (524).

A prospective study of 59 patients admitted within 3 days of SAH and having an outcome assessment at 3 months concluded that the arterial territories involved by infarction hardly reflected the distribution of subarachnoid blood in the basal cisterns. Even the side of the infarcts corresponded only weakly with the side on which most extravasated blood was seen. Infarction occurred twice as often in patients with large amounts of subarachnoid blood. Clearance rate of cisternal blood was not apparently related to the occurrence of infarction. The conclusion that infarction is related to the total amount but not the distribution or clearance rate of extravasated blood is probably not shared by the majority of investigators in this field (525).

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CHAPTER 5

# RADIOLOGY

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## I. Introduction

The fundamental concept of VSP rests on angiographic observations. In the past half century clinical and pathological data have confirmed that reversible arterial narrowing can induce both reversible and irreversible ischemic changes in the brain that can result in transient symptoms, permanent disabilities, or death. It is also abundantly clear that VSP is not the only cause of delayed ischemic symptomatology. Fine judgment is required to select the radiologic study of choice in an attempt to sort out the relative contribution of arterial narrowing and other adverse physiological mechanisms. Digital subtraction angiography is currently the gold standard against which all other methods must be compared. Its disadvantages are obvious. Catheter angiography has its own independent risk of inducing ischemic brain damage and there is also the consideration of moving extremely sick patients and the resourse utilization involved. Unfortunately, there is almost an inverse relationship between the quality of physiological information obtained and the expense and danger of measuring it. Positron emission tomography (PET) provides wonderful information but is generally unattainable, whereas transcranial Doppler ultrasonography is almost universally available but the information obtained is seldom so robust as to be a safe basis for clinical decision making. Computed tomography (CT) angiography is a promising modality.

Angiographic VSP tends to occur in the basal conducting arteries within the subarachnoid space which are surrounded by the most blood clot. It generally takes 3 or 4 days to develop and often becomes maximal about a week following the SAH. Vessel calibers are generally back to normal dimensions 2 or 3 weeks after the SAH. The severity of VSP is roughly proportional to the volume of subarachnoid clot. Acute VSP lasting a few minutes probably occurs in a least some cases of aneurysmal rupture. VSP is usually a diffuse and tapered constriction. The greater the degree of constriction, the more likely infarction is to develop and death to ensue. The rupture of midline aneurysms usually produces the most bilateral VSP. Severe VSP is most likely to occur when a ruptured middle cerebral aneurysm causes a large-volume Sylvian clot. Since angiographic VSP reflects the volume of subarachnoid clot, it is more likely to be found in patients who had more severe hemorrhages and were therefore initially unconscious and in poorer grade on presentation. Actual arterial occlusion with VSP is rare but occurs. In very severe VSP, arterial narrowing may extend outside the subarachnoid space to involve the carotid artery in the skull and neck. VSP is an adverse prognostic factor for outcome. Operative results are worse for patients who are in the early phases of VSP or in whom severe VSP is already established.

There is no generally agreed upon objective standard for diagnosing VSP. Such a diagnosis is most secure when two sets of comparable angiograms are available with one being obtained either prior to or following the period of VSP. Some systems have attempted classification by absolute measurements of diameters, whereas others examined percentage changes between vessels on two sets of films. The likelihood of infarction occurring with clot-induced vessel narrowing depends on numerous physiological variables and very importantly on the age of the brain, the potential for leptomeningeal collaterals to form, and the coexistence of other arterial diseases such as proximal stenosis or occlusion and chronic hypertensive vasculopathy.

The frequency of VSP in a given series depends on the nature of the patient population as well as when angiography was performed. About two-thirds of patients will show significant VSP if angiography is performed 1 week following the SAH. Normally, only a small percentage show VSP in the first 1–3 days and there is a possibility that some of these cases may have had unrecognized prior SAH.

Recent studies suggest that VSP may involve widespread constriction of intraparenchymal vessels. Evidence for this comes from recent PET studies that, contrary to initial reports, do not suggest an increase in cerebral blood volume during severe VSP and from angiographic studies that show a prolongation of circulation times through the brain opposed to a decrease in circulation times in the angiographically demonstrated basal arteries. Why would the small vessels which are not surrounded by clot go into spasm? Would not local ischemic anoxia finally induce vasodilation?

Low-density areas in the territory of distribution of spastic arteries are the first CT evidence of potentially developing infarction. Some of these lesions resolve spontaneously, whereas others go on to bland infarction and a relatively small percentage evolve into hemorrhagic infarction. Hemorrhage is usually delayed for more than a couple of weeks. It may reflect reinstitution of flow to an infarcted area. During periods of intense angiographic VSP, the velocity of blood going through the spastic

segment tends to increase. The cerebral metabolic rate ultimately decreases if VSP is severe enough. The oxygen extraction ratio is also a defensive homeostatic mechanism and more oxygen is extracted by ischemic brain, which can compensate for diminished flow up to a certain point. Very severe VSP is concurrently demonstrable by both magnetic resonance angiography (MRA) and CT angiography. There are limiting technical factors in using these methodologies on a surveillance basis, but technological advances will likely make these more attractive diagnostic methods. Xenon CT shows promise in its ability to be conducted reasonable widely, relatively inexpensively, and with quantitative flow data correlated with anatomical information.

## II. Angiography

### A. Definition and Classification of Angiographic Vasospasm

Ecker and Riemenschneider diagnosed VSP when a vessel was found to be of larger caliber on a subsequent angiogram than it had been initially. They introduced this concept from the study of only 6 cases of anterior circle aneurysms. They had 23 other aneurysm cases in whom VSP was not shown. They noted that VSP was maximal at the site of the aneurysm and was most marked intracranially. Ten cases in whom angiograms were performed more than 26 days following SAH showed no VSP. In an early Japanese study of 109 SAH patients, VSP was classified as diffuse, segmental, nodular, tapering, and centrally sparing. Diffuse narrowing was found to impart a grave prognosis (1).

The mean diameter of the internal carotid artery about a centimeter proximal to its intracranial bifurcation is about 4.6 mm, and the middle cerebral about a centimeter from the bifurcation is approximately 3.8 mm in diameter. The arteries of males are approximately 0.2 mm larger than those of females at these points. There is a variation in the measurement of at least 0.1 mm. If the proximal anterior cerebrals are balanced the diameter is about 3 mm just beyond the carotid bifurcation. A 70% reduction in the internal carotid before the bifurcation would result in a change in diameter from 4.6 to 1.4 mm. Sometimes the easiest way to appreciate the presence of severe VSP is to note a sudden reduction in caliber from where the internal carotid artery enters the subarachnoid space (normally 5.1 mm to one-half or one-third of this dimension by the time the bifurcation is reached) (2).

From a review of 608 angiograms on 266 aneurysmal SAH cases, VSP was classified as diffuse (narrowing more than 2 cm in length), peripheral (narrowing of distal parts more than 2 cm in length), multifocal, and local (single localized narrowing). Diffuse VSP was classified as severe (more than 50% reduction in vessel caliber) and mild (25–50% reduction in vessel caliber). The incidence peaked on days 8–15 at 78%. Initially, VSP was usually diffuse but could develop into local or multifocal types. Severe diffuse VSP was usually associated with diminished cerebral blood flow and focal ischemic deficits (3).

Thirty preoperative angiograms from 30 different patients having SAH were assessed by four trained, experienced observers. Surprisingly, the demonstrated ability to diagnose VSP as present or absent or localized or generalized resulted in agreement that was not much better than chance (4). In this respect, the variability between observers is similar to that which occurs when objective grading of the clinical status of a patient is attempted following SAH. Determining the precise level of consciousness or whether a deficit is present is not always clear-cut (5).

### B. Method of Diagnosis

The gold standard for diagnosing cerebral aneurysms and VSP is biplane digital angiography using nonionic, water-soluble, iodinated contrast. Using 4 French catheters and selective vessel catheterization the complication rate is about 0.5% for persistent new neurological deficits. Angiography can visualize not only arterial but also venous abnormalities related to aneurysms and associated arteriopathies, vascular malformations, fibromuscular dysplasia, dissections, moya moya and venous sinus thrombosis. All of these may coexist with VSP in some patients (6).

Direct measurements of vessel diameters are fraught with difficulty because of the unsharpness of the images at the borders due to such factors as penumbra (geometric unsharpness), absorption unsharpness, blood vessel pulsations, patient or film movement, type of intensifying screen, and adequacy of exposure factors. There is still an element of guess work involved in determining the vessel edge even when using magnification. With current technology the magnitude of vessel unsharpness is approximately 0.5 mm. Despite this unsharpness, changes in vessel caliber of approximately 10% in a 1mm baboon artery or 4% in large cerebral arteries can be distinguished using 0.2-mm focal spot (7). The incidence of angiographic VSP in SAH depends on the timing of angiography and the nature of the patient population. Serial angiography is seldom performed. The diagnosis of angiographic VSP depends on the observation of luminal narrowing during the appropriate time period post-SAH which is not due to atheroma or anatomic variants such

as hypoplasia of the A1 segment of the anterior cerebral artery or streamlining artifacts (7).

### C. Clinical Series

In an early study of 100 aneurysm patients, Fletcher, Taveras, and Pool found that angiographic VSP correlated with poor neurologic status and focal neurologic deficits. VSP was described as segmental or diffuse and found to be present within 3 weeks after presumed aneurysmal rupture. The overall incidence in 100 patients was 39% (8). An early study of 28 patients with aneurysmal SAH showed a correlation between angiographic VSP and outcome. Fifty percent of cases underwent surgery and the mortality rate was 29%. VSP was found in 75% of operated patients who died but only 10% of operated patients who recovered. For the 14 nonsurgical patients the mortality rate was 50%, and all of these cases had VSP. This contrasted with only 29% of spontaneous recovery cases who had VSP. It was surmised that severe intracranial VSP is a crucial factor in determining outcome in patients regardless of whether they were treated conservatively or surgically. Patients with arterial hypertension and increased intracranial pressure seemed to be at particular risk if they had concurrent VSP; the coexistence of these three factors resulted in a mortality rate of 80%. When these factors were not present the mortality rate was only 5%. Another early study of 83 postoperative angiograms found VSP in 51% of patients operated on within 3 days, in 61% of those operated on days 3–10, and in 9% of those operated on more than 10 days after SAH. There was a growing consensus in the 1960s that surgery should be delayed to the time of least likelihood of VSP in order to improve the postoperative mortality (9).

Not all early studies showed a dramatically different mortality rate when VSP was demonstrated. This was sometimes due to the fact that angiography was performed outside the anticipated time for the occurrence of angiographic VSP (10). Gurdjian and Thomas noted that VSP was rare in the first 3 days after SAH and they observed that it was more likely to be seen 5–10 days after the bleed. They hypothesized that chronic VSP resulted from the production of vasoactive substances in the subarachnoid blood undergoing lysis (11). Suzuki and associates studied 44 cases with preoperative VSP and found a hospital mortality rate of 5%. This was not significantly different than that for non-VSP cases. Their policy was therefore to operate even in the face of established VSP, providing that the patient was not comatose or deteriorating. Of 254 aneurysm cases treated prior to 1969, only 2 of 37 patients with VSP died during hospitalization. In the non-VSP patients the mortality rate within 6 months after operation was 10.2% (12).

Seventy-nine patients were studied retrospectively and one-third developed VSP. Surgery had been delayed an average of more than 2 weeks in the majority of these North American cases. Postoperative VSP developed in almost half (13). In patients studied between 1967 and 1970 the smallest arterial measurements following SAH were noted between days 5 and 13 after the most recent SAH (14). One-half of 137 patients with SAH developed VSP. High-grade VSP developed in 10% of the patients in whom there was no or only a very transient loss of consciousness with the ictus. On the other hand, if the patient was initially somnolent or unconscious for several hours one-third had high-grade VSP (15). In one of the earliest cooperative studies about one-fifth of the patients with diffuse VSP died, in contrast to only about one-tenth who died who did not have VSP (16).

Weir and colleagues carried out a retrospective analysis of 274 aneurysm patients treated between 1968 and 1973. The 2-month outcome was correlated with the clinical grade at angiography or surgery, the presence of preoperative VSP, ICH or focal edema, arterial hypertension on admission, a shorter time interval to surgery, and increasing age. The data were believed to support a policy of early surgery on patients in good neurological condition even if there was angiographic VSP preoperatively (17). The role of VSP in determining outcome was questioned in the mid-1970s because outcome appeared to be more directly related to other factors, such as the patient's general state of health, the destructive effects of the initial bleed, the role of increased intracranial pressure, and autonomic and endocrine disturbances (18). Wilkins also reviewed 32 patients with ruptured aneurysms in whom rupture was documented during angiography. In one-third of these ruptures the extravasated dye obscured the major arteries, making a determination of acute VSP impossible. In 40% of cases the VSP was considered more likely related to a prior episode of bleeding. There was no evidence of VSP in the other 25% of patients. It was concluded that there was no definite proof that an acute phase of intracranial VSP occurred following aneurysmal rupture in humans (19).

Fisher and colleagues graded angiographic VSP according to the diameter of the residual lumen of the proximal segments of the anterior and middle cerebral arteries: 0, no narrowing, grade II, dye column 1 mm wide and distinct; grade III, lumen 0.5 mm and indistinct outline; and grade IV, lumen $<0.5$ mm and forward flow of contrast almost halted. For diagnosis of supraclinoid internal carotid artery the figures were 1 mm greater. Of 31 patients with grade III or IV plus VSP, 80% developed delayed ischemic deficit (DID). Of 19 patients with a lesser degree of angiographic narrowing, none developed DID (20).

Ninety-six consecutive ruptured aneurysm cases were studied with repeat angiography. Usually, at least 4 days elapsed between SAH and the onset of VSP, and it was observed to subside 2 weeks after onset. In all cases 4–11 days elapsed between the last SAH and the onset of postoperative VSP regardless of the timing of surgery. Angiographic VSP was associated with depression of consciousness in 19% of patients studied before post-SAH day 6, 73% days 7–14, and 81% day 15 or later. A total of 308 carotid angiograms were performed on 96 cases, an average of 3.2 studies per patient. In all cases but one, angiographic VSP was at sometime associated with neurological signs and symptoms. The case with no neurologic impairment had only slight narrowing of the cerebral arteries. The latest appearance of VSP was on day 16 post-SAH. Sixty-six percent of cases demonstrated symptoms attributed to VSP between days 6 and 9. The average interval between SAH and the onset of VSP was $7.7 \pm 2.6$ days. Angiographic VSP was classified as extensive diffuse, multisegmental, or local. The mortality rates for patients with these types were 45, 19, and 10%, respectively (21).

Weir and associates measured eight arterial points on 627 angiograms from 293 patients with aneurysms. A ratio between the sum of vessel diameters in the subarachnoid and the sum in the base of skull and neck was calculated and plotted against time. Using this ratio VSP appeared to begin about 3 days after SAH, was maximal at days 6–8, and was gone by day 12. There was a tendency for patients in poor clinic grades to have a greater degree of constriction. The patients with the most VSP had significantly higher mortality than those with the least (22). Ito created a similar ratio to assess VSP in 189 aneurysm cases. The mortality rate was 18% in patients with the least degree of VSP, 36% in patients with a moderate degree of VSP, and 41% in patients with the highest degree of VSP (23).

Sano and Saito studied 443 SAH cases. Over 90% had surgical clipping. The mortality rate was only 5%. The highest incidence of fatal postoperative VSP occurred in patients operated on between days 4 and 7 post-SAH. Good results were also obtained in patients operated later than 1 week after SAH. The authors recommended cisternal, ventricular, and epidural drainage after surgical clipping in patients operated on within 3 days after SAH. The incidence of VSP was 17% in the preoperative period and none of these cases occurred within the first 4 days after SAH. The peak incidence occurred between days 6 and 9 and affected two-thirds of the patients. On average, VSP lasted 2 weeks. These authors classified it as follows: type 1, extensive diffuse, which carries the worst prognosis; type 2, multisegmental; and type 3, local. The best prognosis was with type 3 (24).

In the largest aneurysm series studied (797 operated cases), VSP occurred within 3 days after SAH in only 4.2% of 120 cases having angiography in that time frame. VSP peaked on days 10–17; half of the 116 cases studied had it by then. In 39% of 62 patients who had a bleed prior to the index SAH, VSP occurred within 3 days after the index SAH. The presence of established severe VSP within a day or two of SAH therefore raises the likelihood that there has been a prior episode of bleeding (25). In the same large series, VSP tended to occur initially on arteries close to the aneurysm and later extended to other locations. Contralateral VSP or bilateral VSP was more frequent when aneurysms were close to the midsagittal line. Middle cerebral artery aneuryms carried the highest risk of significant VSP. Almost one-third of the vertebral aneurysm cases had angiographic VSP. In multiple aneurysm cases the spasm was usually most severe closer to the offending lesion. VSP was considered to relate to the presence of the hematoma within the subarachnoid space (26). One hundred and fifty-one patients with SAH admitted within 7 days showed a relationship between VSP and poor neurological condition on admission (27).

In one of the earliest careful studies of the extracranial carotid artery, Endo and coworkers found that when angiography reveals severe VSP of the intracranial arteries it could sometimes extend extracranially. They reviewed 23 patients with severe intracranial VSP in whom 50% also had involvement of the extracranial carotid artery system (28). Of 530 patients with single SAH, VSP occurred within the first 3 days in 4% in a subset of 120 studied during this period. The highest incidence was between days 10 and 17 (49% of 116 patients). The incidence of VSP within the first 3 days after the last SAH was 38% in patients with more than one hemorrhage (29).

In eight series with 1849 cases, seven showed mortality rates that were higher when VSP was present. Operations performed between days 3 and 8 in three of these series carried a higher mortality rate than when they were performed in the first 3 days following SAH. Delayed ischemic deficits were documented after day 4 post-SAH. The most common day for the appearance of such deficits was day 8 post-SAH. Only 1 of 45 patients had onset after day 14 post-SAH (30). When angiography was performed 1–3 days before contemplated operation, only 1 of 28 patients in whom VSP was absent or mild had a poor outcome, whereas 4 of 7 good-grade patients with VSP of moderate or severe degree had an unsatisfactory outcome. Seventeen patients who were neurological grade 1 more than 1 week after SAH had moderate or severe VSP. VSP of more than 25% was likely to be diffuse and associated with prolonged circulation times (31).

The failure of some investigators to recognize that VSP alone is sufficient to cause cerebral ischemia may be attributed to variation in the time at which angiographic identification of VSP has been carried out, failure to distinguish mild from severe VSP, and production of ischemic symptoms by unrelated causes such as ICH, surgical manipulation, hypotension, elevated intracranial pressure, direct embolization from intravascular thrombus, and already present vascular disease (32).

Arterial occlusions developed in 7% of 84 patients post-SAH from VSP. It was observed that the affected vessels were branches of the middle cerebral artery (MCA) and occlusions were demonstrated angiographically between days 11 and 30 (33). Another case in which VSP was associated with multiple intraluminal defects has been presented (34).

A strong correlation between the severity of VSP and the outcome was found in patients admitted in the first 2 days after SAH who were clinically grade III on admission. Of 5 such patients without VSP, all made an excellent recovery. Of the 8 patients with severe VSP, none had an excellent recovery, only 2 had a good recovery, and 1 had a fair recovery. Five patients died (35). In a very large series from Denmark (1368 patients prior to 1980) VSP was demonstrated in 53% of patients undergoing angiograms between days 4 and 16 post-SAH. There was no increased morbidity or mortality in the group of patients under the age of 19 years when VSP was present. None of these children developed cerebral infarction. Presumably, the excellent collateral circulatory potential associated with youth protects children against vasospastic infarction (36). Measurement of vessel diameters was made in 56 operated patients studied between 1990 and 1991. Most were in good clinical grade, with 13% being World Federation of Neurological Surgeons grade III and 11% grade IV. Ninety-five percent of the patients showed a reduction in the ratio of summed intracranial vessel diameters to extracranial vessel diameter. The mean decrease was 21% (range, 1–56%). The mean decrease in ratio in those with symptomatic VSP was 24%, and in those without symptomatic VSP the mean decrease was 19%. Symptomatic VSP developed in 30% of the patients. There was a trend for patients with clinical VSP to have worse angiogaphic VSP than asymptomatic patients but this did not achieve statistical significance. Of those developing clinical VSP, 70% achieved independence. Those not achieving independence showed more angiographic VSP in the second week post-SAH. Peak VSP occurred during days 10–14 and resolved by days 15 or 16. Clinical VSP was responsible for 36% of the poor outcomes in this recent series. It accounted for poor outcome in 6% of patients overall (37).

### D. Very Delayed Vasospasm

Occasional cases have been presented in the literature of very delayed cerebral ischemia associated with angiographic evidence of cerebral aterial luminal narrowing. In one report, three such cases were believed to present at 7, 14, and 52 weeks post-SAH. This is far outside the time period in which acute vasoconstriction from subarachnoid clot could possibly occur (38). Another case report involved a 50-year-old male who was operated on day 15 post-SAH and who developed a hemiparesis with concurrent severe angiographic VSP 2 days postop and 17 days post-SAH. It is difficult to believe that operative factors were not at least additive to possibly asymptomatic VSP (39). There was one other case reported of rapid deterioration due to VSP 18 days post-SAH (40).

### E. Nonaneurysmal Vasospasm

#### *1. Traumatic*

Reversible severe VSP in the supraclinoidal internal carotid artery was demonstrated in a 45-year-old female following trauma. Extremely bloody lumbar cerebrospinal fluid (CSF) had been demonstrated. Angiography excluded an aneurysm. Repeat angiography demonstrated that VSP disappeared prior to discharge (41). In 6 patients, vertebrobasilar VSP was observed in the post-traumatic period. The neurological examination suggested a supratentorial mass with herniation in 3 of these cases. Six cases had vertebral angiography demonstrating significant VSP in either the vertebral or the basilar arteries. No patient had an intracranial pressure (ICP) exceeding 25 mmHg (42). Six cases of delayed traumatic symptomatic VSP were studied with CT scans. Initial CT scans were obtained on the day of injury in 4 cases and on the second and third days in the other 2 cases. The severity of SAH was judged to be mild in 4 cases. Five of the 6 had temporal and or frontal contusions. Ischemic symptoms developed between days 5 and 13 post-head injury. Angiography revealed spasm in all patients, and spasm was bilateral in 2. Angiographically, the VSP lasted 2–5 weeks. Two elderly patients with bilateral spasm remained in a vegetative state (43). A 71-year-old patient with a head injury and impairment of consciousness showed a faint SAH in the left Sylvian fissure. Secondary loss of consciousness occurred on the 14th day and carotid angiography demonstrated segmental VSP. It was suggested that a direct mechanical injury to the arterial wall could be a cause of traumatic VSP (44). In a study of 852 severely head-injured patients entered into a prospective trial of nimodipine, there was a trend toward a better outcome in the patients

receiving nimodipine who exhibited traumatic SAH on the initial CT scan (45). Two hundred and eight multiple-trauma patients with head injury were classified as having severe, moderate, and minor head injuries. Subarachnoid hemorrhage occurred in 27% of the severe head-injured group and 12 and 8% of the moderate and minor groups, respectively (46).

#### *2. Arteriovenous Malformations*

VSP was confirmed in 4 of 13 patients with SAH from arteriovenous malformation (AVM). The incidence of VSP following rupture of AVM in this series was higher than that in previous reports in which VSP ranged between 8 and 12% in the AVM population. The existence of VSP was associated with massive SAH when it occurred (47). Fifty patients with AVM with and without SAH were studied with respect to the time course of VSP. It was found to occur in these patients between days 3 and 11 post-SAH. Patients with ICH showed VSP from the first day onwards. There was no observed correlation between angiographic VSP and clinical outcome in these AVM cases (48).

#### *3. Eclampsia*

Cerebral angiography demonstrated spasm of both large- and medium-caliber arteries in the setting of the patient with postpartum eclampsia (49). A fatal case of postpartum cerebral infarction was described in which angiography implicated VSP as the primary etiology (50). Magnetic resonance (MR) angiography has been used to demonstrate cerebral VSP in a case of eclampsia (51). Four patients with eclampsia were investigated with CT and MRI. Low-density areas on CT and $T_2$ high-intensity areas on MRI disappeared within a month in three of the four cases. In one, however, cerebral infarction occurred with associated right hemiparesis and aphasia. Angiography performed in the acute phase demonstrated VSP in all cases and arterial occlusion of the MCA due to VSP in the patient who did not recover. VSP was of several types described as diffuse, peripheral, and multifocal. One patient showed segmental VSP of both vertebral arteries. SAH had been excluded by lumbar puncture in all cases. It was concluded that eclampsia by itself could cause cerebral VSP and that the mechanism did not involve SAH. It was suspected that the angiographic VSP might relate to cytotoxic edema, blood–brain barrier dysfunction, or cerebral autoregulatory dysfunction (52).

### F. Acute Angiographic Vasospasm

A single patient rebled while undergoing angiography 12 hr after the original aneurysmal rupture. The rupture occurred during the injection of contrast material. It was possible to see delayed filling in the MCA complex along with narrowing. A subsequent injection 14 min after the first set of films showed that the initial narrowing had completely disappeared. This is perhaps the best documented case in the literature of an acute transient spasm from aneurysmal rupture. The aneurysm in this case was on the internal carotid artery ipsilateral to the observed acute spasm (53).

### G. Vertebrobasilar Vasospasm

VSP was demonstrated in 75% of 12 vertebral basilar aneurysms. In a review of 24 series the incidence of VSP on preoperative angiograms on vertebral basilar aneurysm cases ranged between 21 and 62%. Eight series of vertebral basilar aneurysm VSP showed the highest frequency in the second week post-SAH (54).

### H. Operation and Vasospasm

The amount of subarachnoid blood clot was noted at surgery in 28 patients, and this was correlated with angiographic VSP. The angiography was performed at intervals ranging between 5 and 17 days and surgery was performed no later than 3 weeks following SAH. Ten patients had no subarachnoid clot at the time of surgery, no or only minor VSP, and no or only mild neurological deficits. Thin clots were found in 8 patients, only 1 of whom had no VSP, 6 had mild VSP, and 1 showed severe vessel narrowing. Major clinical signs were absent in all these cases. Of the 10 patients in whom thick clots were found at surgery, all developed severe VSP and 8 of the 10 had associated severe neurological signs (55).

### I. The Venous System and Vasospasm

The cerebral venous system has seldom been examined in patients who were studied for arterial VSP. In 18 patients with ruptured middle cerebral artery aneurysms operated within 3 days of SAH, Hunt and Hess grades I or II and Fisher's CT grades II or III, arterial VSP was observed in 16 cases—in 9 it was localized and in 7 diffuse. Delayed ischemic neurologic deficits developed in 10 cases; they were transient in 6 and permanent in 4. Of interest was the fact that when the superficial Sylvian veins were visualized and normal on the postoperative angiogram the patients had a good outcome, even if they had diffuse arterial VSP. On the other hand, the outcome was fair at best in those patients who had poor visualization of the superficial Sylvian veins regardless of the presence or not of arterial VSP (56). This is an important observation.

### J. Automated Assessment

In a rabbit model, angiographic VSP was studied using computerized quantitation methods for analysis of digital angiograms. Edge detection algorithms were employed. It was stated that this method would reduce error measurement due to intraobserver variability in assessments (57).

### K. Mean Transit Time and the Intraparenchymal Circulation

Circulation times using conventional subtraction angiography were based on differences in maximal time density curves using two regions of interest—the proximal internal carotid artery and the Rolandic vein. Circulation time in 3 patients with VSP was 3.6 sec, in patients with slight to moderate VSP it was 4.3 sec, and in patients with severe VSP it was 6.8 sec. The circulation time in 10 patients showing cerebral infarction averaged 7 sec (58). Nineteen patients had symptomatic VSP and 6 patients did not. The patients with severe VSP were treated by endovascular balloon angioplasty and superselective infusion of 0.2% papaverine. Symptomatic VSP occurred on days 7–11 post-SAH. The mean transit time just after onset of neurologic deterioration in 10 patients with complete neurologic recovery was 6.92 sec, and in those without complete recovery it was prolonged 7.66 sec. The difference was significant (59).

Circulation (transit) time was divided into proximal (time to traverse the large, basal, angiographically visualized arteries) and peripheral (time to traverse the brain and intraparenchymal small vessels) in a study of 24 SAH cases. Times were assessed by analyzing the time – density curve of the contrast media on digital subtraction angiogram (DSA) images. Regional cerebral blood flow (rCBF) was measured by single photon emission CT. Severe angiographic VSP correlated with a reduced rCBF. A prolongation of the peripheral (intraparenchymal) circulation time was strongly inversely correlated with rCBF. This is consistent with histopathological studies showing luminal narrowing of intraparenchymal vessels after experimental SAH. The proximal circulation time, in contrast, showed a decrease with increasing angiographic VSP. This is consistent with increasing transcranial Doppler ultrasonography (TCD) velocities in VSP (60).

### L. Extradural Vasospasm

A retrospective review was performed in 107 patients having unilateral carotid angiography, 37 patients having bilateral carotid angiography, and 37 patients having carotid angiography who did not have aneurysms, although all patients had SAH. There was a significant reduction in vascular diameters of both intra- and extradural parts of the internal carotid artery in these SAH patients. There was a successive reduction in vascular diameters during the first 5 days after SAH in both the aneurysmal and nonaneurysmal groups. When bilateral angiogaphy was performed there was a significant reduction in the vascular diameters on both sides (61).

## III. CT Scan

### A. Early Demonstration of Subarachnoid Hemorrhage

In 1980, Liliequist and coworkers published results of CT examinations of 39 patients post-SAH. Blood was visualized in 56% of first-generation scans and blood was noted to persist for as long as 5 days post-SAH. They had 6 cases of VSP without evidence of infarction on CT and 2 patients with DID, angiographic VSP, and unilateral low-density expanding lesions (62).

### B. Duration of Subarachnoid Hemorrhage on CT Scan

In a large cooperative study the CT scan showed blood in 92% of studies done on day 0. This rate fell progressively to 58% on day 5 (Table 5.1). Thirty-six grade III and IV patients operated by day 6 post-SAH had multiple CT scans. These were assessed for the rate of diminution of subarachnoid blood. The blood disappeared more quickly when there was a shorter interval between SAH and the initial CT scan, with increasing patient age and with higher SAH grades. The more rapidly the subarachnoid blood disappeared, the less the incidence of angiographic and symptomatic VSP and the fewer the low-density areas on CT scans. Low-density areas on the CT scan with permanent neurological deficits occurred in 5 of

**TABLE 5.1 Findings on First CT Scan of 3451 Patients Post-SAH[a]**

| | Day | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | Total |
| SAH (%) | 92 | 86 | 76 | 68 | 65 | 58 | 85 |
| Diffuse SAH (%) | 56 | 48 | 37 | 31 | 38 | 42 | 50 |
| Thin SAH (%) | 16 | 20 | 21 | 19 | 16 | 15 | 18 |
| Thick SAH (%) | 33 | 31 | 26 | 24 | 20 | 18 | 30 |

[a] Reproduced with permission from Kassell, N. F. *et al.* (1990). *J. Neurosurg.* **73,** 18–36.

9 patients showing no diminution in subarachnoid blood, whereas this occurred in only 5 of the 27 patients showing rapid diminution (63).

One hundred and twenty-five patients had early surgical treatment for ruptured aneurysms. The mean day of surgery was 2.1 days (range, 0–6 days). Symptomatic VSP developed on a mean of 7.4 days (range, 5–12 days). CBF was measured using Xe-enhanced CT. Of the 13% who developed symptomatic VSP, the subarachnoid clots were localized, thick, or associated with ICH in 94%. In only 1 case (6%) was the subarachnoid clot thin on the admission CT scan. Ninety-four percent of the patients who developed VSP showed CT evidence of continuing subarachnoid clot during the time of symptomatic VSP. In 3 patients there were residual cisternal clots remote from the surgical trajectory which resulted in clinical manifestations of VSP appropriate to the site of residual clot. In 8 patients with CBF studies the CBF was reduced in the territory of the arteries involved with VSP. In 57 patients who did not develop VSP the subarachnoid clots were already gone or disappeared very soon after surgery in most patients. It was concluded that the continued presence of subarachnoid clots is an important risk factor for symptomatic VSP (64).

Serial CT scans were first performed on 100 patients post-SAH within the first 2 days. All were positive for blood. The probability of recognizing an aneurysmal SAH on CT scans was 85% at 5 days, 50% after 1 week, and 30% after 2 weeks (most of the blood at 2 weeks would be ICH and not SAH). Occasional cases were noted in which SAH would persist for as long as 3 weeks in the subarachnoid space (65). CT was performed within 3 days of SAH in 1378 patients in a cooperative study. Intracranial blood was detected in 95% on day 0, 91% day 1, and 74% on day 3. CT was normal in only 1 of 284 patients in stupor or coma but was normal in 15% of 638 alert patients (66). CT scans were performed within 7 days of SAH on 242 cases. Fifty-six percent showed angiographic VSP and associated clinical deterioration occurred in 34%. Low-density areas were detected in 20% of cases. There was a strict correlation between the amount of cisternal blood and the subsequent development of VSP. Thick subarachnoid clot was associated with angiographic VSP in 72% and with ischemic symptoms in 51%, as well as subsequent low-density areas on CT in 30%. Patients with blood in multiple cisterns developed angiographic VSP 79% of the time, whereas patients with only frontal hemispheric blood had a 42% incidence. It was suggested that the persistence of SAH beyond 72 hr probably increased the risk of VSP (67). During the days following SAH, blood is redistributed within the subarachnoid space and eventually reabsorbed. It clears first from the basal cisterns during the time when the amount of blood in the cortical sulci may actually increase. It has been suggested that the RBCs act as tracers of the CSF circulation (68).

Figures 5.1–5.3 are examples of cases with subarachnoid clots; the persistence of such clot beyond 5 days is an ominous sign and probably indicates a large initial hemorrhage and/or impaired clearance of RBCs.

### C. Relationship of Subarachnoid Hemorrhage on CT Scan to Angiographic Vasospasm and Infarction

This relationship is illustrated in Fig. 5.4. The first suggestion of a relationship between blood on CT and the development of VSP was made by Katada and coworkers in 1977. The following year 73 patients with SAH were studied by Takemae and associates during the acute stage. Thirty-nine underwent CT within the first 4 days and of these 77% had high-density blood observed in the subarachnoid space. This high density disappeared between days 4 and 22 on the repeat CT scans done on 10 patients who were treated conservatively. It was possible to predict the development of VSP by examining these high-density areas within the first 4 days after SAH. VSP occurred between days 5 and 15 and developed in 83% of the patients with high-density areas in the basal cisterns and in 78% of the patients with blood within the sylvian fissure. No VSP developed in patients without such high-density areas on the CT scan. When the CT scan was performed more than 4 days post-SAH, no relationship was demonstrable between the CT findings and VSP. In addition, they noted that high-density areas on the CT scan within the first 4 days agreed with the distribution of subsequent angiographic VSP. They recommended early surgery to remove blood clot and prevent VSP from developing (69,70). These were landmark observations.

CT scan demonstrated infarction in the territory of spastic arteries in 71% of patients with VSP. Permanent DIDs were always found to accompany VSP affecting one carotid system and the anterior cerebral artery system of the opposite side. When VSP was restricted to only one carotid system or the anterior cerebral arteries, it was usually associated with only temporary symptoms. Infarction on CT scan and clinical symptomotology was always present if VSP involved the ascending branches of the MCA. VSP developed in 37% of 121 patients. VSP developed only in those cerebral arteries immersed in blood-stained cerebrospinal fluid. CT depicted low-density areas or infarction in the territory of the spastic arteries in 71% of cases (71).

VSP developed in 85% of 26 patient who had evidence of blood on CT scans performed within 4 days of the SAH. In 8 patients not having evidence of blood within 4 days of SAH, no VSP developed. This relationship between blood and subsequent clinical VSP was not

demonstrable if the scans were performed more than 5 days post-SAH. The CT scan was positive for blood in 83% of scans performed within the first 4 days (72). Thirty-two patients who were unoperated in the first 2 weeks post-SAH and who did not have IVH, ICH, or initial loss of consciousness, showed no significant blood in the subarachnoid space on the initial CT scan. On the contrary, most of the patients who initially lost consciousness did show high densities of subarachnoid blood. The ones with the initial high-density blood were those who subsequently developed cerebral infarction. Six of the 7 patients with the areas of the highest densities in the Sylvian fissures showed contralateral hemiparesis accompanied by a severe angiographic VSP. Delayed clinical impairment was predicted by the CT density of the subarachnoid clot (Hounsfield numbers >60) (73).

Of 47 cases studied by CT post-SAH, only 1 of 18 patients in whom there was no subarachnoid blood

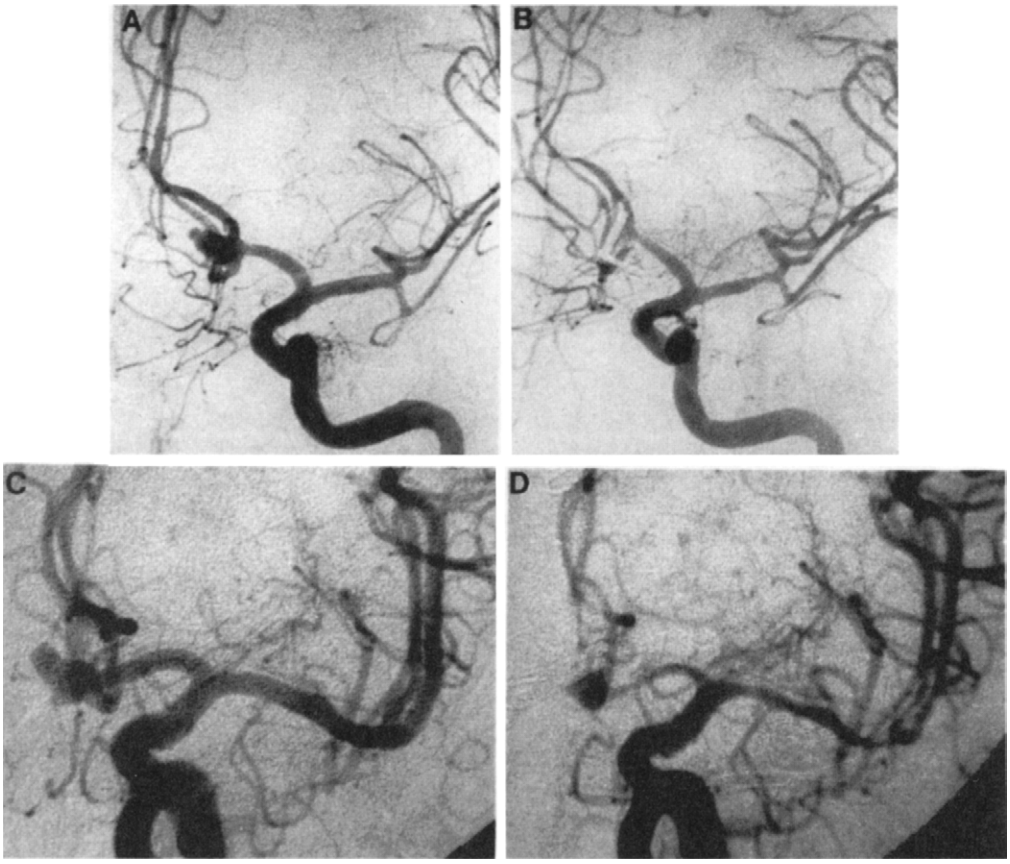



**FIGURE 5.1** (i) Photographs of angiograms of two patients with diffuse thick SAH [same patients as Fig.1(ii)]. The admission left internal carotid angiograms, obtained within 24 hr of SAH, showed that both patients had anterior communicating artery aneurysms (A, patient 1; C, patient 2). There was no vasospasm. Four days later, left internal carotid angiogram of patient 1 (B), who received t-PA, showed only mild vasospasm of proximal middle and anterior cerebral arteries. There was more marked narrowing, however, of the pericallosal arteries. In contrast, left internal carotid angiogram of patient 2 (D), who did not receive t-PA, showed severe vasospasm (>50% reduction in lumen diameter) of proximal middle and anterior cerebral arteries as well as narrowing of more peripheral branches. Transluminal angioplasty was performed to prevent cerebral infarction. (ii) Photographs of CT scans of two patients with diffuse thick SAH. Similar amounts of SAH were present within 24 hr of ictus (A, patient 1; D, patient 2). Patient 1 received 10 mg of intracisternal t-PA after aneurysm clipping and demonstrated extensive clot clearance, particularly in the midline basal cisterns, on CT scan 24 hr later (B). In the second patient, who did not receive t-PA, more clot remained 24 hr later (E). By 4 days after SAH, subarachnoid blood was cleared completely in patient 1 (C), whereas the basal cisterns still contained resolving blood in patient 2 (F) [reproduced with permission from Macdonald, L. R., and Weir, B. K. (1994). In *Concepts of Neurosurgery. Ruptured Cerebral Aneurysms. Perioperative Management* (R. A. Racheson and F. P. Wirth, Eds.), Williams & Wilkins, Baltimore].

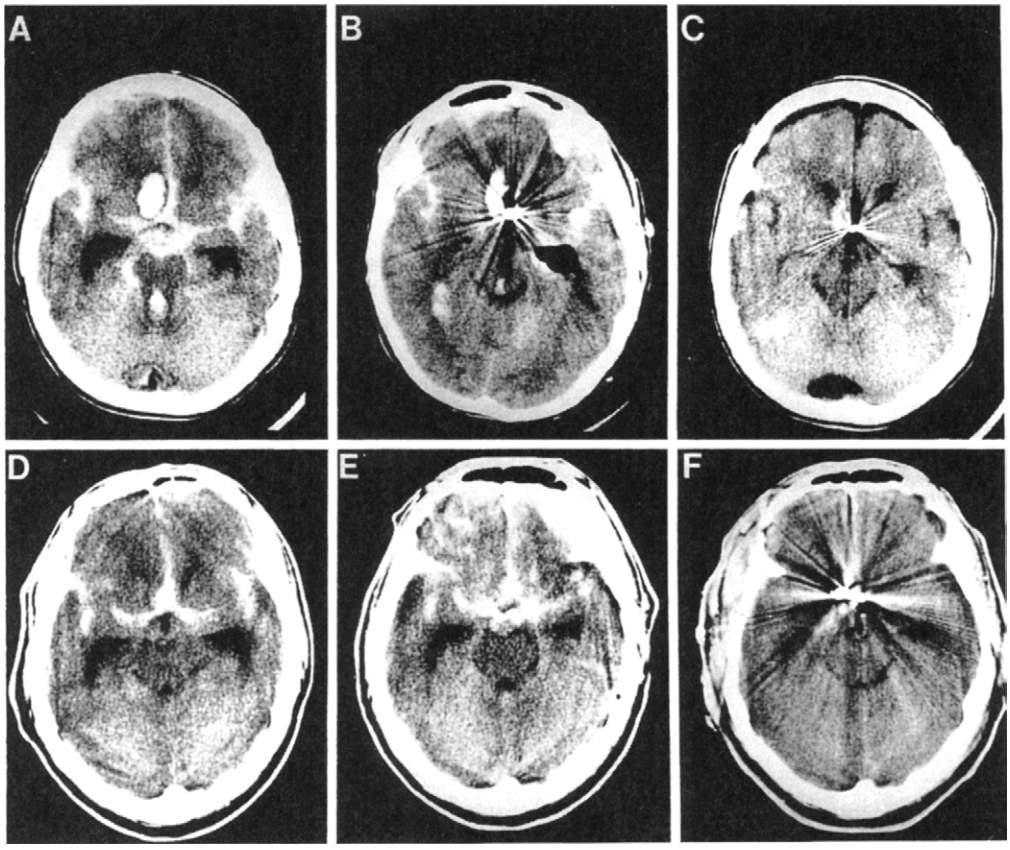



**FIGURE 5.1** (*Continued*)

developed severe VSP. Ninety-six percent of patients with thick subarachnoid clot developed VSP. Every patient with severe VSP manifested delayed symptoms and signs. The very significant conclusion was that blood localized in the subarachnoid space in significant amounts at specific sites is the only important etiological factor in VSP. Of 28 patients with no or mild SAH as judged by CT, 68% developed no worse than mild VSP (74). CTs were classified by the amount of SAH (Table 5.2). Fisher *et al.*'s subsequent prospective study substantiated the initial conclusions (Table 5.3). Abnormal contrast enhancement was not found to be of significance. CT scans were positive for blood post-SAH in 81% of those examined within 2 days and 75% within 5 days. Blood was sometimes noted to be present in the fourth ventricle without being detectable in the lateral ventricles following the rupture of supratentorial aneurysms. This was ascribed to retrograde circulation of CSF. No strict correlation was found between the distribution of blood in the cisterns and the site of a ruptured aneurysm in this early study (62).

The relationship between high-density blood in the subarachnoid space on the CT and the subsequent development of VSP was studied in 177 patients. In the 26 cases with high-density blood noted within the first 4 days post-SAH, VSP occurred in 85%. No VSP was seen when the CT was negative for blood (72). In a study of 110 cases post-SAH, it was considered that subarachnoid blood clot found surrounding the cerebral arteries for at least 3 days after SAH was the most important factor causing VSP (75). Forty-one SAH patients were prospectively studied and a prediction of VSP was made on the basis of CT findings. Fifty-four percent of the patients had large clots or thick layers of hematoma. Ninety-one percent of these subsequently developed severe VSP which was correctly predicted. There were only two false positives. In 46% of patients with no blood, diffuse blood, or ICH, the absence of VSP was correctly predicted in 74%, but there were five false negatives. All the false-positive and false-negative predictions were explained by inadequate CT techniques. This study indicated that the extent and the location of blood in the subarachnoid space determine the severity and location of VSP and indicate which patients are at most jeopardy (76).

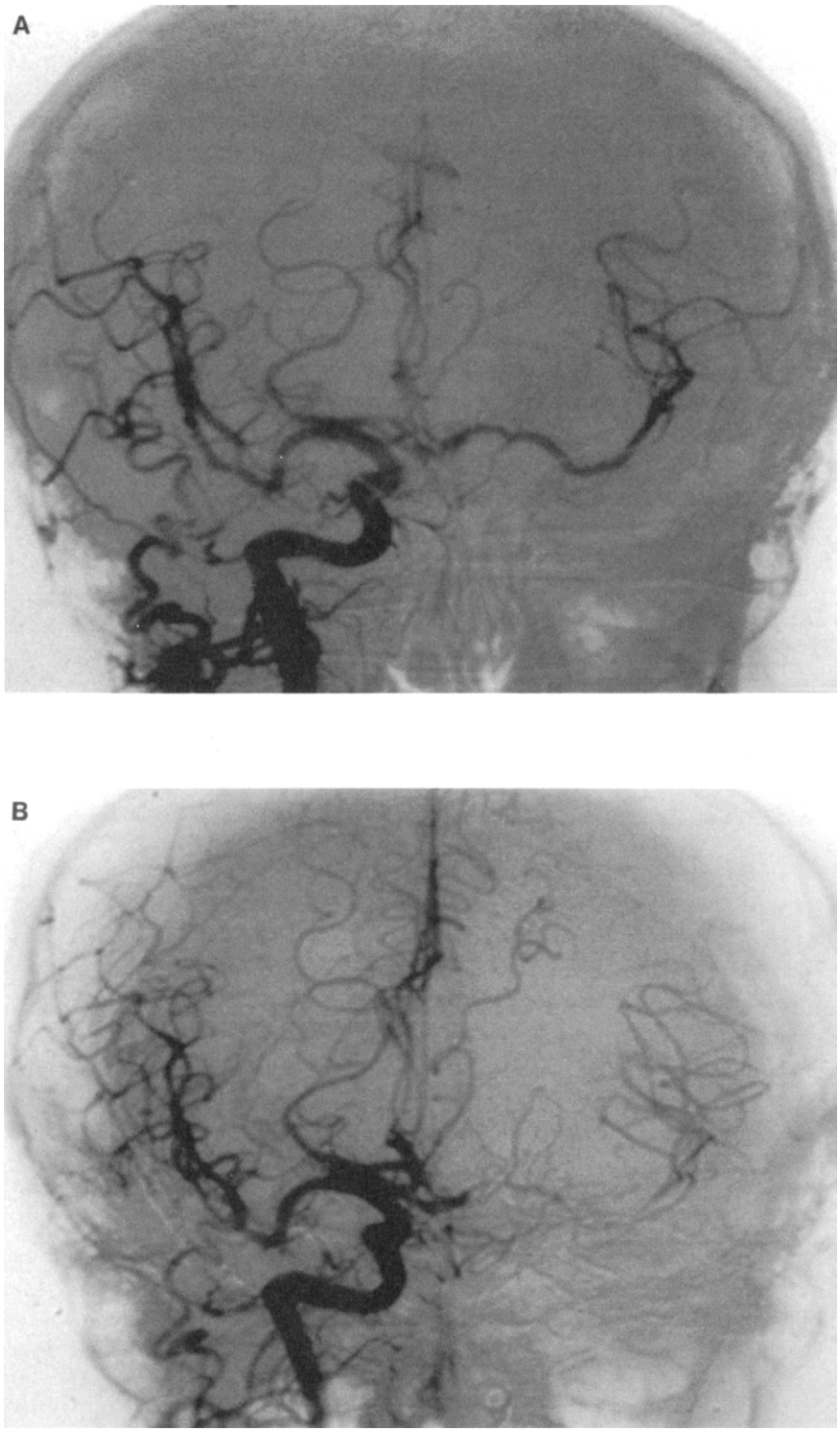
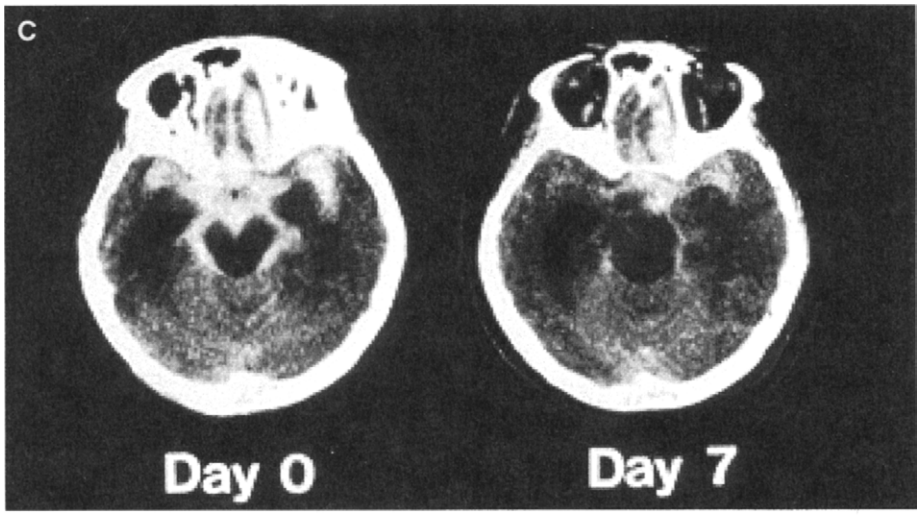



**FIGURE 5.2** (A) Right carotid angiogram on day 0 shows normal vessel caliber. The patient had a remote left carotid occulsion which was well tolerated before the SAH. (B) Repeat angiography on day 7 demonstrates severe diffuse VSP affecting the left anterior carotid circulation—proximal and distal; in the absence of the preexisting structure lesion this was not tolerated. (C) CT scan on day 0 demonstrates a massive SAH (grade III) which is usually followed by VSP. Of further ominous import was the presistence of significant basal clot to day 7. (D) A CT scan done shortly before death demonstrates a massive left hemispheric low density and SAH which has migrated over the hemispheric sulci.



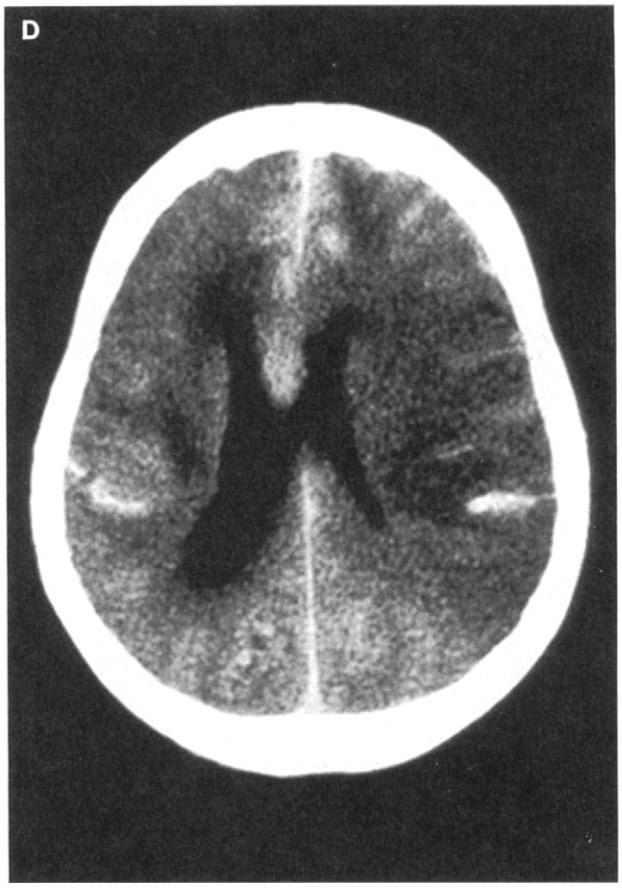




**FIGURE 5.2.** (*Continued*)

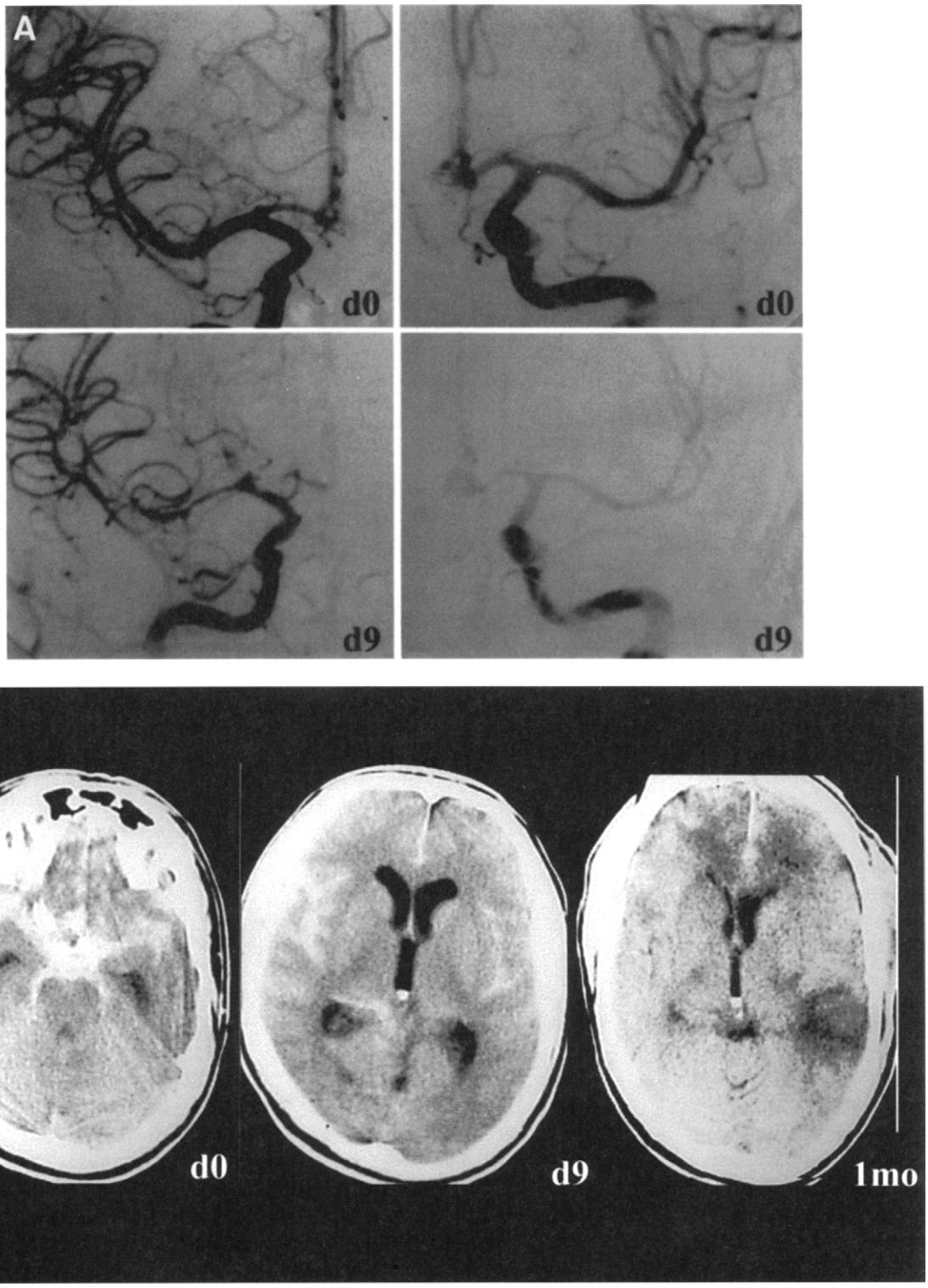



**FIGURE 5.3** (A) Right and left carotid angiograms show normal vessel caliber on day 0 post-SAH. The ruptured anterior communicating aneurysm is not well visualized. By day 9 there is severe diffuse VSP. Note on the left carotid injection that the dye appears "washed out" in the intracranial vessels. This is an ominous sign of severe VSP. (B) CT scans from the same case. The initial SAH is massive (grade III), and this along with persistence of considerable SAH at day 9 are prognostic of a poor outcome. The late CT scan demonstrates bifrontal and left parietal low-density areas.

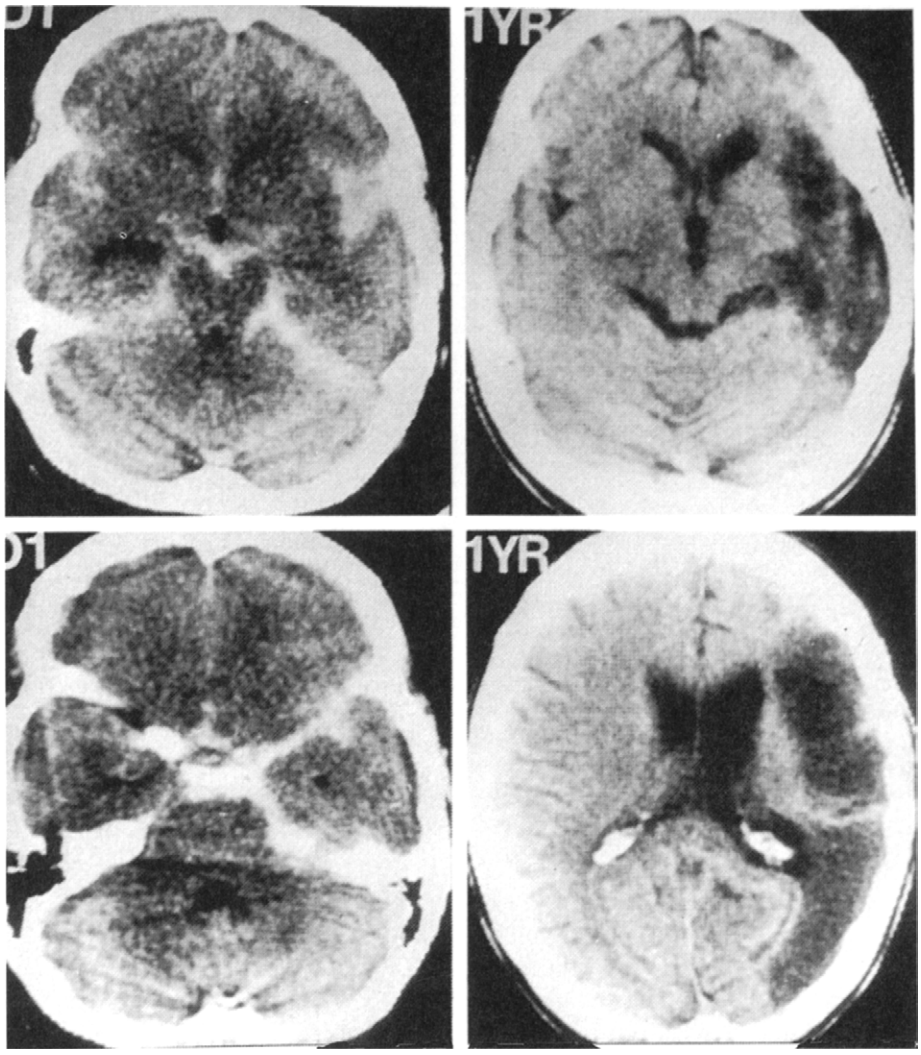


**FIGURE 5.4** CT scans done on day 1 post-SAH (left) show thick clot mainly in the left sylvian cisterns. CT scan 1 year later shows extensive infarction in the left MCA territory in this patient, who recovered to the point of moderate dysphasia and right hemiparesis [from *Subarachnoid Hemorrhage: Causes and Cures* by Bryce Weir, copyright © 1998 by Oxford University Press, Inc. Used by permission of Oxford University Press, Inc.].

In 72 cases post-SAH, there was a correlation between surgical outcome and the development of neurological deficits from VSP and high-density areas in the preoperative CT scan, particularly when they indicated localized thick layers of blood in the subarachnoid space. No relationship between the development of Hyc and the preoperative CT findings was noted (77). One hundred patients admitted within 4 days of SAH were studied. Delayed ischemic deficits developed in one-third of 36 patients with medium SAH on CT (a localized thick layer of blood without diffuse deposition throughout the cisterns or diffuse depositions without a thick layer), and at 2 months post-SAH 25% were conscious but totally dependent, vegetative, or dead. Delayed ischemic deficits developed in 63% of 48 patients with severe SAH (diffuse deposition and localized thick layer), and 63% had the poor outcome noted previously. All 16 patients with minimal SAH had a good outcome. Of 20 patients with the most blood on CT scan and who were treated with antifibrinolytics and developed DID, 80% had a poor outcome. By comparison, of 10 patients with DID and a similar CT appearance but who were not on antifibrinolytics, only 30% did poorly (78). A single patient with ICH and IVH but no SAH by CT or early CSF examination still went on to develop profound clinical and angiographic VSP (79).

**TABLE 5.2 Method of Fisher for Grading the Amount of Subarachnoid Blood on Computed Tomography after Subarachnoid Hemorrhage[a]**

| Grade | Features | Risk of vasospasm |
|---|---|---|
| 1 | No detectable blood on computed tomography | Low |
| 2 | Diffuse blood that does not appear dense enough to represent a large, thick homogenous clot | Low |
| 3 | Dense collection of blood that appears to represent a clot more than 1 mm thick in the vertical plane (interhemispheric fissure, insular cistern, or ambient cistern) or greater than 5 × 3 mm in longitudinal and transverse dimension in horizontal plane (stem of Sylvian fissure, Sylvian cistern, and interpeduncular cistern) | High |
| 4 | Intracerebral or intraventricular clots but with only diffuse blood or no blood in the basal cisterns | Low |

[a]Data from Fisher, C. M., Kistler, J. P., and Davis, J. M. (1980). Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. *Neurosurgery* **6,** 1.

### D. Relationship of Blood on CT Scan to Hydrocephalus

The ventriculocranial ratio (ratio of the distance between the roughly parallel walls of the frontal horns of the lateral ventricles at the head of the caudate nuclei and the inner tables of the skull at that level on the axial CT) is a useful objective measure of developing Hyc (Table 5.4). The following findings were obtained from 47 patients with no or mild diffuse SAH on CT scans; acute Hyc, 29%; delayed VSP, 7%; and chronic Hyc, 14%. In contrast, for the patients with thick diffuse SAH the findings were as follows: acute Hyc, 70%; delayed VSP, 64%; and chronic Hyc, 58%. There was a statistically significantly association between acute Hyc and VSP.

**TABLE 5.4 Upper 95% Confidence Value for Ventriculocranial Ratio, by Age[a]**

| Age (years) | Upper 95% confidence value |
|---|---|
| <30 | 0.16 |
| <50 | 0.18 |
| <80 | 0.21 |
| <100 | 0.25 |

[a]Data from van Gijn, J., Hijdra, A., Wijdicks, E. F. M.,Vermeulen, M., and van Crevel, H. (1985). Acute hydrocephalus after aneurysmal subarachnoid hemorrhage. *J. Neurosurg.* **63,** 355–347.

Most of the patients with chronic Hyc had shown severe SAH on the initial CT scan. These sequella of SAH have a common etiology in the presence of subarachnoid clots (80). Hydrocephalus was diagnosed on admission CT in 15% of patients. Clinically significant Hyc was related to increasing age; preexisting hypertension; admission blood pressure; postoperative hypertension; admission findings of IVH, a diffuse SAH, and thick focal SAH; posterior circulation site of aneurysm; focal ischemic deficit; antifibrinolytic drugs; hyponatremia; poor neurologic grade and a poor outcome. Hypertensive poor-grade patients with posterior circulation aneurysms and large SAH and IVH would be at greatest risk of chronic Hyc (81).

### E. Computed Tomographic Prognostic Factors for Poor Outcome

In 50 patients with aneurysmal SAH, 89% of patients with no or minimal SAH became no worse than a neurological grade III. Fifty percent of patients with significant SAH became grade IV or V. Of 28 patients with no or mild SAH as judged by CT, 68% developed no worse than mild VSP. Of 20 patients with significant SAH on CT scans, 70% developed severe VSP (82). In patients with

**TABLE 5.3 Summary of the Relationship between the Predictive Groups and the Subsequent Development of Vasospasm[a]**

| Group | Subarachnoid blood | No. of cases | Vasospasm | | | |
|---|---|---|---|---|---|---|
| | | | None | Slight to moderate | Severe | Signs of severe spasm |
| 1 | None | 3 | 3 | | | 0 |
| 2 | Diffuse only | 14 | 6 | 3 | 5 | 2 |
| 3 | Clot of thick layer | 22 | 2 | | 20 | 19 |
| 4 | Diffuse or none with cerebral or ventricular blood | 2 | 1 | 1 | | 0 |

[a]From Kistler, J. P., Crowell, R. M., *et al.* (1983). The relationship of cerebral vasospasm to the extent and location of subarachnoid blood visualization by CT scan: A prospective study. *Neurology* **33,** 424–436.

persistent vegetative state for more than 3 months, the finding of diffuse low densities in the cerebral cortex or diffuse low density in the cerebral white matter indicated a poor prognosis. Low-density areas in both basal ganglia were also of poor prognostic import (83). Based on cooperative aneurysm study data, the following was found. If an alert patient had a normal CT, 6 month mortality was 2.8%; if blood was seen mortality was 12.3%. ICH or local, thick, or diffuse SAH on CT among alert patients were particularly related to increase mortality (84). Based on the initial 934 patients in the cooperative study on timing of aneurysm surgery, the initial CT appearance was found to be the best prognostic indicator for the subsequent development of delayed ischemia. A normal CT within 5 days of SAH was associated with a very low risk of DID. Cerebral ischemia was significantly more likely in patients with CT evidence of focal, thick blood collections whether treated with antifibrinolytic drugs (47.4%) or not so treated (38.5%). The subsequent development of ischemic signs was not predicted by CT evidence of Hyc, IVH, or ICH (66). Four hundred and seventy-one consecutive patients with aneurysmal SAH were studied. Factors predicting a poor outcome included low Glasgow Coma Scale, treatment by fluid restriction, older age, initial loss of consciousness, or large amount of subarachnoid blood on CT scan (odds ratio 2.0; 1.3–3.1 confidence interval). Delayed infarction was predicted by a large amount of subarachnoid blood (odds ratio 1.8; 1.2–2.6 confidence interval) or treatment with antifibrinolytics. The total amount of subarachnoid blood on the initial CT was an independent predictor of the occurrence of delayed cerebral ischemia (85).

### F. Computed Tomographic Demonstration of Ischemic and Hemorrhagic Infarction

Ninety-four of 100 patients showed a postoperative increase in cerebral volume, 22% of patients had postoperative CT scan evidence of infarction, and 1 had hemorrhagic infarction. Eighty-six percent of the postoperative low-density areas were ascribed to surgical trauma rather than VSP, which was considered to be the cause in only 1 case (86).

### G. Quantification of Degree of Subarachnoid Hemorrhage on CT Scan

The Hounsfield CT number in the basal cisterns in studies performed within the first 24 hr post-SAH correlated with the severity of the attack as judged by the duration of the initial loss of consciousness. These were presumably functions of the volume of the SAH (87). One hundred and thirty-one patients with aneurysms were studied by CT and a scoring method was devised on the basis of quantification of the largest clot visible. With increasing amounts of subarachnoid blood on the admission CT scan the neurologic grade worsened. Ninety percent of the patients in the category with the highest CT score developed DID. Angiographic VSP was present in 37% of patients with no measurable collections of subarachnoid blood on CT, although this did not imply the development of neurologic deficits. Angiographic VSP was present in 55% of patients with subarachnoid blood of any type (88). Operation within the first 48 hr post-SAH was performed in 36 patients. The Hounsfield numbers in the basal cisterns were studied. In 17 patients without VSP the mean number preoperatively was 65.7, which fell to 62.2 postoperatively. In 19 patients with VSP the comparable numbers were 77.6 preoperatively and 77.5 postoperatively. With numbers below 68, VSP did not occur. In the range of 68–73 half the patients developed VSP and above 73 all of them did so. An attempt at extensive removal of cisternal clot was made (89).

CT attenuation values were compared to MRI signal intensities in mixtures of normal human CSF and normal nonheparinized blood ranging from 0 to 100% by volume. The Hounsfield CT measurements ranged in a nonlinear pattern from 0 with pure CSF to 66 with 100% blood. The $T_1$ times of the mixture decreased with the increasing amounts of blood, ranging from 2200 msec for 100% CSF to 500 msec for 100% blood. There were shorter $T_2$ times at higher concentrations in blood (90). CT scans from 182 consecutive patients were graded by the amount of blood in 10 basal cisterns and fissures in four ventricles. Using this methodology there was good agreement among different observers. Such scores for extravasated blood were considered to be suitable as a baseline variable (91).

Three hundred and eight cases with aneurysmal SAH were evaluated using quantitative CT scale. The amount of blood in the interpenduncular, ambient, and quadrigeminal cistern was graded 0–3. All patients in poor initial condition who were not operated had a mean CT SAH score of 8.2 and all died except 1, who remained vegetative. In poor-condition patients who were operated the SAH CT score was 7.3. Half of these patients remained vegetative or died. In patients who were in good condition when admitted but who improved preoperatively or in patients in good initial condition who were operated the CT SAH score was 2.3–3.7. The duration and level of unconsciousness seemed to correlate with the severity of SAH in the perimesencephalic cisterns (92).

An analysis of disagreement between four neuroradiologists in the assessment of subarachnoid clot and acute hydrocephalus was performed using 59 CT scans in acute SAH. There were systematic interobserver differences in assignment of Fisher grading of the amount of

subarachnoid clot. It was considered that the criteria for specific category levels be further sharpened (93). Although the "Fisher" grading scale is widely quoted to describe the extent of SAH on the CT scan, its original measurements are now meaningless. They related to clot visualization on an early generation EMI scanner and to a specific size of visual reproduction which is no longer used (Table 5.2). The basic concept is still valid; this is, following aneurysmal rupture it is useful to classify the CT scan as showing no, thin, or thick SAH with or without ICH or IVH. From the point of view of predicting VSP and DID, the "Fisher grade III" or large, dense clot in the major cisterns with or without ICH or IVH is the most important (74).

### H. Time Course of Low-Density Areas on CT Scan

Forty-eight percent of supratentorial ischemic infarcts of all causes were detected by CT scanning at the ictus. The CT became increasingly accurate in detecting low-density areas up to a 74% rate on day 11 after infarction. After 1–7 days, 20% of infarcts appeared isodense, and approximately 10% became at least partially hemorrhagic. Twenty-five percent exhibited mass effect. Ultimately, 57% showed dilation of adjacent ventricles (94).

Of 135 patients post-SAH, who did not have associated ICH, angiographic VSP developed in 68%. Cerebral infarction attributed to this VSP occurred in 21% of those studied by CT. The initial low-density areas were first noted on day 9 after SAH. Vasospastic infarction was usually in the cortex and white matter rather than the basal ganglia. In 6 patients the initial low-density areas on the CT subsequently turned into high-density areas. In 5 of the 6 patients the infarction was cortical. The neurologic signs of delayed ischemia appeared on average 7 days following SAH. The low-density areas appeared on average on day 9 (95). A retrospective study of 32 patients post-SAH showed a correlation between CT findings and the development of DID. Poor clinical state correlated with the presence of large blood clots in the subarachnoid space. Subsequent cerebral infarction was also related to these early CT findings (96).

Patients who ultimately developed low-density areas on the CT scan who were evaluated by serial CT scans showed significantly lower average (CBFs) during their course (97). One hundred and sixty-four patients operated within 7 days of aneurysmal SAH were followed by sequential CT scans. Thirty-four percent developed symptomatic VSP and subsequent CT scans showed low-density areas in 26%. The likelihood of low-density areas was higher in patients with poor initial grades and patients with thick subarachnoid clots. Low-density areas usually appeared adjacent to the ruptured aneurysm and in the territory of the artery. In 72% of cases the low densities were unilateral and in 28% they were bilateral. Sixty percent had only one and 40% had two or more density areas. Anterior communicating artery aneurysms were more likely to have bilateral low-density areas. The low-density area can be classified into major territorial, cortical branch type, perforator lacunar, and subcortical white matter including watershed infarction. Of patients with a single low-density area, major territorial infarcts were more likely. In patients with multiple low-density areas subcortical types were more likely (98). Focal hypodense lesions on CT scans do not necessarily represent infarcts and are sometimes fully reversible (99).

### I. Demonstration of Rebleeding on CT Scan

Twenty-two percent of 176 patients had at least one CT-proven rebleed within 4 weeks post-SAH. Rebleeding was fatal in half of the cases. The risk of rebleeding was not predictable based on the patients' clinical condition on admission or the amount of subarachnoid blood on the initial CT scan. Only 18% of 39 patients with rebleeding survived more than 3 months (100).

### J. Effect of Nimodipine on Infarction

Two hundred and sixty-five grade I–III patients were studied prospectively. Factors related to the development of cerebral infarction in order of importance were the amount of blood on the initial CT scan, postoperative angiographic VSP, earlier conduct of operation, and a history of hypertension. The use of nimodipine was associated with a significant reduction in cerebral infarcts visualized by CT (101).

### K. The Basal Cisterns in Subarachnoid Hemorrhage

The probability of a patient developing hypovolemia soon after SAH (36% of the series) was predicted by CT findings of compressed or obliterated basal cisterns as seen in all the hypovolemic patients compared to only 12.5% of the normal volemic patients. A reduction in plasma volume of >10% of normal was 80% when Hyc was present and 100% when the basal cisterns were compressed and there was associated ICH or midline shift (102).

### L. Computed Tomographic Findings in Patients Dying Early from Subarachnoid Hemorrhage

Ten percent of SAH cases were dead on arrival at hospital. These patients showed a significantly higher incidence of pulmonary edema than patients who were

admitted in grade V condition. The dead-on-arrival patients had a significantly higher incidence of pure subarachnoid clot than the poor-grade survivors. Interestingly, the amount of subarachnoid blood in the dead-on-arrival group was significantly less than that in the patients who survived to be admitted in poor condition (103).

### M. Seizures

Nine percent of 181 consecutive patients with SAH had one or more epileptic seizures a median of 18 days post-SAH. Among the variables that predicted this complication were the sum score for the amount of cisternal blood and the presence of IVH on the initial CT scan. The high cisternal blood score and rebleeding proved to be significantly related to epilepsy (104).

### N. Coiling of Aneurysms

Sixty-nine patients with neurologic grades I–III underwent coiling of aneurysms within 72 hr of SAH. Symptomatic VSP occurred in 23%. The clinical grade on admission and the amount of blood on the initial CT scan were both associated with the incidence of subsequent VSP. Of the patients with VSP, 75% had a good recovery, 13% were moderately disabled, and 13% died. This incidence was believed to compare favorably to that found in conventional surgical series (105).

### O. Contrast Enhancement

#### *1. Contrast Enhancement on CT*

Contrast-enhanced CTs were considered to show increased vascular permeability which preceded VSP development in 2 cases (106). After hypoxic events abnormal contrast enhancement was observed within 3 weeks of onset of symptoms in 80% of cases and persisted for up to 4 months in 20% of cases. Eight patients had SAH from aneurysms. Post-SAH ischemic areas following injection of contrast media appeared as ring-shaped regions of gray matter enhancement (107). Fisher *et al.* found no correlation between abnormal enhancement and the subsequent development of VSP (74). Fifty percent of aneurysmal SAH patients in one series showed contrast enhancement at some time either early or late during their course. A diffuse type of subarachnoid enhancement was considered valuable for predicting cerebral infarction due to VSP. Angiography showed VSP in 53% of the patients with positive enhancement, and 75% of these patients actually developed cerebral infarction (108). In a systematic and prospective study of 60 patients with aneurysmal SAH, CT scanning and contrast injection were performed. Patients had immediate and delayed angiograms. A significant relationship between contrast-enhanced CT findings obtained within 3 days post-SAH and the development of VSP was noted. Forty-six percent of the patients undergoing CT enhancement between days 0 and 3 showed prominent increases in densities in the region of the basal cisterns. Severe VSP with neurologic deterioration developed in 76% of these patients. Ninety-five percent of the patients without significant contrast enhancement did not develop severe angiographic or clinical VSP. No relationship between contrast enhancement and VSP was noted beyond day 3 post-SAH (109). In a series of 80 patients, 36% scanned within 3 weeks showed normal contrast enhancement. The abnormal enhancement was usually parenchymal rather than subarachnoid. It involved areas bordering on the CSF collections. Hounsfield numbers were determined for regions in the basal ganglia not directly adjacent to blood-containing spaces. In these regions an increase in attenuation values of 1.2 ± 1.4 Hounsfields units occurred with contrast injection. Post-SAH a mean value increase of 6 Hounsfield units occurred in 14 patients scanned within 5 days of the ictus. Enhancement was greater in poor-grade patients with an unfavorable outcome. Enhancement was considered to be a possible reflection of increased blood volume in the parenchyma due to small vessel dilation (110) (Table 5.5).

**TABLE 5.5 Abnormal Enhancement Correlated with Clinical Conditions and Outcome[a]**

| | Abnormal enhancement (%) | | No abnormal enhancement (%) | |
|---|---|---|---|---|
| Clinical grade and outcome | ≤5 days post-SAH | >5 days post-SAH | ≤5 days post-SAH | >5 days post-SAH |
| Clinical grade | | | | |
| I | 0 | 5 | 40 | 55 |
| II | 21 | 13 | 38 | 29 |
| III | 35 | 19 | 27 | 19 |
| IV | 33 | 22 | 33 | 11 |
| V | 100 | 0 | 0 | 0 |
| Outcome | | | | |
| Excellent | 10 | 7 | 45 | 38 |
| Good | 17 | 17 | 33 | 33 |
| Disabled | 22 | 33 | 11 | 3 |
| Death due to | 50 | 11 | 28 | 11 |
| Rebleed (bleed) | 17 | 0 | 22 | 0 |
| Infarction | 33 | 11 | 0 | 5 |
| Other causes | 0 | 0 | 0 | 5 |

[a]Modified from Doczi, T., Ambrose, J., and O'Laoire, S. (1984). The significance of contrast enhancement in cranial computerized tomography after subarachnoid hemorrhage. *J. Neurosurg.* **60,** 335–342.

It is problematical whether the information gained from augmenting a known ruptured aneurysm case justifies the theoretical risk of delivering potentially neurotoxic contrast agent through a damaged blood–brain barrier. CT scan may of course be performed shortly after angiography, perhaps obviating the need for additional contrast infusion. The rate of clearance of contrast medium within the subarachnoid space following SAH is much slower than the rate of clearance of iodine from circulating blood. In normal subjects contrast medium disappears within 30 min of administration, but in patients post-SAH it may last as long as 2 hr.

#### P. Computed Tomographic Angiographic Direct Demonstration of Vasospasm

CT angiograms have been used to demonstrate convincingly the conventional angiographic findings of VSP (111). Vasospastic changes were revealed in eight patients in whom 3-dimensional repeat scans were performed 1 week after the first ones showed the absence of VSP (112). Seven patients having SAH diagnosed on plain CT scans underwent computerized tomographic angiography (CTA). CTA and digital angiography of four patients were performed within 24 hr of each other, 7–10 days post-SAH. The degree of VSP was categorized as follows: mild, <30% reduction; moderate, 30–50% reduction; severe, >50% reduction. Two separate investigators graded the degree of VSP using these two techniques. There was an overall agreement of 68% between CTA and DSA. By degree of VSP, agreement was more varied: 86% none; 20% mild; 63% moderate; and 64% severe. In 77% of disagreement cases CTA was reported within one category of the DSA classification. CTA correlated with the gold standard angiographic diagnosis for VSP in about two-thirds of cases. The greatest agreement was for the no VSP (86%), and the least was with mild VSP (20%). CTA is incapable of replacing DSA in the routine evaluation of VSP (112), but technical advances in CT may soon alter this judgment.

## IV. Transcranial Doppler Ultrasonography

### A. History

TCD was developed in Bern in 1982 by R. Aaslid and H. Nornes. It consisted of a pulse 2-MHz ultrasound Doppler instrument with a maximum ultrasonic power of about 100 mW and an ultrasound beam focused by an acoustic lens. In 1039 Doppler examinations proximal MCA velocity was recorded bilaterally in 93.7% of women and 99% of men. Systolic, diastolic, and mean velocities can be measured. The mean velocity carries the highest physiological significance because it depends less on cardiovascular factors such as heart rate, contractility, total peripheral resistance, and aortic compliance than do systolic and diastolic values (113). Doppler ultrasound was used to measure the velocity of flowing blood in the peripheral arteries as early as 1959 (114).

### B. Technical Aspects

The technology employs a sound-emitted 2-MHz frequency. A computer calculates the computer shift between the signal reflected at the moving RBCs and the incident frequency. The frequency shift is directly proportional to the velocity of the moving column of RBCs and the cosine of the angle of insonation of the probe. Pulse Doppler transmits and receives bursts of ultrasound at regular intervals from the same crystal source, enabling the recording of velocity signals at preselected depths. Transcranial Doppler uniquely uses the 2 MHz ultrasonic frequency versus the 3–10 MHz used in other applications. The recorded dimension of the velocity is not a direct measurement of flow. To calculate absolute flow one needs to know the velocity and the cross-sectional area of the artery being recorded from, as well as the angle of insonation. Changes in CBF are accurately reflected by changes in velocity in each individual when the arterial diameter remains constant. When the insonant angle is less than 30° the cosine of the angle of insonation is small and the measured blood flow velocity approximates the true blood flow velocity. The angle of insonation is usually least when measuring the MCA velocity and it is the most commonly used index. The highest obtained velocity is normally employed clinically. The most commonly used insonating window is the temporal bone, which can be satisfactorily penetrated in 85–90% of cases (115). The angle of insonation of the posterior cerebral and the anterior cerebral arteries is less favorable than that for the MCA, and collateral flow is more significant than that with the MCA, which is a relatively isolated end vessel. The MCA should be examined between the upwardly directed insular branches at a depth of approximately 35–40 mm to its origin at the internal carotid termination at a depth of 60–65 mm where a bidirectional signal is found in normal circumstances. Flow velocities from the anterior cerebral artery will reverse with ipsilateral carotid compression. The anterior cerebral artery is normally insonated at a depth of 65–70 mm. TCD determination of VSP in the anterior cerebral artery is limited because of the possibility of increased flow across the anterior communicating artery. The correlation of TCD velocity and angiographic vessel caliber was poor in an early study by Aaslid (116). Similarly, VSP involving the

intracranial internal carotid artery may mask MCA narrowing (117). Theoretically, if the diameter of a vessel is reduced to half by VSP, velocity should increase to 400% of the original value. However, if the stenosis reaches a critical level (>70% diameter reduction) volume flow may actually be reduced significantly and therefore velocity may actually decrease with additional reduction in diameters (118). Severe VSP can cause arteries to become so narrow that accurate TCD velocity determination can be difficult or impossible. The sensitivity of TCD varies with the thickness of the temporal bone, the experience of the examiner, and the involvement of readily insonated arteries by VSP.

### C. Normal Values and Indices

The TCD is not a direct measure of vessel diameter but is based on the principle that mean flow velocity should be inversely proportional to change in vessel diameter. In a series of 50 healthy patients, Aaslid *et al.* using TCD established a mean velocity of 62 cm/sec with a range of 33–90 cm/sec for the middle cerebral artery (119).

Various blood flow velocity parameters are determined by TCD: systolic peak flow velocity, diastolic flow velocity, mean flow velocity [(systolic flow velocity plus two times diastolic flow velocity)/3], Gosling pulsatility index [(systolic flow velocity minus diastolic flow velocity)/mean flow velocity], resistivity index [(systolic flow velocity minus diastolic flow velocity)/ systolic flow velocity], and Lindegaard ratio [(intracranial MCA mean flow velocity)/extracranial internal carotid mean flow velocity]. Presumed indicators of VSP include velocities increasing more than 50 cm/sec/day and an increase in the intracranial to extracranial mean flow velocity ratios of $\geq 3$ (Lindegaard ratio). The magnitude of Gosling pulsatility decrease is greater at a given velocity for vasodilation than for stenosis. The exact relationship between velocity and pulsatility changes is complex. There is a degree of overlap in the decrease in pulsatility between the vasodilated and stenotic states (120). Seiler and Grolimund considered the Gosling pulsatility index to be a crude but useful way to estimate the cerebrovascular resistance and intracranial pressure. Their normal mean velocity values were $57.3 \pm 14.8$ for MCA, with a mean side difference of 5.9. MCA values were higher than those for the other intracranial arteries (113).

Laumer *et al.* investigated pulsatility indices which are known to provide typical patterns in cases with extracranial internal carotid stenosis and in the presence of arteriovenous malformations. They established normal reference values for the Gosling pulsatility index, the Pourcelot resistance index, and the first Fourier pulsatility index in 97 normal subjects. As age increases there is also a significant increase in these indices. The various indices were strongly related. There was no side-to-side difference. The indices were inversely related to the flow velocities. In 455 TCD exams performed on 66 SAH patients and classified by the presence or absence of DID, they established that pulsatility indices had a typical time course of elevation but usually normalized by about 10 day after SAH. Surprisingly, a comparison of pulsatility indices in patients with and without DID showed no statistically significantly differences. It was concluded that elevated flow velocities cannot predict DID and that various factors, such as collateral flow and the use of nimodipine, affect the clinical tolerance to clinical VSP and finally that the measurement of pulsatility indices cannot provide further information on impending DID (121).

### D. Time Course of Velocity Changes

Fifty patients with ruptured aneurysms and patients with elective clipping of unruptured aneurysms had daily TCD studies. Surgery for clipping of unruptured aneurysms did not lead to an increase in mean MCA velocity, whereas there is a progressive increase in this parameter post-SAH which peaks between 7 and 10 days. The velocity was higher on the side of the ruptured aneurysm and the degree of increase was greater if blood was seen on the initial CT scan. It was considered unlikely that the patients whose MCA velocity remained <100 cm/sec had a degree of angiographic VSP sufficient to cause clinical symptomatology. On the other hand, patients whose velocities were >200 cm/sec were at greater risk for developing clinical symptomatology. However, there were exceptions to these generalizations (122). TCD was performed within 12 hr post-SAH in 21 patients. Flow velocities did not indicate an early phase of arterial narrowing in any case. Velocities increased only after a delay of at least 4 days (123). Thirty-six patients with proven SAH showed no increased flow velocities in the first 3 days. Half the patients were operated on days 0–2 and these patients showed significantly lower postoperative velocities compared to patients operated later. Two patients out of 36 who were operated on day 4 post-SAH showed the highest velocities and died from VSP and infarction (124). Flow velocities measured by a TCD began to increase on post-SAH day 5 and maximum velocities were recorded between days 9 and 13; normalization occurred within the following 2 weeks. In 5 cases with symptomatic VSP a rapid increase in flow velocities preceded clinical manifestations. In these 5 cases the mean MCA velocity was 149 cm/sec and the range was 119–184 cm/sec. The change in flow velocity was considered to be more important than the value itself. TCD examinations were less

useful when trying to judge whether vessel narrowing was resolving. It was believed that this was so because these velocities are influenced by multiple factors such as blood pressure, blood volume, and therapeutic maneuvers (125). As time progresses post-SAH velocities tend to increase, and in patients with clinical evidence of VSP the velocities may increase sharply prior to clinical development of signs. Fahmy and Smith found that all patients who became symptomatic had velocity increases of >25%/day during the 2 days prior to the onset of symptoms. Only 1 patient in the asymptomatic group had a similar 25% velocity increase (126). Seiler and associates found that all patients post-SAH had velocities >80 cm/sec at some time between days 4 and 10 (127). Hutchison and Weir found that all patients had velocities of >100 cm/sec by day 8. The greatest velocity elevation occurred in the MCA ipsilateral to the ruptured aneurysm in the case of lateralized aneurysms (122). Romner *et al.* found no difference in velocities in patients with surgery in the first 2 days post-SAH compared to those operated between 2 and 4 days post-SAH. Maximum velocities usually occurred between days 8 and 14 (124).

### E. Velocity Changes and Angiographic Vasospasm

Normal MCA velocity is between 30 and 80 cm/sec with a mean of 62 cm/sec (119). In 1984 Aaslid *et al.* studied a consecutive series of 40 patients, of whom 24 had angiographic VSP: All patients with spastic MCA had velocities >120 cm/sec. Elevated velocities may precede clinical deficits (119). Rapidly increasing velocities may indicate impending clinical deterioration (126, 128). In 102 patients with proven aneurysms TCD suggested an incidence of VSP of 93%. In 27 SAH patients with negative angiography the incidence of VSP was 56%. Of 18 patients with angiographically proven MCA spasm, 14 had sonographic VSP; of 61 normal MCA arteries on angiograpy, 9 showed elevated TCD velocities. These data suggested a sensitivity of 80%, but they considered that the 9 MCA cases might not be false positives but actually represent true positive findings due to a higher sensitivity of TCD for direct arterial narrowing (113). We think this unlikely.

Fifty-six angiograms were obtained from 51 patients on days 1–21 post-SAH. In patients investigated on days 1 and 2 the median MCA diameter was 2.8 mm (range, 2.3–3.4 mm) and the median flow velocity was 56 cm/sec (range, 36–88 cm/sec), which were within normal limits. Eleven of the 13 MCAs having diameters 1.5 mm or less showed flow velocities in excess of >140 cm/sec. The ratio of flow velocities of MCA to ipsilateral extracranial internal carotid artery (ICA) was 1.1–2.3 (median 1.7) on days 1 and 2 but rose to >10 in patients with the most severe MCA narrowing (129). Twenty-one patients were admitted within 1 week post-SAH; TCD velocities in MCA and anterior cerebral artery were significantly elevated for the group with VSP on post-SAH days 4–12. The flow velocities for grade V patients were significantly lower than those for grade IV patients. There was poor correlation between TCD velocities and angiographically observed anterior cerebral and MCA radii in 12 instances (130). In 34 consecutive patients having angiography during the period of risk for VSP with technically adequate TCD examinations within a day of the angiogram, the mean flow velocities >120 cm/sec correlated with the presence of angiographic VSP. This occurred in 17 patients and there were no false positives. TCD correctly determined that 5 patients did not have VSP, whereas there were 12 false negatives. False negatives were considered to be due to the involvement of vessels not accessible by TCD. In this group specificity was 100% and sensitivity 59% for the detection of angiographic VSP by TCD (131). In 76 patients post-SAH, MCA velocities were correlated with angiographic diameters. There was moderate agreement between angiographic VSP and the absolute MCA blood velocity. There was substantial agreement between angiographic VSP and the index calculated from dividing the blood velocity in the MCA by the blood velocity in the ipsilateral ICA. When TCD velocities were increased in the proximal anterior cerebral, this suggested that VSP was the explanation rather than a congenital smallness of the vessel (132). Angiograms were assessed for VSP in a four-level scale. Two independent judges categorized the films. There was moderate agreement between angiographic VSP and MCA velocity by TCD. There was higher agreement between angiographic VSP and the index calculated from dividing MCA velocity by the ipsilateral ICA velocity (132).

A correlative study of TCD velocities and angiographic caliber was performed by Newell and published in 1990. By regression analysis the angiographic vasoconstriction correlated well with velocity increases on TCD. Nine MCAs with >50% diameter reduction showed on TCD examination mean velocities >200 cm/sec. The correlation between increased velocity and decreasing vessel diameter was not as close for the proximal segment of the anterior cerebral artery. This is at least in part attributable to the fact that unilateral hyperplasia is common with anterior communicating aneurysms. Another contributing explanation is the fact that if one proximal anterior cerebral artery is in spasm the contralateral one can have an increased flow to compensate for this via the anterior communicating artery. A better regression analysis was obtained when the combined arterial angiographic diameters were plotted against the combined TCD velocities. While patients with severe angiographic VSP could

occasionally be asymptomatic due to compensatory mechanisms, it was believed that patients with true deterioration due to delayed vasospastic ischemia would always show severe vessel narrowing on angiography (118). Forty-five comparisons in 41 patients were made between TCD velocities and angiographic vessel diameters. The angiograms were classified as showing no, mild, moderate, or severe VSP. An upper normal limit of 140 cm/sec for MCA velocity resulted in a good specificity but a poor sensitivity for TCD to detect moderate or severe spasm. Newell and Winn advocated careful interpretation of one-time TCD results because the sensitivity of the method is low. Their severe spasm cases had an angiographic vessel lumen <0.5 mm and a mean flow velocity of 161 ± 64 cm/sec. The 33 patients with no angiographic VSP had velocities of 69 ± 34 cm/sec. TCD was able to detect vessel with MCA diameters <1 mm with a sensitivity of 0.55 and a specificity of 0.9 when a threshold value of 140 cm/sec was used (133).

Observations were made on 56 consecutive patients post-SAH. VSP was diagnosed if the angiographic caliber was reduced 25% or more, and TCD "VSP" was assessed if velocity was ≥ 130 cm/sec. TCD examinations showed 11 true-positive and 0 false-positive results and 97 true-negative and 4 false-negative results (134). Evaluation of the posterior circulation by angiography and TCD examinations were carried out within 24 hr for 42 patients. Mean flow velocities >60 cm/sec were indicative of both vertebral and basilar artery VSP. For the vertebral artery there were 7 true positive tests, 42 true negatives, 6 false positives, and 9 false negatives. Sensitivity was 44% and specificity 88%. For the basilar artery sensitivity was 77% and specificity 79%. When the diagnostic criteria was changed to ≥80 cm/sec for the vertebral and ≥95 cm/sec for the basilar artery, there were no false-positive results. The specificity became 100%. However, the sensitivity at this velocity level was only 13% (135).

In a modeling study the MCA velocity increased as the cross-sectional area decreased up until the point at which CBF started to decrease significantly, at which point velocity started to decrease. This is a possible explanation of false-negative TCD results in very severe VSP (136).

### F. Velocity Changes and Distal Angiographic Vasospasm

TCD cannot detect VSP affecting more distal, vertically oriented branches of the MCA that are outside the standard detection range. In 136 angiograms performed on 68 patients, 40 showed < 25% vessel narrowing, and of these 50% had VSP restricted to the basal vessels and 42.5% had spasm of both the basal and distal segments so that approximately 93% of patients with spasm were considered to be potentially diagnosable by TCD ultrasonography (137) (Table 5.6). Only 7.5% of one series of angiographically studied patients with VSP had spasm only in the distal MCA segments. Correlation of angiographic VSP and TCD velocities of the proximal end of the anterior cerebral arteries has not been close because A1 segments are frequently hypoplastic; owing to the communicating flow connection with the contralateral cerebral artery, the relationship between vessel narrowing and increases in velocity may not be close if the opposite artery is not also in spasm; and in severe VSP the vessel may be so small that little ultrasound will be reflected and it will be difficult to locate and quantify (138).

In an exceptionally revealing study, Okada *et al.* analyzed 50 patients who were operated early and who had both TCD and CCT performed on days 7–14 post-SAH (Table 5.7). Cerebral circulation times (peak density in cavernous carotid to peak density in an ascending vein) were calculated. Significantly prolonged circulation times occurred in patients with severe angiographic VSP, those with the poorest outcomes, those with both proximal and distal MCA VSP, and those who had low-density areas on CT. The TCD velocities did not show significant differences in these groups, except that the velocity was significantly higher in patients with only proximal VSP compared to those with proximal and distal VSP. When severe angiographic VSP developed some patients showed drastic increases in TCD velocities but others did not; therefore, no significant differences were observed between the groups (139).

### G. Velocities, Delayed Ischemic Deficits, and Infarction in Clinical Studies

Seiler *et al.* found no examples of patients with cerebral infarction whose mean velocities had not exceeded 140 cm/sec and usually found delayed ischemic infarction

**TABLE 5.6 Location and Distribution of Angiographic Vasospasm[a]**

| Location and severity | No. of patients | Percentage of entire group | Percentage with significant spasm |
|---|---|---|---|
| None or mild (<25%) | 28 | 41.2 | |
| Basal vessels only | 20 | 29.4 | 50.0 |
| Basal and distal vessels | 17 | 25.0 | 42.5 |
| Distal vessels only | 3 | 4.4 | 7.5 |

[a]Modified from Newell, D. W., Grady, M. S., Eskridge, J. M., and Winn, H. R. (1990). Distribution of angiographic vasospasm after subarachnoid hemorrhage: Implications for diagnosis by transcranial Doppler. *Neurosurgery* **27**, 574–577.

TABLE 5.7 Circulation Time and TCD Velocity[a]

| | | | | |
|---|---|---|---|---|
| | | | **Clinical grade** | |
| | **I (5)[b]** | **II (18)** | **III (14)** | **IV (13)** |
| Mean CCT (sec) | 4.7 | 4.5 | 4.6 | 6.7 |
| TCD mean velocity (cm/sec) | 80 | 111 | 114 | 92 |
| | | | **VSP** | |
| | | **None** | **Slight to moderate (25)** | **Severe (16)** |
| Mean CCT (sec) | | 4.1 | 4.6 | 6.5 |
| TCD MCA velocity (cm/sec) | | 70 | 115 | 105 |
| | | | **DID** | |
| | | | **Absent (34)** | **Present (16)** |
| Mean CCT (sec) | | | 4.5 | 6.5 |
| TCD MCA velocity (cm/sec) | | | 104 | 105 |
| | | | **Outcome** | |
| | | **Good recovery (37)** | **Moderate disability (8)** | **Severe disability or dead (5)** |
| Mean CCT (sec) | | 4.6 | 4.9 | 8.8 |
| TCD MCA velocity (cm/sec) | | 112 | 73 | 97 |
| | | | **Site VSP** | |
| | | | **Proximal (8)** | **Proximal and peripheral (8)** |
| Mean CCT (sec) | | | 5.1 | 7.9 |
| TCD MCA velocity (cm/sec) | | | 128 | 81 |
| | | | **Low density on CT** | |
| | | | **Present (15)** | **Absent (35)** |
| Mean CCT (sec) | | | 6.6 | 4.5 |
| TCD MCA velocity (cm/sec) | | | 107 | 103 |

[a]Data from Okada,Y., Shima,T., Nishida, M., Yamane, K., Hatayama, T., Yamanaka, C., and Yoshida, A. (1999). Comparison of transcranial Doppler investigation of aneurysmal vasospasm with digital subtraction angiographic and clinical findings. *Neurosurgery* **45,** 443–450.
[b]Parentheses indicate the number of patients; CCT, cerebral blood circulation time.

in patients whose mean velocities were >200 cm/sec (127). The highest MCA peak velocities in asymptomatic patients post-SAH ranged between 198 and 302 cm/sec. Symptomatic patients became asymptomatic associated with decreases in peak velocity from 262 to 170 and 306 to 184 cm/sec (140). The same observers also noted velocities of more than 200 cm/sec in 20% of SAH patients and velocities of 140–200 cm/sec in 32%. The velocities tended to be higher in patients who died from VSP than in those who remained asymptomatic, but many patients had the clinical picture that was not obvious from the TCD velocities (113). They also noted that there were several patients, mainly older, in whom low or normal velocities were found despite cerebral infarction proven by CT scans (113).

Forty-nine cases with operated aneurysms, 9 patients with SAH of unknown origin, and 7 patients with SAH and death prior to surgery were studied by TCD. A correlation between clinical status and TCD velocities was only observed in extreme cases when velocities were

>200 cm/sec or increased by 100 cm/sec or more within 3 days prior to clinical VSP (6 patients out of 65) (141). TCDs were performed on 121 unselected patients post-SAH. Of 47 patients who developed DID, the mean MCA velocity was 186 cm/sec, which was more than the 74 patients who did not develop a deficit (149 cm/sec). This was a statistically significant difference. When only those readings made before the onset of neurologic deficits were considered, there was no significant difference between the groups (157 vs 149 cm/sec, respectively). A maximum velocity increase of 65 cm/sec in 24 hr was recorded in patients who later developed deficits compared to 47 cm/sec in patients who did not. This difference was also statistically significant. However, of patients who showed this high increase in velocity 32% did not develop a deficit, and of the patients who did not show this increase in velocity 37% did develop a deficit. The prediction of neurological deficit using TCD velocities is therefore very approximate at best (142). Eighty-eight patients were admitted within 3 days post-SAH, and TCD recordings were performed every other day for 15 days. The Pourcelot resistance index (maximum systolic velocity – end diastolic velocity/maximum systolic velocity) was calculated. The resistance index was significantly higher in grade IV and V cases compared to others up until 9 days post-SAH. Patients in grade IV or V had a significantly lower velocity (61.6 cm/sec) than grade I or II patients (82.4 cm/sec) in the first 3 days post-SAH. In the presence of ICH, velocities were markedly lower. Early Hyc in the first 3 days was also associated with significantly lower velocities. Clinical deterioration from VSP was usually associated with higher velocities and lower resistance indices at 4–9 days but some cases showed normal velocities and 1 case showed a very high resistance index. Patients with severe deterioration in the 4 to 9 day period showed ICA velocities of 142 cm/sec and a resistance index of 0.62 compared to values of 112 cm/sec and 0.67 for patients without clinical deterioration in this time period. Both of these differences were statistically significant (143). Thirty-six percent of 73 patients developed DID, which proved permanent in 6%. Of the patients who developed DID, 62% had rapid increases in TCD velocities preceding the clinical manifestations, whereas 39% showed no such increase. In the patients without rapid increases in velocity, angiography sometimes demonstrated VSP in segments distal to those evaluated by the TCD exam (144).

### H. Clinical Factors Affecting Velocities

Recent reports of patients who were treated with hypervolemia and calcium channel blockers suggest that lower rates of infarction are associated with higher velocities on TCD. Since blood flow through a vessel is equal to the instantaneous average velocity multiplied by the cross-sectional area of the vessel, these factors provide the essential measurements that are needed to estimate blood flow. In 100 consecutive patients treated with intravenous nimodipine and early surgery, TCD velocities peaked by about 7 days post-SAH. Velocities ipsilateral to the side of craniotomy were higher than those on the contralateral side. The peak difference was approximately 20 cm/sec. Patients who developed DID between days 3 and 8 had average increases in velocity of 64 cm/sec in the 3 days prior to demonstrating the deficits. Nine percent of patients developed DID. The velocities in the patients developing DID ranged between 120 and 240 cm/sec. Even when surgery was performed on the first day post-SAH, blood flow velocities were normal when measured within the next 2 days. Younger patients showed higher velocities than older patients. About one-third of these patients developed a peak velocity in the range of 160 cm/sec and fewer than 5% developed velocities >240 cm/sec (145). In 7 patients without ruptured aneurysms the normal side MCA velocity was measured with a $CO_2$ vasoreactivity test with and without intravenous infusion of nimodipine (2 mg/hr). The values were compared to those of 9 patients post-SAH who were being treated with the same dose of nimodipine and who had $CO_2$ vasoreactivity tested during the second week post-SAH, when MCA velocity was increased by at least 50% of the initial value. No significant effect of nimodipine on $CO_2$ vasoreactivity could be demonstrated in any of the tests. In the second week after SAH a significant reduction in the cerebrovascular response to $CO_2$ was found (146).

### I. Effect of Age on Velocities

With advancing age velocities decrease; in one study, 5% of patients with velocities <80 cm/sec following SAH were older than 65 years of age (147). Time-averaged maximum velocities for MCA were 92 cm/sec in children under 10 years of age and 83 cm/sec in older children. Velocities decreased significantly with age in all vessels and did not change significantly during childhood and adolescence. A clear decline occurred from adolescence to adulthood. TCD studies were performed in 38 patients after traumatic SAH. The first investigations were done within a day of injury. Intracranial pressure (ICP) was measured. A significant correlation was found between MCA maximum mean velocities and the quantity of blood seen on CT scanning in the subarachnoid space. Compared to 30 patients with spontaneous SAH, the elevation in velocity over 120 cm/sec was earlier in the traumatic cases. There was only a weak correlation

between maximum velocity and disappearance of post-traumatic SAH (148).

### J. Velocities and Blood Pressure

#### *1. Acute Blood Pressure Changes*

Continuous TCD recordings were performed in 28 patients during the induction and withdrawal of hypertension from phenylephrine. Mean arterial pressure and mean TCD velocities were recorded every minute. Fifty-three percent of the patients had changes of more than 15% in the velocities, and these changes paralleled changes in mean arterial pressure. No clinical differences were identified between the patients whose velocities increased or did not in response to the hypertension (149).

#### *2. Chronic Hypertension*

Twenty-four hypertensive patients were examined daily for 2 weeks. As controls, 24 normotensive patients also post-SAH were matched. MCA velocities were significantly lower in the hypertensive patients. There was no statistically significant difference in pulsatility index. A diastolic notch was noted in 2 of the hypertensive and 6 of the normotensive patients. It was considered that even moderately increased flow velocities in hypertensive patients might represent significant VSP (150).

### K. Velocities and Physiological Parameters

#### *1.* $CO_2$

In normals over the age of 50, a 1 mmHg increase in alveolar $pCO_2$ caused a velocity increase of 1.78 cm/sec. The effect is not linear (151). $CO_2$ reactivity is impaired in patients with VSP compared to a controlled population that showed a 4.7% increase in TCD velocity for each mmHg increase in $pCO_2$. All VSP cases with velocities of 80–120 cm/sec were associated with a 2.3%/mmHg increase in $pCO_2$. Moderate VSP cases with velocities of 120–160 cm/sec were associated with a reactivity of 1.9%. Severe VSP with higher velocities was associated with a reactivity of 0.9%/mmHg $pCO_2$ (151).

Using the rabbit model it was found that there were significant differences in flow velocities and CBF responses to $CO_2$ changes and hypotension so that TCD is not a substitute for CBF measurement in the study of cerebrovascular reactivity and cerebral autoregulation (152). In a two-hemorrhage rabbit model there was a 41% increase in mean flow velocity to 31 cm/sec by day 3 in SAH animals compared to sham controls (153).

#### *2. Hematocrit*

Middle cerebral artery velocity and hematocrit have an inverse relationship (154,155). It has been suggested that MCA velocity does not increase beyond age-adjusted norms unless the hematocrit decreases below 32% in adults (156).

#### *3. Central Conduction Time*

Central somatosensory conduction time was correlated with blood flow velocities and delayed cerebral ischemia post-SAH. Increased conduction times and interhemispheric differences at the time of admission were considered to be poor prognostic signs. Statistically significant elevations of conducting times (>6.7 msec) were found only with velocities above 200 cm/sec (157). An important study of the parameter in SAH is summarized in Table 5.7.

### L. Velocity Changes during Aneurysmal Rupture

An acute SAH occurring during TCD ultrasonography showed a sudden extreme modification of the configuration in the form of an orthograde systolic flow component with zero diastolic flow or an oscillating flow immediately after the occurrence of SAH. Reoccurrence of a diastolic flow component and a markedly raised cerebrovascular resistance index could be demonstrated a short time later. It was implied that acute VSP leads to a delayed normalization of perfusion (158). Aneurysmal rupture occurring during TCD sonography is probably indicated by a gradual decline in diastolic velocity and to a lesser extent systolic velocity. The diastolic component can disappear and even reverse for 3 or 4 min following the onset of rupture (159,160).

### M. Velocity Changes during Brain Death

In brain death TCD sonograms show no forward flow throughout diastole. This is associated with a progressive reduction in MCA velocities (161).

### N. Velocity Changes Correlated with Angiographic Diameter

A highly significant inverse relationship between MCA diameter and velocity ($r = -0.905$) was found by Lindegaard *et al.* (129). Arterial narrowing from VSP could be diagnosed with a sensitivity of 80% using TCD (162).

### O. Velocity Changes Correlated with Single Photon Emission Computed Tomography Studies

A correlative study of TCD and single photon emission computed tomography (SPECT) was performed on

57 patients post-SAH. The mean MCA velocity was evaluated by the ratio in the change of mean flow velocity within 1 day to the previous day mean flow velocity. CBF was measured by SPECT using $^{133}Xe$ and mean CBF was calculated in the MCA territory. On the basis of angiographic classification the TCD velocities in patients with no VSP averaged 49 cm/sec, those in patients with slight to moderate VSP averaged 71 cm/sec, and those in patients with severe VSP averaged 76 cm/sec. The maximum daily flow velocity increase in symptomatic patients was 71% compared to 31% for asymptomatic patients. Delayed ischemic deficits developed within 2 days following the observation of the maximum daily flow velocity increase. However, there were numerous examples of patients with severe VSP who did not develop elevated TCD velocities (163). TCD studies were performed on 34 patients post-SAH. Twenty of them had SPECT with technetium-99 m hexamethylpropylene amine oxime (HMPAO). Of the patients without delayed ischemia, half had evidence of VSP (>120 cm/sec) by Doppler but only 1 had hypoperfusion as evidenced by the SPECT study. In 10 patients with DID and a lateralizing clinical deficit, 75% showed increased TCD velocities and 100% showed hypoperfusion by SPECT. Although simultaneously elevated TCD and hypoperfusion were most often recorded in patients with lateralizing neurological deficits, the fact that discordant results were observed reflects the inherent limitations of TCD, SPECT, and the different levels of the circulation monitored by these techniques (164). Twenty patients had rapid increases in TCD MCA velocities of >50cm/sec in 24 hr. SPECT studies using technetium-99 m HMPAO were carried out for these patients. Ten of the 15 patients studied before the onset of any clinical deficit subsequently developed a focal neurological abnormality. In 14 of these, and a further 5 in whom SPECT CT was performed after the onset of DID, perfusion patterns were abnormal and correlated with the site of increased Doppler velocities. Four patients had zones of cerebral hypoperfusion but did not develop clinical deficits (128).

### P. The Effect of Hyperosmotic Agents on Velocities

In 47 patients TCD velocities were correlated with laser Doppler flow velocities. Mean flow velocities increased significantly by both techniques after infusion of mannitol or glycerol (165).

### Q. The Transient Hyperemic Response

The transient hyperemic response is assessed by measuring TCD velocities before, during, and after a transient compression of the carotid artery in the neck by the medical investigator. The transient increase in velocity over baseline occurs when the carotid compression is relieved as a consequence of compensatory vasodilation. This transient hyperemic response has been described by many investigators (166–175). Giller performed the test by manually occluding the carotid as low as possible in the neck while recording MCA velocities. These velocities decrease 30–50% from baseline values with adequate compression. The compression is continued for 3 sec. An increase in systolic velocity of at least 10% above baseline lasting for longer than one heartbeat was considered to be evidence of intact "autoregulation." At a mean of 8 days post-SAH, the transient hyperemic response was absent more commonly in poor neurological grade patients: grade I, 76%; grade II, 60%; and grades III and IV, 100%. In four patients, as the neurologic status deteriorated or improved the transient hyperemic response was lost or improved. The abnormal response appeared before other indications of VSP. Over these short time intervals the change in velocity presumably directly reflects changes in flow (176). Five of 6 patients, with an increase in MCA peak velocity of < 9% of baseline after 5–9 sec of carotid compression went on to develop DID. None of 14 patients with a greater response got a DID. The 5 affected patients went from normal baseline TCD velocities to levels >150 cm/sec (177). It is probably reasonable to avoid this test in the presence of an unsecured aneurysm.

### R. Intracranial Pressure and Velocities

In 76 patients TCD values were correlated with ICP as measured by an epidural transducer. Importantly, in no case were both high ICP and high mean flow velocity observed simultaneously. It was concluded that with a pronounced increase in ICP evaluation of VSP by TCD based solely on mean flow velocities can lead to false-negative results (178). Fifty-two patients were admitted within 12 hr post-SAH. ICP was recorded along with TCD data. In cases of ICP increases, the resistance index increased and mean flow velocity decreased. Velocities >200 cm/sec were always associated with ICP values <18 mmHg, whereas ICP values higher than 30 mmHg were associated with a velocities <150 cm/sec. Resistance index values <0.5 were found only when ICP was <20 mmHg and mean flow velocities were >120 cm/sec. Resistance index values >0.6 were only found when ICP was >20 mmHg and velocity was <150 cm/sec. The mean flow velocities were as follows: grade I, 119 cm/sec; grade III, 179 cm/sec; and grade IV, 90 cm/sec. Along with the brisk decrease in grade IV velocities was a sharp increase in resistance index (179).

### S. Cerebral Blood Flow and Velocities

A decreased velocity in the extracranial carotid artery coincident with an increased velocity intracranially is thought to reflect a decrease in volume flow secondary to increased intracranial vascular resistance (180). Kontos pointed out the shortcomings of attempting to measure flow without direct observation of vessel caliber. Even with rapid changes in blood pressure it is not known whether there would be almost instantaneous changes in vessel caliber. Applications of TCD based exclusively on the measurements of flow velocity to infer volume flow while perhaps providing useful approximations under steady-state conditions might be misleading in conditions of rapidly changing blood flow and pressure (181). Lindegaard measured MCA TCD velocities while recording carotid arterial flow changes electromagnetically (182). Twenty-one patients post-SAH had serial TCD and rCBF measurement over 3 weeks after SAH. Of patients with thin SAH on CT, only 1 of 6 had a TCD higher than 120 cm/sec. Of 15 patients with thick CT clots, 8 had normal TCD velocities and 3 had reduced hemispheric flow. Regional hyperperfusion was detected in 6 of these 8 cases. In 7 patients with significantly increased TCD values regional hyperperfusion developed in all cases, and 1 patient had a severe clinical deterioration (183). TCDs were evaluated in 14 patients intraoperatively under general anesthesia. There was a poor correlation between the absolute values of hemispheric CBF and the corresponding mean flow velocities. Controlled hyperventilation was associated with a significant decrease in CBF as well as a decrease in TCD flow velocity. In terms of reactivity indices, the correlation between the two methods was poor and not significant (184). TCD changes in 43 patients with symptoms suggesting cerebrovascular diseases were assessed along with rCBF before and after acetazolamide administration. There was significant positive correlation between the absolute increase in CBF in ml/100 g/min and the percentage increase in TCD velocity ($r = 0.63$). An increase of rCBF of 25 ml/100 g/min was associated with an approximately 50% increase in MCA velocity. Normal MCA velocities were approximately 64 cm/sec and showed a 33–35% increase after injection of 1 g acetazolamide (185). In an experimental rabbit model, TCD velocities were correlated with CBF and cerebral perfusion pressure. A two-slope relationship was observed between velocities and perfusion pressure with a breakpoint that correlated closely with the lower limit of CBF autoregulation. Below this cerebral perfusion pressure (CPP) breakpoint the velocity varied directly with CPP, and above it, it varied inversely with CPP. An inverse correlation between velocity and CPP indicates intact CBF autoregulation (186).

In an important study, the relationship of TCD velocities was correlated with CBF as determined by stable Xe CT scanning post-SAH. Fifty patients underwent 94 paired studies. All were treated with nimodipine and hypervolemia/hemodilution. When CBF in the MCA territory was $\leq$31 ml/100 g/min the corresponding peak velocity was 119 cm/sec, whereas those with >31 ml/100 g/min had a velocity of 169 cm/sec. Higher local CBF was associated with higher velocity in all vascular territories ($n = 709$) in all but the internal carotid artery. At the time of each study 41 patients had focal neurological deficits and 53 were nonfocal. The CBF contralateral to the deficit was significantly less in the MCA territory, whereas the systolic velocities in the MCA were not significantly different compared to those of the contralateral side. Territories with increases in velocities >50 cm/sec/24 hr did not have statistically different local CBF. These results indicate that increased TCD velocities correlate with increased local CBF and not with ischemia as most observers had assumed. No difference in local CBF was found in territories with and without rapid increases in velocities in the MCA. While decreased contralateral CBF in the MCA territory corresponds with focal neurological deficits, increasing TCD velocities did not correlate with the neurological findings. The significant conclusion was that therapeutic decisions based exclusively on TCD velocities might be inappropriate and potentially harmful (187).

### T. Velocities in Traumatic Subarachnoid Hemorrhage

High velocities were identified in 68% of 25 patients studied post-head injury. The increased velocities occurred between 12 hr and 4 days and lasted from 12 hr to 14 days. No relationship was apparent between high velocities, blood pressure, intracranial pressure, or outcome. Velocities increased to >100 cm/sec on 10 occasions without neurologic deterioration, and deterioration occurred in 3 instances without change in velocities (188). Saunders and Cledgett performed TCD after head injury and considered that the presence of diastolic flow is typical of a system with low distal resistance. It is the norm in the vessels of the central nervous system, whereas peripheral vascular vessels may show little diastolic flow or even reversal in diastole. In cranial TCD when no diastolic flow is evident there is usually extreme elevation of ICP. The reduction in the systolic spikes is usually an agonal observation. Simultaneous high systolic and high diastolic velocities may represent vasoparalysis (189). MCA velocities following head injuries were obtained from 9 patients. There was a correlation between the velocities and the concurrent pH and $pCO_2$ measurements. The postinjury

day of maximum velocity ranged between 1 and 13 and the maximum velocities ranged between 80 and 180 cm/sec (190). TCDs were performed on 121 patients following head injury. Velocities >100 cm/sec were observed after 3 minor, 3 moderate, and 17 severe injuries but occurred only when the CPP perfusion pressure was >60 mmHg. Six patients with low arterial–jugular venous $O_2$ content differences (<4 ml/dl), indicative of global cerebral hyperemia, had increased mean flow velocities. Four of the 23 patients with increased flow velocities developed noncontusion-related infarction. None of the patients without elevated velocities developed these lesions. Noncontusion-related infarction did not occur in any patient with increased TCD velocities that were associated with global hyperemia (191). Daily TCDs were performed on 67 patients after head injuries. A mean of seven recordings were made between days 1 and 14. Forty percent of the patients demonstrated traumatic SAH on the first CT scan. Forty-one percent of the 27 patients who showed traumatic SAH developed velocities >100 cm/sec compared to 28% of the patients without traumatic SAH on CT. Of the 6 patients whose velocities exceeded 120 cm/sec, SPECT scans verified ischemia in 5 patients but showed a generalized hyperemia in 1. One patient with a thick-layer SAH developed velocities >220 cm/sec bilaterally on day 8. The SPECT scan reflected bilateral ischemia. The patient improved after 5 days of intravenous nimodipine administration (192).

### U. Velocities and Angioplasty

It has been reported that TCD values decrease rapidly following successful angioplasty. Bracard *et al.* reported five cases in which velocities decreased following angioplasty and did not recur (117). Newell *et al.* reported that one of four angioplastied arteries demonstrated recurrent elevated velocities several day following the procedure (193). Smith *et al.* reported normalization of velocity following successful angioplasty (194). Four patients were reported who had serial TCD studies. In two persistent elevated flow velocities were present after angioplasty. Follow-up angiography indicated the appearance of new areas of VSP which led to a second angioplasty of the new regions with significant clinical recovery. The technique was believed to have been a useful aid in the decision to employ repeat angioplasty (195).

### V. The Clinical Value of Transcranial Doppler Ultrasonography

A total of 100 patients treated surgically post-SAH were examined by TCD. Unlike earlier studies, no significant differences were found in velocities in different neurological grades. When the flow velocities of 11 patients who developed DID were compared with those with no deficit, no significant difference was seen. A significant increase in velocity on the day before the onset DID was found in only 3 of 11 cases. High flow velocities did not necessarily indicate impending neurological decline since 8 of 66 patients tolerated flow velocities of over 200 cm/sec. The main difference between this series and earlier investigations was assumed to be the routine administration of nimodipine. Laumer *et al.* (196) concluded that the clinical value of TCD was questionable for any patient since it will be normal in the first 3 days, it will be spuriously low in poor-grade patients, and surgery can be safely performed even in patients that show elevated TCD velocities. Of 11 patients who developed DID, only 3 showed a typical increase in flow velocity on the day before the onset of delayed ischemia. Eight patients showed either constant high or constant low velocities or decreasing time courses. In healthy subjects mean flow velocity decreased from approximately 70 cm/sec in 20-year-olds to 50 cm/sec in 80-year-olds. A study of the systematically measured MCA velocity at the origin, midpoint and bifurcation of the M1 portion of the MCA bilaterally found that the midportion showed velocities of approximately 70 cm/sec compared to the proximal velocities of approximately 60 cm/sec and distal velocities of approximately 50 cm/sec. This study was based on the selection of the highest velocity obtained at each MCA point. Normal velocities of 120 cm/sec were not unusual, particularly in younger subjects (196). Most agree that the higher the velocity, the more likely angiographically proven VSP exists. The specificity of TCD for detection of VSP in the MCA post-SAH ranges from 85 to 100%. Sensitivity of the method is 59–94% (128). Flow velocities in the M1 segment are inadequate for estimating the severity of VSP involving the distal vasculature (which is often involved in the most serious cases). If clinical deterioration suggests VSP, angiography should still be considered even if the TCD is low (139). A lack of TCD and angiographic VSP correlation is illustrated in Fig. 5.5.

## V. Magnetic Resonance Imaging

### A. Basic Mechanisms

Magnetic resonance images are based on the nuclear spin system of hydrogen (protons). Protons have charge and spin and behave as magnetic dipoles. The response of a proton to an external magnetic field and the perturbing influence of an oscillating magnetic field of a radio frequency pulse of electromagnetic energy may be described

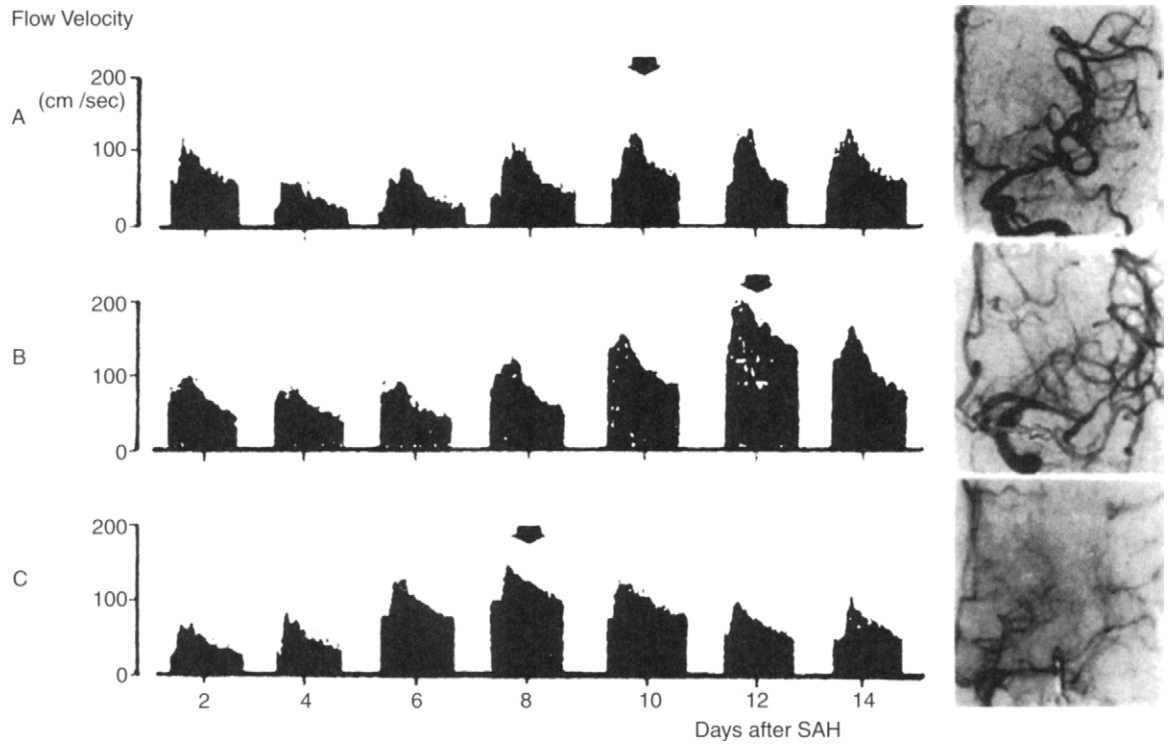



**FIGURE 5.5** TCD and angiographic findings for representative patients with no (A), moderate (B), or severe (C) VSP. (A), TCD ultrasonography showed no obvious increases in the MFV during the 2-week observation period. On day 10, IA-DSA revealed no VSP, a normal CCT (3.5 sec), and a normal MFV (70 cm/sec). (B), The MFV increased significantly during the second week and moderate VSP was observed at the M1 segment on day 12, when the CCT was 4.3 sec and the MFV was 170 cm/sec; the MFV varied from 50 to 110 cm/sec during the first 2 weeks. IA-DSA showed severe diffuse VSP extending from the ICA to the M4 segment, with a prolonged CCT (7.7 sec). Arrows indicate the day when IA-DSA examinations were performed [reproduced with permission from Okada,Y., Shima,T., Nishida, M., Yamane, K., Hatayama, T., Yamanaka, C., and Yoshida, A. (1999). Comparison of transcranial Doppler investigation of aneurysmal vasospasm with digital subtraction angiographic and clinical findings. *Neurosurgery* **45,** 443].

by equations. The nuclear magnetic resonance phenomenon determines the signal measured in the MRI based on the response of the nuclear magnetization to the surrounding magnetic environment. The protein hemoglobin content within an ICH which is nonparamagnetic changes during the process of clot formation, contraction, and necrosis (Table 5.8). The MRI appearance of hemorrhage can be changed by the strength of the magnet, the exact pulse sequence parameters used, and the method of echo formation. The MRI appearance of an ICH depends partly on iron, which is present in high concentrations, because its magnetic properties vary with the biochemical form, oxidation state, and spatial distribution change. In addition, it varies with the amount of edema and gross structural changes within the hematoma. Nonparamagnetic protein concentrations contribute to the patterns as well. RBC volume, thrombus formation, and clot retraction are probably also important. Hyperintensity on $T_1$ images of ICH occurs from several day to months and hyperintensity of $T_2$ occurs moderately in the first several hours and then markedly between several days and months but is otherwise hypointense. OxyHb containing ferrous iron within the intact RBCs is diamagnetic. More than 99% of the body is diamagnetic. Other forms of Hb and iron are all paramagnetic. DeoxyHb and oxyHb have different electron spins states. Immediately after SAH there is a slight decrease in $T_1$ which reflects the increase in hydration-layer $H_2O$ due to the elevated protein content of the bloody CSF. Several days to a week post-SAH, signal intensity increases in the subarachnoid space due to metHb formation. If the SAH is of small volume and RBCs are reabsorbed or removed prior to the time that significant metHb has formed, the anticipated short $T_1$ appearance will not be seen. The short $T_2$ properties of a hematoma require formation of both deoxyHb and retraction (reabsorption of plasma). The short $T_2$

**TABLE 5.8 Magnetic Resonance Characteristics of Intracerebral Hemorrhage[a]**

| Stage | Age | Compartment | Hemoglobin | $T_1$ weighted[b] | $T_2$ weighted[b] |
|---|---|---|---|---|---|
| Hyperacute | < 24 hr | Intracellular | Oxyhemoglobin | Isointense | Slightly hyperintense |
| Acute | 1–3 days | Intracellular | Deoxyhemoglobin | Slightly hypointense | Very hypointensive |
| Subacute: early | >3 days | Intracellular | Methemoglobin | Very hyperintense | Very hypointense |
| Subacute: late | >7 days | Extracellular | Methemoglobin | Very hyperintense | Very hyperintense |
| Chronic: center | >14 days | Extracellular | Hemichromes | Isointense | Sightly hyperintense |
| Chronic: rim | >14 days | Intracellular | Hemosiderin, ferritin | Slightly hypointense | Very hypointense |

[a]From Bradley, W. C. (1993). Magnetic resonance appearance of hemorrhage in the brain. *Radiology* **189,** 15–26.
[b]Intensity compared to brain.

appearance is rarely observed in SAH unless massive bleeding has occurred (197). In the CSF RBCs are phagocytized or lysed by enzymes released from macrophages. Extravasated RBCs have a decline in their energy status. The reductase enzyme systems (NADH – cytochromes $b_5$ reductase and NADPH–flavin reductase) used to maintain heme iron in the ferrous oxidation state become nonfunctional. Hb is oxidized to metHb, in which the heme iron within the globin protein changes to the ferric state with five small *d* electrons. The iron is then paramagnetic. Change to metHb is slowed if the $pO_2$ is either too high or too low (198). When the globin begins to degrade the $Fe^{3+}$ is exposed to the surrounding solvent. The electronic configuration changes from five unpaired electrons, one in each of the five *d* suborbitals, to one unpaired electron as the weak sixth ligand of $H_2O$ is exchanged for a hydroxide and then another imidazole nitrogen of a histidyl residue of the protein. These compounds are hemichromes. Generally, the $T_1$ image is hyperintense (whiter) whenever metHb exists. The differential diagnosis of high intensity on $T_1$ includes fat, very high protein concentrations, paramagnetic cations associated with liver disease or dystrophic calcification or necrosis, melanin, and slow flow and intravenous paramagnetic contrast agents. Extracellular protein degradation releases iron, which is detoxified by chelation to proteins such as transferrin and lactoferrin. Chelated iron is paramagnetic.

The conversion of oxyHb to deoxyHb and subsequently metHb requires a relatively narrow range of oxygen tension. In acute diffuse SAH, the ambient oxygen tension of subarachnoid CSF may be too high for the conversion of oxyHb to metHb to occur with the same speed as it would in parenchymal ICH (197).

Cell-free clots were compared to whole venous blood clots and fresh rat brain standards using a variety of MR field strengths and pulse sequences. The presence of RBCs in the clot had little impact on the images at or below 1.5 T. On $T_2$-weighted scans, the retraction of the RBC-free clot produced a progressive decrease in signal intensity at 2.4 T. Fully retracted RBC-free clots became markedly hypointense relative to serum. Spin density and $T_1$-weighted scans showed no concomitant signal intensity change. The physical basis for these MR effects in RBC-free clots is presumably the concentration of plasma proteins. Because the venous clots with RBCs also have concentrated proteins, the MR appearances are similar (199). Spin echo contrast in clinical MRI is influenced by changes in protein concentration. These presumably result from clot matrix formation, RBC settling, changes in RBC hydration with resultant alteration of intracellular protein, and the contribution of metHb to image contrast. Inhomogeneity present within voxels may reflect clumped RBCs in serum. The state of hydration of the RBCs in the presence or absence of metHb appears to be the principal factor governing contrast on $T_1$ spin echo images (200).

### B. Clinical Series

MRI was performed in 25 patients less than 24 hr after hemorrhage. The SAH appeared as high intensity relative to the surrounding brain on a $T_2$-weighted spin echo image but isointense where the corresponding CT showed blood clot of attenuation value over 60 Hounsfield units. A $T_2$-weighted spin echo image revealed subtle evidence of SAH not apparent on the CT scan. The $T_1$-weighted inversion recovery image was not as sensitive. MRI was sensitive to subacute and chronic SAH in 5 patients. Three of 4 SAHs not evident on CT scan showed high intensities on $T_2$-weighted images (201). $T_2$ values were measured at 0.23 and 4.7 T for deoxygenated blood samples (43–73% $O_2$ saturation) with Hct of 18–100%. As Hct increased there was a marked reduction in $T_2$ at both field strengths. Lysis did not abolish the $T_2$ effects in either field strength. It was speculated that the increase in

Hb concentration caused by formation of a retracted clot causes the hypointense appearance of acute hemorrhage compared to the brain on $T_2$-weighted MRI. Low field strength systems are less sensitive to the $T_2$ shortening effects of paramagnetic intracellular deoxyHb (202).

Gadolinium-enhanced MRI can show meningeal enhancement in the subacute phase following SAH (203–206). Intraparenchymal hematomas studied by $T_1$ MRI may show hypointensity between days 1 and 3 and hyperintensity between days 3 and 14. On $T_2$ studies there may be hyperintensity between days 1 and 7, hypointensities between days 7 and 14, and a rim of hyperintensity after 14 days. In the first 24 hr both regular $T_1$ and $T_2$ studies may show the same intensity as brain (207). Fifty patients with intraventricular bleeding (11 from aneurysms) were studied using 1.5-T MRI. Liquid "layered" blood in the ventricles degraded more slowly (oxy to deoxy to metHb) than clotted IVH or ICH. IVH did not form hemosiderin. SAH cleared faster and was less conspicuous than clotted IVH. Intracellular oxyHb was isointense to bright on $T_1$ and $T_2$, intracellular deoxyHb was dark on $T_1$ and $T_2$, intracellular metHb was bright on $T_1$ and dark on $T_2$, and extracellular metHb was bright on $T_1$ and $T_2$ (208). Twenty-five patients were studied within 72 hr post-SAH using a 0.2-T magnet and inversion recovery and saturation recovery images were obtained. In the acute phase of SAH, subarachnoid spaces close to the aneurysms were insointense on $T_1$-weighted images and hyperintense on saturation recovery images. In the subacute phase the subarachnoid blood became hyperintense on both $T_1$ and saturated recovery images as well as high-intensity $T_2$ (209).

### C. Imaging Techniques

SAH was induced in monkeys and serial CT and MRI imaging were performed. Acute SAH was detected with MRI as isointense signal replacing normal CSF space on $T_1$-weighted images. The signal changes related to protein water binding associated with clotting mechanism rather than oxidative denaturation of oxyHb. After 4 days post-SAH there was a marked increase in signal intensity on $T_1$-weighted images presumably resulting from MetHb formation within clot matrix (210).

Thirty-seven MRIs were performed on 0.5 T magnet in 33 patients 2 hr to 75 days post-SAH. Twenty-four proton-density (long repetitions time and short echo time) images were obtained <72 hr post-SAH. SAH was hyperintense compared to brain and CSF in all cases. The ability to show acute SAH on $T_1$-weighted images (short repetition time and short echo time) was 36% and for $T_2$-weighted images (long repetition time and long echo time) it was 50%. In the later stages (more than 3 days post-SAH) the detectability of SAH on $T_1$, $T_2$, and proton density images was 73, 31, and 83%, respectively. It was concluded that even acute SAH could be reliably demonstrated with MRI using appropriate parameters (211).

Fluid-attenuated inversion recovery (FLAIR) has begun to be applied for the detection of acute SAH. Twenty patients with acute SAH were compared to 27 control subjects using FLAIR sequences in a 0.5-T superconducting unit. The FLAIR images were obtained 2 hr to 2 days after SAH. Acute SAH was clearly demonstrated as an area of signal intensity that was higher relative to normal CSF and surrounding brain. This sequence was particularly useful in the posterior fossa (212). FLAIR sequence suppresses the CSF signal and produces very heavy $T_2$-weighted images. This is useful in identifying lesions at brain margins and in the basal cisterns as well as at the gray white matter junction. Clear visualization of acute SAH was demonstrated in SAH patients in Fisher's group 2 as well as those with greater bleeding. This sequence was also capable of detecting ICH, IVH, and SAH. In addition, aneurysms were found in 57% of 37 cases. The detection rate was 100% for aneurysms >7 mm in diameter (213). Modified versions of FLAIR are currently being developed which will further shorten acquisition times and eliminate pulsation artifacts. FLAIR may ultimately replace conventional spin echo imaging and routine MRI (214).

### D. Diffusion-Weighted Imaging

MR diffusion-weighted imaging (DWI) is a powerful tool in the detection of early brain injury. There is a sharp decline in the apparent diffusion coefficient of water after cerebral ischemia that relates to cellular swelling due to ischemically induced depolarization. This can occur in the first couple of minutes after the onset of ischemia. The reduction in the apparent diffusion coefficient is indicated by hyperintensity on diffusion-weighted MRI. The rat model of SAH induced by the perforation of a MCA or one of its branches by a suture induced a sharp decline in apparent diffusion coefficient within 2 min. This decrease in diffusion spreads quickly over the ipsilateral hemisphere. After a further 1–3 min similar decreases appeared in the contralateral hemisphere. After 30 min the extent of the diffusion abnormality began to decrease. In a group of animals that was heparinized and that presumably had larger volume hemorrhages, no recovery occurred during this experiment. The spatial and temporal pattern changes were considered to be due to acute VSP and spreading depolarization of brain tissue (53,215,216).

Combined DWI and hemodynamically weighted MRI studies were performed on six patients with clinical and angiographic VSP, one patient with only angiographic VSP, and one post-SAH patient without VSP. The protocol required 30 min of patient time within the magnet. There was a minimal artifact from the nonferromagnetic aneurysm clips. Trace DWI and relative cerebral blood volume (CBV) images were computed within 10 min of study completion, whereas the CBF and tissue mean transit time images required additional postprocessing that took approximately 20 min. Hemodynamic imaging comes from spin echo-planar imaging during the injection of 0.2 mmol/kg of gadodiamide or gadopentetate. Contrast was administered at 5 ml/sec. Small, sometimes multiple, ischemic lesions were seen on the DWI encircled by large areas of decreased rCBF and increased tissue mean transit time in all patients with symptomatic VSP. No prominent decreases occurred in rCBV. The hemodynamic abnormalities were appropriate to the territories of the vessel in angiographic VSP or their watershed areas. The zones of abnormal transit were much larger than the area of DWI abnormalities. In the patient with asymptomatic angiographic VSP, the MRI images were normal, as they were in the post-SAH patient without any evidence of VSP. Previous studies have shown weighted image abnormalities as early as 40 min after ischemic strokes. These images are highly sensitive and specific for irreversible ischemic injury. In the ischemic stroke patient rCBV is generally significantly reduced in the region of the DWI abnormality or $T_2$ abnormality in a more mature infarct. The ultimate infarction may be larger than the initial regions of abnormal DWI or rCBV. In acute ischemic stroke decreased rCBF and increased tissue transit time regions are often found to include areas of DWI and rCBV abnormality. The abnormalities in patients with VSP post-SAH were less severe than usually seen with acute ischemic stroke. The decrease in rCBF was less and rCBV was relatively normal. During this study, all patients were given phenylephrine through very long tubing leading to injection equipment outside the room with the magnet (217).

A case of focal, reversible decrease in apparent diffusion coefficient from rupture of a MCA aneurysm was reported (218).

### E. Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) was used to evaluate the result of complete cerebral ischemia in dogs. The brain stem was spared. Sodium signal intensity decreased in step with the decrease in ATP. After 12 min of ischemia, reperfusion resulted in a more rapid recovery of $Na^+$ intensity than either ATP or intracellular pH. A rapid recovery of $Na^+$ intensity during early reperfusion might represent $Na^+$ efflux, although it could also be attributable to increased plasma volume and $Na^+$ uptake from plasma. It was hypothesized that the later recovery of ATP could be due to its utilization for the restoration of the transcellular $Na^+$ gradient (219). MRS was used to evaluate the chemical changes occurring in the basal ganglia of a baboon following MCA occlusion. No significant CBF remained within the basal ganglia region of interest after arterial occlusion. Proton MRS demonstrated increasing cerebral lactate and decreasing *N*-acetyl aspartate within 30 min of MCA occlusion. Changes in the MRI signal intensity were observed as early as 3.1 hr on $T_2$-weighted, 3.3 hr on $T_1$-weighted, 6.1 hr on spin density-weighted images. Increasing cerebral lactate and decreasing *N*-acetyl aspartate preceded changes in MRI signal intensity (220). In a human case of MCA stroke, MRIs at 3 hr showed no abnormalities. MRS at 24 hr showed localized elevation of cerebral lactate levels and in most of these regions infarction was subsequently documented. Five months following the infarction there was a reduced *N*-acetyl aspartate level, increased choline levels, and the absence of lactate (221). For 20 patients with aneurysmal SAH showing normal neurologic examination in the chronic stage, proton MRS was performed in the intervals 7–10 days post-SAH or >4 weeks post-SAH. The peak areas for the creatine and phosophocreatine, choline compounds (composing cell membrane structure), and *N*-acetyl-aspartate (a marker for neurons) were calculated from the proton spectrum and the ratios of choline: phosphocreatine and *N*-acetyl-aspartate:creatine were evaluated. A control group of 11 volunteers was evaluated. All SAH patients were free from any significant infarction on MRI. The mean ratio of choline: creatine was significantly increased in the acute stage (1.25) compared to the control value (1.14). Changes in choline:creatine ratio significantly correlated with the SAH grade. The mean ratio of *N*-acetyl-aspartate: creatine more than a month later was 1.88, which was significantly decreased compared to the control value of 2.15. This ratio was not significantly changed in the acute stage of SAH patients. The *N*-acetyl-aspartate: creatine ratio in the chronic phase significantly correlated with the dementia score. Intracerebral elevation of choline in the first week or so after SAH probably correlates with the extent of brain injury and the impairment of circulation. A decrease in the neuronal marker in the chronic stage indicates a delayed neuronal loss. This can occur even when MRI shows no apparent infarction (222).

### F. Magnetic Resonance Angiography

Serial evaluation of VSP following SAH was attempted in 11 patients using MRA angiography. VSP was shown

in 3 patients as a segmental narrowing or loss of flow signal usually accompanied by a decreased distal flow signal. MRA showed no VSP in 5 patients without clinical evidence of VSP. No signal was seen in 1 patient, which was attributed to the iatrogenic clipping of a unilateral cerebral artery. MRA was considered to have potential for the detection of VSP if further technical improvements occur (223). MRA was performed 5 days after symptoms of preeclampsia. Diffuse intracranial VSP was clearly demonstrated by this technique (224). Delayed cerebral VSP was detected by MRA (225).

### G. Advantages and Disadvantages

MRI is superior to CT after aneurysmal rupture in demonstrating IVH and posterior fossa SAH, transependymal migration of CSF, and early ischemic changes. Newer nonferromagnetic clips showed smaller areas of artifacts on MRI than CT scans (226). MRI and CT were obtained on 31 patients in the first day after acute cerebral infarction. Follow-up exams were performed 7–10 days later. MR imaged the infarct in 82% cases and CT in 58%. The region of hyperintensity on proton density in $T_2$-weighted scans corresponded to acute infarcts, but proton-density weighted scans often showed better definition of the lesion in terms of the anatomy. At follow-up, MRI showed 88% of the subacute strokes and CT 54%. Hemorrhagic characteristics were seen on at least 1 examination in follow-up studies of MRI (227). Late follow-up MRI shows a poor correlation between evident tissue loss and neurobehavioral deficits assessed by neuropsychological examination. Some patients with pronounced pathological changes on MRI show little evidence of cognitive dysfunction and vice versa (228). Magnetic resonance techniques are capable of very early detection of ischemic regions that will ultimately infarct, the direct visualization of cerebral blood vessels, and the correlative demonstration of brain metabolites and anatomy. However, the cost, complexity, lack of general availability, difficulty of discerning acute SAH, and incompatibility with certain medical devices have prevented MRI from replacing CT as the main imaging device in SAH (229).

## VI. Positron Emission Tomography

### A. Changes with Vasospasm

The classic delineation of the pathophysiological response to VSP using PET scanning came from the Saint Louis group and was published in 1977. Their studies demonstrated that CBF decreased progressively with poorer neurological grades as did the $CMRO_2$. The CBV showed a reverse change increasing as neurological grade deteriorated. The decrease in CBF and $CMRO_2$ and increase in CBV were aggravated by the presence of VSP. The grade III and IV patients with VSP showed a reduction in CBF to <20 ml/100 g/min, a decrease of $CMRO_2$ of more than 1 ml/100 g/min, and an increase in CBV of more than 2 ml/100 g/min (230). A young patient who developed hemiplegia postoperatively, attributable to VSP, was studied using PET scanning methodology. The weakness was reversible by dopamine-induced systemic hypertension. CBF was low in both hemispheres but CBV was elevated only on the side of the VSP. The oxygen extraction ratio (OER) was significantly elevated in both hemispheres; this was interpreted as showing a generalized impairment of $O_2$ delivery to the brain. Control values were as follows: CBF, 55 ml/100 g/min; $CMRO_2$, 3.5 ml/100 g/min; OER, 0.44, ml/100 g/min; CBV, 3.5 ml/100 g/min. Corresponding values from the vasospastic hemisphere were as follows: 30, 2.5, 0.53, and 5.5 ml/100 g/min. There were significant differences in the values of CBV and CBF between vasospastic and nonvasospastic hemispheres (231).

Contrary to the findings of the previous studies, it was later suggested that CBV may decrease during VSP. The normal vasodilating capacity of distal intraparenchymal small arteries may be impaired post-SAH. There was significantly reduced CBV in patients with VSP compared to normals. The apparent increase of CBV in earlier studies may have reflected failure to target the intraparenchymal vessels (232,233).

### B. Flow and Metabolism with Infarction

Four hemiparetic patients with VSP were studied post-SAH. The two patients who recovered had minimal contralateral hemispheric rCBF of 15 and 16.2 ml/100 g/min; the values from the two who did not recover were 12 and 11.7 ml/100 g/min. The comparable values for $rCMRO_2$ were 1.34 and 2.60 vs 0.72 and 1.66 ml/100 g/min. Measurement of OER and CBV showed no consistent pattern. All patients showed higher CBF before and after VSP than during it (234).

### C. Oxygen Delivery

Thirteen SAH patients studied with PET scans during the time of anticipated VSP were compared with 10 volunteers. The patients had decreased Hb concentrations and decreased total $CaO_2$. The patients also had reduced $CMRO_2$ and tissue oxygen supply, even in apparently normal cortex. Regional reductions in CBF and perfusion reserve were seen in cortical areas perfused by spastic vessels. Patients (controls) had the following values:

$CaO_2$, 14.1 ml/dl (18.3); hemoglobin, 10.8 g/dl (14); CBF, 48.3 ml/100 g/min (41.4); OEF, 45.6% (44.3%); $CMRO_2$, 2.91 ml/100 g/min (3.31); CBF $\times CaO_2$, 6.4 ml/100 g/min (7.5); and CBV, 4.67 ml/100 g/min (4.45) (235).

## VII. Single Photon Emission Computed Tomography

### A. History

Planar or SPECT imaging with the radiolabeled amine *N*-isopropyl-*p*-[$^{123}$I]iodoamphetamine (IMP) was first used in the early 1980s (236). *N,N,N* '-trimethyl-*N* '-2-hydroxyl-3-methyl-I-[$^{123}$ I]5-iodobenzyl-1,3-propane diamine was introduced about the same time (237).

### B. Technique

SPECT with either [$^{123}$I]-amphetamine or 99mTc-HMPAO usually provides nonquantitative images of CBF. These studies cannot be readily repeated (238).

### C. Findings in Vasospasm

Mickey's group used SPECT to obtain a noninvasive three-dimensional estimate of rCBF. Five patients with normal studies on admission developed DID in the second week after SAH. Four patients had large areas of well-defined regional ischemia. Angiography carried out in 3 of these patients demonstrated severe angiographic VSP. The CBF decrease was directly related to VSP and the onset of ischemic deficits in patients who were in poor grade neurologically. Some impaired patients developed global reductions in CBF later in the course of their illness. Four patients who had delayed onset of hemiparesis showed a 26% reduction in CBF in the appropriate brain areas (239). Eleven patients with symptomatic VSP following early surgery were studied with SPECT. Ten patients were shown to have angiographic VSP in studies performed within 2 weeks of early surgery. Severe angiographic VSP was defined as a more than 50% narrowing of the lumen. In the 5 patients whose ischemic symptoms from VSP reversed, the early SPECT/IMP revealed moderate hypoactive areas within the territories of the constricted vessels in the second or third week post-SAH. The delayed image at this stage showed mild hypoactive areas within the same vascular territories but no low-density areas on CT scan. Both early and delayed images normalized with neurological recovery. In the 4 patients with irreversible VSP the moderately hypoactive areas on the early images turned into large and markedly active or inactive areas due to progressive ischemia. Low-density areas developed on CT scan. IMP is a lipid-soluble substance that penetrates the normal blood–brain barrier and has first-pass extraction efficiency in the brain (240).

### D. Activation Studies (Induced Parenchymal Vasodilation)

In 114 patients undergoing early surgery, weekly postoperative SPECT/IMP studies were performed under acetazolamide activation. This substance causes cerebral vasodilation. Fifty-five percent of the patients in preoperative grades III and IV had moderately impaired vasodilation compared to only 29% of patients who were preoperative grades I or II. Of the patients with such moderate impairment, 18% were severely disabled and 10% died, compared to zero disability or death in the patients with normal vasodilatory capacity (241). Of 17 patients with moderately impaired SPECT dilation to Diamox, disability or death were attributed to VSP in 11 of the cases. Three poor outcomes were attributed to initial damage, 2 to operative complications, and 1 to rebleeding (242).

### E. Postoperative Changes

A total of 20 HMPAO/SPECT scans were performed in nine patients who did not develop clinical VSP post-SAH. Areas of diminished rCBF were found near the operative site in 17 of 20 studies which did not correlate with the patients' neurological condition and were suggestive of postoperative edema. Such postoperative changes should not be attributed to VSP (243).

### F. Angioplasty

Brain SPECT with 99mTc-HMPAO was obtained before and after angioplasty of 17 anterior circulation vessels in 10 patients. Visual interpretation using an internal reference (cerebellum) and manual, semi quantitative region of interest analysis revealed improvement of rCBF in 9 of 10. For the 9 patients who improved, the average increase was 10.5% in the region of interest analysis (244).

### G. Attempted Quantification

An attempt at blood flow measurement using IMP/SPECT autoradiography was made by Hatazawa and colleagues. They employed the IMP autoradiographic method with SPECT. They used a standard arterial input, a single static scan, a fixed distribution volume, and one-point arterial sampling. rCBF was measured in 39 normal volunteers and 16 patients with SAH. rCBF

was measured 1 or 2 weeks post-SAH. VSP was evaluated by angiography. In SAH patients the vasospastic area showed significantly lower rCBF than normal cortical rCBF and vasospastic areas. Brain regions with rCBF <20 ml/100 g/min showed infarction on follow-up CT scans. The IMP autoradiographic method was found to be reproducible, sensitive to hypoperfusion, and feasible for the quantitative evaluation of rCBF (245).

### H. Eclampsia

Sixty-five women with eclampsia were studied within 2 days postpartum. 99mTc-HMPAO was used as a tracer of rCBF. SPECT scanning revealed perfusion deficits in the watershed areas in 100% of the women, 75% of whom had concomitant deficits in the parietooccipital areas of the brain. Hypodensities were seen in the corresponding CT scans in 59%, with parietooccipital involvement in 97% of the patients with the low densities. Increasing flow velocity measurements in the middle cerebral and posterior cerebral arteries were recorded in 86% of patients in whom TCD was performed. The study was thought to demonstrate that the pathophysiological mechanism of the eclamptic seizure is primary cerebral VSP, with resultant ischemia and cerebral edema involving mainly the watershed areas and the parietooccipital lobes of the brain (246).

### I. Comparative Studies

Correlative studies were carried out in 25 patients using DSA and SPECT. Thirteen patients had symptomatic VSP and 15 had angiographic VSP. SPECT showed hypoperfusion in 22 of 25 patients. CT clot had predicted VSP in 8 of these 22 patients. Varying degrees of hypoperfusion on the SPECT studies were shown in patients with DID (247). A comparison of CT and SPECT IMP was carried out, and all the sites of infarction identified by CT were picked up by SPECT. SPECT studies also detected reduced rCBF in areas that were normal by CT. The major advantage of SPECT/IMP is its visualization of the entire brain in transverse, coronal, and sagittal sections. The major advantage of Xe CT is its greater resolution and noninvasive quantitation (248).

## VIII. Cerebral Blood Flow Studies

### A. $^{133}$Xe Studies

#### *1. Clinical Series*

Kågström and associates first delineated the fact that CBF tended to decrease after an initial delay of several days following SAH. James examined 36 patients and found a general reduction in CBF that correlated with impairment of consciousness and the radiologic appearance of VSP. The reduction in flow was frequently bilateral and usually greater on the side of the aneurysm. A reduction in CBF was associated with hyperventilation and arterial hypertension (249). As early as 1973, Bergvall and colleagues showed that CBF could reach very low values after SAH, particularly in the presence of VSP or hematoma (250). Ishii studied 49 patients who were in poor grade with diffuse VSP. Flows were reduced to less than half their control values with focal areas of decreased flow <30 ml/100 g/min, in addition to a reduction in mean values. Serial studies demonstrated that the disappearance of VSP was associated with an increase in rCBF in the ischemic focus and mean CBF. Postoperative results correlated with rCBF: excellent, 42 ml/100 g/min; good, 40 ml/100 g/min; fair, 31 ml/100 g/min; and poor, 25 ml/100 g/min. Mean rCBF reached its nadir in the second week post SAH. During this interval the values of mean rCBF correlated well with the severity of angiographic VSP and the development of DID. Fifteen patients with symptomatic VSP had mean rCBF of 27 ml/100 g/min compared with 41 ml/100 g/min in 11 patients with asymptomatic VSP (251). Meyer carried out frequent determinations of CBF in 116 patients in the first 3 weeks post-SAH. Older patients showed greater impairment of CBF (252). Yamakami and coinvestigators formed serial studies in 35 patients. The mean CBF of those who developed DID from VSP was 37 compared to 52 ml/100 g/min for patients who did not develop these deficits. They found that CBF was most reduced in the week following surgery and on the side ipsilateral to the operation. Severe VSP, but not mild, was associated with flow reduction. Two-thirds of their patients with severe angiographic VSP developed DID and the mean value in this group was 38 compared with 52 ml/100 g/min for those who did not develop such deficits. SAH tended to decrease globally. Poor clinical grades correlated with reduced CBF (253). Koike and associates studied 84 patients and found a good correlation between the amount of subarachnoid clot within the first 3 days following SAH and the occurrence of severe diffuse angiographic VSP. Patients with no VSP had a mean CBF of 42 ml/100 g/min compared to those with VSP whose flows averaged 30 ml/100 g/min. None of the patients without severe diffuse VSP showed focal ischemia or impaired $CO_2$ responses in their blood flow studies. Ninety percent of those with severe diffuse VSP showed focal ischemia and 100% showed impairment of $CO_2$ responses. Sixty percent of patients with VSP developed low-density areas on CT scans (254). In another study mean hemispheric CBF on the side ipsilateral to the

operation was lower than the contralateral hemisphere CBF (253).

In the studies by Voldby and coworkers severe diffuse VSP was always associated with global ischemia (average flow 21 ml/100 g/min) and was always followed by cerebral infarction (255). In the first week post-SAH, 13 good-grade patients had a mean CBF of <40 ml/100 g/min: 54% developed DID, and 43% of these died (256). Thirty-three similar patients had a CBF greater than this; one-fourth of them showed evidence of ischemia and about one-third of this group died. No patient without blood on the initial CT scan developed DID, but 43% of those with such evidence did. The early reduction in flow that was presumably not due to VSP or increased ICP in these good-grade patients studied early was perhaps a reflection of reduced cerebral metabolism following SAH. Geraud and coworkers noted an average CBF of 41 ml/100 g/min in patients who recovered, 37 ml/100 g/min in those who became disabled, and 33 ml/100 g/min in those who died. About one-fourth of the patients who showed oligemic foci ultimately recovered; such foci were found in two-thirds of those who were disabled or died. Patients with an alternation of consciousness or motor deficit showed a mean decrease of CBF of 30% compared with the remainder. Disparities were sometimes found in patients between good neurological grade but with abnormally low CBF and vice versa (257). In 6 patients with poor outcomes CBF was studied. Two of the patients died of severe VSP demonstrated angiographically. One of these patients had both carotid arteries occluded presumably from prior disease, and this had clearly predisposed to inadequate cerebral flow when VSP was superimposed on the original insult (258).

Eighteen studies of CBF done between 1968 and 1984 showed a decrease in flow following SAH in 89% of studies. There was a correlation between angiographic VSP and reduction in flow in 67% of investigations. The failure to demonstrate a positive correlation was probably due to technical inadequacies or timing differences between CBF and angiographic studies (259). The poorer the neurological grade post-SAH, the lower the CBF, but there is a wide range of flows within each neurological grade. There is a tendency for flows to decrease in the first few days after SAH (259). In 11 reports of CBF in grade I and II patients the flow averaged 85% of normal. In poor grade patients it was 69%. Patients with impairment of consciousness had an additional reduction in flow averaging 19% compared to alert patients (259).

#### *2. Cerebral Blood Flow and Intracranial Pressure*

rCBF was measured in a patient postoperatively. An acute increase in rCBF coincided with clinical improvement following administration of mannitol, CSF drainage, and induced hypertension. Later, further elevations to normal levels occurred with ventriculoperitoneal shunting (260).

#### *3. Cerebral Blood Flow and Arterial Diameter*

In 45 patients post-SAH $^{133}$Xe CBF studies were performed. A positive correlation was found between rCBF and the diameter of the major conducting vessels. In 13 cases with focal VSP the reduction of CBF was global and not restricted to the area of the spastic vessel. Cerebral OER was reduced but independently of the degree of VSP. It was suggested that metabolic depression from SAH might be responsible for both reduction in cerebral $O_2$ uptake and the degree of angiographic VSP (261).

#### *4. Disadvantages*

Limitations of $^{133}$Xe CBF measurements include lack of three-dimensional anatomical correlation, poor resolution due to isotope scatter, see-through phenomenon that obscures low-flow regions, the fact that $^{133}$Xe produces such a weak signal that it provides little information on deep brain regions, and radiation exposure. The clinical value is probably insufficient to justify the continued use of this technique.

### B. Xe CT Studies

#### *1. History*

Drayer *et al.* introduced Xe CT for analysis of cerebral perfusion and blood flow in 1978 (262).

#### *2. Technique*

Xe is a highly soluble inert gas, which makes it an ideal tracer for measuring tissue perfusion. It is rapidly eliminated from the body. Studies can be repeated after approximately 30 min. A subanesthetic concentration of nonradioactive Xe gas is used in conjunction with CT scanning to measure CBF. The gas mixture includes $O_2$ (68%) and Xe (32%) for just over 4 min. There are few side effects to this concentration. CBF is calculated by integrating the buildup of xenon within the tissue and within the arterial blood to solve the Kety equation for each of the 24,000 voxels per CT level. Xe/CT flow values are more accurate than those obtained with other methods in disease conditions because the local partition coefficient is integrated into each flow measurement (263). It compares favorably to other blood flow techniques (Table 5.9).

#### *3. Clinical Studies*

In 51 patients post-SAH from aneurysms, Xe CT studies were performed. Fourteen had symptomatic VSP. In

**TABLE 5.9 Cerebral Blood Flow Imaging Techniques**[a]

| | PET | $^{99}$Tc-HMPAO SPECT | $^{133}$Xe | Xe/CT |
|---|---|---|---|---|
| Resolution | 5–6 mm | 8 mm | 10–20 mm | <4 mm |
| Anatomic reference | No | No | No | Yes |
| Quantitative | Yes | No | Yes | Yes |
| Image acquisition | <3 min | 24 min | 6 min | 6 min |
| Repeatable | 12 min | 6–24 hr | 20 min | 20 min |
| Cost of equipment | $4–6 million | >$500,000 | >$500,000 | $70,000 |
| Cost per study | $1500–2000 | $400–1000 | $400–1000 | $650 |

[a]From Beristain, X., Dujovny, M., and Gaviria, M. (1996). Xenon/CT quantitative local cerebral blood flow. *Surg. Neurol.* **46**, 437–440, with permission from Elsevier Science.

all of these patients the first postdeficit Xe CT study found abruptly reduced CBF either regionally or globally. In 9 of these 14 patients flows decreased below 15 ml/100 g/min in two or more adjacent 2-cm cortical regions of interest. CT scan follow-up showed infarction in all these regions. No patient with CBF >18 ml/100 g/min developed infarction (264). Thirteen patients with a clinical diagnosis of VSP were studied using Xe/CT scans. Nine of 13 patients had CBF of <25 ml/100 g/min. When the mean arterial blood pressure was increased from 90 to 111 mmHg via dopamine administration, the local CBF increased above the ischemic range in more than 90% of the uninfarcted territories identified on CT scans, and the local CBF decreased in one-third of the nonischemic territories. These changes in CBF after hypertension correlated with the resting CBF at normotension and were unrelated to the change in blood pressure. Only one-half of the patients with presumed clinical VSP had identifiable reversible ischemia. Since the flow changes to dopamine-induced hypertension were not entirely proportional to the change in blood pressure, it was considered that the direct cerebral vascular effects of dopamine might have important and unpredictable effects on CBF in the post-SAH state. The actual measurement of local CBF was believed to be a useful adjunct (265).

#### *4. Disadvantages*

The Xe concentrations are usually in the range of 30–33%. The radiation dose to tissues examined is relatively high (15–20 rads) (266). Xe/CT is a very useful way of evaluating whether or not induced hypertension is actually increasing regional blood flow. Yonas's group established beyond doubt that induced hypertension on occasion is harmful. It would be of interest to determine whether close clinical observation would provide the same or less information as the Xe/CT scan. Clearly, having both types of data would be ideal.

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CHAPTER 6

# PHARMACOLOGY

## I. Introduction

The ability of a compound to produce contraction of isolated arterial segments is only relevant to its potential as a mediator of clinical vasospasm if the slow-onset VSP arises from the slow liberation of a spasmogen, which then reacts with a blood vessel that is approximately normal. A putative spasmogen for chronic VSP should be liberated sometime after the hemorrhage and have a very prolonged duration of action or be liberated continuously during days and weeks (Fig. 6.1; Table 6.1). Cook pointed out that while irreversible antagonists are relatively common in pharmacology, irreversible agonists are essentially unknown. He also noted that nearly all receptor systems show some measure of tachyphylaxis, desensitization, or autoregulation which interferes with what would otherwise be an indefinitely sustained contraction. For these reasons, he concluded that the unique properties of cerebral VSP would make any purely pharmacological mechanism unlikely (1). Have studies of tension developed by isolated fresh blood vessels contributed significantly to our knowledge of VSP? The answer to this question depends on the mechanism by which the delay of onset in chronic VSP occurs. If the delay is due to a prolonged series of events within the smooth muscle cell leading to contraction only after several days, then acute studies are not as likely to be helpful. However, if the delay is due to a rapidly acting spasmogen released slowly and in a cumulative fashion, the study of isolated vessels would be useful. There is substantial and growing evidence that the latter situation prevails. The evidence is

**TABLE 6.1 Vasoactive Substances in CSF Post-SAH**

| |
|---|
| Substances increasing over days |
| OxyHb, deoxyHb, bilirubin, heme, hemin |
| Fibrinogen, fibrin degradation products |
| PAI-1, thrombin–antithrombin, tPAPAI-1 complexes, prothrombin fragments $F_1$ and $F_2$ |
| Interleukins, TNF-α |
| Lactate |
| NE |
| ET |
| Substances decreasing over days |
| ATP |
| Complement proteins |
| BK |
| Adhesion molecules |
| Glucose |
| $K^+$, $Mg^{2+}$ |
| ACh |
| Fibrinopeptide A, platelet-secreting protein |
| Substances unchanged |
| 5-HT |
| CPK |

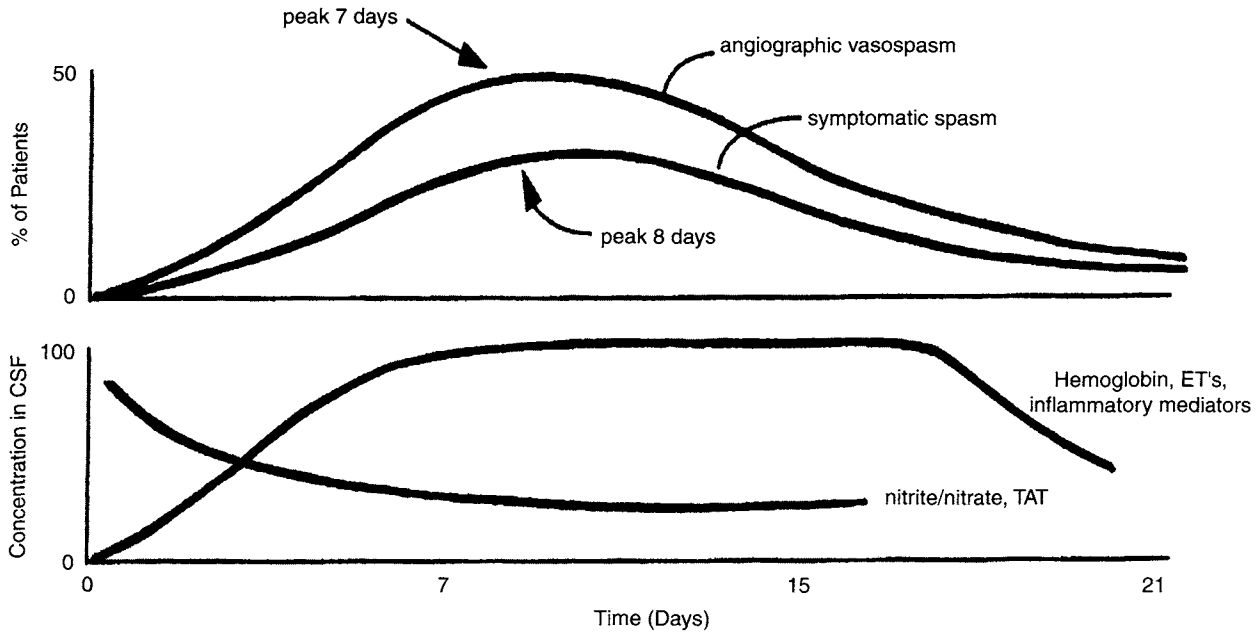


**FIGURE 6.1** Time course of vasospasm and CSF concentrations of compounds possibly involved in its genesis [reproduced with permission from Weir, B., Stoodley, M., and Macdonald, R. L., (1999). Etiology of cerebral vasospasm. *Acta Neurochir.* **72,** 27–46. Copyright © Springer-Verlag GmbH & Co.].

that the delay arises from the slow release of material from red blood vessels (RBCs) trapped in the clot surrounding the vessel (2). Acute studies of the response of cerebral blood vessels to constrictors have been carried out using pressure recordings from perfused vessels, helically cut strips, rings, and other tissues. Isolated vascular smooth muscle cells have also been studied (3). The search for putative spasmogens has involved the use of inhibitors or blocking agents (Table 6.2). There is a wide range of physiological vasoactive mediators of cerebrovascular tone that may be altered following SAH (Table 6.3).

Vasospasm is an alteration in vasomotor tone in which sustained contraction predominates (4). This state may result from a deficiency in vasorelaxing substances or an increase in vasocontracting ones. Some authors have defined true VSP as a state of contraction refractory to relaxation by the addition of potent relaxants (refractory to "active relaxation") and refractory to removal of vasoconstrictors (refractory to "passive relaxation")(5). VSP can be viewed as a state inherent to the vascular smooth muscle (4). It implies a primary alteration of smooth muscle cell signaling events that modulate the state of contraction and relaxation (4,5). Systemic examples of VSP include extremity arteries damaged by fractures or dislocations, occlusive or nonocclusive mesenteric ischemia (6), spasm in autologuous vein grafts in peripheral vascular reconstructions (7), anginal pain unrelated to exercise associated with ST-segment elevations (Prinzmetal's angina), ergonovine sensitivity (8), spasm associated with coronary thrombosis, Raynaud's phenomenon, and artherosclerosis associated with enhanced contractile responses (9). Knowledge of VSP in noncerebral arteries is rudimentary, with the exception of coronary VSP. The cerebral arteries may be relatively susceptible to vasconstrictors acting over a long time frame (1).

The prolonged nature of VSP may be partly due to necrotic damage to the blood vessel wall resulting from ischemia. The resolution of VSP might therefore be due to tissue repair and not reversal of vasoconstriction. Eldevik *et al.* failed to demonstrate specific ultrastructural changes in spastic blood vessels following SAH, regardless of whether they were taken from human autopsy material or animal models (10). It is possible that ultrastructural changes are neither necessary nor sufficient to produce VSP.

**TABLE 6.2 Vasoactive Sites and Substances and Their Inhibitors**

| Compound | Blocker, antagonist, or selective inhibitor |
|---|---|
| 5-HT (serotonin) | Methysergide, cinanserin |
| Histamine | Chlorpheniramine, mepyramine ($H_1$ blockers), cimetidine ($H_2$ blocker) |
| Thrombin | Hirudin |
| Acetylcholine | Atropine |
| Norepinephrine | Phentolamine (adrenergic blocker), phenoxybenzamine, prazosin ($\alpha$ blockers), propranolol ($\beta$ blocker) |
| Angiotensin | Sarcosine-alanine-angiotensin |
| Prostaglandins | Polyphoretin phosphate, DPP |
| Thromboxane $A_2$ | OKY-1581 |
| Thromboxane synthetase | OKY-046 |
| Cyclooxygenase | Indomethacin, aspirin |
| Phospholipase $A_2$ | Glucocorticoids |
| Phospholipase C | Neomycin |
| Phosphatidylinositol 3-kinase | Wortmannin, LY-294002 |
| NO synthase | L-NAME, L-NNA |
| Tyrosine kinase | Genistein, triphostin |
| Guanylate cyclase | Methylene blue, hemoglobin |
| Protein kinase C | Staurosporine, H7, bisindolylmaleimide I HCl |
| All $Ca^{2+}$ entry channels | Lanthanum |
| L-type $Ca^{2+}$ channel | Nimodipine, nifedpine |
| Voltage-independent $Ca^{2+}$ channel | Lanthanum, econazole, SKF 96365 |
| $P_1$ purinoceptor | Theophylline |
| $P_{2\mu}$ purinoceptor | Suramin, quinidine |
| Sarcoplasmic reticulum ATPase | Thapsigargin, cyclopiazonic acid |
| Sarcoplasmic reticulum $Ca^{2+}$ release | Ryanodine, dantrolene |
| | **Increased by** |
| cAMP | Forskolin, 8-bromo-cAMP |
| Protein Kinase C | Phorbol esters |
| ET-1 | Thrombin, phorbol esters |
| cGMP | ACh, A23187 |
| Guanylate cyclase | NO, nitrovasodilators |
| NO | L-Arginine, nitrovasodilators |

## II. General Considerations

### A. Experimental Variables

An enormous volume of experimental evidence has accumulated with respect to the pharmacological basis for chronic VSP. Most generalizations must be interpreted with caution. The onset and resolution of human clinical VSP over many days, the gradual removal of thick clot, and an active repair response are seldom replicated in experimental studies. Studies should be analyzed with several questions in mind:

**TABLE 6.3 Physiological Vasoactive Mediators of Cerebrovascular Tone That May Be Altered after SAH and Contribute to Vasospasm[a]**

| Compound | Action |
|---|---|
| *Amines* | |
| Norepinephrine | Perivascular sympathetic nerves originating from cervical sympathetics; tone is balanced between α and β receptor activation; causes nerves to degenerate after SAH; time course lasts longer than vasospasm |
| Serotonin | Innervates intrinsic vessels from brain stem nuclei; vasoconstricts large arteries although existence of serotoninergic nerve fibers controversial |
| Histamine | Acts via $H_2$ receptors to cause vasodilation of distal arteries and increased permeability; $H_1$ receptor activation causes constriction of proximal cerebral arteries |
| Dopamine | Increased cerebral blood flow probably by indirect action on brain neurons |
| Acetylcholine | Parasympathetic neurotransmitter; causes endothelium-dependent relaxation |
| *Lipids* | |
| Eicosanoids | $PGI_2$ relaxes via increased cAMP; thromboxane $A_2$ and prostaglandin $F_{2\alpha}$ are vasoconstrictors; $PGE_2$ is a vasodilator |
| Leukotrienes | Potent vasoconstrictors of large vessels, increase vascular permeability |
| Platelet-activating factor | No apparent direct effect |
| *Peptides* | |
| Sympathetic | Neuropeptide Y; causes vasoconstriction |
| Other constrictors | Angiotensin 2; also causes release of thromboxane $A_2$ from endothelium and endothelins; vasopressin may cause direct smooth muscle contraction and endothelium-dependent relaxation |
| Parasympathetic | Vasoactive intestinal peptide; peptide histidine isoleucine; pituitary adenylate cyclase-activating peptide vasodilate by acting directly on vascular smooth muscle; increasing cAMP |
| Trigeminal sensory | Calcitonin gene-related peptide is a vasodilator that acts via receptor to increase cAMP; substance P vasodilates possibly by releasing NO and/or increasing cAMP; neurokinin A also vasodilates |
| Other vasodilators | Adrenomedullin is a vasodilator and may increase vessel permeability |
| *Purine nucleotides* | |
| Adenosine | Vasodilator, acts by receptor-mediated increase of cAMP |
| ADP and ATP | Complex effects; may vasodilate by endothelium-dependent or independent mechanisms and may vasoconstrict by direct effect on smooth muscle |
| *Gases* | |
| Nitric oxide | Free radical; vasodilates by stimulating production of cGMP by activating guanylate cyclase |
| Carbon monoxide | May be vasodilator; increases cGMP |

[a]From Weir, B., Stoodley, M., and Macdonald, R. (1999). Etiology of cerebral vasospasm. *Acta Neurochir.* **72,** 27–42. 

1. What was the time frame? Seconds, minutes, hours, days, or weeks.
2. What species was studied? There are vast differences in the pharmacological responsiveness between species.
3. What tissue was studied? Arteries, smaller vessels, veins, cerebral or systemic, proximal or distal, intrasubarachnoid or extrasubarachnoid.
4. How were the studies done? Intact organisms, *in vivo*, *ex vivo*, *in vitro*, vessel rings, strips or perfusion with or without endothelium preparations, freshly dissociated cells, or cell cultures.
5. How was SAH replicated? Autologous blood clot, blood from a different species, fresh or aged hemolysate.
6. Was the experiment protected from light? Human VSP develops in the dark.
7. Was it conducted at room temperature or body temperature? The majority of patients with VSP are febrile, and the majority of *in vitro* experiments have been performed at room temperature.
8. How were putative agonists and antagonists prepared? Were their concentrations known and within a physiological range?

9. Were extraneous substances employed such as non-physiologic buffers or antibiotics? These might have intrinsic vasoactivity.

10. Was the electrolytic and gaseous composition of bloody cerebrospinal fluid (CSF) faithfully replicated? Most *in vitro* experiments had far higher $pO_2$ than would occur naturally.

11. How was "hemoglobin" prepared? Was it oxy or deoxy? Was metHb present? Was hemin? Was it free of trace quantities of toxic impurities? If it was "ultrapure," was the molecular configuration greatly altered?

12. In what sequence were agonists and antagonists added? Were agents delivered intraluminally or extraluminally? Could agents have interacted?

13. Were appropriate statistical techniques utilized and were numbers adequate? Notwithstanding the absence of many perfect experiments, a coherent explanation for the etiology of VSP has emerged in recent years.

### B. Hypoxia

We think it is likely that the vessel wall embedded in thick clot and CSF will be hypoxic compared to normal vessels. It is not known whether extreme constriction and abluminal clot would interfere with $O_2$ transport from the blood or CSF into the vessel wall. There have been relatively few systematic studies of anoxia on vasoconstriction. Hypoxia augmented the contractile responses to KCl, $PGF_{2\alpha}$, and Hb ($10^{-6}$ M). The tension generated by Hb was increased almost fivefold by hypoxia. Removal of the endothelium did not affect the hypoxic potentiation of canine basilar artery constriction (11). Relative hypoxia may contribute to VSP by suppressing endothelium-dependent relaxation (12). In dogs, basilar arteries were removed on day 7 post-SAH. Endothelium-dependent relaxation was abolished in response to arginine vasopressin and was significantly reduced in response to thrombin. Relaxation to nitrovasodilators was relatively preserved, as was endothelium-dependent contraction in response to certain drugs, mechanical stretching, and hypoxia (13). ACh-induced relaxation in isolated canine femoral arteries was reduced by hypoxia (14), although this reduction in $O_2$ did not change relaxation in canine intrapulmonary arteries (15). Also in canine coronary arteries, both ACh and ADP produced relaxation that was not inhibited by hypoxia (16). Endothelium-dependent contractions occurred in response to hypoxia in both control and SAH feline arteries. These contractions were highly dependent on the presence of endothelium (17).

## III. Neurogenic Factors

### A. Adrenergic Nerves

The adrenergic nerves around cerebral arteries originate both in the superior cervical sympathetic ganglia and in the intrinsic intracerebral systems (18). The cerebral vascular system receives well-developed innervation by nerve fibers originating in the superior cervical sympathetic ganglia which are adrenergic as well as cholinergic nerves. Middle cerebral artery (MCA) innervation of sympathetic nerves originates in the superior cervical and trigeminal ganglia (19).

### B. Cholinergic Nerves

The cholinergic nerve fibers appear to run in the facial nerve and enter the geniculate ganglion to continue in the greater superficial petrosal nerve to the plexus around the internal carotid artery where they synapse (18). The parasympathetic innervation of the cerebral arteries in the rat are derived from the sphenopalatine ganglion via the middle arteries that connect in a collateral anastomotic fashion with the intracranial arteries (20).

### C. Intracerebral Pathways

Intracerebral noradrenergic nerves originate in the locus ceruleus. The density of innervation of intraparenchymal arterioles varies between different brain regions (18). The release of norepinephrine (NE) from sympathetic nerves around the brain vessels can be induced by the administration of indirectly acting sympathomimetic amines such as tyramine or by electrical stimulation of the perivascular nerves, producing vasoconstriction (18). Monoamine metabolites were measured in rat lower brain stem using dialysis probes implanted in the nucleus tractus solitarius. 3,4-dihydrophenyl acetic acid, homovanilic acid, and 5-hydroxyindolacetic acid were nonspecifically increased in the acute phase after cisternal injection of blood or saline. Noradrenergic and serotonergic neurons in this region may show disturbed function following induced VSP (21). In the squirrel monkey SAH model 6 days post-SAH there was a decrease in cerebral blood flow (CBF) of 25% and a corresponding increase in glucose uptake of between 30 and 50%. Lesioning of the A2 nucleus, its ascending pathway, or the median eminence prevented the occurrence of VSP. Also, a unilateral postganglionic trigeminal lesion caused an ipsilateral constriction of cerebral arteries of 27%, whereas a preganglionic lesion did not affect baseline vessel diameters (22).

### D. Effect of Subarachnoid Hemorrhage on Nerves

Cholinergic, adrenergic, and peptidergic innervation is found on the adventitia of cerebral blood vessels. Many neurotransmitters are found in the cerebral blood vessel nerves. The disappearance of characteristic staining of nerves that occurs following exposure to blood has not temporally correlated well with VSP in rats or primates (23,24). A sympathectomy has not been reported to prevent or reverse VSP in experimental animals and humans (25,26).

In the rat SAH model constriction occurs 2 days post-induction without any obvious change in sympathetic or parasympathetic perivascular neural networks (27). The effect of Hb on sympathetic adrenergic transmission was studied in isolated pig cerebral arteries. Hb blocked neuronal [$^3$H]NE uptake in a dose-dependent manner. The mechanism underlying the [$^3$H]NE uptake blockade involved the binding of NE to Hb rather than a specific blockade of the uptake pump. Hb did not influence the electrically stimulated, tetrodotoxin-sensitive [$^3$H]NE release from cerebral arteries. Altered neurogenic responses in the presence of Hb may be influenced by the affinity of NE for this protein (28). Using dog MCA *in vitro* relaxations were induced and the effects of oxyHb studied. Relaxation to papaverine was unaffected by oxyHb, the calcium ionophore A23187 ($10^{-7}$ M), or the stable $PGI_2$ analog TRK-100. The relaxation induced by nicotine of strips precontracted with $PGF_{2\alpha}$ was completely prevented by oxyHb. It was concluded that vasodilation mediated by vasodilator nerves was impaired in dog cerebral artery after exposure to oxyHb (29).

### E. Effect of Electrical Stimulation

Lende induced local constriction in epicerebral arteries by mechanical and electrical stimulation. Phentolamine, an adrenergic blocker, was found to be the most effective drug in preventing this type of VSP. It was effective in dilutions of 0.05%. The effect persisted for only 5–10 min, however (30). Transmural electrical stimulation dilated six of eight porcine cerebral arteries. The addition of $5 \times 10^{-6}$ M oxyHb constricted all these vessels as evidenced by increase perfusion pressure. OxyHb had no effect on neuronal [$^3$H]choline uptake in arteries. OxyHb also did not alter the release. It was concluded that oxyHb inhibited porcine neurogenic vasodilation which was not mediated by a direct action of ACh (31).

Transmural nerve stimulation by trains of 100 biphasic square-wave pulses of 0.3 msec that were 160 mA across the electrodes was applied to isolated dog cerebral arteries. This electrical stimulation relaxed MCAs (10/12), basilar artery (0/7), anterior cerebral arteries (2/3), and internal carotid artery (0/2). Tetrodotoxin abolished all responses to electrical stimulation suggesting that they were of neurogenic origin. Hemolysate from dog RBCs abolished the vasodilator responses to electrical stimulation and in some cases converted them into constrictor responses. Active vessel tone was induced by uridine-5′-triphosphate prior to the application of electrical stimulation. The vessels were still capable of relaxation after the exposure to tetrodotoxin (32). In a pig model CBF decreased 7 days after induced SAH. Continuous electrical stimulation of the trigeminal ganglion preformed in six animals with severely reduced CBF induced a remarkable cerebral vascular dilation and increase in CBF lasted over 3 hr. Electrical stimulation of the trigeminal ganglion produced a similar vasodilation in pigs in which no SAH was induced (33). Our impression of the overall evidence is that VSP is much more likely to be due to a direct effect on the vessel wall of local agonists than to result from a central mechanism.

## IV. Biogenic Amines

### A. Definitions

The biogenic amines include 5-HT, histamine, NE, and ACh (Fig. 6.2). The blood vessel nerve fibers can be classified as sympathetic, containing NE; parasympathetic [ACh, vasoactive intestinal polypeptide (VIP), and neuropeptide Y (NPY)]; and sensory [tachykinins, calcitonin gene-related peptide (CGRP), substance P (SP), and neurokinin A] (34). Amines, R-$NH_2$, are organic derivatives of $NH_3$ and are classified by the number of alkyl (R) (carbon and hydrogen-containing) groups bonded to the nitrogen (primary, secondary, or tertiary). Catecholamines have a 6-carbon ring.

### B. Catecholamines

Epi and NE are water-soluble amines derived from tyrosine via 3,4-dihydroxyphenylamine. An intermediate in this conversion is dopamine (Fig. 6.2). The metabolites of the catecholamines are for NE 3-methoxy-4-hydroxymandelate, for Epi 4-methoxy-4-hydroxyphenylglycol, for DA homovanillic acid, and for 5-HT 5-hydroxyindole-3-acetate. The molecular weight of 3,4-dihydroxyphenylamine is 153, that of Epi is 183, that of NE is 169, and that of 5-HT is 176. Hydroxytyramine is converted to NE by dopamine-β-hydroxylase and NE is in turn converted to Epi by *N*-methyl transferase. In plasma the concentration of Epi is 0.06 μg/liter and the concentration of NE is 0.3 μg/liter. Both compounds are metabolized via O-methylation by catechol *O*-methyl

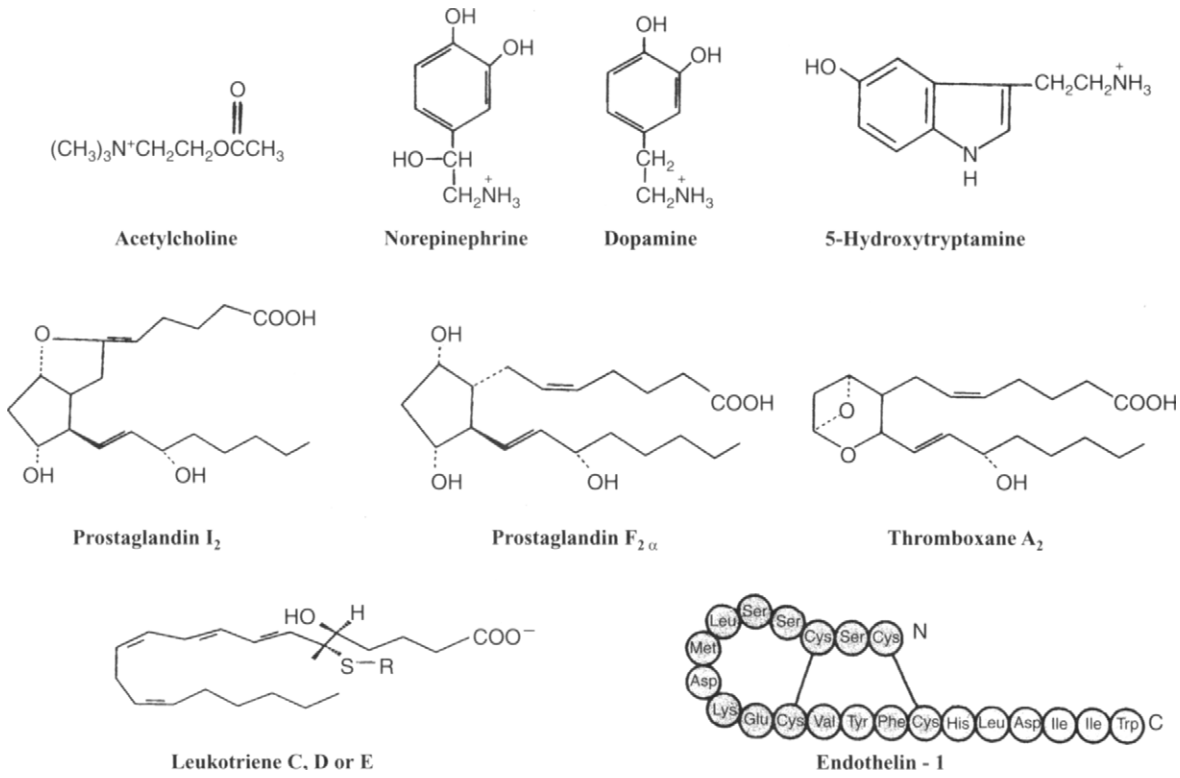


**FIGURE 6.2** Structure of potential agents involved in vasospasm.

transferase; oxidatitive deamination occurs via monoamine oxidase and conjugation (35).

Adrenergic receptors are divided into classes by relative sensitivity to different agonists and antagonists. α Receptors mediate vasoconstriction and are generally more sensitive to Epi than NE and unresponsive to isoprel. In contradistinction isoprel is the most potent for β receptors, which mediate vasorelaxation. α and β effects on different blood vessels vary with the location and type of the blood vessel. Some drugs can elicit contraction without a change in membrane potential and this is referred to as pharmacomechanical coupling. $\alpha_2$ Receptors may cause vasoconstriction by inhibiting adenylate cyclase and decreasing cAMP rather than increasing $[Ca^{2+}]i$ (35).

There are multiple catecholaminergic neurotransmitters with various origins and mechanisms of action. They have both neural and hormonal origins. In contrast, the dopaminergic system, which has a purely neural origin, involves a limited number of specific functions. NE and Epi are of peripheral, sympathetic, and central origin and play a role in many autonomic functions. DA is primarily located in three midbrain nuclei (36). NE, Epi, and sympathomimetic amines have a wide variety of actions on all types of muscle tissue. Differences in action depend in part on the distribution and density of α and β receptors on the responding tissues. As a general rule, α receptors on smooth muscle tend to cause vasconstriction, whereas β receptors trigger vasodilation. The responses to Epi resemble the stimulation of adrenergic nerves. Epi and the adrenergic nerve mediator NE differ in their proportion of α to β receptor activity. Epi increases the strength of ventricular contraction, increases heart rate, and causes a profound vasoconstriction in many vessels. Blood pressure, however, is seldom greatly elevated. This is in contradistinction to NE, which produces arterial hypertension. Epi does not have a significant constrictor action on cerebral arteries but can affect CBF and $O_2$ uptake without altering cerebral vascular resistance. Total peripheral resistance is not increased by Epi and is modestly increased by NE (37). NE is the chemical mediator liberated by postganglionic adrenergic nerves. The pharmacologically active form is the L form; the D isomer is much less active. NE constitutes 10–20% of the catecholamine content of human adrenal medulla. Intravenous infusion of 10 μg of NE/min elevates mean arterial blood pressure (MABP) and raises total peripheral resistance. The heart is usually slowed (37). Peripheral arterial constriction is readily induced by NE with an α-adrenoceptor-mediated contraction. Stimulation of β receptors usually leads to relaxation. There is significant adrenergic innervation of

cerebral blood vessels (38,39). The $\alpha_2$ subtype occurs in microvessels but the $\beta_2$ subtype is more frequent. The second messenger of the β-adrenergic receptor is cAMP. The different receptors have a heterogeneous distribution in the cerebral vasculature and in the vessel wall. The abluminal layer particularly of the media is richly endowed with contractile α-adrenergic receptors. The perivascular NE nerve endings tend to innervate capillaries rather than arterioles of penetrating arteries (36).

The main source of central NE neurons is the locus ceruleus, a small pigmented nucleus in the caudal pontine gray matter. It is the site of increased NE synthesis under acute stress, via enhanced activity of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis. The Epi which affects cerebral circulation originates mainly from the adrenal medulla. Immunohistochemical studies of phenylethanolamine *N*-methyltransferase have shown the existence of two groups of epi-synthesizing neurons in the medullary brain stem (36). Activation of the locus ceruleus induces blood flow changes of small amplitude only. These nuclei may be activated in a protective constricting effort during the stress of hypertension. Compared to DA, NE given iv, although producing similar blood pressure changes, causes only minimal change in CBF. In the rat, NE and Epi tend to decrease CBF in the anterior brain, whereas isoprenaline, a β-adrenergic agonist, moderately increases flow in the posterior brain. Enzymatic barriers significantly contribute to the exclusion of plasma catecholamines from the brain in normal circumstances. Stimulation of the dorsal medullary reticular formation induces CBF increases that are suppressed by adrenalectomy. The cerebral vascular effects of plasma catecholamines depend in part on a central mechanism situated in the reticular system that can sensitize the brain. Epi can elicit cerebral metabolic activation in concentrations that would normally be without effect (36). There is catecholaminergic innervation of brain microvessels. The fibers do not penetrate further than the Virchow–Robin space. Nerve fibers from the locus ceruleus closely relate to intraparenchymal blood vessels such as the capillaries in the hypothalamus. These nerves can be stained using fluorescent antibodies to dopamine β-hydroxylase (36). DA is present in sympathetic nerves as a precursor to NE and in perivascular mast cells of some species. It is a potent *in vitro* pial cerebral constrictor. Although DA does not cross the blood–brain barrier (BBB), its precursor L-3,4-dihydroxyphenylalanine and various DA receptor agonists or antagonist do cross it (36).

### C. Norepinephrine as a Potential Spasminogen

NE was suggested by early workers to be a possible spasmogen (40,41). Raynor *et al.* suggested 5-HT, as did Chow *et al.* and Allen *et al.* (42–45). The constriction produced by these catecholamines is generally short lived. Shigeno found elevated cisternal levels of NE in patients with VSP (Fig. 6.3) (46). The possibility of blood inducing denervation hypersensitivity to catecholamines was proposed by Fraser *et al.* (47) and Peerless and Kendall (48). The depletion of catecholamines in perivascular nerves was demonstrated by several workers (23,47–50) and increased sensitivity to NE and 5-HT was also shown (49, 51). Fraser and coworkers demonstrated that an abundant NE periarterial nerve plexus in the adventitia of major intracranial vessels was depleted of fluorescence by repeated induced VSP. Phenoxybenzamine was found to dilate both spastic and normal cerebral vessels and make them refractory to constriction to blood, 5-HT, or NE. Bilateral superior cervical ganglionectomy did not affect constriction (47). The effect of blood in depleting NE content of perivascular nerves was recently demonstrated by Lobato and colleagues. The uptake of [$^3$H]-NE by cerebral arteries was decreased to about 60% of control 3–7 days after SAH and did not normalize for 15 days (23).

Loss of catecholamine histofluorescence, increased sensitivity to NE, and changes in $\alpha_1$ receptor binding all supported the proposal that denervation hypersensitivity may play a role in chronic VSP. Electron microscopy post-SAH in experimental animals has demonstrated disintegration of both clear- and dense-core vesicles, fragmentation of varicosities, and axonal degeneration that is most pronounced 7 days post-SAH (52). Attempts at correlating the time course of VSP with the degree of innervation have been made (53). Cat basilar artery, exposed *in vivo*, constricted in a dose-dependent fashion to $2.75 \times 10^{-3}$ M oxyHb, NE, and $PGF_{2\alpha}$. α Blockers did not significantly alter the oxyHb-induced contraction. Adrenergic denervation by sympathectomy, reserpine, or 6-OH DA increased the constriction to NE but did not alter oxyHb-induced constriction. It appeared as though oxyHb did not act on α receptors and did not act via an adrenergic system (54).

Selective lesions of the medullary catecholamine nuclei have been shown to prevent experimental cerebral VSP in rat model (55). Also in a rat SAH model, reduced tyrosine hydroxylase-like immunoreactivity around cerebral arteries was found after experimental SAH but there was no alteration in the activity in the superior cervical ganglion or in the nerve bundles around the internal carotid artery outside the subarachnoid space. It was concluded that SAH impairs the initial step of catecholamine synthesis in the adrenergic nerve within the subarachnoid space only (56). Hb blocks neuronal [$^3$H]NE uptake into anterior cerebral arteries in a dose-dependent fashion post-SAH. The blockade involves binding of NE to Hb rather

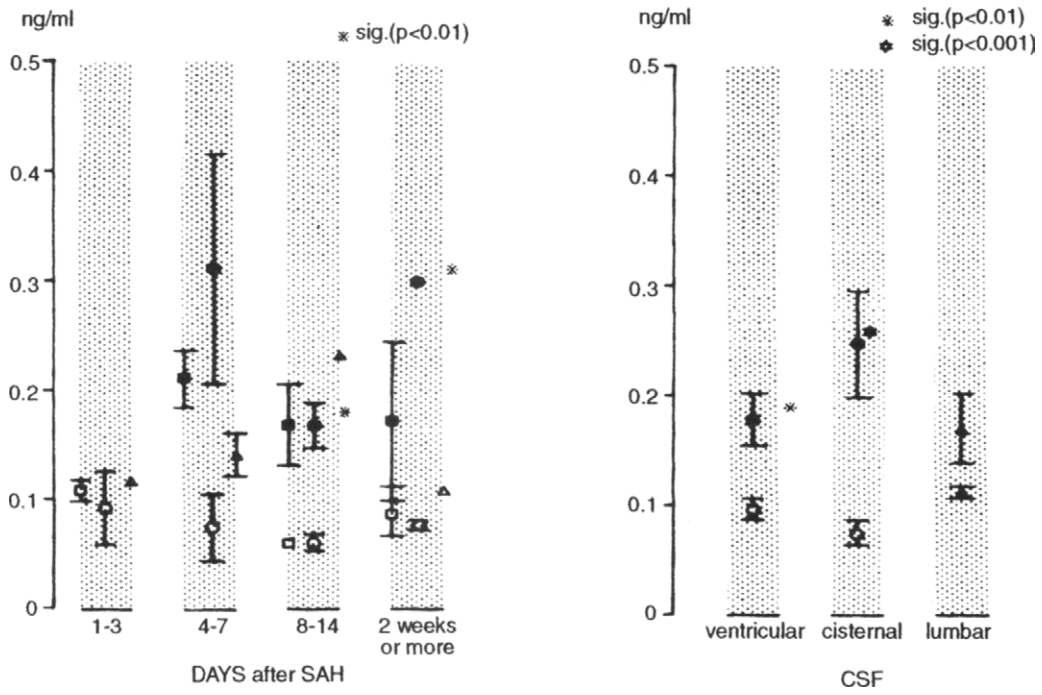


**FIGURE 6.3** Norepinephrine concentrations in the CSF. Squares, ventricular CSF; circles, cisternal CSF; triangles, lumbar CSF; white symbols, without vasospasm; black symbols, with spasm. (Left) Changes in relation to days after subarachnoid hemorrhage. (Right) Pooled values in relation to the site of CSF sampling [reproduced with permission from Shigeno, T. (1982). Norepinephrine in cerebrospinal fluid of patients with cerebral vasospasm. *J. Neurosurg.* **56,** 344–349].

than a specific blockade of the uptake pump. Although Hb blocked [$^3$H]NE uptake, it did not influence electrically stimulated, tetrodotoxin-sensitive [$^3$H]NE release from these arteries (28). Goats were subjected to SAH. Cerebral arteries were preloaded with [$^3$H]NE. When these arteries were subjected to electrical stimulation there was a reduced release of [$^3$H] post-SAH compared to controls. Microscopic studies showed a reduction in catecholamine fluorescence and signs of sympathetic degeneration in some perivascular axons (57).

Drugs that block α- and β-adrenergic or muscarinic receptors have little effect on VSP induced by whole blood (58) or oxyHb (29,54,59–63). When denervation is induced with 6-hydroxydopamine there is little difference in reactivity of the vessels on subsequent exposure to catecholamines, 5-HT, or whole blood (64). The contractility of vasospastic vessels from dogs did not differ from control preparations in the response to NE, 5-HT, histamine, or high $K^+$ (65). Phenoxybenzamine has not gained widespread acceptance as a means of alleviating VSP (44, 66–68). It appears unlikely that enhanced sensitivity of arteries is involved in VSP. Combinations of systemic vasoconstrictors with the general (including cerebral) vasodilators had intellectual appeal but were similarly disappointing in primate models and have not obtained clinical acceptance (69,70). *In vitro* experiments were performed to determine the contractile activity of adrenergic agents on canine arteries in six locations. Cumulative log-dose responses were obtained for phenylephrine, NE, Epi, and DOPA. For both the basilar artery and the internal carotid the response to these agents was much less than that of systemic arteries. Sodium nitroprusside (SNP) was 100-fold more effective in relaxing the basilar artery after it was precontracted by 5-HT when applied to the luminal rather than the adventitial surface. Allen suggested that simultaneous use of SNP intravenously in conjunction with an α-adrenergic agonist such as phenylephrine could be rational therapy for VSP (71). This work has not been confirmed in primates or humans, nor has it reached general clinical acceptance.

### D. Human Studies

Segmental VSP was demonstrated in a patient with pheochromocytoma (72). Isolated human basilar artery was shown to contract to 5-HT, tryptamine, NE, and histamine (59). Further studies showed that human cerebral arteries contracted to 5-HT, histamine, NE, methoxamine, and isoproterenol in a dose-dependent fashion. ACh and carbachol ($10^{-5}$–$10^{-9}$ M) caused a negligible

response. Tyramine ($10^{-4}$ M) caused tonic contraction. Nicotine ($10^{-4}$ M) and electrical transmural stimulation produced phasic contractions of arteries which were antagonized by adrenergic blocking agents. High concentrations of adenosine caused a minimal relaxation. Papaverine and nitroglycerine caused marked relaxation. The contractile efficacy of 5-HT was similar to that of NE in the anterior circulation but greater in the basilar artery (73). NE content of human arteries was analyzed. Following SAH the content was only 5% of the control group. Studies using a selective α antagonist showed that in control arteries there were two classes of binding sites, whereas post-SAH there was only one. Sympathetic denervation and subsequent alteration in $\alpha_2$-adrenergic receptors occurred post-SAH (74).

In 25 patients CSF was analyzed post-SAH by high-performance liquid chromatography (HPLC). Measured catecholamines or indolealkylamines and their metabolites (in parentheses) were as follows: Epi (dihydroxyphenylacetic acid), NE (3′-methoxy-4-hydroxyphenylglycol), DA (homovanillic acid), and 5-HT (5-hydroxyindolacetic acid). Activation of CSF NE and 5-HT metabolism was noted in the patients with VSP during days 4–19. The 5-HT values were below the detectable levels in all cases. The 5-HIAA was high but not significantly so in the second sampling. The elevated ratio of MHPG to NE in the VSP group in the second sampling seemed to indicate more activation of dopamine β-hydroxylase, monoamine oxidase, and catechol-*O*-methyltransferase from days 4 to 19 in the VSP group (75).

Phenoxybenzamine was injected into the carotid artery of patients and angiography was performed. Three patients were considered to show rapid improvement in their neurologic findings. Twenty other patients were treated prophylactically. The immediate improvement may have been coincidental and this therapy has not found general favor (76). Handa and coinvestigators also injected phenoxybenzamine into the carotid artery in doses of 20–40 mg in 3 patients with ruptured aneurysms and severe VSP. No reversal of VSP was noted (77). Adrenergic blockade has never been shown to successfully prevent chronic VSP (78).

### E. Serotonin (5-Hydroxytryptamine)

5-HT is released from the destruction of platelets (79). The molecular weight is 176 Da. 5-HT injected into the subarachnoid space has been shown to cause spasm of cerebral vessels (42,43). Thirty-four monkeys were subjected to injections into the subarachnoid space of CSF, blood, and 5-HT. When 5-HT was injected at concentrations of $5 \times 10^{-6}$ M, CBF and arterial vessel caliber were unaffected. However, *in vitro* 5-HT concentrations of $5 \times 10^{-6}$ M produced maximal contraction of cerebral arteries. The VSP induced by these high concentrations of 5-HT was of shorter duration than that obtained with blood (80). In a study in which the antibiotic kanamycin was employed in monkeys the levels of 5-HT decreased. This was associated with the absence of VSP following SAH (81). Based on such animal experiments a human trial was undertaken by Zervas and colleagues (82). It was suggested that ischemic signs were more common in the control group than in those receiving reserpine and kanamycin, which were designed to lower 5-HT levels. Despite the suggestive evidence this therapy has not gained widespread acceptance.

Cerebral arteries are substantially more sensitive to 5-HT than peripheral arteries (18,83). When exposed to SAH they become more sensitive to the constrictor effects of 5-HT as evidenced by both animal experiments and human autopsy samples (23,84,85). However, 5-HT does not seem to be substantially elevated in CSF post-SAH (86). Chronic VSP was insensitive to any dilating effect of the 5-HT receptor antagonist cyproheptadine. The acute constriction of cerebral arteries in dogs resulting from intraventricular injection of 5-HT was blocked by phenoxybenzamine, as was the constriction induced by blood (44). In 1978, Boullin *et al.* found that the 5-HT antagonist (BW501C67) reversed the constriction produced by the introduction of 5-HT into CSF of baboons but not the constriction resulting from blood (87). Blood-induced contractions in isolated vessels have a rapid phase antagonized by methysergide and a more protracted phase which was insensitive (88). In canine basilar arteries continued exposure to 5-HT produced almost complete autoinhibition which occurred within 30 min (89). While a role for 5-HT in the acute phase of VSP is possible, its ongoing contribution to chronic VSP is highly unlikely (1). In a study of CSF concentrations of 5-HT post-SAH the 5-HT concentrations did not differ from those in control patients (86). In a monkey SAH experiment anti-5-HT medications were given which were effective in reducing 5-HT levels, but despite reductions of 5-HT to 19 and 26% of control values, treated animals experienced no difference in the frequency or severity of VSP (90). Using a perfusion canine basilar artery model it was shown that pressure increases resulting from 5-HT infusion could be attenuated with the calcium blocker nimodipine. The 5-HT response was enhanced in vessels treated with blood application (91). The uptake of [$^3$H] 5-HT was markedly diminished following SAH in rabbit basilar arteries (62).

Platelet factors responsible for the release of relaxing factors from the vascular endothelium include adenine nucleotides, ADP, and ATP, which activate $P_{2y}$-purinergic receptors on the endothelial cells, and 5-HT,

which stimulates 5-HT1-like serotonergic receptors. The 5-HT response is mediated by a pertussis toxin-sensitive mechanism (92). Extracted $^3$H-amines from rabbit basilar arteries following incubation with [$^3$H]5-HT showed reductions after denervation, treatment with pargyline, and SAH. It was concluded that the neuronal uptake of 5-HT was impaired post-SAH, although monoamine oxidase activity is relatively preserved (93). The contractile responses of rat basilar artery were studied after SAH *in vitro* incubation with 5-HT and during electrical field stimulation of perivascular nerve following experimental SAH. The electrical stimulation caused a tetrodotoxin and ketanserin-blockable contractile response. There was no such response in vessels from rats treated with 6-hydroxydopamine or after blockade of 5-HT uptake. After SAH a pronounced network of 5-HT immunoreactive nerve fibers was demonstrated in the vessel wall. In vessels from control rats, no 5-HT fibers were seen. The perivascular sympathetic nerves were shown to have capacity to take up 5-HT both *in vitro* and during the early phase of SAH in this model (94). In rats the subependymal ventricular surface is richly innervated with 5-HT immunoreactive nerves. There is a marked reduction in the 5-HT immunostaining observed as early as 6 hr post-SAH which lasts for between 14 and 28 days (95). The contractile responses to 5-HT were studied *in vitro* in vessels removed from goats with or without SAH and with or without intact endothelium. The contractile responses were increased by removal of the endothelium and the addition of competitive inhibitors of NO synthesis. 5-HT contracted arteries with or without endothelium. SAH increased the contractions. An inhibitor of NO synthesis enhanced the contractile response to 5-HT after SAH. VSP post-SAH was attributed to a hyperreactive response to 5-HT resulting from the absence of a modulatory role of endothelial NO (96).

### F. Acetylcholine

Parasympathomimetic agents such as ACh cause excitatory inhibition of autonomic effector cells innervated by postganglionic parasympathetic or cholinergic nerve impulses. ACh is rapidly hydrolyzed by both acetyl cholinesterase and nonspecific cholinesterase. The muscarinic or parasympathomimetic actions of ACh are practically equivalent to the effects of postganglionic parasympathetic stimulation. These actions are blocked by atropine. ACh produces dilatation of virtually all vascular beds but there are little data on a physiological role with respect to the cerebral circulation (34). The calcium ionophore A23187 is 10–30 times more potent than ACh in producing an endothelial-dependent relaxation of rabbit aorta. This drug resembles ACh except that it is not inhibited by quinacrine. Relaxations to ACh, A23187, ATP, SP, and bradykinin (BK) are all strictly dependent on the presence of endothelial cells and are not blocked by cyclooxygenase inhibitors (97). Endothelial-derived relaxant factor (EDRF) was studied from a variety of arteries in rabbits and dogs. Dose response using isometric tension recording differed according to the vasodilator employed, the site, and the species. It was concluded that ATP has a more important role than ACh in the regulation of vascular tone of the major cerebral arteries in rabbit and dogs (98). Endothelial-derived relaxation to ACh, ATP, and thrombin varies between species and location of arteries (98). The effect of drugs not only varies with the species and concentration used but also varies depending on whether they are applied extraluminally or intraluminally. Extraluminal ACh was 1/44 potent as intraluminal ACh ($10^{-7}$ M). Extraluminal application of oxyHb reduced the relaxation to ACh by one-half when ACh was applied intraluminally. Both ACh and oxyHb appeared to be translocated to the endothelium when applied extraluminally (99). ACh-induced relaxations of 5-HT-induced contractions of rabbit basilar arteries were more significantly impaired by intraluminal than extraluminal application of Hb. Hb inhibited both ACh- and ATP-induced relaxations more significantly with intraluminal than extraluminal application. Immunohistochemical studies revealed Hb in the outer layers of the smooth muscle and in the adventitia when $10^{-5}$ M Hb was applied extraluminally for only 5 min. When Hb was applied intraluminally, Hb was observed on the surface of endothelial cells immediately (100). Rabbit carotid arteries were studied using isometric tension recording methods. In vessels obtained from animals 7 days post-SAH, ACh-induced relaxation was suppressed and the degree of relaxation in this group was 50% of the initial contractile tone in response to $10^{-5}$ M ACh. These relaxant responses did not return to control values in carotid arteries obtained from animals treated with deferoxamine and subjected to sympathectomy. In these preparations the contractile responses to ET-1 were significantly enhanced after SAH (101).

Pharmacological studies were performed on monkey MCA which had been surrounded by blood clot for 7 days, and these reactions were compared to those of the contralateral arteries. Relaxations induced by histamine and A23187 in MCA from the clot side were substantially reduced. The small component of ACh relaxation was also abolished. Endothelium-independent relaxation induced by GTN occurred in arteries from both the control and clot sides. Constrictions induced by KCl and $PGF_{2\alpha}$ were reduced on the clot side of the MCA. The nonclot control arteries precontracted to $PGF_{2\alpha}$ were relaxed by histamine and A23187. These relaxations

were abolished by removal of the endothelium. ACh-induced relaxations were greater in the proximal internal carotid artery than in the MCA of the nonclot side (102).

ACh induced endothelium-dependent and dose-dependent relaxation of normal human cerebral arteries precontracted by $10^{-5}$ M 5-HT. No ACh-induced relaxation was observed after SAH. Muscarinic cholinergic receptor sites on cerebral arteries were sought using radio ligand-binding assays. A marked decrease of endothelial receptor density was observed after SAH (103).

### G. Histamine

Although most frequent in the leptomeninges, mast cells also occur in the hypothalamus and cortex. Depending on the species the cells may also contain 5-HT or dopamine (104). Blood basophils are a source of histamine (105). Histamine is present in non-mast cells found within smooth muscle layers of cerebral vessels (106). Histamine in high doses produces a cerebral vasoconstriction which does not involve specific histamine receptors. This response can be blocked by mepyramine. Histamine is able to dilate isolated brain vessels (18). In extracranial arteries histamine produces remarkable vasoconstriction which is much less marked in intracranial arteries. An increase in the mast cell population of cerebral arterial walls after SAH has been reported (107).

## V. Neuropeptide Transmitters

Neuropeptides are usually derived from larger proteins which are cleaved by proteolysis. They are synthesized in neuronal cell bodies and transported down the axon. Potentially vasoactive peptides found in the brain include SP, NPY, neurotensin, angiotensins I and II, and VIP. There was an explosive increase in knowledge of neuropeptide transmitters in the 1980s. Many neuropeptides belonging to structurally related superfamilies have been identified and their distributions in the CSF characterized. It has been suggested that there is a resting dynamic balance between the endothelium-derived vasodilator tone and the sympathetic vasoconstrictor tone which can be modified under pathologic conditions. Perivascular sympathetic nerves cotransmit NE, ATP, and NPY. Parasympathetic nerves contain ACh, VIP, peptide histidine-methionine, and NPY. "Sensory motor" nerves contain SP, CGRP, tachykinin, and ATP (108). The two vasodilator systems supplying the cerebral circulation are parasympathetic and sensory. The parasympathetic system stores VIP, peptide histidine isoleucine, ACh, and NPY. The sensory system mainly originating in the trigeminal ganglion stores SP, neurokinin A, and CGRP (109). NPY causes concentration-dependent, potent contractions of cerebral vessels both with and without endothelium. In human pial vessels the NPY constriction was not affected by changes in $[Ca^{2+}]e$. NPY-evoked contraction was effectively antagonized by the $Ca^{2+}$ antagonist nifedipine. CGRP and SP caused relaxation of precontracted cerebral arteries with intact endothelium. The potent dilatory effect of CGRP was not affected by removal of endothelium. The potency of NPY was greater than that of NE. Sensory nerve fibers of trigeminal origin which contain CGRP and SP are present at the junction of the media and adventitia. The peptides presumably must diffuse through the media and elastica to reach the endothelium in order to produce their relaxation effects. The potent endothelium-independent vasoconstrictor effects of NPY on cerebral arteries are sensitive to $Ca^{2+}$ antagonists but are not influenced by changes in $[Ca^{2+}]e$ concentration, which is the opposite of NE- and $K^+$-induced vasoconstriction (110). CGRP is a neurotransmitter candidate together with the tachykinins in sensory fibers of the cerebral vasculature (111).

### A. Tension Experiments

#### *1. CGRP*

In isometric tension experiments of rat basilar and common carotid arteries with or without SAH exposure, it was found that CGRP relaxed the basilar but not the common carotid after the vessels were precontracted with $10^{-5}$ M 5-HT (112). Similar tension measurements were performed *in vitro* on strips of basilar arteries obtained from rabbits subjected to artificial SAH as well as control animals. Vessels preconstricted with 5-HT showed an attenuated vasorelaxant response to CGRP. The effect of forskolin, which activates adenylate cyclase directly, was slightly enhanced after SAH. GTN, which activates soluble guanylate cyclase directly, was not affected by SAH. The capacity of CGRP to raise cAMP levels was not affected by SAH. The resting levels of cAMP and the forskolin-induced elevations of cAMP were not different between SAH and control animals. In the rabbit model of SAH, CGRP-induced vasodilation is attenuated during VSP, even though the vasodilatory responses to cAMP and cGMP are intact (113). Tension measurements were performed on rings of rabbit basilar artery *in vitro*. The arteries had not been exposed to SAH. CGRP reversed constriction induced by phorbol 12,13-dibutyrate (PDB; 2 n*M*) and histamine (3 μ*M*) in a dose-dependent fashion. CGRP (100 n*M*) could not relax arteries constricted by the maximum effective dose of PDB. Opening of the ATP-sensitive $K^+$ channels did not seem to be the mechanism by which CGRP mediated dilation since inhibitors of

these channels—glibenclamide and tolbutamide—did not block the CGRP-induced relaxation (114).

#### 2. NPY

Dural nerves in rats showed substantial reductions in SP-like immunostaining after SAH. NPY-like fiber innervation was also markedly reduced after SAH; although immunostaining intensity increased, it had not returned to control levels by day 6. The 5-HT content of dural mast cells also markedly decreased at 6 and 24 hr and returned to control levels at 48 hr. CGRP immunostaining was unchanged (115). NPY produced contraction in isolated canine basilar arteries in a concentration-dependent manner which was independent of endothelium. The NPY-induced vasoconstriction was strongly potentiated by an increase in the $K^+$ concentration in the medium by up to 20 m*M* or by pretreatment with a $K^+$ channel blocker and hemolysate containing oxyHb. NPY augmented the contractile response to $PGF_{2\alpha}$, NE, and histamine but not to 5-HT. The contractile response to NPY was attenuated by a $Ca^{2+}$ antagonist (116).

#### 3. VIP

VIP is a 28-amino acid polypeptide. It is found in perivascular nerve fibers of intracerebral origin which also contain the polypeptide precursor to VIP (117). VIP ($10^{-6}$–$10^{-11}$ M) evoked dose-dependent relaxation of rabbit basilar arteries. VIP-induced relaxation was suppressed significantly post-SAH. The cAMP content was significantly higher in basilar arteries 2 days post-SAH. In normal arteries the cAMP content was increased by VIP, whereas the increase was less in arteries evaluated 2 days post-SAH (118).

#### 4. SP

Whereas the relaxations from A23187 and vasopressin were abolished by removal of endothelium, that from SP was not. The function of vasopressin receptors in endothelial cells was markedly different than that in basilar and MCAs in the dog (119).

### B. Effects of SAH

#### 1. CGRP

In the single SAH dog model, immunohistochemical studies showed a supression of CGRP-like immunoreactivity in cerebral vascular nerve fibers beginning on the third day post-SAH and this was most marked on days 7–14. Recovery to normal levels occurred by day 42 post-SAH (120). Dog basilar arteries from controls and post-SAH were examined *in vitro* during exposure to CGRP and VIP. In the two-hemorrhage model on days 7 and 14 there was a decrease in induced relaxation to these peptides. The vasorelaxant effect, however, was significantly enhanced on days 28 and 42 post-SAH (121). Two days post-SAH in the rat there was a significant decrease in CGRP-like immunoreactivity. This reduction in neurotransmitter content, which was assumed to be due to denervation, was not associated with a decreased sensitivity of the basilar artery to CGRP when the vessels were studied *in vitro* (122). Following the induction of VSP in rabbits post-SAH, some animals were infused with 100 mg/kg/min of CGRP for 2 hr immediately prior to perfusion–fixation sacrifice. Basilar artery diameters established by morphometry were significantly greater in the CGRP group than in control groups. Intravenous but not intracarotid CGRP caused significant hypotension (123).

#### 2. SP

SAH was produced in dogs and cerebral vessels were examined by immunohistochemistry. Many VIP, SP, and NPY perivascular fibers were present in control and sham-operated animals, but these were reduced to up to 40% of control values within 24 hr post-SAH and remained <60% of control during the first week post-SAH. VIP was most prominently reduced. Evidence of recovery was seen after 3 weeks. The immunoreactivity of VIP and SP in the sphenopalantine and trigeminal ganglia and that of NPY in the superior cervical ganglia were unchanged (124). Sensory projections from the trigeminal ganglion innervate cerebral blood vessels and use preprotachykinin gene products, SP, and neurokinin A as putative neurotransmitters conveying pain. Marked reductions in SP levels were observed in basilar artery segments within 4 hr after SAH. The depression persisted for 2 days and recovered by 7 days. The decreases in SP peptide levels were accompanied by an increase in trigeminal ganglionic content of preprotachykinin mRNA that codes for the peptide (125).

#### 3. NPY

Following single blood injection into the CSF of rats there was an increase in cerebrovascular sympathetic nerve content of 5-HT arising from the uptake from subarachnoid clot. NPY decreased from 3 to 48 hr post-SAH. 5-HT and NPY content were normal 3 days post-SAH (126). In the primate SAH model, blood and CSF were sampled for NPY before SAH and 7 days after clot application. Later sampling was also performed on days 12 and 28. SAH did not evoke changes in CSF for plasma levels of NPY. NPY levels did not change with the development of VSP or following its resolution, and NPY levels were substantially higher in CSF than in arterial plasma. NPY is a 36-amino acid peptide (127).

### C. Intracisternal Injections

#### *1. CGRP AND SP*

In dogs after both single and double injection of blood the administration of $10^{-10}$ M/kg of CGRP intracisternally reversed the VSP and the effect continued for a few hours after administration. In the two-hemorrhage model, on day 7 intracisternal administration of $10^{-11}$ to $2 \times 10^{-10}$ M/kg dilated the arteries in a dose-dependent manner (128). CGRP and VIP were able to relax the spastic basilar artery of dogs after single or double SAH injections. The *in vitro* effect was depressed on days 7 and 14 in the double-injection model but was significantly enhanced on days 28 and 42, subsequently returning to control levels on day 63 (121). Following the injection of blood or washed RBCs there was a suppression of CGRP and VIP immunoreactivity, whereas the injection of platelet-rich or platelet-poor plasma had no effect (129). CGRP is expressed and secreted a few minutes after injection of blood into the cisterna magna of rabbits. The marked increase in CGRP immunoreactivity in perivascular nerves observed after SAH is due to compensatory secretion of this peptide (130). The basilar arteries of rabbits subjected to SAH dilated to 117% of their pre-SAH levels following intrathecal administration of $10 \times 10^{-10}$ M/kg CGRP and the dilatory effect lasted for up to 6 hr following treatment. VIP injections also dilated the arteries to 115% of pre-SAH levels, although the duration of effect was less than 3 hr. Intrathecal administration of CGRP or VIP did not produce any apparent adverse effect (131). Also in a rabbit SAH model, intrathecal administration of CGRP 3 days post-SAH at $10^{-10}$ M/kg CGRP dilated the basilar artery 217%, which was significantly more than the 67% in the vehicle group (132). In rats following an injection of SP into the cisternal magna there was an acute opening of the blood–arterial wall barrier to horseradish peroxidase (133).

SAH was induced in squirrel monkeys. Gamma globulins against SP or CGRP were injected into the cisterna magna prior to and for 5 days following the SAH. Five of nine animals treated with CGRP anti-gamma globulin died from respiratory failure. SP anti-gamma globulin had no effect on baseline arterial diameter, while CGRP anti-gamma globulin significantly reduced arterial diameter. SP anti-gamma globulin prevented acute VSP and significantly reduced late VSP. It also ameliorated the reduction in CBF noted in control SAH animals (134).

### D. Human Studies

In 10 healthy male volunteers receiving a 3-hr iv infusion of 0.6 μg/min of α-CGRP, CBF and MCA transcranial Doppler ultrasonography (TCD) velocities were unaffected. Diastolic blood pressure fell significantly. In rabbits post-SAH TCD velocities fell significantly in the basilar artery following CGRP infusion (135). Infusions of CGRP were given to 15 patients with neurologic deficits following aneurysm surgery for SAH. In 9 patients the Glasgow Coma Scale (GCS) improved with no apparent adverse effects. After a placebo infusion only 2 of the 15 patients showed improvement. Patients were enrolled if they had postoperative deficit defined as impairment of at least one point on a GCS modified to record the worst arm motor response. Patients received CGRP or placebo in random order 24 hr apart. The infusion delivered 0.035 μg/min CGRP and was doubled every 10 min until there was a clinical response or a maximum dose of 1.15 μg/min was reached at 1 hr. In the absence of deterioration the maximum infusion rate was continued for another 20 min. Patients were studied for 20 min prior to infusion and during the 60–80 min of infusion and for 60 min afterwards. Four patients improved on CGRP but not on placebo and 1 showed no change on placebo so that all 4 patients who showed a treatment preference favored CGRP. Improvements consisted of such changes as improved eye opening, motor responses, or verbal responses. The duration of neurologic improvement ranged between 15 and 50 min. Facial flushing occurred in 60% of patients, 7 of whom showed neurologic improvement. Two patients improved without facial flushing. Eight of the 9 patients who improved with CGRP had tachycardia and hypotension, and 7 or these showed facial flushing. These changes were not observed with infusion of placebo. Short-term infusions of CGRP in volunteers were known to increase cardiac output and internal carotid artery (ICA) blood flow. While the most significant changes in blood pressure were noted in volunteers, there were significant changes in both systolic blood pressure and diastolic blood pressure and pulse rate in the patients. Volunteer studies had shown that although systemic hypotension could be induced by CGRP, ICA blood flow was still increased (136). In a single case report, infusion of 1.15 μg/min CGRP was associated with a significant decrease in peak and mean TCD velocities in the symptomatic MCA. There was a moderate decrease in MABP and an increase in heart rate. No neurologic improvement was observed (137). Human cerebral vessels were shown to contain CGRP-like immunoreactivity. The level was significantly lower in arteries removed from patients who had died from SAH compared to those who died from coronary infarction (138). Following surgery for aneurysms, 12 SAH patients who had an ischemia-induced reduction of 2 or more points in GCS were randomly assigned to infusion of 0.6 μg/min CGRP for 4 hr and then up to a maximum of 10 days and 55 patients received standard best management.

Three-month outcomes were good in 66% of CGRP patients and 60% of controls. The relative risk of a poor outcome in CGRP-treated patients was 0.88 [95% confidence interval (CI), 0.60–1.28]. Hypotension was commonly induced by CGRP infusion. Sixty-six percent of the CGRP group did not complete treatment because of adverse events or lack of improvement at 4 hr or later. Because only one-third of the patients completed the treatment and due to the fact that there was a wide confidence interval for the risk of a poor outcome, it was concluded that a clinical useful benefit could not be ruled out (139). CGRP, SP, and VIP were studied in cerebral venous outflow and CSF of 32 acute SAH patients. TCDs were studied. The highest CGRP-like immunoreactivity levels were found in patients with VSP as judged by TCD velocity index values. CSF from 14 patients was analyzed and showed lower VIP levels following SAH than in control patients. CGRP was present in the SAH CSF but not in the CSF of controls. Patients with the most marked VSP showed increased levels of CGRP and NPY. It was assumed that these dilatory peptides were part of a dynamic homeostatic reflex aimed at counterbalancing the vasoconstriction due to SAH (140). Five patients post-SAH received eight infusions of CGRP. There was an apparent dilation of constricted MCA as judged by TCD. There was no measurable change in the hemodynamic index on the contralateral unaffected side. No significant changes were observed in TCD pulsatility index, blood pressure, or consciousness during the infusion of the peptide (141).

### E. Bradykinin

BK is a potent stimulus for release of EDRF factors. Perfused canine basilar arteries dilated in response to BK, SP, and vasopressin. At higher doses vasoconstriction followed the initial vasodilation. After the endothelium was removed the dilatations were attenuated and vasoconstriction was enhanced. The vasoconstriction was inhibited by inhibitors of thromboxane synthesis (142). Angiotensin-converting enzyme (ACE) inhibitors potentiate EDRF release by BK, augment local production, and inhibit the breakdown of BK into inactive peptides by ACE. BK, through activation of $B_2$-kinin receptors on endothelial cells, causes the release of EDHF and/or NO with resultant relaxation (143). Vessels from dogs having single induced SAH were contracted with $PGF_{2\alpha}$ and then treated with increasing doses of the endothelium-dependent dilators ATP and BK. Animals with angiographically proven severe VSP showed a reduced or absent dilator response to these agents or had the response converted to a contraction. The results suggested a correlation between the impairment of endothelium-dependent vasorelaxation and the existence of VSP (144). In some smooth muscle preparations NO or cGMP can activate BK receptors, and $Ca^{2+}$-activated $K^+$ channels which result in hyperpolarization from $K^+$ influx (145). Forty-eight hours after the induction of SAH in rats the cerebral arteries were studied *in vitro*. BK (endothelial-dependent) relaxation was impaired on the SAH side only, whereas SNP (endothelium-independent relaxation) was depressed bilaterally, although to a lesser extent. Both endothelium and smooth muscle dysfunction may contribute to VSP post-SAH (146).

## VI. Eicosanoids

### A. Biochemistry

Eicosanoids are produced by the enzymatic metabolism of arachidonic acid (AA) and include prostaglandins (PGs), thromboxanes, and leukotrienes (147). Prostaglandins and thromboxanes are derivatives of 20-carbon, monocarboxylic acids (Fig. 6.2). Unesterfied AA is metabolized through the cyclooxygenase pathway leading to prostaglandins and thromboxanes (Fig. 6.4). PGs contract smooth muscle. They are produced by nearly all cells except RBCs, and they are released immediately upon synthesis. There are three major classes of PG; the A, E, and F. Since AA and many of its metabolites contain 20 carbon atoms, they are referred to as eicosanoids (icos = 20). AA is derived from membrane phospholipids by the hydrolase phospholipase $A_2$. In its transformation to various PGs, AA is cyclized and takes up $O_2$, and is catalyzed by PG synthase. Cyclooxygenase catalyzes the production of $PGG_2$ which is converted to PGH2 by glutathione-dependent peroxidase. PG synthase occurs in platelets and endothelium as well as other tissues. The enzyme thromboxane A synthase replaces the cyclopentane ring of $PGG_2$ and $PGH_2$ by a six-membered oxygen-containing ring which makes it highly active. The name derives from its thrombogenic capacity. $PGH_2$ is converted into the short-lived thromboxane $A_2$ (half-life in aqueous solutions $<1$ min), which then converts into the stable thromboxane $B_2$. Both steroids and nonsteroidal anti-inflammatory drugs such as aspirin block PG synthesis. Leucotrienes are substituted derivatives of AA without an internal ring, and R is variable.

### B. Prostaglandins

The cerebral blood vessels synthesize both prostaglandin endoperoxide ($PGH_2$) and prostacyclin ($PGI_2$) (148). Elevations of different prostaglandins have been reported in CSF post-SAH (Fig. 6.5) (149–151) Cerebral blood vessels *in vitro* are constricted by prostaglandins $F_{2\alpha}$ and

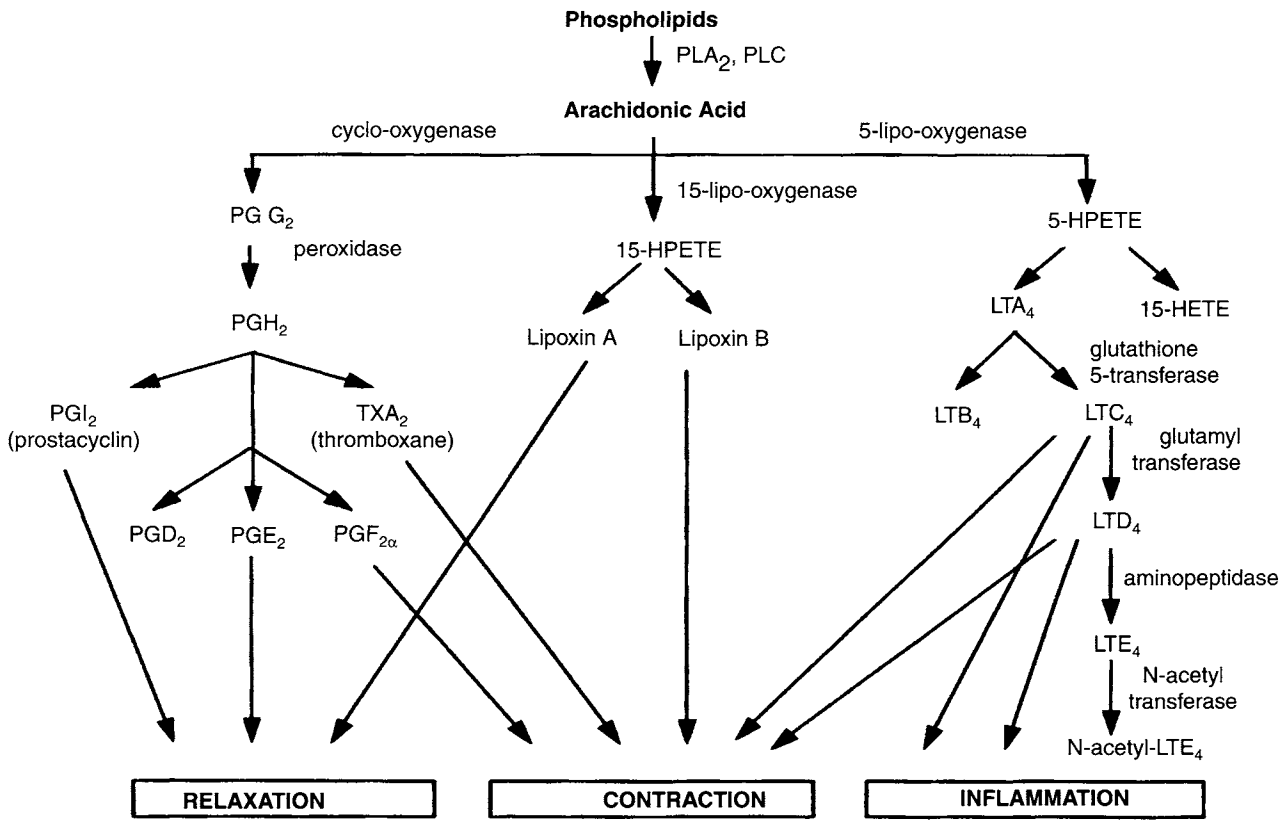


**FIGURE 6.4** Diagram of metabolic pathway of arachidonic acid production of eicosanoids (prostaglandin, thromboxanes, and leukotrienes). $PLA_2$, phospholipase $A_2$; PLC, phospholipase C; PG, prostaglandins; HPETE, hydroperoxyeicosatetranoic acid; LT, leukotriene [reproduced with permission from Weir, B., Stoodley, M., and Macdonald, R. L. (1999). Etiology of cerebral vasospasm. *Acta Neurochir.* **72,** 27–46. Copyright © Springer-Verlag GmbH & Co.].

$E_2$ (69), A1 and B1 (152), and B2 (1). Prostaglandin $F_{2\alpha}$ has a MW of 354 kDa. There is general agreement that prostacyclin ($PGI_2$) is a powerful vasodilator of cerebral arteries (Fig. 6.6) (153–155). It is possible that a reduction in prostacylcin production might result in an imbalance in favor of constriction (156). There is some experimental support for this conclusion (157,158). However, prostaglandin synthetase inhibitors have provided equivocal results. Sudoxicam has a marked inhibitory effect on the development of VSP after intracisternal injection of blood in the dog; meclofenamate has a similar effect but not aspirin (148). Indomethacin seemed only slightly effective in blocking contractions induced in isolated basilar arteries by whole blood (88), whereas *in vivo* indomethacin potentiated constriction (159). Inhibitors of thromboxane synthetase have been claimed to ameliorate VSP in animal models and patients (160,161). Prostaglandin synthesis inhibitors are not very effective in reversing VSP, but these agents have multiple actions. It is possible that some of these inhibitors inhibit both constrictor and dilator pathways (1). Blood vessels can make a variety of eicosanoids either from endogenous substrates or from endogenously administered arachidonic acid (162–164). In VSP the amount of vasorelaxant prostacyclin, manufactured mostly by the endothelium, is reduced (165–167). The amount of vasoconstrictor component thromboxane $A_2$, low under normal conditions, also remained low in spastic vessels (166).

### C. Thromboxanes

Cerebral blood vessels are constricted by thromboxane $A_2$ and its carbocyclic analog (156,168), thromboxane $B_2$ (155), and various other related compounds including leukotriene D4 (169), linoleic and arachidonic acids, and similar compounds (157,170,171). *In vitro* studies of canine basilar artery rings showed endothelium-dependent contractions to A23187, AA, and ACh could be blocked by a thromboxane synthetase inhibitor. Presumably, the vasoconstrictors were produced in the endothelial cells via the cyclooxygenase pathway (172).

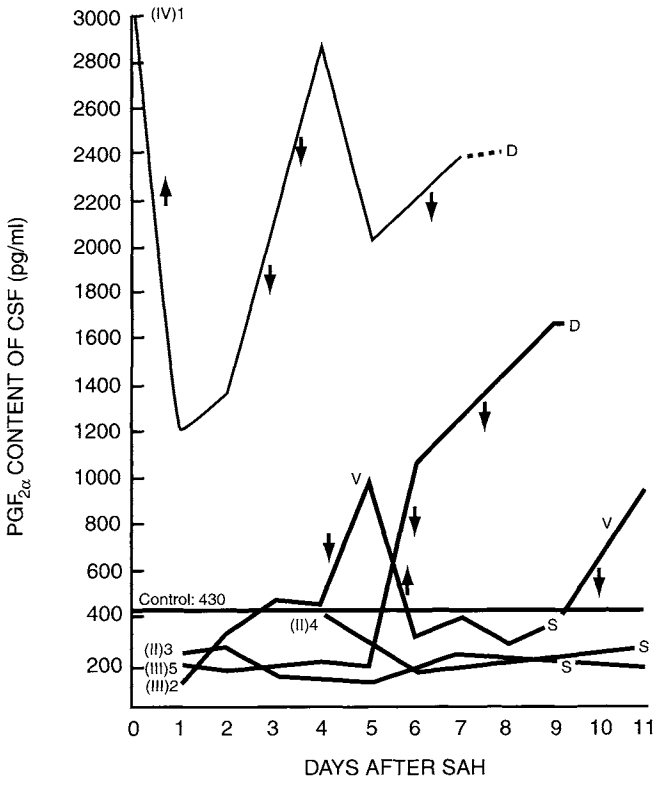


**FIGURE 6.5** Changes in prostaglandin $F_{2}\alpha$ concentration in lumbar CSF after SAH. Numerals 1–5 represent values for corresponding patients. SAH occurred on day 0. Control: baseline concentrations from three healthy volunteers, three patients with incidental aneurysms, and three asymptomatic SAH patients 9 months after surgery. S, surgery; D, death secondary to VSP; V, onset of right hemiplegia and aphasia due to VSP. Arrows show direction of changes in level of consciousness associated with fluctuations in severity of clinical vasospasm. Neurologic grade on admission in indicated by Roman numerals in parentheses [reproduced with permission from Chehrazi, B. B., Giri, S., and Joy, R. M. (1989). Prostaglandins and vasoactive amines in cerebral vasospasm after aneurysmal subarachnoid hemorrhage. *Stroke* **20,** 217–224].

### D. Leukotrienes

Leukotrienes are products of lipoxygenase rather than cyclooxygenase and their activity is not attenuated by the inhibition of cyclooxygenase. Cerebral arteries can manufacture small amounts of leukotriene $C_4$ and/or $D_4$ (173) but the amounts produced are low and only modestly elevated in experimental VSP models. There are no detectable changes of leukotrienes in CSF post-SAH (174,175). Leukotrienes do not have potent vasoconstricting effects (176,177). Intermediates in the production of leukotrienes may be more potent spasmogens. As AA is converted to leukotrienes unstable hydroxyeicosatetraenoic acid compounds are produced. Intraventricular injection of such compounds into dogs has produced VSP (167). It has been claimed that the hydroperoxy acids are vasoactive *in vitro* and *in vivo* and are capable of participating in processes which could result in cellular damage (178). The sensitivity of cerebral arteries to ACh and A23187 was considerably different from that of femoral arteries in dogs. Lipoxygenase inhibitors did not affect endothelium-independent relaxation induced by GTN (179).

## VII. Endothelin

### A. History

Furchgott and Zawadzki discovered endothelium-dependent vasodilation in 1980. The importance of vascular endothelium as a functional unit regulating vascular smooth muscle tonus stimulated many subsequent discoveries. An extremely significant series of investigations was performed by Yanagisawa and coworkers, who isolated a potent vasoconstrictor peptide from the culture supernatant of porcine aortic endothelial cells, determined its amino acid sequence, and molecularly cloned the peptide precursor (Fig. 6.2). This work was recorded in 1988 (180). The peptide, endothelin (ET), did not belong to any previously known peptide family. The ET sequence, however, showed local homologies to certain groups of peptide neurotoxins that act on voltage-dependent $Na^{+}$ channels suggesting that ET might act directly on membrane channels (181). Three isotypes of ET have been described: ET-1, ET-2 and ET-3.

The cellular mechanisms of action appear similar to those of other classic constrictors such as angiotensin II and NE. In the slow time course of the regulatory mechanisms for its biosynthesis and method of secretion, it is more like an inflammatory cytokine than other vasoconstrictor agonists (182). It is a likely factor in the genesis of VSP (Table 6.4).

### B. Basic Science

#### *1. Sites of Production*

The active forms are produced from the corresponding 200-residue prepropolypeptides that are encoded by three separate genes. These prepropeptides are first cleaved by a protease into biological inactive intermediates called big ET-1, -2, and -3. Big ETs are then proteolytically activated via cleavage at the common Trp21 residue by a highly specific endopeptidase called ET-converting enzyme (Fig. 6.7) (183–185). ET production is regulated at the level of mRNA transcription. In the vascular endothelium the secretion of ET-1 is constitutive without further regulation at the level of exocytosis. ETs are expressed following appropriate stimulation in many

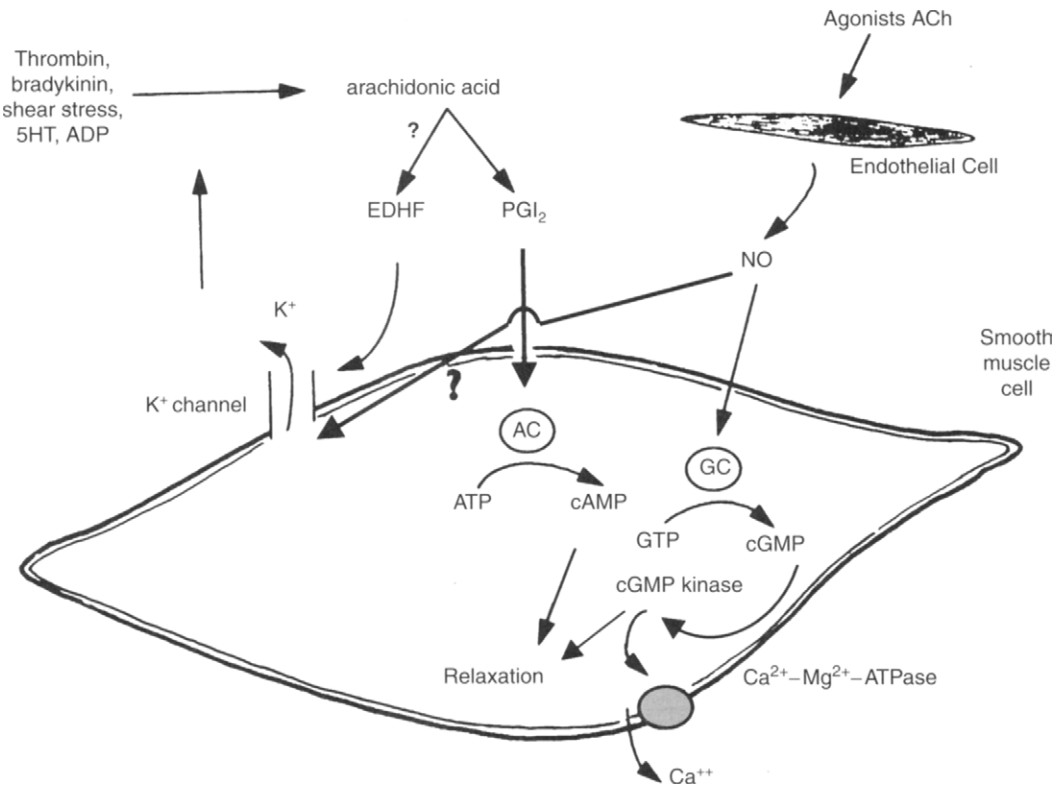


**FIGURE 6.6** Diagram of pathways of smooth muscle relaxation. EDHF, endothelium-derived hyperpolarization factor; AC, adenylate cyclase; GC, guanylate cyclase; ATP, adenosine triphosphate; cGMP, cyclic guanosine triphosphate [reproduced with permission from Weir, B., Stoodley, M., and Macdonald, R. L. (1999). Etiology of cerebral vasospasm. *Acta Neurochir.* **72,** 27–46. Copyright © Springer-Verlag GmbH & Co.].

**TABLE 6.4 Endothelin (ET)**

| |
|---|
| ET consists of three distinct 21-amino acid peptides: ET-1, ET-2, and ET-3 |
| ET has two main receptors: $ET_A$ (affinity ET-1 > ET-2) and $ET_B$ (ET-1 =2 =3) |
| Synthesized in blood vessels, brain, and other mammalian cells |
| Produces prolonged and profound vasoconstriction at low doses |
| Hemolysate, hemoglobin, thrombin, angiotensin II, and vasopressin can cause increased production and release of ET from endothelium |
| Increased concentrations are found in CSF post-SAH |
| Animal models show decrease in VSP by treatment with<br>RNA synthesis inhibitors<br>ET-converting enzyme inhibitors<br>ET antisense DNA<br>ET receptor antagonists |
| Activation of $ET_A$ and $ET_B$ can stimulate increased production of ET-1 (vasoconstrictor) and NO (vasodilator) |
| ET-1 causes $Ca^{2+}$ influx, $[Ca^{2+}]i$ mobilization, activation of PKC and phospholipases $A_2$ and C, $Na^+/H^+$ exchange, and inhibits $Na^+$, $K^+$ – ATPase |

other cell types. They act primarily as local hormones because the receptor-bearing cells are usually found within the same tissue as the producer cells. The circulating plasma levels of ET are generally much lower than the threshold concentrations for pharmacological activities, and circulating ETs are efficiently cleared by multiple organs. It is likely that cytosolic plasma concentrations correlate poorly with local levels of production (182,186). The endothelins are three 21-amino acid peptides synthesized by endothelium and other tissues. ET-1 and-3 occur in endothelial cells in brain. Plasma is almost devoid of ET. ET is not stored in significant amounts in cellular vesicles. They become active on synthesis. Their production is stimulated by factors such as hypoxia, ischemia, and thrombin, and various receptor agonists (187). Studies with preproendothelin cDNA revealed that the precursor peptide is proteolytically processed in an unusual fashion and its biosynthesis is regulated at the transcriptional level in response to various chemical and mechanical stimuli. ET had a molecular mass of 2492 and is composed of 21 amino

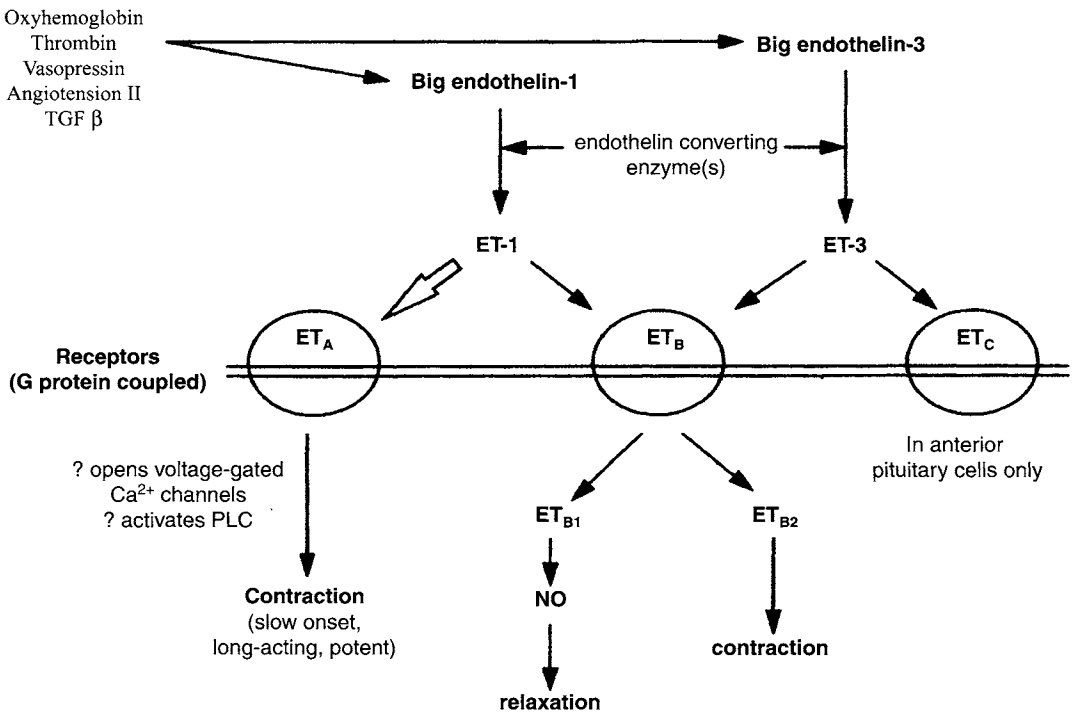


**FIGURE 6.7** Diagram of pathways for ET synthesis and actions of $ET_A$ and $ET_B$ receptors. TGF-β, transforming growth factor β; PLC, phospholipase C [reproduced with permission from Weir, B., Stoodley, M., and Macdonald, R. L. (1999). Etiology of cerebral vasospasm. *Acta Neurochir.* **72,** 27–46. Copyright © Springer-Verlag GmbH & Co.].

acid residues with free amino and carboxy termini. PrepromRNA is expressed not only in cultured endothelial cells but also in porcine aortic intima *in situ*. Endothelial cells have few secretory granules so that it is unlikely that ET is accumulated in granules and released in response to stimuli. ET-induced vasoconstriction is absolutely dependent on the presence of $[Ca^{2+}]e$. ET mRNA is actively expressed in endothelial cells *in situ*. It is produced in response to various chemical and hemodynamic conditions (180).

Preproendothelin has a half-life of only 15 min. Since ET production is stimulated by the calcium ionophore A23187 and phorbol ester, it is possible that phosopholipase C (PLC) is involved in the release of endothelin (188). A variety of growth factors such as TGF-β or platelet-derived growth factor-A (PDGF-A) as well as vasoactive hormones could induce ET-specific transcripts in quiescent cultures of vascular smooth muscle cells. Downregulation of protein kinase C (PKC) by 48 hr of pretreatment with phorbol ester markedly reduced the subsequent ability of the cells to express ET-1 transcripts and secrete ET-1 peptide. Inducible prepro ET-1 mRNA expression was accompanied by a cycloheximide-inhibitable release of ET-1 peptide into the bathing medium (189).

The pressor effect of ET is a direct one on vascular smooth muscle. ETs have baseline circulating levels of 1 or 2 pg/ml (190). Elevation of ET levels occurs in pathologic states such as myocardial infarction, renal failure, and shock and also in endotoxic states (190–192).

### 2. *Sites of Action*

#### *Receptors*

The site of synthesis of mRNA for ET is in close proximity to the binding sites of ET-1 in organs such as lung, kidney, and intestine as well as the vascular walls. ET acts by at least three different receptor subtypes: $ET_A$ (50–70 kDa) and $ET_B$-1 and $ET_B$-2 (30–40 kDa). The $ET_A$ receptor is localized in vascular smooth muscle cells and mediates the vasoconstrictor effects of ET (193,194). The $ET_{B1}$ subtype also occurs on vascular endothelial cells and mediates the endothelium-dependent vasodilator effects of ET, and this effect is equal for ET-1 and ET-3. The $ET_{B2}$ receptor subtype on vascular smooth muscle cells causes vasoconstriction. The binding of ET-1 to the $ET_A$ receptor is not inhibited by other vasoactive factors or $Ca^{2+}$ antagonist, and once bound to its receptor there is only a very slow dissociation. The ET-dependent contraction is dependent on extracellular $Ca^{2+}$ but does not act directly on voltage-dependent $Ca^{2+}$ channels (195).

When ET binds to its receptors a similar set of intracellular signaling systems are set in motion. Heterotrimeric G protein are activated which may activate PLC-β. This leads to a variety of cellular actions depending on the target cell. The affinity rank order for the $ET_A$ receptor is ET-1 ≥ ET-2 ≥ ET-3. The $ET_B$ receptor accepts all three isopeptides equally avidly. It used to be thought that in vascular tissues the $ET_A$ receptors mediated vasoconstrictor ET actions, whereas the $ET_B$ receptor would produce vasodilatory effects. This is an inaccurate oversimplification (182). Both $ET_A$ and $ET_B$ receptors are involved in the vasoconstrictor action of ET on human blood vessels. ET-dependent relaxation is always mediated by $ET_B$ receptors. The relative contribution of the $ET_A$ and $ET_B$ subtypes to the vasopressor responses varies depending on the species and particular vasculature. There is a striking species-related variation. The $ET_B$ receptors appear to play a more important role in the vasoconstriction resulting from very low concentrations of ET. Two ET receptors have been cloned: $ET_A$, which preferentially binds ET-1, and $ET_B$ receptor, which equally binds ET-1 and ET-3 and preferentially sarafotoxin S6c. Vascular smooth muscle cells of human internal mammary artery, internal mammary vein, and porcine coronary artery do not express functional $ET_B$ receptors linked to NO and/or prostacyclin production; therefore, inhibition of ET-induced contraction in patients requires the use of combined $ET_A/ET_B$ antagonist (196).

Subtype-selective radiolabeled ligands (PD151242 for $ET_A$ and BQ3020 for $ET_B$ receptors) were measured in the media of human blood vessels including those in brain. In the brain, resistance vessels with diameters <300 μm within the cortex and the pial arteries expressed only $ET_A$ receptors. A small population of $ET_B$ receptors were detectable in larger diameter vessels in other organs. It was suggested that ET-1-induced constriction would occur via the $ET_A$ subtype in the smaller resistance vessels because $ET_B$ receptors could not be detected (197). $ET_B$ receptors can act either as vasoconstrictors or as vasodilators depending on the species and the specific type and location of the vasculature (196). Endothelin-converting enzyme (ECE) is an metalloprotease that has a strict substrate specificity (198).

ET-1 acts from the adventitial but not the luminal side of cerebral arteries. *In vivo* and *in vitro* ET-1 causes a dose-dependent and long-lasting vasoconstriction (186). ET-1 mRNA was found during VSP after SAH in monkeys using the unilateral clot model. There was an increase in $ET_B$ receptors in vasospastic arteries and in both $ET_A$ and $ET_B$ receptors in the cerebral cortex (199). Similar changes were reported in ET receptor binding after SAH in dogs (200). Alterations in ET and ET receptors may occur after SAH but they may be secondary to the ischemia resulting from VSP rather than causing the VSP in the first instance.

ET is a potent and long-lasting constrictor (180,201) which produces vasoconstriction of cerebral blood vessels. ET acts by a $Ca^{2+}$-dependent mechanism (202–211) partly mediated by the activation of PKC (212). The vascular smooth muscle contraction resulting from ET develops slowly and relaxes slowly. After complete depletion of $[Ca^{2+}]i$ ET induces a contraction which can be inhibited by the PKC inhibitior H7. Phorbol esters can elicit $Ca^{2+}$-independent vascular smooth muscle contraction. PKCε and ζ, which require relatively little $Ca^{2+}$ for activation, are present in vascular smooth muscle. It has been hypothesized that calcium-independent isoforms of PKC are activated by ET and thereby produce sustained, irreversible contractions (213).

*Channels*

A predominant inward rectifier and a small outward $K^+$ current were obtained in whole cell patch-clamp recordings from cultured bovine pulmonary artery endothelial cells. ET-1 (10–100 nmol/liter) inhibited the inward rectifier current. In cell attached studies ET-1 (1 nmol/liter) inhibited single channel activity of the inward rectifier and in some patches enhanced activity of the outward $K^+$ current without changing conductance. ET-1 (1 nmol/liter) increased activity of the nonspecific cation channel in cultured human umbilical vein endothelial cells (214). In certain blood vessels the ET receptor is linked to voltage-operated $Ca^{2+}$ channels via a G protein. This linkage is thought to explain the inhibition of $Ca^{2+}$ antagonist of ET-induced contractions in certain blood vessels, particularly small ones (215).

### *3. Antagonists*

*Animal Studies*

The development of specific ET receptor antagonists began in the early 1990s. They are listed in Table 6.5. Clozel and Watanabe used BQ-123, a cyclic pentapeptide, to prevent a decrease in CBF at 1 and 2 hr following SAH in rats. Intracisternal but not intravenous injections were effective, suggesting that this compound does not cross the blood–brain barrier (BBB) (216). Numerous studies have shown an apparent benefit to ET receptor antagonists in preventing or decreasing experimental VSP (200,217) (Table 6.6)

The $ET_A$ receptor antagonist FR139317 was administered intracisternally (0.1 mg) on day 7 post-SAH in the dog two-hemorrhage model. Vasoconstriction in the control group was 62% and in the treated group 76% (218). A nonpeptide ET antagonist SB 209670 caused a dose-related inhibition of contraction of dog basilar arteries mediated by ET. The effects were assumed to

result from inhibition of $ET_A$ receptors since the $ET_B$ selective antagonist sarafotoxin 6c did not contract these posterior cerebral vessels. In the canine SAH model the ET antagonist was administered in a dose of 360 μg/day via osmotic minipump for 7 days. On day 7 the cross-sectional area in the treated animals was significantly greater than that in the vehicle group (68 vs 27%). There were no differences in blood pressure or heart rate (219). Another peptide ET antagonist (ET-ant) was tested by Foley *et al.* using a rabbit model in which the basilar artery was exposed surgically. Arterial diameters were measured by video microscopy. In non-SAH rabbits, ET-ant had little effect on resting tone and did not inhibit $K^+$ constrictions. ET-ant reversed SAH-induced constriction by 71% (220). The maximum contraction of canine basilar artery in response to ET-1 was markedly decreased following SAH. Treatment with $10^{-8}$ M phorbol ester reduced the contractile responses to ET-1 in the basilar arteries from control dogs. Treatment with phorbol ester also reduced the contractile responses to $Ca^{2+}$. It was suggested that the decreased responsiveness of the basilar arteries following SAH was due to decreased activity of PKC (221).

The calcium antagonist HA1077 lead to significant dilation of the basilar arteries put into spasm by intracisternal ET. The rank order of *in vitro* contractile activity in canine cerebral arteries was thromboxane $A_2$ analog > ET > 5-HT > $PGF_{2\alpha}$ > histamine > NE. HA1077 antagonized ET-induced contraction of dog basilar artery strips in both calcium-containing and calcium-free medium (222). Oral treatment with the $ET_{A/B}$ receptor antagonist RO 47–0203 resulted in a lessened degree of constriction (9% reduction vs 34% in controls). The $ET_A$

**TABLE 6.5 Endothelin-Related Drugs**

| Drug | |
|---|---|
| BQ 123, BQ 485 | $ET_A$ antagonist |
| PD 155080, PD 156707 | |
| RO 61–1790, SB 209670 | |
| FR 139317 | |
| RO 46–2005 | $ET_A$ and $ET_B$ antagonist |
| Bosentan (RO 47–0203) | |
| Sarafotoxin 6 c | $ET_B$ agonist |
| Res-701–1 | $ET_{B1}$ antagonist |
| BQ 788 | $ET_{B1}/ET_{B2}$ antagonist |
| Phosphoramidon | ECE inhibitor |
| CGS 26303 | |
| Actinomycin D | RNA synthase inhibitor |

**TABLE 6.6 ET Receptor Antagonists: Effects on Chronic Vasospasm (6 or 7 days) VSP in Different Studies[a]**

| Agent | Drug | Animal | Route | Control VSP(%) | Treated VSP(%) |
|---|---|---|---|---|---|
| $ET_A$ and $ET_B$ antagonists | PD 145065 | Rabbit | Superfused | 65 | 96 |
| | Bosentan | Dog | iv | 34 | 40 |
| | Bosentan | Dog | iv | 52 | 80 |
| | Bosentan | Monkey | I cisternal | 63–71 | 54–70 |
| | Ro 47–0203 | Dog | po | 69 | 87 |
| | Bosentan | Rabbit | po | 66 | 91 |
| | | | Mean | 60 | 74 |
| $ET_A$ antagonists | BQ-485 | Dog | sq | 60 | 75 |
| | BQ-123 | Dog | ic | 56 | 62 |
| | FR139317 | Dog | ic | 62 | 76 |
| | BQ-610 | Rabbitt | ic | 62 | 89 |
| | BQ-610 | Rabbit | Superfused | 65 | 80 |
| | SB209670 | Dog | iv | 27 | 68 |
| | BQ-123 | Monkey | ic | 63–71 | 85–95 |
| | PD155080 | Rabbit | po | 66 | 84 |
| | | | Mean | 59 | 84 |

[a]Modified from Wanebo, J. E., *et al.* (1998). Systemic administration of the endothelin-A receptor antagonist TBC 11251 attenuates cerebral vasospasm after experimental subarachnoid hemorrhage: Dose study and review of endothelin-based therapies in the literature on cerebral vasospasm. *Neurosurgery* **43,** 1409–1418. VSP = days 6 or 7 diameter/initial diameter.

receptor antagonist (PD155080) was slightly less effective, being associated with a reduction of 16% of the control diameter (223). RO 47–0203 was administered orally in two single doses of 30 mg/kg/day. Vessel diameter decreased 13% in the treatment group and 31% in the control group. Concentrations of ET-1 in CSF significantly increased with time after SAH (224). In a feline model of focal cerebral ischemia the intravenous administration of PD156707 ($ET_A$ receptor antagonist) progressively increased cerebral perfusion up to normal levels following MCA occlusion. The intravenous administration of PD156707 reduced the hemispheric volume of ischemic damage by 45% in treated animals (225).

Swiss workers developed a water-soluble ET antagonist. This compound is in a class of trifunctionalized heteroarylsulfonamide pyrimidines designed especially for high water solubility (RO 61–1790). It is a competitive ET antagonist with an affinity to $ET_A$ receptors in the subnanomolar range. It has a 1000 fold greater selectivity for $ET_A$ than $ET_B$ receptors. It has high functional potency for inhibiting contraction induced by ET-1 in isolated rat aorta. In a double-hemorrhage canine model this compound prevented and reversed VSP in a dose-dependent fashion. When mild VSP was already established RO 61–1790 in a dose of 3 mg/kg intravenously was half as effective as intravascular papaverine. In a dose of 20 mg/kg/day the drug totally prevented the occurrence of VSP. In the prevention study the low-dose RO 61–1790 VSP was not significantly different from the control group. However, the high-dose treated group did not show any VSP. ET-1 concentrations were increased in the CSF after SAH from 1.2 to 5.4 pg/ml by day 4 post-SAH. The day 4 levels in the low-dose and high-dose drug groups were 8.9 and 6.8 pg/ml, respectively (226). The nonpeptide competitive antagonist of ET-A receptors (PD156707) was effective in significantly decreasing the degree of basilar artery VSP. CSF levels of the drug were substantially lower than the plasma levels. The drug was administered by intravenous infusion following the SAH. Direct infusion of PD156707 into the basilar artery on day 7 caused a 10% increase in diameter compared to placebo (217). In an acute rabbit model involving sacrifice 48 hr post-SAH, the $ET_A$ receptor antagonist TBC 11251 was given intravenously beginning at the time of SAH. There was a significant reduction in VSP in animals receiving 5 mg/kg and an even greater reduction in animals receiving 10 mg/kg BID. However, animals receiving 20 mg/kg BID did not achieve a significant amelioration of VSP. Also, animals given the drug beginning 24 hr after SAH and again at 36 hr post-SAH achieved a lesser degree of reduction in VSP which was not statistically significant (227).

The ET receptor antagonist BQ-123 attenuates the early decrease in cerebral blood flow post-SAH (216). Intracisternal BQ-123 (10 nmol) completely prevented the decrease in CBF at 60 and 120 min post-SAH in a rat model (216). BQ-123 ($10^{-5}$ mol/liter) inhibited contractions caused by ET-1 in canine basilar artery. Daily intracisternal administration in a concentration 10 times higher than the concentration *in vitro* abolished the contractile effect of ET-1 and did not prevent experimentally induced cerebral VSP (228). On the other hand, the intracisternal injections of BQ-123 or an ECE inhibitor failed to significantly affect angiographic VSP in the canine two-hemorrhage model. In isolated dog arteries BQ-123 ($10^{-5}$ mol/liter) selectively inhibited concentration-dependent contractions to ET-1. Levels of ET in the CSF and plasma did not correlate with the development of VSP (229). In the double-hemorrhage canine model saline controls were associated with a reduction in basilar artery diameter to 56% of controls, whereas diameters were 62% with BQ-123 and 56% with phosphoramidon. In contrast, in isolated basilar arteries BQ-123 ($10^{-5}$ M) inhibited concentration-dependent contractions to ET-1 ($10^{-11}$–$3 \times 10^{-8}$ M). The development of VSP did not affect levels of immunoreactive ET in plasma or CSF (229). Itoh *et al.* used the canine two-hemorrhage SAH model to show that continuous intrathecal administration of BQ-123 ($5 \times 10^{-6}$ M/day) prevented day 7 VSP of the basilar artery. As percentages of controls the treated animals had a 98% diameter versus 71% in the untreated animals. Expression of $ET_A$ receptor mRNA was not detectable in control animals but markedly increased on day 3 post-SAH and was still detectable on day 7 (230). Hino *et al.*, using the primate model, compared BQ-123 administered intracisternally by continuous infusion at a dosage of 6 mg/kg/day with bosentan administered intracisternally twice a day into an Ommaya reservoir with a catheter along the right MCA at a dosage of 5 mg/kg/day. Both placebo and bosentan groups showed significant reductions in the diameter of the MCA underneath the blood clot (34 and 46%, respectively). In contrast, animals injected with BQ-123 did not show significant reduction in the right MCA. CSF examination on day 7 post-SAH did not show any bosentan, whereas BQ-123 was detected in two animals. Multiple dosages were not employed (231).

BQ-485 ($ET_A$ receptor antagonist) in the two-hemorrhage dog model was administered by systemic continuous administration of 120 mg/day. The diameter of the basilar arteries from treated animals was 75% of baseline compared to 60% in controls, who only had the SAH (232). Zuccarello *et al.* used the double-hemorrhage rabbit model to study the effect of the intracisternal infusion (10 μl/hr) of the $ET_B/_{B2}$ receptor antagonist BQ788 and

10 μmol/liter of RES-701–1, an $ET_{B1}$ receptor antagonist. Both drugs reduced VSP by 10%. *In situ* superfusion with 1 μmol/liter BQ788 reversed the spasm by 40% and 1 μmol/liter RES-701–1 reversed the spasm by 50%. Both these compounds enhanced by approximately one-half the ET-1-induced constriction elicited in spastic vessels previously relaxed with 0.1 mmol/liter phosphoramidon. There has been little study of $ET_B$ receptor antagonists because the cerebral vasculature is largely $ET_A$ receptor mediated and $ET_B$ receptor blockade might actually enhance the spasm by prevention of $ET_B$ receptor-mediated NO release. However, in a rabbit model of completely relaxed spastic vessels it was shown that $ET_{A/B}$ receptor antagonists were required in addition to selective $ET_A$ receptor antagonists. Following SAH there is also an increase in $ET_B$ receptors and mRNA levels (199,200). It has been suggested that $ET_B$ receptor antagonist could prevent and reverse SAH-induced spasm and that attenuation of the VSP results from blockade of smooth muscle $ET_{B2}$ receptor-mediated constriction and/or endothelial $ET_{B1}$ receptor-mediated ET-1-induced ET-1 release. ET-1 was assumed to be able to cause chronic VSP by stimulating not only $ET_A$ receptors but also $ET_{B2}$ receptors on smooth muscle (233).

A nonpeptide antagonist of both $ET_A$ and $ET_B$ receptors (RO 47–0203, bosentan) was a derivative of a less potent RO 46–2005. A bosentan inhibited the pressor response to ET-1 after intravenous or oral administration. At the time of introduction it was the most potent orally active antagonist of ET receptors (234). In rabbits subjected to single SAH, Bosentan was capable of reversing basilar VSP to the same extent as SNP. In the dog double-SAH model it could only reverse VSP by approximately half as much as papaverine. Bosentan did not induce hypotension. In the basilar artery SAH was associated with increased ET-1 concentrations, big ET-1 concentrations, and levels of ECE. Receptor-binding studies of canine basilar arteries showed a shift in ET receptor distribution from $ET_A$ to $ET_B$ subtypes after SAH. In CSF the SAH increased ET-1 by 6-fold, and in the basilar artery SAH increased ET-1 concentrations 1.3-fold (200). In the dog SAH model the twice-daily intravenous injection of bosentan immediately after the first SAH and continuing for 6 days resulted in a lesser degree of VSP than in untreated animals (21 vs 49%). ET-1 concentrations did not differ between the two groups (235). Bosentan efficacy was compared to the synthetic $ET_A$ receptor antagonist PD155080 in the rat double-hemorrhage model. Control animals showed a 34% reduction from control diameters compared to only 9 and 16% reductions in animals receiving bosentan or PD155080, respectively (223). Oral bosentan (30 mg/day) was used in the dog two-hemorrhage model. No VSP occurred in the treated group, whereas in the control group there was significant reduction in diameter. No differences in CSF levels of ET-1 were measured between the treated and control groups. There was a slight increase in ET-1 in plasma from day 1 to day 8 (224).

Phosphoramidon is an inhibitor of the metalloproteinase ECE. Intracisternal pretreatment with phosphoramidon, a nonspecific ECE inhibitor, potently suppressed the decrease in diameter of basilar artery following double-hemorrhage in canines. Despite the administration of phosphoramidon ET levels were still markedly increased on day 2 post-SAH, although they significantly declined by day 7 (201,236–240). Phosphoramidon antagonizes ET pressor activity *in vivo* (239).

Phosphoramidon intracisternally has been found to decrease VSP in experimental SAH (236,241,242). In a study of an ECE inhibitor its topical application was found to block vasoconstriction resulting from the application of big ET but not ET-1. Intraperitoneal administration of this substance reduced the delayed spastic response of basilar artery (243). However, in a canine two-hemorrhage model daily intracisternal injections of phosphoramidon did not prevent SAH-induced VSP (229).

ET produced an intense, sustained vasoconstriction over a dose range similar to that seen with 5-HT and $PGF_{2\alpha}$. The $ED_{50}$ was equal to $10^{-8}$ M. The constriction was resistant to selective antagonists of NE, 5-HT, isopoterenol, histamine, acetylcholine, and angiotensin II. The constriction was antagonized by SNP, verapamil, and a disulfide bond reducing agent (dithiothreitol) (209).

The basilar artery of rabbits was exposed transclivally and measured using video microscopy. Intravenous or topical application of the ECE inhibitor CGS 26303 blocked vasoconstrictor responses to topically applied big ET-1 but not ET-1. A structurally related compound which does not inhibit ECE (CGS 24592) was not effective in blocking the vasoconstriction to the topical application of big ET-1. Rabbits pretreated with intraperitoneal CGS 26303 for 24 hr before SAH showed attenuation of the delayed spastic responses of the basilar artery (243). In the rabbit model the ECE inhibitor CGS 26303 was injected intravenously after induced SAH in the rabbit. Basilar diameters were measured at sacrifice on day 2 post-SAH. Treatment with CGS 26303 attenuated the arterial narrowing which followed SAH in animals receiving the drug either 1 or 24 hr post-SAH. The protective effect of the drug was achieved in statistical significance at dosages of 3,10, or 30 mg/kg (244).

There was no significant correlation between the occurrence of VSP and plasma or CSF levels of ET in patients or dogs with induced SAH. Since ET synthesis is regulated at the level of mRNA transcription the effect of

actinomycin D was examined as a means of preventing VSP. It was found that actinomycin D treatment completely inhibited development of VSP in dogs (245). Antisense oligoDNA for preproendothelin-1 (ppET-1) mRNA was studied in a rat model of VSP. Phosphorothioate antisense oligoDNAs for ppE-1 were injected into the cisterna magna. There was a striking inhibitory effect on contractions of the basilar artery to hemolysate after the oligoDNAs were injected into the cisterna magna. The antisense oligoDNAs were proven to be incorporated into the vascular wall. The VSP was significantly inhibited after 20 min of exposure to hemolysate, which suggested that ET synthesis started approximately 20 min after hemolysate stimulation. Expression of ppET-1 in the basilar artery in which the VSP was inhibited was suppressed at the level of transcription (246).

*Human Studies*

ET-1-induced concentration-dependent constriction of human cerebral arteries. This was significantly antagonized by both bosentan and FR139317, a selective $ET_A$ receptor antagonist. Sarafotoxin 6c did not cause contraction of human cerebral arteries. In precontracted vessels this substance did induce dilatation that was significantly inhibited by bosentan (10 μmol/liter). mRNA encoding human $ET_A$ and $ET_B$ receptors was detected in human cerebral arteries both with and without endothelium. It was concluded that ET-1 induced constriction in human cerebral arteries by activating $ET_A$ receptors and that sarafotoxin 6c-induced vasodilation was mediated via $ET_B$ receptors (247).

In human pial arteries ET-1 was more potent than ET-3 as a vasoconstrictor, indicating an $ET_A$-mediated effect. Sarafotoxin 6c had no effect on contractile action at concentrations up to 30 nmol/liter. The nonpeptide $ET_A$ receptor antagonist PD156707 (3–30 nmol/liter) caused a parallel rightward shift of the ET-1-induced response. The $ET_A$ receptor antagonist PD156707 (30 nmol/liter) fully reversed established constriction in pial arteries induced by ET-1, whereas the selective $ET_B$ receptor antagonist BQ788 had little effect. The maximum response to ET-1 was significantly attenuated by the $Ca^{2+}$ channel blocker nimodipine (0.3–3 μmol/liter). A radioisotope-labeled $ET_A$ receptor antagonist localized to the smooth muscle layer of intracerebral blood vessels and pia. A labeled selective $ET_B$ receptor antagonist did not result in detectable labeling. It was suggested that $ET_A$ receptor antagonist could provide additional dilatory benefit in cerebrovascular disorders associated with raised ET levels. These pial and intracerebral arteries were obtained from human tissues obtained during neurosurgical procedures for deep-seeded gliomas or epilepsy control (248).

In human vascular tissue reverse transcriptase-polymerase chain reaction (RT-PCR) with nested oligonucleotide primers detected the presence of mRNA encoding both $ET_A$ and $ET_B$ in the media from aorta, pulmonary, and coronary arteries. BQ-123 was 9000-fold more effective against human vascular $ET_A$ subtype than $ET_B$ receptor. An $ET_B$ selective ligand, BQ3020, was selective for the human $ET_B$ receptor in binding assays and was a potent constrictor of some animal vessels *in vivo*; however, it had no detectable agonist activity in any of the human vessels tested. The responsiveness of $ET_B$ sometimes could vary in different vascular beds. The vasoconstrictor activity may be lost in pathophysiological tissue (249).

Yanagisawa, the discoverer of ET (180), stated that the challenge in the field is to find the right animal models for potential target human diseases and to match these models with antagonists having the right receptor subtype selectivity. Therapeutic possibilities would advance with the discovery of orally active nonpeptidic antagonists of ET receptors.

## C. Putative Spasmogens

### *1. Cause of Vasospasm*

Since ETs are potent and long-lasting vasoconstrictors both *in vivo* and *in vitro* in a wide variety of models, it has been hypothesized that they are a key factor in the generation of VSP (180,209,250–255).

### *2. Levels in CSF and Plasma Post-Subarachnoid Hemorrhage*

Various studies have suggested elevation of ET levels in the CSF following SAH (228,256,257) (Figs. 6.8 and 6.9). Some workers have found no such correlation (199,258). ET is elevated in some conditions which are not associated with VSP (217). In the two-hemorrhage dog model Yamaura *et al.* found that ET-1 levels increased up until the second day and then decreased to the seventh day after experimental SAH. VSP was slightly reversed by the application of antibodies to ET-1 on day 2 but not day 7 (259). In a primate model Hino *et al.* did not find elevation of ET-1 levels in CSF post-SAH. Levels of $ET_B$ receptor and mRNA were higher in the areas of the VSP of the clot side arteries. There were no significant differences in the levels of prepro- ET-1, prepro- ET-3, or $ET_A$ receptor mRNA in cerebral arteries. The levels of $ET_A$ and $ET_B$ receptor mRNA were more than two fold higher in the vasospastic than in the contralateral cerebral cortex (199).

ET-1 was found in both plasma and CSF following SAH. The plasma levels did not change. ET-1 concentrations apparently did not correlate with angiographic

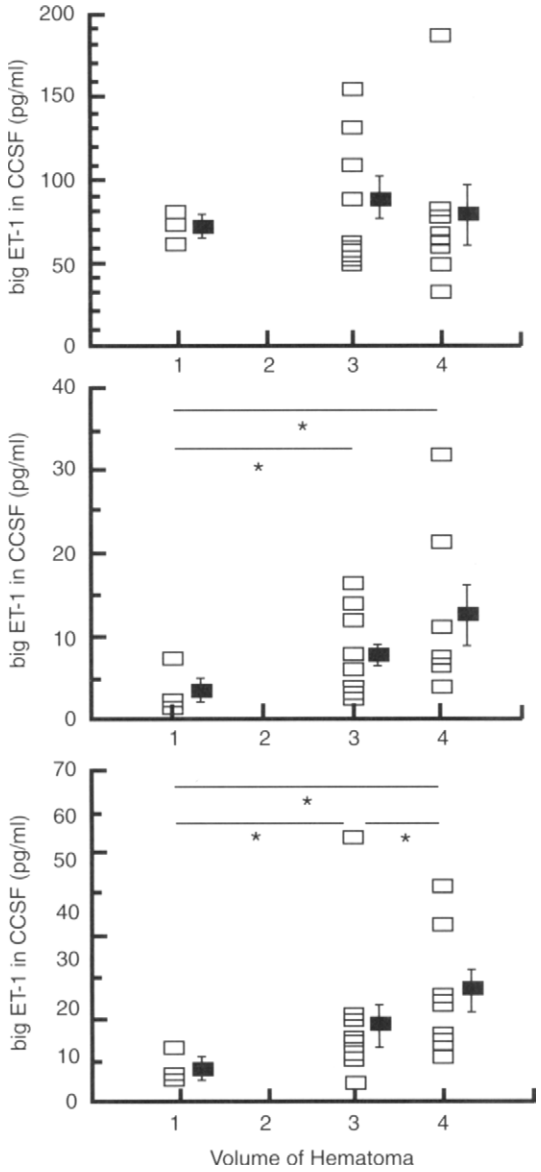


**FIGURE 6.8** Graphs showing correlation of the concentrations of ET in the CSF of patients with SAH according to their Fisher classification. Individual (open squares) and mean ± standard error of the mean (closed squares) are presented. The groups did not differ significantly with regard to mean age and Hunt and Hess grade or the occurrence of VSP. $^{*}p \leq 0.05$ [reproduced with permission from Seifert, V., Löffler, B. M., Zimmerman, M., Roux, S., and Stolke, D. (1995). Endothelin concentrations in patients with aneurysmal subarachnoid hemorrhage. *J. Neurosurg.* **82,** 55–62].

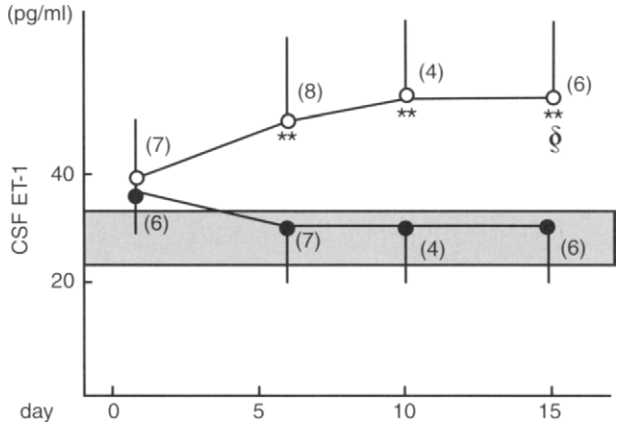


**FIGURE 6.9** Graph showing serial changes in CSF ET-1-like immunoactivity levels in eight patients with angiographic and/or symptomatic VSP (open circles) and those in seven patients without VSP (closed circles). The CSF ET-1-like immunoactivity levels in patients with VSP were close to normal on days 1–3; thereafter, they were significantly elevated until the end of the second week. Significance: $^{**}p < 0.01$ compared with the normal value; §, $p < 0.01$ for the difference between the two groups. The CSF ET-1-like immunoactivity levels in the patients without VSP remained in the normal range during the entire study period. The shaded area indicates the normal range of CSF ET-1 (27.9 ± 5.0 pg/ml), the perpendicular lines represent standard deviations, and the number of patients is shown in parentheses [reproduced with permission from Suzuki, R., Masaoka, H., Hirata, Y., Marumo, F., Isotani, E., and Hirakawa, K. (1992). The role of endothelin-1 in the origin of cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage. *J. Neurosurg.* **77,** 96–100].

VSP (260). ET-1 plasma levels were measured in 7 patients with symptomatic VSP. Average concentrations were higher than in controls at the onset. Plasma ET-1 concentrations in patients with VSP were higher than those without on day 3 post-SAH and on day 7. The onset of VSP appeared to correlate with the peaks in concentration (261). Big ET-1 was the major isoform in human CSF. Only very small amounts of ET-1 and ET-3 were found in CSF. The same substances were also identified in plasma (262). A positive correlation between the plasma ET-1 concentrations and symptomatic VSP was reported in patients with symptomatic VSP on days 3 and 7. The CSF ET-1 levels in patients with SAH were higher than those with healthy controls, although the ET-1 levels did not apparently coincide with the clinical course (263). One hundred and sixteen CSF samples were obtained from 26 patients post-SAH. Concentrations of ET increased from 0.4 on days 0 and 1 to 2.2 pmol/liter on day 6 and then declined gradually. Most patients showed the highest concentrations between days 4 and d 6 (264).

CSF levels were analyzed for up to 18 days post-SAH. The ET levels increased following SAH (265). ET-1 and ET-3 levels were followed post-SAH and in severe head injury. Levels were higher than those in control patients. ET-3 as well as ET-1 levels were significantly raised (266). Serial measurements of ET-1 levels were performed in CSF post-SAH and a progressive rise in the first few days was found. The time course for the

occurrence of VSP and the increase in CSF ET-1 appeared to coincide (257).

Plasma and CSF ET-1-like immunoreactivity was measured serially by radioimmunoassay for 2 weeks post-SAH in CSF from drains in 27 patients. Mean ET-1-like immunoreactivity levels in the plasma of these patients was highly elevated during the whole study, whereas the levels in CSF were not. Patients with Fisher CT group 3 CT scans showed high levels of plasma immunoreactivity. There were no significant differences in these plasma levels when patients were stratified according to neurological grade. The plasma ET-1-like immunoreactivity levels in 12 patients with VSP were higher than those in 15 patients without if during the first week. CSF ET-1-like immunoreactivity levels in patients with VSP were normal on days 0–3 but became elevated on days 5–7 and remained high until the end of the second week. Patients without VSP had normal levels throughout the entire study (257). It is impossible to exclude the possibility that increased ET-1 levels in CSF postoperatively and post-SAH might not be simply the consequence of surgically induced disturbances (228).

Plasma and CSF concentrations of big ET, ET-1, and ET-3 were studied for up to 2 weeks in 22 patients. The major component in CSF was big ET-1, with ET-3 and ET-1 being found in lesser amounts. ET-3 correlated with the age of the patient. The concentrations of big ET-1, ET-1, and ET-3 appeared to decrease with time post-SAH in contrast to previously reported increases. The concentrations of ET-1 and ET-3 appeared to correlate with the volume of hematoma in the basilar cisterns (256). The postoperative concentrations of ET in the CSF were higher than those measured before surgery. In SAH patients without VSP, the concentrations of ET in the CSF decreased with time, whereas the time course of VSP coincided with the increase in concentrations of big ET-1 and ET-1. The time course of changes in patients with and without VSP was different for the concentrations of big ET-1 and ET-1. Whereas the patients with VSP had higher levels of CSF ET-1, big ET-1, and ET-3, there was no difference between patients with or without VSP and the plasma levels of these compounds (256). Concentrations of ET-1 in CSF were measured by radioimmunoassay in 22 SAH patients. CSF samples were collected daily for 10 days after the surgical procedure via the cisternal drainage tubes inserted at operation. Concentrations of ET-1 on postoperative day 1 were all increased compared to control levels. Angiographic VSP was observed in 8 of 22 patients and in 7 of them the concentrations of ET-1 had increased prior to the clinical observation of angiographic VSP. The elevated levels decreased before the disappearance of VSP. In 10 of 12 patients without VSP the concentrations of ET-1 in CSF decreased with time (267).

### *3. Levels in Clot and Tissue Post-SAH*

In the primate clot model serial ET-1 levels were measured from the perivascular space using microdialysis technique. ET-1 levels were also measured in plasma and CSF after transient cerebral ischemia. ET-1 levels were measured in cultures of endothelial cells and astrocytes exposed to oxyHb (10 μM), metHb (10 μM), and hypoxia (11% oxygen). The perivascular levels of ET-1 did not correlate with the development of VSP or its resolution. CSF and plasma levels of ET-1 were not affected by VSP. CSF ET-1 levels were 9 pg/ml before SAH and remained unchanged when VSP developed (7 pg/ml). Transient cerebral edema increased ET-1 levels in CSF (1 pg/ml at occlusion vs 3 pg/ml 4 hr after reperfusion). The elevation returned to normal after 24 hr. Endothelial cells and astrocytes showed inhibition of ET-1 production 6 hr after exposure to Hb. Hypoxia inhibited ET-1 release by endothelial cells at 24 and 48 hr. The ET-1 was released by astrocytes but not endothelial cells during hypoxia (258). This study provides persuasive evidence that ET-1 is not a prime cause of VSP.

ET levels within the basilar artery as well as the CSF are increased post-SAH (259,261,265,266). In the canine two-hemorrhage model basilar arteries were quickly frozen following removal of surrounding blood clot 7 days after SAH. Immunoreactive ET-1 was measured by sandwich immunoassay. The level was 113 pg/mg protein prior to VSP, 180 pg/mg on day 2 after VSP, and 115 pg/mg on day 7. VSP was moderately reversed by the topical application of monolonal antibody against ET-1 on day 2 but was resistant on day 7. Yamaura *et al.* supposed that ET-1 may be a trigger for VSP but not responsible for maintenance (259).

### *4. Effect of CSF Injection*

ET-1 causes a dose-dependent contraction in isolated dog basilar arteries (250,253,254). This contraction is reversed by nicardipine and papaverine (250,253). In a feline model intracisternal but not intraarterial injection of ET-1 causes constriction. Similarly, in dogs the compound is more potent from the adventitial side (255). Vasoconstriction from ET-1 intracisternal injection has been observed as late as 3 days after injection (250). Intracisternal injections of $1.2 \times 10^{-12}$ mol/kg of ET caused basilar artery contraction lasting for more than 24 hr. Intracisternal injection of $2 \times 10^{-12}$ mol/kg ET induced acute contractions of the basilar artery. This concentration, however, produced sustained respiratory insufficiency and death (253). Intracisternal injections of ET in cats and dogs caused basilar artery contraction,

whereas infusions into the cerebral artery had no appreciable effect. Vasoconstriction was maintained for as long as 12 hr. It was shown that ET acts on cerebral vessels from the adventitial and not the luminal side, possibly due to the presence of the BBB (255). Sasaki and Kassell found that ET at concentrations of $10^{-12}$ to $10^{-7}$ M elicited dose-dependent contractions in canine, rabbit, and monkey cerebral arteries (268). The maximal contractile responses to ET were much stronger than those induced by 40 m*M* KCl in all arteries studied. Nicardipine ($10^{-8}$ M) antagonized the constriction produced by $10^{-8}$ M ET. In animal experiments intracisternal injection of 0.6–1.2 $\times 10^{-12}$ M/kg of ET caused biphasic contraction of the basilar artery lasting more than 1 day. The initial vasoconstriction was associated with hypertension, bradycardia, and respiratory arrest. The intracisternal injection of $2 \times 10^{-12}$ M/kg of ET induced acute contraction of the basilar artery. This dose of ET was lethal (269).

### D. Vasoconstriction

#### *1. In Vitro Studies*

In isometric tension experiments on canine basilar artery removal of endothelium significantly augmented the constrictor response to ET. Both papaverine and nicardipine almost completely inhibited contraction from $10^{-8}$ M ET (270). ET in a muscle bath caused dose-dependent contraction of the rat basilar artery from both control animals and those having prior SAH. The maximum contractile response to ET was much stronger in the animals having had SAH. There was a strong tachyphylaxis, and repeated exposures to ET within 2 hr of the first exposure and separated by repeated washings with return to resting tone resulted in a very weak subsequent or no response to ET (271). ET-1 produced potent and long-lasting contractions of normal cerebral arteries from monkeys, dogs, and rabbits. ET-1-induced contraction was increased in canine basilar arteries exposed to SAH. The constriction at 7 days post-SAH was less than the constriction at 2 days post-SAH in response to ET (272). ET at concentrations of as little as $10^{-12}$ M produced contractions in canine basilar arteries *in vitro*. Maximum tensions were larger than those induced by 40 n*M* KCl (253). ET produced a dose-dependent contraction of canine and bovine arterial smooth muscle with $ED_{50}$ values ranging from 4 to 8 n*M*. The response to ET developed slowly and was persistent. Maximum contraction required the presence of extracellular $Ca^{2+}$ and was independent of the presence of endothelium. The maximum contraction was two or three times greater than that produced by neuropeptide Y or angiotensin II (273). The goat model was also used to provide MCA rings for *in vitro* studies. ET-1 elicited dose-dependent contraction which was significantly potentiated after endothelium denudation or incubation with a NO inhibitor. The latter effect was reversed by L-arginine. Hyperreactivity to ET-1 was observed after SAH. Endothelial denudation did not alter the enhanced response to ET-1 but it was further significantly increased after incubation with L-NOArg. It was concluded that the absence of endothelial NO after SAH may contribute to the hyperreactivity of cerebral arteries to ET-1. L-NOArg is an inhibitor of NOS (274).

#### *2. In Vivo Studies*

Topical suffusion of ET at $10^{-10}$ M produced 5% dilatation of pial arterials. At concentrations of $10^{-8}$ and $10^{-7}$ M there was a reduction in caliber of 22%. The basilar artery was not dilated by low concentrations of ET but higher concentrations constricted it up to 56%. ET did not alter the BBB permeability as judged by fluorescein (204). Continuous injection of ET-1 for 7 days intrathecally using an osmotic pump resulted in severe degenerative changes in endothelial cells and smooth muscle cells from the cerebral arteries of dogs. Severe angiographic vasoconstriction was evident on day 7 post-SAH (254). In a goat model of SAH, on the third day post-SAH CBF was reduced by 28% and cerebral vascular resistance increased by 39%. At this point, ET-1 reductions in CBF and the constricting effect of ET-1 *in vitro* were enhanced. Nicardipine was less efficacious in blocking the effects of ET. By day 7 post-SAH, however, CBF and cerebrovascular resistance were returned to normal and the constricting effect of ET-1 was no longer enhanced (275).

### E. Pharmacological Interactions

#### *1. Vasoconstrictors*

Stimuli inducing the synthesis of ET-1 include TGF, angiotensin II (276), oxyHb, and interleukin-1 (277). The synthesis of ET-1 is inhibited by NO. Both thrombin and Hb, which are present in the thrombus and CSF, are known to induce ET gene expression and stimulate its synthesis (180,201,278–286).

In *in vitro* studies of human cerebral arterial segments the contraction is resistant to antagonists of other biologically active vasoconstrictors. Only SNP verapamil, and dithiothreitol inhibit the ET-1-induced constriction (209). Both intraluminal and extraluminal ET showed vasoconstricting effects in perfused rabbit basilar arteries. The vessels most sensitive to intraluminal ET were femoral or mesenteric arteries. The constrictor effect of

intraluminal ET was enhanced by the presence of extraluminal oxyHb (287). Confluent cultures of bovine aortic endothelium in serum-free medium were exposed to concentrations of freshly prepared RBC lysate. Hb increased ET-1 secretion into the medium in a dose-dependent manner after 24 hr. This enhanced ET-1 production was sustained for 72 hr (288). ET contracted bovine cerebral arteries under isometric conditions. This contraction was inhibited by $Ca^{2+}$ antagonists or the substitution of a $Ca^{2+}$-free medium. Inhibitors for PLC and PKC suppressed the ET-induced contractions. ET markedly augmented contractions induced by other spasmogens, such as $K^+$, oxyHb, $PGF_{2\alpha}$, and 5-HT. The augmenting effect of ET was also blocked by a PKC inhibitor (289).

#### 2. *Vasodilators*

Inhibition of NO and cGMP can result in increased ET-1 production from stimulated endothelium (290). Although ET may not be elevated after SAH, it is possible that a selective inhibition of vasodilator mechanisms (NO and/or related NO-containing compounds and $PGI_2$) would permit unopposed ET action and resultant VSP.

We believe that the evidence for RBC derivatives and substances formed during clotting and fibrinolysis is stronger than for any other vasoactive substances in the etiology of VSP. Other compounds may play a supportive role. Table 6.7 summarizes some of these data.

## VIII. Blood and Cerebrospinal Fluid

### A. Circulating Factors

Circulating vasoactive substances which are endothelial-independent vasoconstrictors include endothelin, vasopressin, thromboxane, thrombin, 5 HT, angiotensin II, 5-HT, histamine, ACh, and NPY. Endothelial-independent vasorelaxant agents include adenosine and atrial natriuretic peptide, and prostacyclin. Synthetic agents include calcium channel blockers, nitrosovasodilators and isoproteronol (213).

### B. Blood Derivatives

#### 1. *In Vitro Studies*

In an early study Zucker found that by using various smooth muscles including ox carotid artery, constriction could be induced by defibrinated blood, lysed platelets, and lysed RBCs (291). Numerous compounds have been isolated from blood which have been shown to be vasoconstrictors in various animal models (69). Boullin *et al.* in 1976 showed that material from humans post-SAH could contract human cerebral blood vessels obtained at autopsy (292).

Osaka found that fresh, intact RBCs had no vasoactivity but gained this following incubation for up to 7 days. The VSP induced by RBC breakdown products did not relax but increased in severity during a period of observation up to 24 hr. Vasoconstrictors from fresh serum and platelet-rich plasma (PRP) lost their reactivity after incubation (2). Fresh blood and supernatants of blood CSF mixtures incubated for up to 15 days were applied to the basilar artery of adult cats. The most powerful and long-lasting activity was from supernatants incubated for 7 days. Mixtures incubated for 15 days had little or no activity. In the 15-day mixture the oxyHb had spontaneously converted to metHb, which had no vasoconstrictor activity. Sonobe and Suzuki prevented experimental VSP by oxidizing oxyHb into metHb with sodium nitrite. Using a variety of purification techniques Sonobe and Suzuki suggested that extracellular oxyHb was the spas-

**TABLE 6.7 Possible Etiologic Agents (Other Than RBC Derived) for Vasospasm**

| | Increase in constriction | | | | | Decrease in contraction | | |
|---|---|---|---|---|---|---|---|---|
| | NE | 5-HT | $PGF_{2\alpha}$ | NPY | ET | CGRP, VIP | $PGI_2$ | NO |
| Present in circulating blood | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Present in clot | No | Yes | No | No | No | No | No | No |
| Present in vessel wall | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Appropriate time course in CSF | Yes | No | Yes | No | Yes | No | No | Yes |
| Vasoconstrictior | Yes | Yes | Yes | Yes | Yes | No | No | No |
| Vasodilator | No | No | No | No | No | Yes | Yes | Yes |
| Readily antagonized | Yes | Yes | Yes | Yes | Yes | No | Yes | Yes |
| VSP prevented by removal or blockade | No | No | No | No | ?Yes | No | No | No |

minogen (293). Endo and colleagues induced VSP in cats by a topical blood application. The arteries were fixed and extirpated and the condition of nerve terminals in the vessel wall was assessed by electron microscopy. Disappearance of small core vesicles was evident in arteries exposed to aged blood–CSF mixtures but did not occur following application of fresh arterial blood, lysed platelets in saline, or following mechanical stimulation or bilateral superior ganglionectomy (294).

Canine cerebral, coronary, renal, and mesenteric arteries were studied *in vitro*. Dog blood hemolysate was prepared by hypotonic shock. Dose-related contractions of the cerebral arteries were caused by hemolysate. The contractions of systemic arteries were less than half those of cerebral arteries. The contractions of cerebral arteries to metHb in the same concentrations as the Hb in the hemolysate were markedly less (19 vs 154%). The constriction to hemolysate was slightly attenuated by aspirin, polyphloretin phosphate (a PG antagonist), and $10^{-7}$ M cinanserin (5-HT antagonist). Phentolamine ($10^{-7}$ M) was ineffective in blocking the response (154). Dog basilar artery and mesenteric arteries were studied *in vitro*. Intact RBCs and RBC ghosts had no effect. Hemolysate contracted basilar arteries in a dose-dependent fashion but had only contracted mesenteric arteries in very high concentrations. The basilar artery contraction was significantly attenuated by $5 \times 10^{-5}$ M aspirin, $3 \times 10^{-5}$ g/ml polyphloretin phosphate, $10^{-6}$ M cinanserin, $1.1 \times 10^{-3}$ U/dl superoxide dismutase (SOD), $10^{-4}$ U/dl catalase, and $10^{-4}$ M/*dl* α-tocopherol (295). A vasospastic activity probably resides in the RBCs and is released by lysis. The evidence for this is that fresh, intact RBCs are inactive, whereas lysed RBCs produce large contractions (2). The vasospastic activity of stored blood follows a time course similar to that for the onset of VSP (88,293,296). The vasocontractile potency of incubated RBCs increased over 7 days. Thiobarbituric acid assays in incubated samples showed progressive increases and spectrophotometry demonstrated increasing conversion of oxyHB to metHb. $H_2O$ and linoleate hydroperoxide were shown to possess significant vasocontractile activity (297). On the basis of biochemical analysis of incubated blood in one study it was suggested that the mechanism of Hb-induced contraction involved the release of endogenous prostaglandins (88,298).

The vasocontractile activities of hemolyzed RBCs on canine basilar arteries was studied *in vitro*. Chromatography demonstrated the vasoactive fraction to have a molecular weight of 40–45 kDa. Biochemical characterization suggested that contractile activity resided in a protein. Enzymatic digestion of the crude fraction enhanced its contractile activity. This was the only study to suggest a protein other than Hb might be involved (299).

Dog basilar artery was studied in tissue baths. Various blood fractions were prepared from blood. Fresh PRP and serum were most vasoactive and this vasoactivity decreased with incubation. The reverse occurred with RBCs, D-600 ($10^{-5}$ M) blocked all contractions and $10^{-6}$ M methysergide partially blocked incubated RBC contraction. Incubated RBC fractions passed through sephacryl S-200 column showed an active band at $5.5 \times 10^4$ Da. Electrophoresis provided a similar picture to that of Hb. Hb was progressively released into the incubated RBC fraction as judged by spectrophotometry (88). Ghost-free hemolysate of RBCs was found to constrict the basilar artery of cats. Both hemolysate and purified Hb constricted the basilar artery in a concentration-dependent fashion (300). Structural changes have been observed, including the presence of lysing RBCs in the external adventitia of dogs following induced SAH (301). Evidence does not exist that the RBCs could actually penetrate through the adventitia into the media.

Fresh dog and human pial arteries were exposed to various hemolysate preparations. Dose-dependent contraction of vessels to hemolysate was as follows: monkey > dog > human. Storage of arteries for 3 days at 40 °C reduced the constriction to 5-HT and hemolysate more than the constriction to $K^+$. The changes in tension were greater in more proximal arterial segments than in distal ones. Larger arteries also constricted more extensively than smaller ones. The apparent lesser responsiveness of the human arteries might have been due to the fact that, unlike the animal ones, they were taken from distal locations (302). Studying dog basilar arteries *in vitro*, it was concluded that hemolysate-induced contractions were independent of endothelium and were inhibited by hydroquinone, which is an inhibitor of endoplasmic reticulum $Ca^{2+}$ ATPase-like thapsigargin (303). Canine basilar arteries were studied *in vitro*. Hemolysate of washed RBCs was prepared by hypotonic shock, oxyHb by ion-exchange chromatography, and metHb by oxidation of Hb by $NaNO_2$. The hemolysate was shown by spectrophotometry to contain oxyHb. Vasoconstriction induced by hemolysate was abolished by treating it with $NaNO_2$. The hemolysate contraction was 30% of the maximum resulting from $K^+$. Hemolysate contractions were not affected by $10^{-6}$ M phentolamine, $10^{-6}$ M atropine, $10^{-6}$ M cholorpheniramine, $10^{-6}$ M ketanserin, and $3 \times 10^{-7}$ M tetrodotoxin. Endothelial removal reduced the response to hemolysate (62). In Peterson *et al.*'s *in vitro* studies of both control and vasospastic canine basilar arteries, the vasoactivity of hemolysate was variable. Most vessels were somewhat reactive but some were completely unreactive to high concentrations. They considered that some modest vasoactivity could be ascribed

to non-Hb factor in hemolysate but believed that Hb is principally responsible (304).

The contractions in rabbit basilar arteries were considerably less to purified oxyHb than to hemolysate containing oxyHb. A low-molecular-weight fraction of hemolysate, ranging from 0.5 to 2.0 kDa, elicited only a mild contraction on its own, but combined with purified oxyHb significant contractions were generated. It was suggested that low-molecular-weight components in hemolysate of this weight range, although incapable of inducing a potent contraction alone, could act in concert with oxyHb (305). Mitogen-activated protein kinase is a signaling factor in vascular contraction and proliferation. Preincubation of rabbit arteries with the kinase inhibitor PD-98059 markedly reduced contractions normally induced by hemolysate. The tyrosine kinase inhibitor AG-490 also reduced the contractile response to hemolysate when used to preincubate the arterial rings. Hemolysate produced a time-dependent elevation of mitogen-activated protein kinase immunoreactivity as seen on Western blots of rabbit basilar artery. Enhancement of kinase was maximal 5 min after exposure of the rings to hemolysate (306).

### 2. *In Vivo Studies*

It seems likely that the arterial response to subarachnoid blood is dependent on both the duration of exposure and the amount of blood (307). Autologous cat blood was injected into the cisterna magna and 3 days later the basilar was exposed and tested with applications of various compounds. There was a 30–40% constriction which resulted from incubated blood–CSF mixture, methemalbumin, catalase, NADH, and metHb. A greater constriction (50%) resulted from fresh, lysed RBCs. Hematin, hemin, or bilirubin had no vasoconstrictor activity (308). Prepontine injection of whole blood or RBC blood was performed in cats. Seven or 10 days later the vessels were perfused and fixed. Instillation of whole blood produced lumenal narrowing associated with profound ultrastructural changes in all layers of the vascular wall. There were no significant alterations in the wall of arteries bathed in RBC free blood (309). PRP formed durable clots in the basal cisterns surrounding the basilar artery and provoked no vascular reaction in 3 days or more. Freshly isolated autologous RBCs resuspended in PRP likewise produced no VSP. Hemolyzed fresh RBCs led to a severe vascular response after introduction into the basal cistern using PRP as the carrier/clotting medium. The same response resulted from the injection of intact RBCs incubated *ex vivo* for 72 hr. Resolution of the initial reaction was rapid for hemolysate but slow and, depending on the hematocrit, incomplete for intact "aged" RBCs. It was concluded that the rate of lysis of RBCs in the subarachnoid clot is a major factor in the genesis of VSP post-SAH (310) (Fig. 6.10). In Peterson *et al.*'s experiments, PRP with a reconstituted hematocrit of isolated, washed RBCs produced VSP with a time course and severity like that of whole blood (310). They noted that severe cerebral arterial constriction could be produced *in vivo* using subarachnoid clot of PRP if exogenous pyrogenic materials were added. These promoted a severe inflammatory reaction to the subarachnoid clot (304) (Fig. 6.11). In all their studies the cytosolic component of lysed RBCs appeared solely responsible for the vasoactivity.

Suzuki and colleagues centrifuged blood in the presence of sodium citrate. The RBCs were divided into three groups and diluted to achieve hematocrits of 30, 50, and 70%. The PRP fraction was adjusted to contain approximately $7.5 \times 10^8$ platelets. Acute and chronic VSP was induced by the double injections of these preparations on days 1 and 3. The VSP was demonstrated angiographically on day 7. Whole blood produced a greater amount of constriction than either the RBC or PRP fractions. The constriction produced by the RBC fraction increased progressively with the hematocrit (311). In the rat femoral artery model whole blood, washed RBC, or WBCs in PRP were selectively applied to the adventitial surface of the femoral artery for 7 days. There was a prominent reduction in lumenal cross-sectional area after 7 days in vessels exposed to whole blood or washed RBCs but not in those exposed to WBCs in PRP. Arterial narrowing increased progressively until 7–10 days and returned to nearly control levels by 20 days. The presence of ultrastructural changes corresponded to the degree of arterial narrowing (312). Using injections of blood components, in dogs PRP and platelet-poor plasma produced only early VSP and no arterial narrowing was observed on days 1, 3, or 7 postinjection. On the contrary, intracisternal injections of washed RBCs (0.1 ml/kg body weight) produced no arterial narrowing 6 hr after injection and induced moderate arterial narrowing on days 1, 3, and 7 after injection. Hemolysate containing 10 g/dl concentration of Hb produced prolonged monophasic arterial narrowing after injection. The results supported the concept that RBCs are required for late, prolonged arterial narrowing post-SAH (313). Also, from Mayberg's laboratory a porcine model of SAH was developed. Washed RBCs, WBCs plus PRP, Hb/cytosol, and RBC membranes were selectively applied to the MCA of pigs for 10 days. Chronic VSP as judged by marked reduction in MCA lumen cross-sectional area occurred after selective application of RBCs or Hb/cytosol but not after leukocyte/PRP or RBC membranes. In both RBC–Hb/cytosol-treated vessels, lumenal narrowing was associated with a relative increase in wall thickness adjacent to the subarachnoid space compared to wall thickness adjacent to the

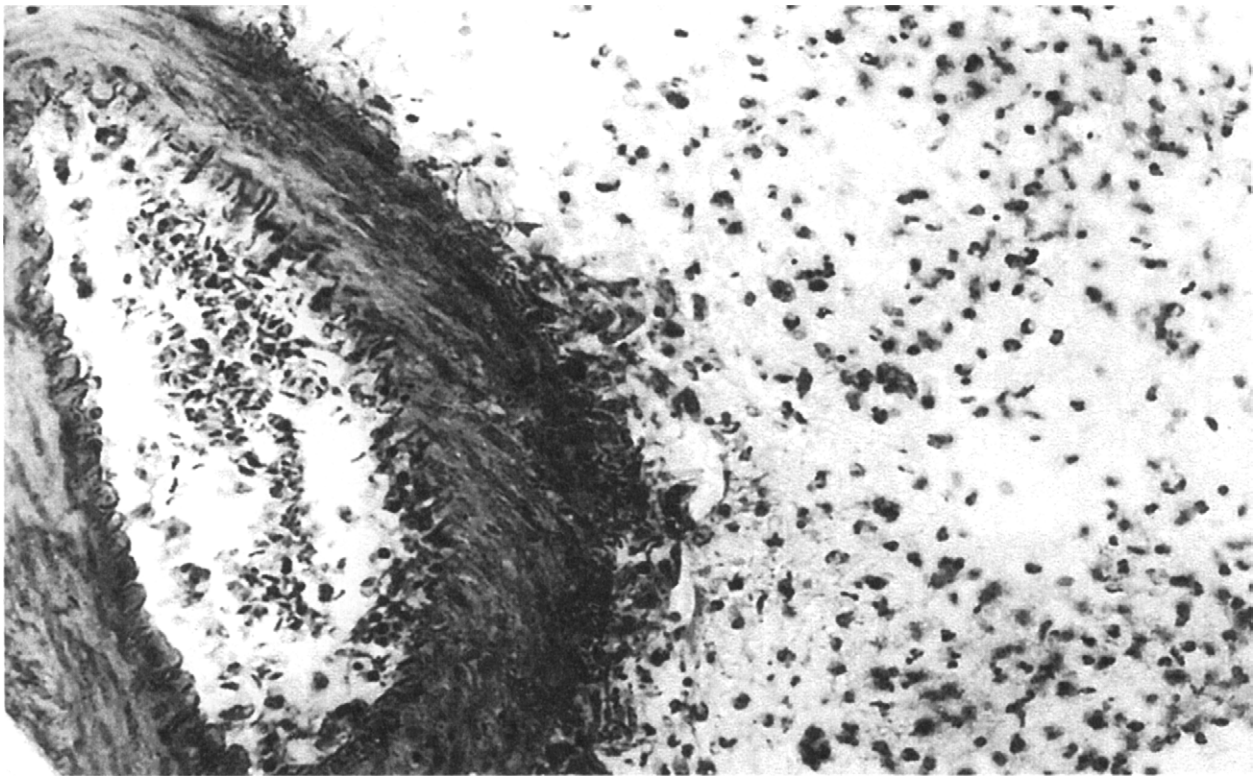

**FIGURE 6.10** Photomicrograph of the perivascular space of a branch of the basilar artery in an animal 48 hr after subarachnoid injection of a 15% hematocrit of "aged" erythrocytes. There is massive infiltration by inflammatory and immunoreactive cell types and almost a complete absence of intact subarachnoid erythrocytes [reproduced with permission from Peterson, J. W., Roussos, L., Kwun, B. D., Hackett, J. D., Owen, C. J., and Zervas, N. T. (1990). Evidence of the role of hemolysis in experimental cerebral vasospasm. *J. Neurosurg.* **72,** 775–781].

brain. Selective application of commercially available Hb to the MCA produced similar structural and morphometric changes. It was concluded that the degree of VSP is proportional to the volume of the RBC mass adjacent to the vessel at sacrifice (314).

### C. Studies of CSF after Subarachnoid Hemorrhage

The main studies of CSF contents conducted post-SAH were reviewed in detail in Chapter 4. The main candidates thought to cause VSP are summarized in Table 6.1. The vasoactive substance present in the greatest amount and released into the CSF around spastic blood vessels in the time course of VSP is oxyHb. OxyHb-induced contraction shows pharmacological properties similar to those of the agent found in xanthochromic CSF (315). Wilkins *et al.* conducted a series of experiments on rabbit aorta *in vitro*. Clear CSF showed only one example of vasoconstriction, whereas 10 of 30 xanthochromic CSF samples caused constriction (316). It was established using human basilar arteries that tachyphylaxis to 5-HT and NA developed rapidly. Cell-free plasma was inactive. Sonified PRP, which presumably released 5-HT, contracted arteries and this effect was abolished by a 5-HT blocker. The contractile effect of intact RBCs was greatly increased following cell lysis. Normal clear CSF was devoid of contractile activity (59). Numerous other studies have demonstrated that bloody CSF obtained after aneurysmal rupture can cause vasoconstriction in a large variety of experimental preparations *in vitro* and *in vivo*. The most vasoactive samples have generally been obtained around the time when VSP is maximal. Hb in most experiments has been measured as oxyHb and metHb. The latter has been detected later and in smaller amounts than oxyHb (45,292,298,317–329).

Two to four milliliters of autologous blood was injected into the cisterna magna of cats. Three days later, Ohmoto *et al.* exposed the basilar artery and tested 5-min applications of various substances. The most constriction occurred with oxyHb from fresh lyse

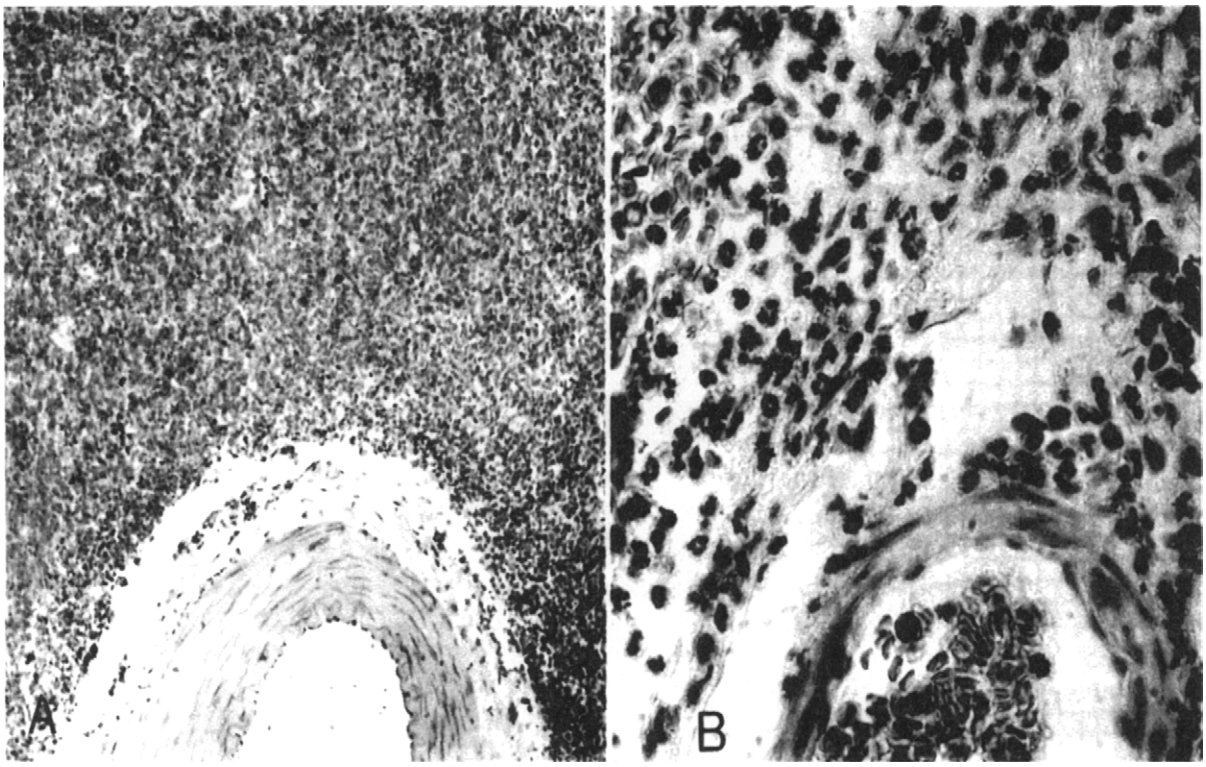


**FIGURE 6.11** Photomicrographs showing the perivascular space of the basilar artery 72 hr after subarachnoid injection of autologous whole blood. (A) Essentially normal populations of blood cells are seen. (B) There is some indication of inflammatory infiltration around smaller vessels [reproduced with permission from Peterson, J. W., Kwun, B. D., Hackett, J. D., and Zervas, N. T. (1990). The role of inflammation in experimental cerebral vasospasm. *J. Neurosurg.* **72,** 767–774].

RBCs (50%). A lesser degree of constriction (30–40%) followed the application of metHb, NADH, catalase, methemalbumin, and incubated blood–CSF mixtures. No constriction occurred with this short-term application of hematin, hemin, or bilirubin (308). Intracisternal injection of blood lacking RBCs does not produce angiographic VSP or structural narrowing (309). Some earlier workers isolated materials from bloody CSF which was vasoactive but did not appear to be identical to Hb (299,330).

When blood was incubated at body temperature in artificial CSF the rate of spontaneous hemolysis of RBCs was only 1% per day, becoming somewhat more rapid after 4 days. The rate of hemolysis of aging RBCs was dramatically increased, up to 1000-fold, by the addition of plasma proteins but only after the RBCs had aged 2 or 3 days or more *in vitro*. The age-dependent, plasma-induced hemolysis of originally autologous RBCs presumably involved activation of the plasma complement protein pathway (331).

Intraparenchymal injections of various blood components were performed in the basal ganglia of rats. Lysed autologous RBCs but not packed RBCs produced marked edema 24 hr after infusion, and this edema formation was mimicked by Hb infusion. Although infusion of packed RBCs did not produce dramatic brain edema during the first 2 days, there was a marked increase in brain water content 3 days postinfusion. Edema following thrombin infusion peaked at 1 or 2 days. This is earlier than the peak in edema formation that follows ICH, which suggests that there is a delayed, nonthrombin-mediated, hemogenic component of ICH that depends on the lysis of RBCs (332).

Samples of bloody CSF from patients with SDH, SAH, or postcraniotomy for tumor were all shown to be active vasoconstrictors but clear human CSF obtained during myelography was devoid of activity (88). Bloody CSF appears to be able to contract cerebral arteries studied *in vitro* regardless of the presence of VSP. Blood products have a spasmogenic activity on cerebral arteries, although other arteries may be less sensitive (44,333). ATP and A23187 but not ACh caused potent endothelium-dependent relaxations in monkey and human cerebral arteries. Pretreatment with CSF from SAH patients

suppressed the relaxations to ATP. The degree of inhibition was greater when the CSF was obtained from patients with symptomatic VSP (334). Intracisternal injections of various blood components were performed in dogs and the resulting degree of meningeal irritation, fever, and cellular response in spinal fluid was assessed. Autologous fresh whole blood and autologous serum of plasma incubated at body temperatures for 4–9 days caused no clinical signs of meningeal irritation and only a mild cellular response in the CSF. Autologous whole blood or packed RBCs incubated at body temperature produced profound responses which increased as the incubation period exceeded 3 days. OxyHb and bilirubin both caused severe responses. It was concluded that the agent in blood causing the greatest meningeal response was present in the heme component (335).

A wealth of experimental data support the observation that the duration of exposure of blood to the vessel adventitia is of importance for the development of pathologic changes within the vessel wall. Intracisternal blood injections seldom produce morphologic changes in vessels unless multiple injections over several days are employed (301,336–338) or clot is formed which persists to the time of angiography and sacrifice (301,337). Partially purified protein from hemolyzed RBCs was injected into the cisterna magna of dogs and produced marked angiographic VSP of the basilar artery. Relaxation occurred by days 10–14 (339). The prepontine injection of various whole blood components was performed in cats which were studied to sacrifice at 7 and 10 days. Whole blood caused ultrastructural degenerative changes in the smooth muscle cells of feline arteries. Injection of serum and RBC-free blood did not produce any ultrastructural changes (309). Adult cats were subjected to prepontine injection of autogenous whole blood or RBC-free blood containing latex beads as markers. Profound ultrastructural changes in all layers of the muscle wall resulted from the whole blood injections but no significant alterations occurred in arteries bathed in RBC-free blood, showing that the other blood elements did not have an autogenous role (309). In a porcine model various blood components were applied to the MCA for 10 days. Morphometric analysis showed significant reduction in lumenal area with Hb, RBC cytosol, RBC hemolysate, and whole blood. There was no chronic VSP with WBCs plus PRP. The reduction in lumenal diameter was accompanied by a minimal (12%) increase in wall area and this only occurred with the whole blood fraction (314).

Single intracisternal injections of different blood components were performed in dogs. PRP and platelet-poor plasma produced decreases up to 80% of control diameter by 30 and 60 min but were normal by day 1. Whole blood produced VSP of 61% by 30 min and then relaxation and a subsequent 50% constriction by day 3. RBCs also caused biphasic change with 67% reduction by 30 min, relaxation, then 67% VSP by day 3 which did not release until 21 days. Hemolysate produced monophasic constriction to 63% which lasted for 14 days (313). Cisterna magna injections of various blood products were performed in dogs and the basilar artery diameter was determined by angiography on day 3. PRP produced no vascular reaction. Fresh autologous RBCs only produced VSP following a second injection on day 3. Hemolysate of RBCs produced immediate severe constriction, as did intact RBCs incubated *ex vivo* for 72 hr. *In vitro* measurements of hemolysis showed variable rates of cell dissolution but in general a progressive hemolysis over time corresponded to the way in which clots disappear from the subarachnoid space. It was concluded that hemolysis was the key to VSP (310). Local application of various blood or tissue components was performed on the MCA of primates. Sham-operated and WBC groups had no VSP. Collagen gave 17% reduction in diameter, which was not significant. PRP induced a 33% decrease in lumen by day 2 with a slow return to normal by day 14. Severe VSP was the result of whole blood (51%) and RBC (72%) application. It was concluded that RBCs were essential for VSP in primates (340).

### D. Thrombin

Thrombin can produce sustained contraction of smooth muscle more intense than that from 5-HT. Thrombin contraction is insensitive to most blocking agents (68,151,341). In rabbit cerebral arteries, however, there was only minimal response to thrombin (342). Increased concentrations of thrombin elevated ET-1 production in pig endothelial cells in a dose- and time-dependent fashion. Treatment of cells with cycloheximide, a protein synthesis inhibitor, inhibited the extracellular accumulation of ET-1 (278). ET was detected in the incubating medium of unstimulated pig aortae but not if the endothelium was removed. In preparations with endothelium, thrombin and the calcium ionophore A23187 stimulated the release of ET. The basal and thrombin-stimulated production of ET was prevented by the protein synthesis inhibitor cycloheximide. The production of ET upon stimulation with thrombin was potentiated by $N^G$-monomethyl-L-arginine (L-NMMA) and methylene blue and reduced by cGMP. The basal release of the ET peptide was unaffected. It was concluded that endothelium-derived NO released during stimulation with thrombin inhibits the production of ET via a cGMP-dependent pathway (343). The normal life span of an adult human endothelial cell is considered to be 30 years. Regenerated endothelial cells lose some of their ability to release

EDRF, in particular in response to platelet aggregation in thrombin. Hypertension and atherosclerosis may cause a decrease in EDRF release in response to a variety of stimuli (143).

### E. Fibrin and Fibrinogen Degradation Products

A dramatic rise in fibrinogen occurs with a similar time course to VSP (344). Fibrin degradation products in a concentration of $6 \times 10^{-6}$ to $5 \times 10^{-2}$ g/ml were vasoactive on canine basilar arteries. Low-molecular-weight fractions were more potent than higher molecular weight fractions. The vasoactivity of supernatants of blood CSF combinations following 7 days of 37°C incubation was attenuated by a fibrin degradation product (345). Vasospastic activities may result in the degradation products of fibrin and fibrinogen, and these products may interact with other spasmogens resulting in mutual enhancement (346–348).

### F. Bilirubin

RBCs mixed *in vitro* with CSF did not produce bilirubin (349). OxyHb released after SAH is transformed to bilirubin by an *in vivo* heme oxygenase produced by the cells of the arachnoid and choroid plexus. It has been hypothesized that if the volume of SAH is too great the heme oxygenase system is overwhelmed and nonenzymatic autooxidation of oxyHb to metHb with the release of superoxide radical occurs (350). Injection of 1 ml of blood into the cisterna magna of dogs results in bilirubin appearing quickly in the CSF, and its level peaks about day 8 postinjection (351). Bilirubin in the CSF is present in the same ratio to CSF proteins as that of serum bilirubin to serum proteins. CSF bilirubin is bound to albumin almost exclusively (352). Bilirubin is normally found in CSF (0.07 μm/liter), whereas there is no Hb (353). It is increased after SAH, below subarachnoid space blocks and in jaundice. It was found to be present by days 3 or 4 post-SAH, increasing over the next week and disappearing by weeks 2 or 3 (354). Bilirubin injected into subarachnoid space of dogs in doses of 4–9 mg caused severe clinical meningism, fever, and leukocytosis in the CSF. Smaller doses were ineffective (335).

Made in distilled water, bilirubin induced strong constriction of cat, dog and pig cerebral arteries (355). Different concentrations of bilirubin suspended in ringer's lactate solution and topically applied to exposed cat arteries produced vasoconstriction and widespread histologic changes in endothelium and smooth muscle. The effect did not seem to vary with the dose of bilirubin employed (356). Topical application of bilirubin to exposed rat basilar artery caused little constriction (357). Bilirubin is relatively insoluble in water (358). Injection of bilirubin into the CSF of primates failed to produce chronic VSP (315). Elevated levels of bilirubin occur in various forms of jaundice without the development of VSP.

### G. Iron

Exposed dog basilar artery was used to study various blood derivatives. Ferrous chloride caused vasodilation and ferric chloride caused mild but definite constriction. This is not consistent with the known vasoconstriction resulting from oxyHb but not metHb (359). In early studies, it was postulated that Hb breakdown products including iron may be important in the genesis of VSP (360,361). The iron chelating agent deferoxamine inhibited delayed VSP in the rat femoral artery model (362). This compound can also inhibit the effects of oxyHb on intracellular $Ca^{2+}$ levels in vascular smooth muscle cells (363).

### H. Adenosine Triphosphate

RBCs are rich in this compound. Evidence for a potential role in VSP is given in Table 6.8.

## IX. Hemoglobin

### A. Overview with Subarachnoid Hemorrhage

Oxyhemoglobin is likely to be the principal pathogenetic agent of VSP (364) (Table 6.9). When blood is incubated *in vitro* there is a slow hemolysis of the RBCs with a resultant release of oxyHb into the supernatant fluid. This breakdown becomes more intense over a couple of days. *In vivo* heme is transformed into bilirubin with time, whereas *in vitro* oxyHb is oxidized to metHb alone. The chemical mechanism by which heme groups are converted into bilirubin is still not precisely defined. There is no doubt that RBCs contain a vasoactive substance or substances that are released by hemolysis. There has clearly been an evolutionary advantage to the vasoconstriction results from a breech in the continuity of the vascular system. Many investigations have demonstrated that incubated and mixtures of whole blood, blood components, and CSF will constrict a wide variety of blood vessels from many different species. Serum, PRP, and lysed RBCs have significant vasoactivity, whereas fresh, intact RBCs are inert. With prolonged incubation, however, lysed RBCs develop contractile activity, whereas serum and PRP lose their effect. The vasoacitvity of blood incubated *in vitro* for a similar time course to the development and duration of VSP in man has been shown to reside in

**TABLE 6.8 Evidence of a Low-Molecular-Weight Spasmogen, Possibly ATP[a]**

| |
|---|
| **For** |
| Isolated rat basilar artery smooth muscle cells develop increased $[Ca^{2+}]i$ in a dose-dependent fashion when exposed to a low-molecular-weight fraction from fresh human erythrocyte hemolysate, but the effect diminishes with time and is absent after 21 days of incubation |
| Effect was potentiated by a high-molecular-weight fraction of hemolysate and also by pure hemoglobin that did not affect $[Ca^{2+}]i$ independently |
| Erythrocytes contain ATP (1.6 mmol/liter) in concentrations that contract vascular smooth muscle |
| Rat femoral arteries contract after 7 days of exposure to substances containing ATP such as dog hemolysate or ATP itself but not pure hemoglobin |
| Chronic vasospasm in monkeys resulted from subarachnoid placement of ATP, hemolysate, or pure hemoglobin |
| Dog hemolysate containing ATP (34 μmol/liter) produced concentration-dependent contractions of dog basilar artery that were inhibited by suramin, a $P_{2u}$ purinoceptor antagonist |
| Hemolysate increases $[Ca^{2+}]i$ in both rat basilar artery smooth muscle and bovine middle cerebral artery smooth muscle by releasing $Ca^{2+}$ from internal stores and causing $Ca^{2+}$ entry by voltage-independent $Ca^{2+}$ influx—effects that are identical to those of ATP |
| Nucleotides such as ATP and UTP activate G proteins coupled to $P_{2u}$ purinoceptors to mobilize $[Ca^{2+}]i$ in rat basilar artery smooth muscle cells |
| **Against** |
| Levels in human CSF after SAH decrease rapidly and after several days are negligible |

[a]From Weir, B., Stoodley, M., and Macdonald, R. (1999). Etiology of cerebral vasospasm. *Acta Neurochir.* **72,** 27–42. Copyright © Springer-Verlag GmbH & Co.

the RBCs. Selective application of washed RBCs, RBC cytosol, and pure Hb all cause significant VSP, whereas leukocytes and PRP do not. Many experiments have shown that the component in RBCs responsible for vasoconstriction is a peptide with a molecular weight, spectrophotometric absorption pattern, and electrophoretic movements similar or identical to those of oxyHb (315, 364).

**TABLE 6.9 Evidence for and against a Role for Oxyhemoglobin in Vasospasm[a]**

| |
|---|
| **For** |
| Thick perivascular blood clot causes severe chronic vasospasm and hemoglobin is the principal component which is progressively released as erythrocytes lyse within CSF |
| Oxyhemoglobin inhibits endothelium-derived relaxing factor (NO) by binding to it and/or by producing $O_2^{-\bullet}$ that destroys it |
| Oxyhemoglobin stimulates release of vasoconstricting ET from endothelial cells |
| Oxyhemoglobin stimulates release of vasoconstricting prostaglandins from endothelial cells |
| Oxyhemoglobin, even from different species, can constrict arterial rings and strips of both cerebral and systemic arteries *in vitro* |
| Oxyhemoglobin can autooxidize to release $O_2^{-\bullet}$ that can produce $OH^{\bullet}$ by reacting with iron released from hemoglobin |
| Hemoglobin damages perivascular nerves of all types |
| Hemoglobin has synergistic effect with other vasoconstrictors, such as $K^+$, ATP, serotonin, fibrin degradation products, and hypoxia |
| Hemoglobin increases $[Ca^{2+}]i$ and can cause isolated vascular smooth muscle cells to contract |
| Hemoglobin has been shown immunohistochemically within spastic vessel walls after periadventitial blood injection |
| As oxyhemoglobin is metabolized it can produce other potential vasoconstrictors such as hemin, iron, and bilirubin |
| **Against** |
| Hemoglobin usually contains trace amounts of endotoxin, stromal proteins, and phospholipids that can also cause vasoconstriction and inflammation |
| Most studies have been done on vessel rings or strips *in vitro* using impure hemoglobin |
| Studies show that hemoglobin is not a very potent contractile agent but that its potency can be increased by combination with low-molecular-weight components of erythrocytes |
| Pure human oxyhemoglobin did not produce severe vasospasm in monkeys |

[a]From Weir, B., Stoodley, M., and Macdonald, R. (1999). Etiology of cerebral vasospasm. *Acta Neurochir.* **72,** 27–42. Copyright © Springer-Verlag GmbH & Co.

Cerebral arteries and isolated smooth muscle cells from many different species contract when in contact with Hb *in vitro* (297,298,345,365–369). Hb causes slowly developing and long-lasting contraction not only of cerebral arteries but also of many systemic arteries and other smooth muscle tissues (60). OxyHb has been shown to be a much more potent vasoconstrictor than metHb (61, 62, 154, 297, 365, 370). In comparative studies cerebral arteries have usually been found to be more responsive to oxyHb than systemic ones (1,61,154,371). OxyHb in pure form can contract cerebral arteries (61,369,372). The contraction is sustained. MetHb is essentially devoid of activity (154). Commercial samples of Hb are often contaminated by metHb. It has become common practice to use dithionite reduction followed by dialysis to prepare oxyHb in a relatively pure form (373).

### B. Biochemistry

Hemoglobin is a protein in which polypeptide chains are folded into a compact, globular shape. In this respect it resembles nearly all of the other 2000 or more enzymes. Hemoglobin possesses single iron-porphyrin or heme groups and is the oxygen-binding protein of RBCs and imparts the deep red-brown color to hemoglobin. Heme consists of a complex organic ring structure, protoporphyrin, to which an atom in the ferrous ($Fe^{2+}$) state is bound. The iron atom has six coordination bonds. Four of these connect to the flat porphyrin molecule and two are perpendicular to it. One of these is filled by a nitrogen atom of a histidine residue of the globin and the other is the binding site for an oxygen molecule. The tertiary structure of globular proteins is maintained by four different forces: hydrogen bonding between R groups of residues in adjacent loops of the chain, ionic attraction between oppositely charged R groups, hydrophobic interactions, and covalent crosslinkages. Hemoglobin contains disulfide crosslinkages, and it is an oligomeric protein containing four separate polypeptide chains. Its molecular weight is 64.5 kDa (374). The protein portion which is the globin consists of two α chains of 141 residues each and two β chains with 146 residues each. The hemoglobin molecule is roughly spherical with a diameter of about 5.5 nm. Each of the four chains has a specific tertiary structure in which the chain is folded. The four chains fit together in an approximately tetrahedral arrangement to constitute the characteristic quaternary structure of Hb. One heme group is bound to each chain. The hemes are situated 2.5 nm from each other and tilted at different angles. They are partially buried in a pocket lined with hydrophobic R groups. The heme is bound to its polypeptide chain through a coordination bond of the iron atom to the R group of a histidine residue. The six coordination bonds of iron atom in each heme are available to bind a molecule of $O_2$. There is little direct contact between the two α chains or between the two β chains, although there are many contact points between the α and β chains of the dissimilar chain pairs $\alpha_1\beta_1$ and $\alpha_2\beta_2$. These contacts are mainly hydrophobic R groups of amino acid residues. The α and β chains of Hb have nearly identical tertiary structures. Both are over 70% α-helical in character. The Hbs of many different vertebrates have similar tertiary structure of their polypeptide chains and the quaternary structures also closely resemble each other. The Hb of 100 ml of whole blood binds about 20 ml of gaseous $O_2$. In addition to transporting $O_2$, Hb also carries two end products of tissue respiration, $H^+$ and $CO_2$. Hb transports approximately 20% of total $H^+$ and $CO_2$ formed in the tissues to the lungs and the kidneys. $CO_2$ is bound by the α-amino group at the amino-terminal end of each of the four polypeptide chains of Hb to form carbaminoHb. DeoxyHb has a slightly different shape than oxyHb. When $O_2$ is bound to the heme groups of deoxyHb the $\alpha_1\beta_1$ and $\alpha_2\beta_2$ halves of the molecule, while remaining rigid, change their position slightly with respect to each other and come closer together. There is an inverse relationship between the binding of $O_2$ and the binding of 2,3-BPG. In binding to deoxyHb, 2,3-BPG forms a crosslink or bridge between the two β subunits. The four ligands for Hb are therefore $O_2$, $CO_2$, $H^+$, and 2,3-BPG. The other three ligands influence the affinity of Hb for $O_2$ (374).

The symbol Hb is used for unoxygenated or reduced Hb, and $HbO_2$ is used for oxyhemoglobin. The same ferroprotoporphyrin is present in all vertebrates. Differences between Hbs from various species depend on the protein moiety which confers differences in crystalline form, solubility, amino acid content, oxygen affinity, and absorption spectra. All the myriad of hemoglobins are tetramers consisting of four peptide chains, to each of which is bound a heme group. Human Hb is usually constructed by the combination of two α chains with two β, γ, or δ chains. Normal human adult hemoglobin, called Hb A, consists of two α and β chains (375).

### C. Heme, Hemin, and Hematin

$HbO_2$ derives its stability of iron in the $Fe^{2+}$ form because the heme moiety lies within a cover of hydrophobic groups of the globin. When Hb is oxidized metHb containing $Fe^{3+}$ is formed. MetHb cannot bind $O_2$ or $CO_2$. Since it is positively charged because of the additional charge on the $Fe^{3+}$ iron, it can combine with hydroxide ion in alkaline solutions or with chloride or

other anions in acidic solutions (heme becoming hemin). The treatment of oxyHb with oxidizing agents such as ferricyanide, quinones, or peroxides causes the complete liberation of the bound $O_2$ and conversion to metHb (375).

The iron compounds of Hb can exist in different forms which depend on the valence of the iron. In heme, the ferroprotoporphyrin contains divalent iron and the group possesses no net charge. Heme exists in a square-planar form. Hemin is ferriprotorphyrin, the iron is $Fe^{3+}$, and the group has a net positive charge and adds an extra ligand to it. It is usually obtained as the chloride. The resulting penta coordinate complex is square–pyramidal, with the extra ligand situated perpendicular to the porphyrin plane. The chloride is bound coordinately to the $Fe^{3+}$. Free heme is unstable and rapidly oxidizes to hemin. If hemin is dissolved in excess alkali and then titrated with acid, the resulting neutral compound is known as hematin (375). In biological systems oxyHb tends to spontaneously oxidize to metHb, from which hemin readily dissociates (376,377).

The effect of NO synthesis on rat aortic smooth muscle cell culture was estimated by measuring accumulation of nitrite and nitrate, oxidative products of NO. Hemin in a dosage of 1–100 μm increased the levels of nitrite and nitrate in a dose- and time-dependent fashion. Both NOS inhibitors and protein synthesis inhibitors significantly inhibited the hemin-induced elevations of nitrite and nitrate. Apparently, hemin is capable of stimulating the expression of an inducible isoform of NOS in vascular smooth muscle (379). Hb is capable of augmenting IL-1β-induced NO production in vascular smooth muscle cells (380). Once released from RBCs deoxyHb undergoes spontaneous, nonenzymatic oxidation (not oxygenation) to metHb. When the iron becomes oxidized to the $Fe^{3+}$ state the resulting hemin more rapidly separates from globin than heme would from globin (376,377). In a canine model, clot aged for 7 days in the subarachnoid space showed a total (bound and unbound) hemin content of 390 ± 247 μ*M* (378).

### D. *In Vitro* Studies

#### *1. Animal*

The short-term effects of oxyHb on the cerebral arteries of many different preparations have been universally reported as vasoconstriction (54,293,298,300,308, 357,359,381–385). OxyHb has always been known to be more vasoactive than metHb. Hemolysate is usually more potent than oxyHb. There are many more effective vasoconstrictors than oxyHb, but it is present in enormous concentrations for many days adjacent to vessels which are in VSP. The concentrations of oxyHb which have been used in most short-term *in vitro* and *in vivo* studies have ranged from $10^{-8}$ to $10^{-2}$ M. Concentrations of $\geq 10^{-3}$ M usually produce maximal contraction (11,60,61,154,295, 302,365,370,372,386–388). By actual measurement Hb concentrations in subarachnoid hematomas have been shown to be high near spastic arteries (298).

Lysed RBCs produce long-lasting vasoconstriction of exposed rat basilar arteries. This was 30% for hemolysate and 19% for $3.7 \times 10^{-4}$ M oxyHb. The constriction was directly proportional to the concentration of the solution. In this key early study from experiments with gel filtration, Chokyu concluded that heme component is the cause of VSP and that bilirubin causes little constriction (357). Using dog basilar artery *in vitro* it was demonstrated that serum was vasoactive in fresh state but became inactive after 4 days of incubation. Methysergide also blocked the vasoactivity of the fresh serum. Vasoactivity resulting from whole blood incubation and incubated blood CSF mixture showed progressive increases in activity peaking after approximately 7 days. This constriction was a tonic tension on which vasocontractions were superimposed. These were observed to last for hours. Hypoxia markedly enhanced the contractions. Hb concentration as measured by spectrophotometry increased steadily following incubation. The 7-day incubate was purified in Sephadex G-100 columns. Injection of isolated peak 3 with a positive ninhydrin reaction into the cisterna magna of dogs caused marked VSP lasting over 2 weeks. Further biochemical analysis of peak 3 using cellulose ion-exchange resin and disc electrophoresis showed that the vasoactivity was due to a polypeptide closely allied to or identical with oxyHb (389).

In studies of exposed cat basilar artery, vessels were observed for 60 min. RBCs and serum were inactive and fresh RBCs and PRP caused transient vasoconstriction only. Supernatant from stored RBC mixtures was most active after 7 days of incubation and minimally active at 3 and 15 days and following the start of storage. Seven-day supernatant lost its activity when heated for 10 min to 100°C. Ultrafiltration showed the spasmogen to have a molecular weight of >10 kDa. Chromatographic analysis suggested that the spasmogen had a similar spectrophotometric spectrum to oxyHb. It also showed the same movement on polyacrylamide gel disc electrophoresis as generic Hb, day-7 supernatant had an Hb concentration of 5.8 g/dl. Thirty-seven percent of spasm resulted from the topical application of Hb compared to only 1% with metHb (293). Dog cerebral arteries and other smooth muscle preparations were exposed to bovine Hb $10^{-4}$ to $10^{-6}$ M which contracted all except vas deferens in a dose-dependent fashion. 5-HT was 7.5 times as potent as Hb. The Hb contractions developed slowly and were long-

lasting as opposed to the tachyphylaxis which occurred with 5-HT. Hb contractions were not blocked by atropine or tetrodotoxin but were partially blocked by $5 \times 10^{-7}$ M methysergide, 16 μg/ml indomethacin, and $10^{-7}$ M D-600, a calcium channel blocker (60). A series of early *in vitro* experiments concluded that the spasmogen is oxyHb and that cerebral arteries are particularly sensitive to it (61).

Human Hb, prepared from human RBCs and shown to be pure oxyHb by spectrophotometry, was applied to canine arteries *in vitro*. OxyHb ($10^{-8}$ to $10^{-4}$ M) produced a dose-dependent contraction of cerebral but not systemic arteries. The dose–response curve for pure Hb was the same as that for hemolysate. It was suggested that most of the effect of hemolysate is due to Hb. There was no contraction from Hb in $Ca^{2+}$-free fluid. Papaverine ($10^{-6}$ M) also inhibited Hb contractions. MetHb and cyanometHb (ferric compounds) had no vasoconstrictor effect and neither did ferric or ferrous chlorides, globin, protoporphyrin, or hematin after 5-min applications of up to $10^{-4}$ M (61). Rabbit and canine basilar arteries were studied *in vitro* and dose-dependent contractions were induced by human Hb solutions. The dog arteries were more responsive than the rabbit ones. ACh evoked a dose-dependent vasodilation of rabbit basilar artery precontracted with $10^{-6}$ M 5-HT. This vasodilation was endothelium dependent. Hb inhibited this ACh-induced, endothelium-dependent vasodilation in proportion to the dose. ATP relaxed isolated rabbit basilar artery previously precontracted with $10^{-6}$ M 5-HT. ATP-induced vasodilation was thought to be composed of ATP, which is an endothelium -dependent and ATP-degradation products such as AMP and adenosine that may be endothelium-independent. Hb markedly inhibited ATP-induced vasodilation (390).

Cat MCA was shown to be more responsive to Hb than mesenteric artery. The reverse was the case with NE and 5-HT. A strict relationship was observed between ACh- and Hb-induced contraction of both MCA and mesenteric arteries. It was postulated that a muscarinic cholinergic mechanism may be involved in the vasospastic effect of Hb. The usage of a pharmacologically high concentration of ACh was necessary to overcome the indirect, endothelium-mediated vasodilatory effect of ACh. Atropine significantly attenuated the constrictor effects of Hb in this preparation (391). Japanese monkey and dog cerebral arteries were compared to systemic vessels *in vitro*. Removal of endothelium by rubbing was performed. OxyHb caused dose-dependent contraction of monkey and dog cerebral arteries. Monkey systemic arteries were almost insensitive to oxyHb and dog systemic arteries were only minimally reactive. Removal of endothelium markedly diminished the contractions. In arteries with endothelium, SOD and catalase alone or together did not affect the oxyHb-induced constriction. Contraction to oxyHb by those arteries possessing endothelium was markedly decreased by indomethacin and diphloretin. Toda suggested that oxyHb-induced contraction is associated with a mechanism dependent on the endothelium but not related to EDRF (366). Slowly developing contractions to oxyHb occurred in isolated monkey, dog, and bovine cerebral arterial strips. Indomethacin ($10^{-6}$ M) inhibited the oxyHb-induced monkey arterial contraction. The same degree of inhibition occurred with diphloretin phosphate ($10^{-5}$ M) but there was only a slight reduction with aspirin ($10^{-5}$ M) (392).

### 2. *Human Studies*

The *in vitro* responses of basilar arteries from dog, rabbit, and man were compared using concentrations of $10^{-9}$ to $10^{-5}$ M Hb. Compared with maximum 5-HT contraction, the relative constriction caused by $10^{-5}$ M Hb was greatest in rabbit and least in man. These differences could be due to postmortem changes in human arteries (372). Dose responses of canine basilar arteries to human Hb, rabbit Hb, horse myoglobin, and human metHb and cyanometHb were compared. The *in vitro* arterial segments showed similar responses to Hb and myoglobin when doses were based on the Hb dimer rather than on the tetramer (its normal state). Superoxide radicals produced by autoxidation of Hb to metHb do not seem to be involved in the mechanism of Hb-induced contraction since the contraction was not blocked by SOD or other agents known to react with superoxide-generated products. The authors rejected the possibility that heme proteins are taken up into smooth muscle cells by pinocytosis. They calculated that insufficient Hb dimer could be delivered to smooth muscle cells to account for observed rates of contraction (370). Human basilar arteries obtained many hours postmortem and canine basilar arteries were studied *in vitro* and shown to contract to 5-HT and $PGF_{2\alpha}$. The human basilar artery contracted to NE but not oxyHb, whereas dog basilar artery showed the opposite response (388). Cerebral arteries from dogs and monkeys and human pial arteries obtained during a lobectomy were exposed to hemolysate from lysed RBCs. Canine basilar artery responded *in vitro* to 5-HT, $K^+$, and hemolysate but after 3 days in the bath only contracted to $K^+$. All three species contracted well to hemolysate. Proximal monkey MCA contracted more vigorously than distal MCA (302). Surgically isolated penetrating intracerebral arterioles constricted and then dilated with application of ATP and ADP after both intraluminal and extraluminal application. Pretreatment with oxyHb constricted these arterioles to an average of 87% of control and blunted extraluminally induced dilation to ATP and ADP at low concentrations. Propagated vasodilation

along the arterioles was significantly attenuated by oxyHb (393).

### 3. *Intra- and Extraluminal Applications*

Canine internal and common carotid arteries were perfused *in vitro*. Abluminal application of $10^{-5}$ M oxyHb led to progressively increased perfusion pressures in the internal carotid but not common carotid artery. Intraluminal 5-HT-induced vasoconstriction was potentiated by extraluminal application of oxyHb (371). A perfusion system was created for canine basilar artery permitting intra- and extraluminal drug application *in vitro*. Bovine oxyHb ($2.5 \times 10^{-5}$ M) caused vasoconstriction as measured by increased perfusion pressure. This was slowly developing and difficult to reverse by washing. Addition of ACh did not cause vasodilation, and using saponin to destroy the endothelium did not change the drug responses suggesting that oxyHb had already inhibited the function of the endothelium. The responses of A23187 and thimerosal, which are EDRF agonists, were inhibited by oxyHb. It appeared as though oxyHb impairs some functions of EDRFs and independently causes vasoconstricition (369). A perfusion system was developed for the rabbit basilar artery. Intraluminal drug application was more potent than extraluminal application for Hb inhibition of endothelium-dependent relaxation and for calcium antagonist inhibition of constriction. Hb inhibition of endothelium-dependent relaxation was observed by either intra- or extraluminal application (394).

## E. Hemoglobin and Isolated Cells

### 1. *Hemoglobin and Vascular Smooth Muscle Cells*

Smooth muscle cells in culture undergo permanent ultrastructural changes following exposure to oxyHb for periods longer than 3 days (368). Using rat aortic media smooth muscle cells oxyHb and other agents were added during 2 weeks of incubation. OxyHb produced progressive contraction of the cells and also ultrastructural changes of myonecrosis. The culture medium was changed every 2 days. The myonecrosis was assumed to be due to some intrinsic process initiated by oxyHb (368).

Cultured rat arterial smooth muscle cells were exposed to $10^{-6}$ M oxyHb. Progressive changes in ultrastructure were noted starting after 24 hr characterized by shortening, vacuolation, degeneration, and loss of internal structure. Washout of oxyHb at 3 hr reversed the changes but had no effect after 24 hr of exposure. 5-HT, NE, and angiotensin II caused short-lived contraction but no ultrastructural changes (368). Rat aortic vascular smooth muscle cells were exposed to oxyHb or metHb following incubation with [$^3$H]-myoinositol. The various inositol polyphosphates were separated using HPLC and peaks were identified by use of label standards. OxyHb produced an elevation in Ins(1,4,5) $P_3$ which corresponded approximately to its ability to produce contraction. There were subsequent elevations in $InsP_4$ and Ins (1,3,4)$P_3$. It was suggested that the initial response to oxyHb arises at least in part from release of [$Ca^{2+}$] i by $InsP_3$ (395). Similar cells were exposed to $5 \times 10^{-6}$ M oxyHb which caused contraction, an increase in $Ca^{2+}$-dependent $K^+$ currents, an increase in plasma membrane permeability to ions, and cell death. MetHb was without effect. Catalase, which removed $H_2O_2$ and OH$^\bullet$, leaving only superoxide $O_2^{-\bullet}$ prevented the effect of oxyHb. SOD, which removed only $O_2^{-\bullet}$ and xanthine oxidase–xanthine systems, had no effect. Cells were also damaged from the generation of OH$^\bullet$ radicals. OxyHb had an apparent effect on neuroblastoma cells in culture (396). Such cells were exposed to human CSF samples. [$Ca^{2+}$]i was measured using the $Ca^{2+}$ indicator fura-2/AM. All samples induced higher transient [$Ca^{2+}$]i compared to control CSF. The elevations in [$Ca^{2+}$]i were greatest with CSF obtained on day 2 post-SAH (397). Rat basilar arterial smooth muscle cells were exposed to hemolysate, 5-HT, and leukotriene $C_4$, and contraction was evident. The PLC blocking agent NCDC and a myosin light chain kinase blocking agent ML-9 suppressed this augmented response. PKC activity in the cells did not increase during the period of culture with hemolysate. Hemolysate therefore caused acute but gradual cell contraction and augmentation of the cellular responses to vasoactive agents (398). The advantages of studying contractile mechanisms in cultured vascular cells are somewhat limited since the cells tend to undergo phenotypic changes that likely alter their physiologic responses. Cultured vascular smooth muscles usually change from a spindle-shape "contractile" phenotype to a more pleomorphic "synthetic" phenotype (213).

Cultured rat basilar smooth muscle cells were exposed to oxyHb and other substances. OxyHb and reactive oxygen species generated by xanthine plus xanthine oxidase elevated [$Ca^{2+}$]i. Procaine, which blocks $Ca^{2+}$-induced $Ca^{2+}$ release, did not inhibit the oxyHb-induced elevation of [$Ca^{2+}$]i. Ryanodine, which may open or close $Ca^{2+}$ release channels in the sarcoplasmit reticulum, plus oxyHb caused a markedly greater elevation in [$Ca^{2+}$]i than ryanodine alone. Thapsigargin, an ATP-dependent pump inhibitor, plus oxyHb had no additional effect when compared with thapsigargin alone. The oxyHb-induced elevation of [$Ca^{2+}$]i was blocked by an $Fe^{2+}$ chelator (ferene) but not by an $Fe^{3+}$ chelator (deferoxamine mesylate). The addition of a thio-reducing agent

caused a significant reduction in the peak oxyHb-induced increase in $[Ca^{2+}]i$. This suggested that $OH^{\bullet}$ radicals induced the elevation in $[Ca^{2+}]i$ by inhibiting the ATP-dependent $Ca^{2+}$ pump in the sacroplasmic reticulum, and that treatment with thiols may prevent an activation of this pump by inhibiting the oxidation of membrane sulfhydryl groups. OxyHb did not affect the levels of NO metabolites in the culture medium (399). When rat aortic smooth muscle cells were exposed to RBC hemolysate *in vitro*, mRNA levels were determined by competitive RT-PCR. Message levels for c-*fos*, *jun*B, and c-*jun* were increased in the presence of hemolysate and reached maximum expression at 30 and 60 min. The level of *jun*B mRNA was unaffected. The c-*fos* and *jun*B levels were increased with increasing doses of hemolysate. Whole hemolysate caused larger increases in c-*fos* expression than ATP or Hb alone. All of the c-*fos* mRNA-inducing activity of hemolysate was shown by size fractionation to reside in the molecules greater than 6 kDa. Multiple high-molecular-weight components present in RBCs have synergistic effects on gene expression in these cells (400).

### 2. *Hemoglobin and Endothelial Cells*

Cultured bovine cerebral endothelial cells exposed to various concentrations of oxyHb showed a dose-dependent increase in ET in the supernatant. This occurred after 4 hr for carotid endothelial cells but took 24 hr for cerebral artery endothelial cells (401). When these cells were treated with oxyHb cell density was significantly decreased in a time- and dose-dependent manner. SOD provided partial protection against the cytotoxic effect of oxyHb. The release of radiolabel from the $[^3H]$ arachidonic acid-treated cells was increased by oxyHb also in a time- and dose-dependent manner. Phospholipase $A_2$ inhibitors or $Ca^{2+}$ chelator reduced the effect of oxyHb on AA release and cellular viability. The direct cytotoxic effect of oxyHb on cultured endothelial cells was associated with an apparent increase in AA. Phospholipase $A_2$ and free radicals appeared to participate in this cell damage (402). Using bovine MCA endothelial cells, the incorporation of $[^3H]$ leucine into the cells was used as an index of cellular viability. CSF preincubated with blood for 3 days or longer prior to treatment elicited significant reductions in leucine incorporation. Treatment with CSF preincubated with blood for 5–7 days resulted in rapid destruction of cells with large numbers of cells detaching immediately. The blood and CSF mixture incubated for longer than 3 days had profound elevations in Hb (403). Fura-2 $[Ca^{2+}]i$ microfluorimetry was employed using cultured bovine pulmonary and cerebral arterial endothelial cells. RBC lysate and bloody CSF produced a biphasic $[Ca^{2+}]i$ response, an initial peak followed by a plateau. This response was attenuated by the endoplasmic reticulum $Ca^{2+}$ pump inhibitors thapsigargin and cyclopiazonic acid, by the voltage-independent $Ca^{2+}$ channel blocker SK&F96365, by the P450 cytochrome inhibitors econazole and miconazole, and by the inorganic $Ca^{2+}$ pathway blockers lanthanum, nickel, and cobalt. It was concluded that RBC lysate releases $Ca^{2+}$ from internal stores and promotes $Ca^{2+}$ influx from voltage-independent $Ca^{2+}$ channels. The active component in the lysate appeared to have a molecular weight $< 1$ kDa. Fractions containing oxyHb did not effect the $[Ca^{2+}]i$. Adenosine nucleotide mimicked the effect of the lysate and bloody CSF on $[Ca^{2+}]i$. The P2-purinoceptor antagonist suramin attenuated the effect of ATP, lysate, and the small fraction (404). The effect of RBC hemolysate and its low-molecular-weight and high-molecular-weight fractions on $[Ca^{2+}]i$ in freshly isolated rat basilar artery smooth muscle cells was studied. The effect of hemolysate declined with increasing incubation time. The high-molecular-weight fraction and purified oxyHb did not invoke an elevation of $[Ca^{2+}]i$. The effect of the hemolysate on $[Ca^{2+}]i$ declined over time (405). Using confluent bovine aortic endothelial cells exposed to various oxyHb concentrations, it was shown that cell density was decreased in a concentration- and time-dependent manner. Analysis of DNA showed a pattern of intranucleosomal cleavage characteristic of apoptosis, the DNA ladder. Transmission electron microscopy demonstrated condensation of nuclei and apoptotic bodies. Western blotting with PARP antibody revealed that 116-kDa PARP was cleaved to an 85-kDa apoptosis-related fragment. It was concluded that oxyHb induces apoptosis in cultured endothelial cells (406). It will be interesting to learn if similar results occur with smooth muscle cultures.

## F. *In Vivo* Long-Term Studies

Prolonged VSP was produced in feline basilar arteries by applying oxyHb. Myonecrosis was observed within 1 or 2 hr. Smooth muscle cells showed vesicles and granules slowly increased in number for 24 hr following application. Intrusion of myointimal cells, vesicles, and granules into the basement membrane-like substance of the tunica intima, detachment of endothelial cells, and invasion of blood-borne cells several layers into the subendothelial gaps developed massively 24 hr after the oxyHb application. Constriction of the arterial wall resulted in reduction of luminal size (407).

OxyHb and metHb caused less severe but longer lasting constriction than whole blood following topical application to exposed rat basilar artery. If given within the first 3 hr of exposure, papaverine mildly dilated contracted

arteries (298). Exposed cat basilar arteries were studied over the course of 2 hr *in vivo*. Lysate of cat RBCs was prepared using freezing. The produced Hb was mainly oxyHb with a concentration of 5.5 m*M* Hb. MetHb was produced by oxidation of the oxyHb with sodium nitrite. OxyHb produced a 32% decrease in basilar diameter compared to only 15% for metHb. Addition of SOD to oxyHb decreased its vasoconstricting effect to 15% or about the same as that of metHb. Once the artery had constricted to oxyHb, however, SOD did not reverse the constriction. Catalase also decreased the effect of oxyHb to about that of metHb. A system which was believed to generate hydroxyl radical had no effect on the basilar artery except when it was added with metHb, in which case it produced a constriction comparable to that of oxyHb (383). Also, using the transclivally exposed feline basilar artery it was shown that oxyHb produced a 30% constriction following exposure for 6 hr. Following treatment with intravenous $PGI_2$ the vasoconstriction was lessened from 10 to 14%. The $PGI_2$ infusion was started 30 min after oxyHb was placed onto the artery (384). In the same model components of RBC hemolysate were isolated by gel column chromatography. The active part of the hemolysate was found in the peak, which contained mostly oxyHb. Activity was lost by heating or filtering that excluded molecules with a weight >20 kDa. The constriction resulting from hemolysate was significantly suppressed by $5 \times 10^{-5}$ M aspirin or $3 \times 10^{-5}$ g/ml polyphloretin phosphate, a PG antagonist (300). Hemolysate caused dose-dependent contraction of dog basilar artery as well. Also, SOD and catalase did not affect hemolysate contraction (300).

OxyHb ($2.75 \times 10^{-3}$ M), NE, and $PGF_{2\alpha}$ caused dose-dependent vasoconstriction in the exposed basilar artery. The oxyHb-induced constriction was severe and long-lasting. This contraction was not statistically significantly affected by 10 mg/kg phenoxybenzamine or 0.5 mg/kg prazosin, both of which are α-adrenergic blockers. Also, adrenergic denervation by sympathectomy, reserpine, or 6-OHDA slightly increased constriction to NE but did not alter oxyHb-induced constriction. It was concluded that oxyHb did not act by α-adrenergic stimulation (54). Helical strips of dog cerebral artery were exposed to oxyHb for 5 hr and then stored overnight after being washed. Vasodilations mediated by vasodilator nerves, the electrogenic sodium pump, EDRF, and $PGI_2$ were all impaired in dog cerebral arteries previously exposed to oxyHb. Vascular endothelium appears to participate in cerebral VSP via release of vasoconstrictor PGs according to Onoue *et al.* (29). Aged clot was applied to cat basilar artery *in vivo*. Following 10 days of incubation the Hb concentration was 10.95 g/dl and the ratio of oxyHb to metHb was 1:3. Application of this material caused concentration-dependent contractions with $ED_{50}$ of $4 \times 10^{-6}$ M. The decrease in luminal diameter following a 4 hr-exposure to $10^{-3}$ M (6.4 g/dl) to this solution was 22.5% (409).

Single intracisternal injections of low doses of oxyHb have in some experiments failed to produce chronic VSP. This is presumably related to the fact that in the spinal fluid dilution occurred and significant, long-lasting exposure to oxyHb did not occur (298, 389, 408, 410–412). The constriction of dog basilar artery *in vivo* to oxyHb was reversed by addition of haptoglobin. The injection of 50 mg oxyHb into the cisterna magna of dogs produced VSP lasting 7 days. At this point, haptoglobin was still able to relieve VSP (410). Hb and fibrin degradation products produced more VSP when injected together into the cisterna magna of dogs than when given separately (345). Human oxyHb was injected into the cisterna magna of three baboons and one was given metHb. Only single injections were given and the amount of injectate was only 53.3–58.6 mg/kg oxyHb and 50 mg/kg metHb. This did not result in significant long-lasting VSP (408).

Intracisternal injections of Hb caused concentration-dependent decreases in diameters of intra-but not extrathecal porcine arteries. There was a slight dilatory response intrathecally to the infusion of ACh but this dilator response was converted to frank constriction after cisternal injection of Hb. The findings were consistent with the hypothesis that subarachnoid Hb can induce cerebral VSP by acting as an extraluminal "sink" for intimally released EDRF (385). Also, in the pig very high concentrations of Hb and other blood components were maintained adjacent to cerebral arteries for 10 days. Porcine RBCs were used to prepare various blood fractions, including white blood cells plus PRP, RBCs, Hb containing cytosol, or RBC membranes. Eighty-six percent of the Hb in the RBC cytosol was metHb. Morphometric analysis showed significantly decreased luminal area with Hb, RBC cytosol, RBCs, and whole blood. An increase in wall area only occurred with whole blood (12%) and volume densities of endothelium, internal elastic lamina, media, and perivascular axons were not significantly different from controls (314).

We performed a study on monkeys in which catheters were placed along the right MCA and connected to subcutaneous CSF reservoirs used for the injection of various compounds. Multiple intrathecal injections of oxyHb, metHb, bilirubin, mock CSF, or supernatant fluid from an incubated mixture of autologous blood and mock CSF were given for 6 days. Significant VSP of the adjacent MCA developed chronically in those animals receiving the oxyHb and supernatant from incubated blood. Pure metHb produced no significant arterial narrowing and neither did the mock CSF (315).

### G. Spectrophotometric Experiments

Spectrophotometric investigations of CSF in the near-ultraviolet region revealed that the most powerful absorption of oxyHb is at 415 nm (Soret band). MetHb has a corresponding peak at 406 nm. The maximum of the single broad absorption band for bilirubin is 455 nm. OxyHb and metHb have isobestic points at 412 and 480 nm (413). The spectrophotometric analysis of hemoglobin mixtures must take into account that extinction coefficients for deoxyHb and oxyHb as well as metHb can vary with pH (414). Spectrophotometric analysis of CSF post-SAH showed that oxyHb peaked on day 2 and stayed relatively high for 8 days with detection still possible up to 13 days. Bilirubin peaked on about day 8 (351). The xanthochromic index is the sum of absorption at 415 nm (oxyHb) and at 460 nm (bilirubin). Some xanthochromia is present for as long as 3 weeks and the pattern of increase and decrease of xanthochromia varied considerably between patients (415).

The total of oxyHb, metHb, and bilirubin in subdural hematomas of patients ranged between 13.9 and 55 μmol/liter and from 0.1 to 8.2 μmol/liter in the CSF. When the totals were <1 μmol/liter bilirubin was the major constituent. It was suggested that when the concentration of oxyHb is very high, all of it cannot be enzymatically converted *in vivo* by heme oxygenase to biliverdin and therefore spontaneous oxidation to metHb occurs. The three hemoglobin derivatives in a couple of subdural hematomas were present in higher concentration than normal male Hb levels in blood (140–170 g/liter or 8–10 mmol/liter). This was explained by the packing of RBCs in hematomas and their subsequent lysis (350).

Using the primate unilateral clot application model and placement of a semipermeable microdialysis catheter adjacent to the middle cerebral artery in spasm, Pluta and colleagues showed that perivascular concentrations of oxyHb and deoxyHb peaked on day 7 in the SAH group, at which time the concentrations in the dialysate were 100-fold higher than those in any sample obtained from the control animals (415a). MetHb increased only slightly, peaking between days 7 and 12, at which time the concentrations in the dialysate were 10-fold higher than those in samples from the control animals. The concentrations of the various Hbs were measured by spectrophotometry. Late peaks in concentration of oxyHb and deoxyHb occurred 7 days post-clot application, whereas metHb slowly increased and was maximal on day 12. Peak oxyHb concentration was $0.5 \times 10^{-4}$ M, for deoxyHb it was $0.2 \times 10^{-4}$ M, and for metHb the peak on day 12 was approximately $0.35 \times 10^{-4}$ M. The microdialysis catheters used in this experiment had a high-molecular-weight cut-off (100 kDa). This method of spectophotometric analysis measured about 70% of known Hb concentrations in *in vitro* solution tests. The demonstration of deoxyHb in this experiment was important. In most previous studies deoxyHb had been converted to oxyHb by the measurement methods employed in CSF, which has an oxygen tension of 31–47 mmHg (416,417). At least 75% of the deoxyHb could be expected to be converted to oxyHb (416). The level of deoxyHb in this experiment was significantly higher than earlier reported values of $0.003–5 \times 10^{-5}$ M (329). In addition, magnetic resonance imaging suggests that magnetic oxyHb is converted to diamagnetic deoxyHb within hours of SAH (418)

### H. Attempted Reversal of Hemoglobin-Induced Vasoconstriction

The reversal of VSP and normalization of arterial caliber correspond to the time of clearance of the subarachnoid space of the disintegrating RBCs. The disappearance of oxyHb from the periarterial space also corresponds to this time of clearance. It is possible that oxyHb works as a continuous agonist of constriction. Contraction caused by receptor-operated systems usually diminishes after awhile due to tachyphylaxis, desensitization, and/or autoregulation (1,324). It is not clear what receptor would be activated by the huge oxyHb molecule. Other putative constrictors, such as biogenic amines, ET, and eicosanoids, are all much smaller. Specific pharmacological antagonists of receptor-operated vasoconstrictors have generally failed to reverse oxyHb-induced contractions. These antagonists include atropine, methysegide, cinanserin, ketanserin, phenoxybenzamine, phentolamine, mepyramine, chlorpheniramine, propranolol, salbutamol, angiotensin, sarcosine, alanine, theophylline, and quinine (11,29,54,60–63,154,295,298,300,308,330,381, 382,390,419). Vasoconstriction induced by oxyHb can be reversed by papaverine, some calcium channel antagonists in certain circumstances, and some inhibitors of eicosanoid synthesis (29,60,61,63,295,381,382,419). The calcium antagonist nimodipine can partly reverse oxyHb contraction *in vitro* but has not been efficacious for this purpose in primates (420) or humans (421, 422). Bloody CSF produced contractions of human basilar artery *in vitro* that were not blocked by 5-HT blockers (BOL and methysergide), mepyramine ($H_1$ histamine blocker), atropine (anticholinergic), or phentolamine (α blocker) in 10 μmol/liter concentration (292). Contractions of dog cerebral, rabbit ear arteries, and rat stomach fundus resulted from *in vitro* exposure to bovine Hb $10^{-4}$–$10^{-6}$ M (80% metHb). Hb contraction was not blocked by atropine or tetrodotoxin but was partially blocked by $5 \times 10^{-7}$ M methysergide, 16 μg/ml

indomethacin, and $10^{-7}$ M D-600 (a calcium channel blocker) (60).

Fresh RBCs were lysed to produce oxyHb, which constricted exposed cat basilar arteries. Apparently fusaric acid (10 mg/ml), methylprednisolone (4 mg/ml), ascorbic acid (10 mg/ml), and salbutamol (1 mg/ml) released the vasoconstriction (308). Using cerebral and systemic arteries of dogs, *in vitro* constriction was produced by exposure to Hb. The contractile responses were reduced by aspirin ($10^{-5}$ to $2 \times 10^{-4}$ M), polyphloretin phosphate ($3 \times 10^{-5}$ g/ml), and cinanserin ($10^{-7}$ M). Combined treatment with all three agents failed to suppress the response to Hb-containing solutions and only slightly attenuated the responses induced by $K^+$. Also ineffective was phentolamine. The contractile responses to Hb-containing solutions were attenuated by aspirin and by polyphloretin phosphate in concentrations that did not significantly inhibit the contraction induced by $K^+$. Hb-containing test solutions released vasoconstricting PG from the cerebral arterial wall but vasodilating PG from mesenteric arteries (154). The constriction of dog basilar artery *in vitro* to RBC lysate was not affected by $10^{-6}$ phenoxybenzamine, although this was effective in shifting the curves of constriction to fresh serum and fresh PRP (330). In a similar experiment Hb-induced contraction of dog basilar artery was not abolished by methysergide, phentolamine, mepyramine, or aspirin, indicating that 5-HT, NE, histamine, and PG were not involved in the contractile response to Hb application. There was no response of the basilar artery to Hb in $Ca^{2+}$-free solutions. Nifedipine was a potent inhibitor of Hb-induced contraction. The effect was considered to be even greater than that of papaverine (423). Using human Hb prepared by chromatography and shown to be pure by spectrophotometry, dog basilar artery was tested *in vitro*. The dose–response curve for pure Hb was the same as that for hemolysate, suggesting most of the effect in hemolysate is due to Hb. Nifedipine ($10^{-7}$ M) blocked $10^{-4}$ M Hb-induced contractions but no effect was demonstrated by methysergide ($10^{-6}$ M), phentolamine ($10^{-6}$ M), mepyramine ($10^{-6}$ M), or aspirin ($10^{-5}$ M). Hb had no effect in calcium-free medium. Papaverine ($10^{-6}$M) also inhibited Hb. MetHb and cyanometHb (ferric compounds) had no vasoconstricting effect and neither did ferric or ferrous chlorides. Globin, protoporphyrin, and hematin in concentrations of up to $10^{-4}$ M produced no constriction after 5 min of application (61).

The constrictor effect of bloody CSF, which contracted human basilar artery *in vitro*, was not antagonized by methysergide (2000 nmol/liter), mepyramine (1000 nmol/liter), phenoxybenzamine (1000 nmol/liter), propranolol (3000 nmol/liter), atropine (340 nmol/liter), and sarcosine–alanine–angiotensin (20 nmol/liter) (321). The vasoconstrictor effect of bloody human CSF on canine cerebral artery was reduced by methysergide $5 \times 10^{-7}$ M, which was sufficient to entirely abolish responses to 5-HT (324).

Guinea pig basilar arteries were exposed to hemolysate. The original concentration of Hb was $0.83 \times 10^{-3}$ M, which was subsequently diluted to about $10^{-5}$ M. Hemolysate-induced contraction was not affected by phentolamine ($10^{-6}$ M), methysergide ($10^{-6}$ M), mepyramine ($10^{-6}$ M), atropine ($10^{-6}$ M), theophylline ($10^{-6}$ M), quinidine ($10^{-6}$ M), OKY-1581 ($10^{-5}$ M), and apamine ($10^{-7}$ M). Also, the constriction was not affected by removing endothelium (63). *In vitro* contractions to RBC hemolysate of basilar but not mesenteric artery were significantly attenuated with $5 \times 10^{-5}$ M aspirin, $3 \times 10^{-5}$ g/ml polyphloretin phosphate, and $10^{-6}$ M cinanserin. SOD ($1.1 \times 10^3$ U/dl), catalase ($10^4$ U/dl), and dl-α-tocopherol ($10^{-4}$ M) did not antagonize the contraction (295). The contractile responses of canine arterial strips to bloody CSF were not antagonized by phenoxybenzamine ($10^{-5}$ M), porpanolol ($10^{-6}$ M), methysergide ($10^{-7}$ M), mepyramine ($10^{-7}$ M), and atropine ($10^{-6}$ M). Disulfide bond reducing agents dithiothreitol ($10^{-4}$ M), and dithioerythritol ($10^{-4}$ M), did not affect KCl-induced arterial contraction but they did inhibit the action of bloody CSF on the artery. This effect was antagonized by DTNB ($10^{-4}$ M), a sulphydril group oxidizing agent, in five of six CSF samples tested. These agents may have affected some component in the bloody CSF or the contractile apparatus in the mucle (328). Hb ($10^{-7}$ to $10^{-5}$) contracted rings of rabbit basilar or dog cerebral arteries dilated with ACh ($10^{-7}$ to $10^{-4}$ M) or ATP. It took 45 min after washout of the Hb before ACh again induced relaxation. Papaverine ($10^{-7}$ to $10^{-4}$ M) was capable of relaxing vessels precontracted with Hb (390). Exposed cat basilar artery was contracted by oxyHb; this was not significantly reversed by phenoxybenzamine (10 mg/kg) or prazosin (0.5 mg/kg). These α-adrenergic blockers did not statistically significantly alter the oxyHb contraction (424). Canine basilar artery was contracted *in vitro* using hemolysate from washed RBCs prepared by hypotonic shock. Spectrophotometry showed the active agent to be oxyHb. Hemolysate treated with $NaNO_2$ (which converted Hb to metHb) had no vasoconstrictor activity. The contraction was not affected by $10^{-6}$ M phentolamine, $10^{-6}$ M atropine, $10^{-6}$ M chlorpheniramine, $10^{-6}$ M ketanserin, or $3 \times 10^{-7}$ M tetrodotoxin. The response was slightly attenuated by removal of endothelium. Hemolysate also potentiated the contractile response to quick stretch of the artery. Aspirin ($10^{-4}$ M) and OKY-046 ($10^{-5}$ M) attenuated the basal effect of hemolysate but had no effect on stretch potentiation. Indomethacin ($10^{-5}$ M) inhibited both effects (62). OxyHb was applied to dog cerebral arteries

*in vitro* for 5 hr. This exposure prevented nicotine-induced relaxation of strips precontracted with prostaglandin $F_{2\alpha}$. The stable $PGI_2$ analog TRK-100 had a relaxant effect which was also attenuated by oxyHb. Relaxation by the $Ca^{2+}$ ionophore A23187 ($10^{-7}$ M) was less effective after oxyHb treatment (29).

Toda showed a greater contraction of monkey cerebral arteries than those of dog to oxyHb. The contraction was attenuated by endothelial denudation and treatment with indomethacin, aspirin, and diphloretin phosphate. OKY-046 also inhibited oxyHb contractions. Treatment with SOD and catalase failed to reduce the response to oxyHb or AA (366).

OxyHb produced a concentration-dependent contraction of monkey, dog, and bovine cerebral arteries, although a combination of oxyHb and ascorbic acid failed to contract the arteries. OxyHb did not produce canine basilar artery constriction when animals had previously been treated with ascorbic acid. The incubation of oxyHb with ascorbic acid markedly diminished its biological activity (392).

The PLC inhibitor neomycin inhibited the elevation of $[Ca^{2+}]i$ produced by oxyHb in cultured monkey cerebral vascular smooth muscle cells. In isolated rings of canine basilar arteries neomycin attenuated the contractions to Hb. The contraction was further inhibited by procaine, which is an inhibitor of calcium-induced calcium release. Hb apparently activates PLC, possibly by a mechanism involved in the production of free radicals (363).

### I. Hemoglobin Interactions

#### *1. Prostaglandins*

The contraction of dog cerebral arteries to a combination of metHb and oxyHb was partially blocked by indomethacin (16 μg/ml) (60). Such cerebral arteries exposed to hemolysate were contracted and this response was also attenuated by $10^{-5}$ M aspirin and $3 \times 10^{-5}$ g/ml polyphloretin phosphate (PG antagonist) (154). Dog cerebral arteries contracted to xanthrochromic and bloody CSF and this was not affected by indomethacin ($5 \times 10^{-5}$ M) (324). Contraction of canine basilar artery *in vitro* was induced by oxyHb inhibited by $10^{-6}$ M $PGI_2$, although the contraction that took $10^{-5}$ M oxyHb was not completely abolished (386). Cat basilar artery exposed transclivally was contracted by infusion of oxyHb over 6 hr. Treatment with intravenous $PGI_2$ decreased the VSP from 30% to the 10–14% range (384). In *in vitro* studies of canine basilar artery, crude dog hemolysate $10^{-4}$ M oxyHb caused dose-dependent contraction. $PGI_2$ dose dependently inhibited the oxyHb contraction, although $10^{-6}$ M $PGI_2$ did not completely abolish the $10^{-5}$ M oxyHb contraction (386). Guinea pig basilar artery was contracted by hemolysate containing about $10^{-5}$ M Hb. Indomethacin, even at $10^{-5}$ M, was only able to partly block this contraction (63). Dog basilar artery, but not mesenteric arteries, was significantly contracted by hemolysate. This contraction was attenuated by treatment with $5 \times 10^{-5}$ M aspirin and $3 \times 10^{-5}$ g/ml polyphloretin phosphate (295). The injection of oxyHb into the cisterna magna of dogs decreased $PGI_2$ levels in the basilar artery 4 and 7 days later, although there was no change in the measured level of thromboxane $A_2$ in the arterial wall (425).

In a bioassay cerebral arteries were superfused with 10 g/dl Hb. The superfusate was then run on to rat stomach strips, dog ileal strips, and coronary artery strips. This produced contraction of the stomach strip, relaxation of the coronary, and no response from the ileal strip. Direct application of hemolysate onto these assay organs caused no response. Pretreatment of the cerebral vessels with indomethacin ($3 \times 10^{-7}$ M) abolished or attenuated the perfusate response in the assay organs, so it was suggested that the cerebral arteries were releasing PG-like substances in response to hemolysate (426). Canine basilar artery *in vitro* was exposed to oxyHb (0.01–2 mg/ml) and a concentration-dependent tension increase occurred. The hemolysate potentiated a contractile response to quick stretch. This response was dependent on intact endothelium. ASA ($10^{-4}$ M) and OKY-046 ($10^{-5}$ M) attenuated the basal effect of hemolysate but had no effect on stretch potentiation. Indomethacin ($10^{-5}$ M) inhibited both effects (62).

Monkey and dog cerebral arteries contracted to oxyHb in a dose-dependent fashion. Removal of endothelium markedly diminished the contractions. In endothelium-denuded arteries, indomethacin and DPP slightly attenuated the contractions of oxyHb. In arteries with endothelium, indomethacin and DPP markedly decreased oxyHb-induced contraction (366). In many experiments, however, indomethacin and aspirin, which are inhibitors of cyclooxygenase, have little or no effect on contractions of the dog basilar artery to oxyHb (60–63,154,295,427). OxyHb may affect vessel wall eicosanoid production and alter CSF levels of these substances post-SAH, but inhibitors of the synthesis of prostaglandins and thromboxanes do not prevent VSP.

#### *2. Endothelins*

OxyHb may cause VSP by releasing endothelin (245, 263,264,401). OxyHb (0.01–100 μ*M*) produced concentration-dependent increases in ET levels in bovine pulmonary artery endothelial cell conditioning medium. The maximum stimulation of immunoreactive ET level

was 5.5-fold greater than that under basal conditions. An L-arginine analog inhibitor of NO synthase, L-NNMA (200 $\mu M$), did not change the basal ET levels but significantly augmented platelet-induced ET production. Methylene blue (10 $\mu M$), an inhibitor of soluble guanylate cyclase, did not significantly affect ET levels either basally or after platelet-mediated stimulation of ET production from cultured endothelial cells (280). Kasuya found that oxyHb produced a concentration- and time-dependent increase in ET-1 in the conditioning medium of bovine arterial cells. In rat aortic smooth muscle cells oxyHb also induced ET-1 production, although the rate was much less than that for endothelial cells. OxyHb was a more effective stimulant of ET-1 production than compounds such as thrombin and phorbol esters. ET-1 mRNA was induced in the endothelial cells by this exposure. Staurosporine, a PKC inhibitor, inhibited oxyHb-induced ET-1 production in both endothelial and smooth muscle cells. Increasing cAMP by forskolin or 8-bromo-cAMP only inhibited the effect on smooth muscle cells and not endothelium (279). Northern blot analysis of total RNA from endothelial cells showed ET-1 mRNA induction by oxyHb in cultured endothelial cells (428).

Bovine Hb constricted rabbit aorta *in vitro*. The endothelium-dependent relaxation induced by ACh was completely blocked at $10^{-5}$ oxyHb. Bovine ferric porphyrin, alkaline hematin, non-iron-containing porphyrin, and protoporphyrin 9 had no effect on the ACh-induced relaxation. OxyHb ($10^{-6}$ M) completely blocked A23187-induced relaxation and substantially blocked glycerol trinitrate relaxation. OxyHb but not metHb prevented the increase in cGMP associated with relaxation induced by ACh, A23187, or glycerol trinitrate (429). ACh induced vasodilation of precontracted dog basilar artery rings which was endothelium dependent. Hb ($10^{-7}$ to $10^{-5}$ M) contracted rings dilated by ACh ($10^{-7}$ to $10^{-4}$ M) or ATP. It took 45 min after washout of Hb before ACh-induced relaxations could again be elicited. Papaverine ($10^{-7}$ to $10^{-4}$ M) relaxed vessels independently of endothelium and also relaxed vessels precontracted with Hb (430). Hb inhibited ACh and ATP-induced relaxation of perfused rat basilar arteries in a significantly greater fashion when given intraluminally rather than extraluminally. Immunohistochemical stains showed Hb in the outer layer of the smooth muscle and in the adventitia when $10^{-5}$ M Hb was applied extraluminally for 5 min, whereas Hb was immediately observed on the surface of the endothelial cells after intraluminal application (100). The vasoconstriction induced by the extraluminal administration of oxyHb could be partly counteracted in the basilar artery by administration of ACh in a dose-dependent fashion. Relaxation of the artery by ACh was abolished and vasoconstriction was induced during the intraluminal infusion of $10^{-5}$ M oxyHb. This constriction was potentiated by removal of the endothelium. Intraluminal perfusion of $10^{-5}$ M oxyHb completely inhibited endothelium-dependent vasodilation (431).

When arteries were precontracted with oxyHb or 5-HT applied extraluminally and ACh ($10^{-5}$ to 1 M) was applied intraluminally, ACh caused a dose-dependent relaxation which was similar in both groups. Intraluminally applied oxyHb $10^{-5}$ M completely abolished ACh-induced relaxation. This suggested that impairment of endothelium-dependent vasodilation of arteries exposed to subarachnoid clot was due to damage to the endothelium and not to inactivation of a relaxing factor by oxyHb (431). Dog femoral arteries were studied in a bioassay. Bovine oxyHb applied extraluminally to the femoral artery attenuated the vasodilator response to intraluminal ACh by 50% (99). Hb was injected into the cisterna magna of pigs in a dosage of 10–40 $\times 10^{-6}$ M and angiography was performed within 15 min. The most concentrated injectates caused decreases of 26% of control diameter. ACh infusion performed before Hb injections induced minor degrees of dilation in the intrathecal vessels but only at concentrations of $10^{-4}$ M (385).

Hb inhibits endothelium-dependent relaxation to ACh in human coronary arteries *in vivo*. In seven patients, the drugs were infused into the coronary arteries during angiography. ACh increased coronary artery diameter. Hb infused at concentrations of $10^{-6}$ or $10^{-5}$ M reversed this vasodilator effect. Isosorbide dinitrate in the presence of Hb caused dilation of the coronary arteries in all cases. Hb infusion alone had no effect on coronary artery diameter. These results suggested that basal EDRF release does not play an important role in the maintenance of human artery diameter *in vivo* but is responsible for ACh dilatation (432).

Using a canine basilar artery model, the vasoconstrictor component of the response to ACh was significantly enhanced by exposure to extraluminal oxyHb. The enhanced constriction to ACh after endothelial removal was blocked by a thromboxane synthetase inhibitor and a dihydropyridine $Ca^{2+}$ antagonist, although not by a lipoxygenase inhibitor (433).

### *3. Nitric Oxide*

The reaction between NO and oxyHb rapidly produces metHb. The reaction of NO and deoxyHb does not make metHb but does make NO-ferrous Hb. NO-ferrous Hb can be detected by electron paramagnetic resonance since NO has an unpaired electron. NO-ferrous Hb is slowly

oxidized in the presence of $O_2$. NO not only relaxes blood vessels but also increases $O_2$ release from Hb (434). NO combines with the heme group of Hb with a 1500 times greater affinity than CO. The reaction of the combination of NO and deoxyHb is much faster than the reaction of $O_2$ with deoxyHb (435).

Constrictor responses of larger cerebral arteries to 5-HT, thromboxane, and $PGE_2$ are enhanced in atherosclerosis. NO inhibits the responses of large cerebral arteries to several vasoconstrictors, including 5-HT and $PGE_2$. Impairment of this normal inhibitory influence may account for augmented constrictor responses in larger atherosclerotic arteries. Similarly, relaxation is impaired in larger cerebral arteries from humans and animal models of SAH (13,102,436–441). Impaired relaxation post-SAH may result from impaired endothelium-dependent relaxation associated with reduced activity of guanylate cyclase and the loss of cGMP. On the contrary, some studies have indicated that the responses to nitrovasodilators are normal after SAH suggesting that the activity of guanylate cyclase is unaltered (102,436, 438). Hb may constrict the cerebral arteries after SAH by direct effects on vascular smooth muscle as well as by inhibiting the basal activity of NO. Hb may also destroy NO by generating superoxide anion (364). In intact cerebral arteries Hb decreases basal levels of cGMP to levels similar to that produced by L-NAME or the removal of endothelium (442). WBCs may be activated following SAH. Activated neutrophils may impair NO (443,444). DeoxyHb as well as oxyHb scavenge NO (445,446) in a reaction that produces a stable nitrosyl-Hb (445,447). Both hemoglobins can eliminate the action of the vasodilator (369,390,445,446).

OxyHb and other ferrous hemoproteins (although not metHb or ferric hemoproteins) can inhibit endothelium-dependent relaxation in many vascular preparations *in vitro* (29,31,99,100,369,390,429,431,448–453) Nitric oxide is probably the principal endothelium-derived relaxing factor. NO-induced relaxation is associated with raised levels of cGMP in the arterial wall (429). Nitrosamines and NO were capable of activating soluble guanylate cyclase in the absence of catalase. The activation was blocked by $Fe^{2+}$ Hb but not $Fe^{3+}$ Hb (454). Hb but not metHb inhibited azide, catalase, cytochrome $b_2$, and cytochrome c reductase-induced activation of partially purified guanylate cyclase. Heme apparently scavenged the NO (455). Hb and methylene blue selectively inhibit relaxation of rabbit aorta induced by agents that increase cGMP levels (46).

Furchgott *et al.* showed that Hb blocked endothelium-dependent vasodilation and suggested it was a possible factor in the VSP associated with SAH (456). Bowman *et al.* also suggested that Hb may act to block the effects of ACh or other circulating or neurally released vasodilators, and that Hb may also potentiate vasoconstrictor substances. It may act as a negative modulator or vasoconstrictor by blocking the action of NO. Hb may impair the action of certain nonadrenergic, noncholinergic vasodilators. Neurogenic vasodilation in penile arteries, for instance, is blocked by Hb. This vasodilation is also impaired by hypoxia. It was hypothesized that Hb and hypoxia acting in concert would produce a powerful synergistic impairment of vasodilator mechanisms (457).

Haptoglobin–Hb complex from human plasma inhibited NO in rabbit aortic strips (451). Hb selectively inhibited endothelium-dependent vasodilation in the rabbit basilar artery preparation (390). At $10^{-6}$ M, Hb partially inhibits relaxation induced by A23817 in canine basilar artery rings. This inhibition of relaxation was dose dependent. Pretreatment of these arterial rings with CSF from SAH patients also resulted in a dose-dependent inhibition of A23187-induced relaxation. It was considered that Hb released from lysed RBCs inhibits NO of canine basilar arteries and may play an important role in the pathogenesis of VSP (419). Nitroglycerin and human vascular smooth muscle cell suspensions produce a substance (presumably NO) which profoundly inhibits platelet aggregation. This effect is prevented by Hb (458). Incubation of rat aortic rings with endotoxin induces a delayed and prolonged release of NO which results in a decrease in the contractile response to phenylephrine. Hb significantly potentiates these contractions. Lysed RBCs but not whole RBCs shift the concentration–contraction response curve to phenylephrine significantly to the right in endotoxin-treated preparations. The binding of Hb and NO requires a cell-free form of Hb capable of undergoing redox reactions (459).

Cytokines produced by inflammatory cells such as interleukin-1β (IL-1β) can increase NO production via the inducible form of NO synthase. The effect of Hb on IL-1β-induced NO production was studied in cultured rat vascular smooth muscle cells. The production of NO was estimated from the accumulation of nitrite, an oxidative product of NO, in the culture medium. Nitrite accumulation was slightly but significantly increased by exposing culture medium to purified human Hb in the presence of IL-1β. This augmentation persisted even after removal of Hb from the culture medium (380). NO levels can be reduced by scavenging with Hb or inhibiting NOS by *N*-nitro-L-arginine. Using a closed-window preparation in rats, when artificial CSF concentrations were increased in $K^+$ content at 35 mmol/liter there was a sudden spontaneous transient ischemic event with a decrease in CBF. Cortical spreading depression associated with long-lasting ischemia occurred at lower $K^+$ levels in

artificial CSF when it was combined with Hb or L-NA (460).

#### *4. Other Agents*

The contractions resulting from hypoxia (389,461), 5-HT (371,437), $K^+$ (449), and fibrin degradation product (335) are all accentuated by oxyHb. *In vitro* studies of rabbit basilar arteries showed that Hb ($10^{-5}$ M) markedly augmented contractions induced by 5-HT ($10^{-9}$ to $10^{-6}$ M) and slightly augmented those induced by KCl (20–80 m*M*) in arteries with intact endothelium. The augmentation by Hb of the vasoconstrictor response was dependent on the presence of intact endothelium. This suggested that Hb acted by inhibiting spontaneously released NO. Arteries removed after SAH showed a significant reduction in Hb-induced augmentation compared to that seen in control arteries with intact endothelium. It was concluded that NO spontaneous release may be inhibited after SAH (437).

It is unknown if vasoconstriction can be conducted in a wavelike fashion from regions initially in spasm. The observation of constriction in petrous and cervical segments of the internal carotid appears to support this concept. Gap junctions might be a means for waves of increased $[Ca^{2+}]i$ to induce spreading VSP. In the microcirculation, conducted vasomotor responses have been observed. Initial constriction in arterioles to ATP, for instance, cause a secondary conducted vasodilation (462). In this model oxyHb constricted arterioles an average of 87% and attenuated propagated vasodilation in response to ATP, ADP, and adenosine (393).

### J. Ultrapure Hemoglobin

One problem with Hb solutions has been to produce normal colloid osmotic pressures. Synthetic Hb has been subjected to polymerization in an effort to achieve this. The preparation of Hb solutions has demonstrated the occurrence of stromal contaminants. One of the problems with much of the early research on Hb and cerebral VSP was that the investigators did not distinguish between oxyHb and deoxyHb and did not specify whether reagents contained metHb or other forms of Hb. In addition, most reagents would have had stromal contaminants. Most commercially available hemoglobins are listed as being purified to 99%. Commercial ferrohemoglobin ($Fe^{2+}$ Hb) has to be treated to be fully converted to oxyHb (463).

Diaspirin crosslinked hemoglobin (DCLHb) was developed as a potential $O_2$-carrying solution. Its use is associated with a self-limiting increase in mean arterial pressure. Administration of cyanomet DCLHb, a DCLHb molecule that is unable to react with NO, was not associated with an elevation of MAP. L-Arginine, the substrate for NO synthesis, and nitroglycerin, an NO donor, significantly reduced MAP when infused 15 min after DCLHb administration. Phosphoramidon attenuated the elevation of MAP when administered before DCLHb. It was concluded that the DCLHb-induced elevation in MAP *in vivo* was mediated at least in part by ET and the inhibition of NO (464).

Human Hb cross-linked between α chains with bis-3,5-dibromosalicyl fumarate (α,α-Hb) was perfused in isolated perfused rabbit hearts. The findings were consistent with the concept that both NO binding to Hb and Fe-mediated $O_2$ free radical generation contribute to an altered coronary vasomotor responsiveness induced by cell-free Hb (465). A genetically engineered recombinant human Hb (rHb1.1) rapidly and reversibly inhibited both ACh and IL-1β induced decreases in phenylephrine contractile responses. The inhibitory effect of rHb1.1 was equipotent to that of purified, cell-free human Hb. The soluble Hb forms were at least 10 times more potent than Hb from RBCs (466). DCL Hb produces an immediate increase in blood pressure and marked regional circulatory changes in various experimental animals. The endothelin-A receptor antagonist BQ-123 attenuates the systemic hemodynamic and regional circulatory effects of both DCL Hb and stroma-reduced Hb. The increase in blood flow to the heart induced by stroma-reduced Hb was not attenuated by BQ-123. A purified Hb is still capable of inducing vasoconstriction. The effect varies with the site of blood vessels studied. Human umbilical arteries and veins are relaxed by $PGI_2$ rather than NO. Substance P and nitroglycerin, but not ACh, caused relaxation of both umbilical arteries and veins. The NO synthase inhibitor L-NA did not significantly affect this relaxation. DCL-Hb did not alter 5-HT preconstricted tension of either umbilical arteries or veins. The basal cGMP content of these vessels was low (467). These findings support the view that DCL Hb-induced vasoconstriction in isolated vessels is dependent primarily on the binding of NO by Hb (468).

Isolated femoral and renal dog arteries studied *in vitro* relaxed to ACh and the calcium ionophore A23187. Crosslinked Hb inhibited this endothelium-dependent relaxation. In the same concentration range, purified bovine Hb exerted a similar inhibitory effect on relaxations mediated by activation of endothelial cells. Crosslinked Hb reduced basal production of cGMP but not cAMP. ACh stimulated production of cGMP, although this effect was abolished by crosslinked Hb. Inhibition of the endothelial L-arginine-NO biosynthetic pathway with a subsequent decrease in cGMP in vascular smooth muscle may explain the vasoconstrictor and pressor effects of crosslinked Hb (469). Porcine pulmonary veins were

relaxed by ACh, SNP, and papaverine and contracted by increasing concentrations of α,α-Hb and Hb. Cyanomet α,α-Hb in human serum albumin did not cause constriction. The maximum responses to Hb and α,α-Hb were significantly increased during relaxation with ACh and SNP but not with papaverine. It was suggested that the Hb-induced vascular contraction is primarily mediated by inactivation of the vasodilator NO *in vitro*. This mechanism is common to cellular hemoglobins in which the ligand binding site is unimpaired and in which iron is in the $Fe^{2+}$ state (470). The subarachnoid administration of α,α DCL Hb caused a reduction in CBF in rats and an area of hypoperfusion. The area of hypoperfusion was less with the synthetic Hb than with whole blood (471).

Recent evidence suggests that blood pressure increases developing during exchange transfusions with cell-free Hb solutions do not result from NO scavenging reactions at the heme but must be due to a different mechanism (472). Concerted efforts to provide stroma-free Hb solutions which could be used for organ perfusion or systemic infusion to carry oxygen have been made (473). Acellular Hb blood substitutes have not reached clinical usefulness because of side effects, including pulmonary hypertension and systemic vasoconstriction (474).

Rat aortic rings relaxed by ACh were contracted by human stroma-free Hb. The contraction induced by the latter was reversed by GTN, a NO donor. Preincubation with the NO synthase inhibitor $N^G$-nitro-L-arginine methyl ester (L-NAME) almost completely inhibited the Hb vasoconstrictor ability. Prenitrosylated Hb or ferric Hb derivatives (metHb or HbCN) did not elicit constriction. These results also suggest that the primary mechanism of Hb vasoconstrictor activity is ferrous Hb scavenging of endothelium-derived NO which signals cGMP-mediated smooth muscle relaxation. Hb-induced vasoactivities may be modulated by NO-independent vasodilators such as GTN (475).

Pulmonary vasoconstriction was studied in isolated rabbit lungs. Hb in perfusate resulted in a hypertensive response. This vasoconstriction was significantly blunted after GTN, L-arginine (NO precursor), and BQ788 ($ET_B$ receptor antagonist). Pretreatment with L-NAME, which blocks NO production, or BQ123 ($ET_A$ receptor antagonist) did not show significant changes in pulmonary artery pressure. The reduction of Hb-induced pulmonary hypertension by NO donors pointed toward the inactivation of NO by free Hb. $ET_B$ receptor-mediated vasoconstriction without changes in NO concentrations suggested that ET agonist might play a pathogenetic role in Hb-induced pulmonary vasoconstriction (476).

Various recombinant Hbs which varied in their rate of reaction with NO were constructed. It was suggested that the rapid reactions of oxyHb and deoxyHb with NO are the fundamental causes of hypertension resulting from Hb infusion. The magnitude of the blood pressure effects correlated directly with the *in vivo* rate of NO oxidation (477). The type of pressor response to Hb may depend on the reactivity of the particular Hb type with NO. Hb solutions that produce either transient or no significant increase in blood pressure showed tighter NO binding affinities than Hb solutions that exhibited sustained increases. It was hypothesized that blood pressure increases observed upon exchange transfusion with cell-free Hb solution cannot be the result of NO scavenging reactions of the heme but rather must be due to alternative physiological mechanisms (472). Unmodified highly purified human Hb and Hb crosslinked with *O*-raffinose were compared for their effects on increasing systemic vascular resistance and mean arterial pressure. The extent to which these two preparations inactivated NO was compared using three separate *in vitro* assays: platelet NO release, NO-stimulated platelet cGMP production, and EDRF-mediated inhibition of platelet aggregation. Unmodified Hb inactivated or oxidized NO to a greater extent than the *O*-raffinose crosslinked Hb solutions in all three assays. It was considered that *O*-raffinose crosslinking reduced the degree of oxidation of NO which contributed to the reduced vasoactivity of this modified Hb (478).

It seems, therefore, that the problems stemming from the use of blood substitutes—modified Hb placed intraluminally—are very similar to those of SAH-unmodified Hb positioned extraluminally. The Hb catalyzes numerous oxidative and peroxidative reactions manifest as severe vasoconstriction (induced hypertension), neutrophil and macrophage activation, microthrombi formation, platelet aggregation, complement activation, and induction of various enzymes systems. Hb and its breakdown products have cytolytic activity which is a function of substrate concentrations such as free radicals. The autooxidation of oxyHb to metHb produces $O_2^{-\bullet}$, which is dismutated to produce $H_2O_2$. The cytolytic effects of Hb on cells can be potentiated by $H_2O_2$. Also, $H_2O_2$ can react with metHb to produce $Fe^{4+}$ (ferryl) and protein radicals which can oxidize other proteins and lipids. Similar therapeutic strategies have been considered for adverse responses to Hb transfusions and VSP resulting from SAH:Fe binding and Hb binding by ferritin and haptoglobin, NO induction, antioxidants such as uric acid and ascorbate, and free radical scavengers such as SOD and catalase.

### K. Heme Oxygenase

Heme oxygenase exists as HO-1 inducible and HO-2 constitutive isoforms which catalyze the degradation of heme to biliverdin and CO. It has recently been consid-

ered that the latter may play a role similar to NO in controlling vascular tone. α,α-Hb, a chemically modified Hb, or L-NAME, a competitive NOS inhibitor, produced an acute hypertensive response 1 day after injection if a HO inhibitor (zinc protophorphyrin IX) were given. If it were not, HO-1 was expressed in aorta and heart, there were increased cGMP levels and aortic CO production, and the hypertensive response was prevented (479). VSP of the rabbit basilar artery was induced by the injection of ET, hemolysate, or CO–hemolysate. There was no significant difference in the degree of VSP induced by these three injections. The HO-1 induced throughout the glial cells of the brain resulted only from hemolysate or CO–hemolysate and not from ET or saline. Since ET produced VSP as well as the hemolysate, but not the increase in HO-1, it was deduced that the stimulus for the latter might be heme per se rather than ischemia (480).

### L. Hemoglobin and Arterial Wall

Even brief exposures to moderate concentrations of oxyHb can cause morphologic changes in vascular smooth muscle and endothelial cells of the type seen in human cerebral VSP (314,368,381,407,481). Using fluorescent antibodies and the ferritin antibody method, Hb was found distributed in the adventitia and smooth muscle layer of the media following induced SAH. The serum constituent haptoglobin, which normally binds Hb, was able to release VSP both *in vitro* and *in vivo*. Human Hb (50 mg) was injected into the cisterna magna of dogs and angiograms were performed 3 days later. The vessels were then perfused and fixed. Using fluorescent antibody technique, Hb was demonstrated to be present in the vessel wall down to the media (412). Using immunohistochemistry the distribution of Hb in rabbit basilar artery was studied following intracisternal injection of autologous blood. Hb immunofluorescence was most prominent in the adventitia but was also seen in the media and endothelium in 8 of 11 rabbits. The degree of constriction at 2 and 4 days post-SAH correlated with the total amount of fluorescence present in the cell wall (482) (Fig. 6.12).

### M. Endothelin and Hemoglobin

The discovery of specific endothelial-derived contracting (endothelin) and relaxing (NO) factors changed our understanding of the interaction between endothelium and underlying vascular smooth muscle (213). Vascular smooth muscle contraction and relaxation is controlled by a wide variety of factors, including autocrine, paracrine, and endocrine vasoactive substances, the ionic milieu, and physical factors resulting from blood flow. In the presence of intact endothelium many substances interact with surface receptors which result in the release of vasoactive compounds that influence the adjacent vascular smooth muscle in paracrine fashion. These responses are endothelium dependent. Examples include BK, NE, 5-HT, histamine, angiotensin II, and thrombin, which cause the endothelium to release NO that subsequently causes vascular smooth muscle relaxation. In the absence of endothelium the same substances interact with receptors directly on the smooth muscle and generate contraction. Endothelial injury might be involved in the pathogenesis of chronic VSP. It is also likely that the vascular smooth muscle cells can exert an influence on the endothelial cells. Parathyroid hormone-related peptides are released from vascular smooth muscle cells and promote muscle relaxation and the inhibition of the release of endothelin from the endothelium (483). Vasomotor tone in cerebral blood vessels can be affected by substances in contact with the adventitia (213).

Endothelial cells of canine arteries were shown to release a substance mediating vascular smooth muscle relaxation by Furchgott and colleagues. After the removal of endothelial cells the relaxation elicited by BK was abolished in canine arteries. Since endothelium-dependent relaxation remained intact after treatment with cyclooxygenase inhibitors, it was argued that the relaxation was independent of prostaglandin synthesis (484). It is well established that relaxation by ACh of isolated preparations of arteries is dependent on the presence of endothelial cells (485).

Peterson and colleagues concluded in the mid-1980s that cerebral vascular tone *in situ* is largely the result of plasma-borne vasoactive substances interacting with vascular smooth muscle in the media, although this reaction is mediated and/or modulated by the endothelium. They concluded that a functional rather than anatomical deendothelialization of the effected cerebral blood vessels could result from prolonged exposure to adventitial clot with the development of irreversible VSP (486).

Using dog basilar arteries *in vitro* it was shown that contraction elicited by Hb was the same whether or not endothelium had been removed by Triton X-100. The Hb used was mainly in the metHb form (487). On the other hand, the importance of endothelium for the maintenance of VSP in the two-hemorrhage dog model was shown, and spastic segments observed *in vivo* were shown to redilate when perfused *in vitro*. Interestingly, intraluminal plasma perfusion resulted in maintained constriction of these isolated vessels studied *in vitro*. It was concluded that normal *in vivo* cerebral vascular tone is at least partly due to interactions of vessel endothelium with materials in the systemic circulation (486).

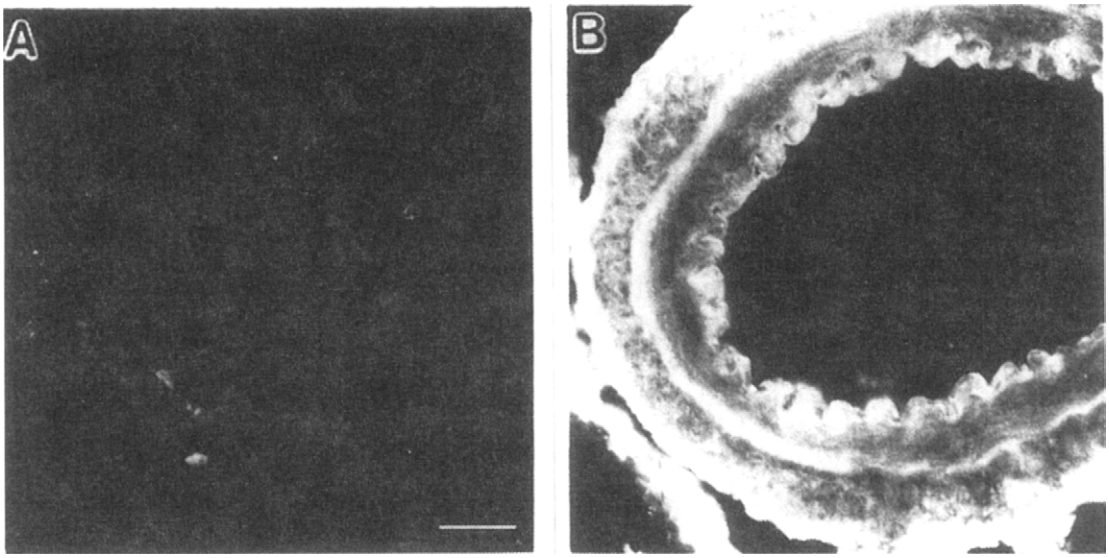


**FIGURE 6.12** (A) Control. (B) Hemoglobin immunofluorescence was most pronounced in the adventitia but was also seen in the smooth muscle and endothelial cell layers of 8 or 11 animals. The degree of vasoconstriction correlated with the total amount of fluorescence present in the vessel wall [reproduced with permission from Foley, P. L., Kassell, N. F., Hudson, S. B., and Lee, K. S. (1993). Hemoglobin penetration in the wall of the basilar artery after subarachnoid hemorrhage. In *Cerebral Vasospasm. Proceedings of the 5th International Conference, Edmonton* (J. M. Findlay, Ed.), Elsevier, Amsterdam].

Triton X-100 was used to remove endothelium in some dog basilar arteries tested *in vitro*. Canine Hb was used as the constricting agent. Hb (10 n*M* to 30 $\mu M$) caused dose-dependent contraction of both control and Triton X-100 arteries and both responses were of the same magnitude. The Hb employed was mostly metHb (487). Endothelium was removed by gas drying or rubbing from rabbit and dog basilar arteries. Hb prepared from human blood caused contractions which were dose dependent and independent of the presence or absence of endothelium, respectively (488). SAH was induced in rabbits and basilar arteries were removed 2 days later. These arteries showed a significant reduction in Hb-induced augmentation of contraction compared to control arteries with intact endothelium. It was suggested that spontaneously released EDRF is significantly reduced post-SAH (437).

The surface of normal endothelium is kept nonthrombogenic by at least three mediators. First, prostacyclin is a product of arachidonic acid metabolism discovered in 1976. It is a labile prostanoid with a half-life of approximately 3 min and relaxes vascular smooth muscle as well as inhibits platelet aggregation. Second EDRF (presumably NO), discovered in 1980, also relaxes smooth muscle and inhibits the aggregation and adhesion of platelets. Its production is stimulated by ACh, BK, and ADP. It is more labile than prostacyclin, with a half-life of about 6 sec. Third, there is evidence that 13-hydroxy-9,11-octadecadienoic acid is an intracellular mediator which contributes to the nonadhesiveness of the endothelial surface (489).

Basilar arteries were harvested 7 days following double SAH. Rings with intact endothelium contracted with uridine triphosphate and relaxed to arginine vasopressin and thrombin in a dose–dependent fashion. This did not occur in arteries without endothelium. The response was also lost after SAH. Nitrite relaxation was significantly decreased after SAH compared to controls but papaverine relaxation was unaffected (490). OxyHb caused dose-dependent contraction of dog or rabbit basilar artery independently of the presence of endothelium (488). With intact endothelium ACh caused transient hyperpolarization and sustained relaxation of rat aorta and pulmonary artery which had been precontracted with NE. Removal of endothelium led to a loss of hyperpolarization and relaxation. Hb and methylene blue both inhibited ACh-induced relaxation but not hyperpolarization (453). Hb ($10^{-5}$ M) augmented the contractile responses of rabbit basilar arteries to 5-HT when the endothelium was intact, but when it was removed by saponin there was no such effect (437). In canine basilar arteries the removal of endothelium increased the vasoconstrictor response to 5-HT, $PGF_{2\alpha}$, $PGD_2$, and $PGE_2$ but not to KCl, Hb, or NE. Contraction induced by 5-HT and NE following the endothelium removal had a greater effect on rabbit basilar than on canine basilar arteries (488). The removal of

endothelium sensitizes smooth muscle to the constrictor effect of endothelin (491).

Pharmacological studies of arteries removed 7 days post-SAH from dogs showed that endothelium-dependent relaxation to arginine vasopressin was abolished and that to thrombin was significantly reduced. Endothelium-independent relaxation in response to papaverine and SNP was essentially preserved. Endothelium-dependent contraction in response to mechanical stretching and hypoxia persisted in the SAH group. There was a significant correlation between the degree of VSP present angiographically and the loss of endothelium-dependent relaxation (13). Hb induced contraction in canine basilar artery ring preparations, as did nitro-L-arginine. Removal of endothelium by rubbing or precontraction induced by oxyHb both profoundly reduced nitro-L-arginine – induced contraction. Similarly, endothelium rubbing and precontraction by nitro-L-arginine reduced the contraction to oxyHb (492). Monkey cerebral arteries but not systemic arteries contracted to oxyHb. These contractions were markedly diminished by removing the endothelium. In endothelium-denuded arteries, indomethacin and diphloretin phosphate slightly attenuated the contractions of oxyHb. In arteries with endothelium, indomethacin and diphloretin phosphate markedly decreased oxyHb-induced contractions (366).

Endothelium-dependent relaxations are abolished in canine basilar artery after SAH. The release of EDRF toward the lumen was not reduced, however. Therefore, it was suggested that the responsiveness of smooth muscle to EDRF is impaired during VSP post-SAH (17).

Endothelial cells can produce several relaxing substances including EDRF (most likely NO) or a nitroso-derivative-releasing NO, derived from L-arginine. Endothelium can also release contracting factors which include superoxide anions, thromboxane $A_2$, and ET. NO relaxes vascular smooth muscle by activating the soluble form of guanylate cyclase, which leads to an accumulation of cGMP. This effect is synergistic with the inhibition evoked by prostacyclin. Thrombin from aggregating platelets is a potent stimuli for the release of NO. Other platelet products are ADP and ATP, which activate $P_{2y}$ – purinergic receptors on the endothelial cells, and 5-HT, which stimulates serotonergic receptors. The response to 5-HT is mediated by a pertussis toxin-sensitive mechanism, whereas the response to the adenine nucleotides is not (92). Endothelial cells therefore play a key role in the local regulation of vascular smooth muscle tone by producing and releasing relaxing and contracting factors (493–495). Shear stress on the arterial wall is one of the factors modulating the release of EDRFs. Flow-induced vasodilation is endothelium dependent in the intact organism (143).

Isometric tension studies were performed on human basilar arteries removed within a day of death from SAH and other patients. Vasoconstriction to KCl, NE, and 5-HT did not differ between groups. Endothelium-dependent relaxation to thrombin, BK, and A23187 was less in the SAH group. Endothelium-dependent responses to SNP did not differ between groups. These results were consistent with the hypothesis that decreased relaxation to thrombin and BK occurred at the level of endothelial cells and not smooth muscle cells and that decreased relaxation may be involved in vasoconstriction in VSP post-SAH (436).

### N. Endothelium-Derived Hyperpolarizing Factor

Endothelial cells inhibit the tone of underlying vascular smooth muscle cells by releasing NO and endothelium-derived hyperpolarizing factor (EDHF). The latter has not yet been identified. The importance of EDHF varies along the vascular tree. The basal release of EDHF is not likely to occur *in vitro*. Its production and release are regulated by the cytosolic $Ca^{2+}$ concentration derived both from the extracellular space and from intracellular stores. Calmodulin may be involved in its production and/or release. EDHF hyperpolarizes the vascular smooth muscle by opening $K^+$ channels. The hyperpolarization closes voltage-dependent $Ca^{2+}$ channels and as a consequence relaxes blood vessels (496).

EDHF is presumed to be a labile metabolite of arachidonic acid formed through the P450 pathway. It acts on smooth muscle by being one of the few physiological openers of the $K^+$ channels (143).

## X. Nitric Oxide

The modulation of vasomotor tone is complex and multifactorial (Fig. 6.13), (213). An important factor is the natural vasodilator NO. The binding or destruction of NO by Hb might remove a tonic vasodilator influence and be a significant causative factor for VSP.

### A. Nitric Oxide as a Vasodilator

NO has a wide variety of signaling roles in biological systems. It is one of the most ubiquitous messenger molecules in mammals and regulates multiple functions. It exerts its effect by diffusing across cell membranes instead of interacting with membrane receptors (497). The early 1980s witnessed the emergence of the concept that vascular endothelium controls the tone of blood vessels (Fig. 6.14). Endothelial-derived messenger molecules control the contractile activity of vascular smooth muscle cells.

Since the endothelium can sense changes in flow, the presence of humoral factors, and adherence of formed elements, it is an ideal sensor to regulate blood flow. It does so by altering vascular smooth muscle tone. Around the same time the NO free radical was identified as the active moiety produced by nitrovasodilators, NO was found to be the activator of soluble guanylate cyclase in vascular smooth muscle which leads to the increase in cGMP and the eventual relaxation of contracted smooth muscle. It was only later in the 1980s that it was appreciated that NO was a biologically produced messenger and not simply pharmacologically produced by medications. By the late 1980s it was established that L-arginine was the precursor of NO in activated macrophages and subsequently the concept was developed that NO is biologically produced from L-arginine in vascular endothelial cells. NO is a highly diffusible molecule which enter groups of cells within a defined radius (145). In 1986, Furchgott (498) and Ignarro *et al.* (499) independently suggested that EDRF may be NO or a closely related compound.

EDRF has since been identified as NO. The pharmacology of these two substances is indistinguishable and sufficient NO is release from endothelial cells to account for the biological activities of EDRF. Organic nitrates exert their vasodilatory activity following conversion to NO in vascular smooth muscle cells. Moncada and his group considered that NO is the endogenous nitrovasodilator. NO is synthesized by the vascular endothelium from the terminal guanido nitrogen atom(s) of the amino acid L-arginine, which is the endogenous precursor (Fig. 6.15) (500). NO is a labile humoral agent mediating the action of some vasodilators. Palmer *et al.* first demonstrated that NO release accounted for the biological activity of EDRF experimentally. They studied the release of EDRF and NO from endothelial cells in culture. NO was determined as the chemiluminescent product of its reaction with ozone. Biological activity of EDRF and NO was

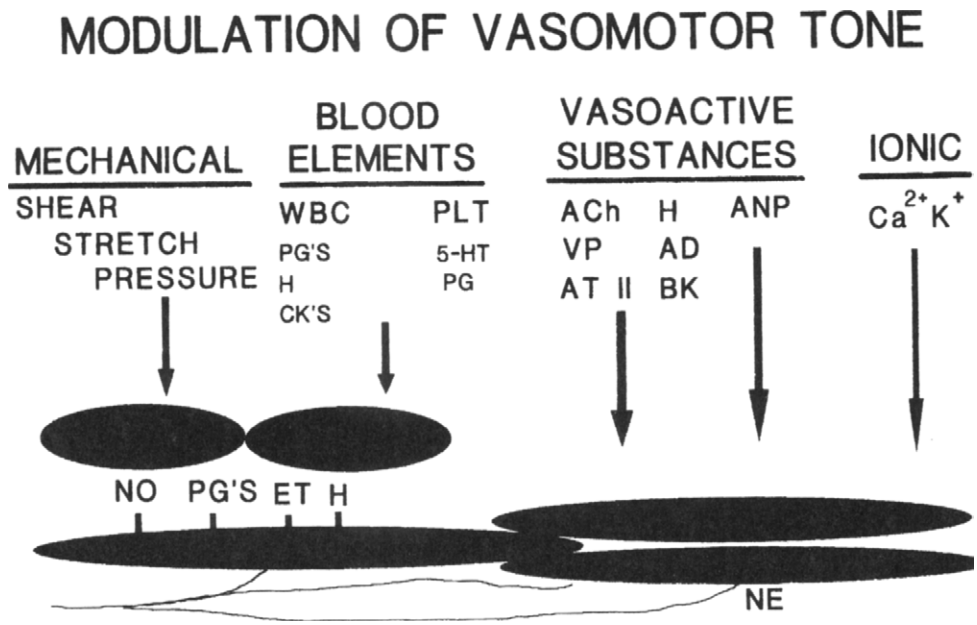


**FIGURE 6.13** Modulation of vasomotor tone. This schematic oversimplifies some of the numerous factors and forces that modulate vasomotor tone. These signals originate from the lumen of the vessel, from cells in the vessel wall, and from the adventitial aspect of the vessel. The vasomotor response to many substances depends on whether the endothelium is present or absent. Endothelial cells respond to many intraluminal influences with the release of NO, PG, ET, and histamine. Mechanical forces such as sheer stress and pressure resulting from the pulsatility of blood flow impact on vasomotor tone. Blood elements such as WBCs and platelets release PG, histamine, cytokines, and 5-HT. Circulating vasoactive substances include ACh, arginine vasopressin, angiotensin II, histamine, adenosine, BK, and ANP. The extracellular concentration of ions such as $Ca^{2+}$ and $K^{+}$ also influences vascular tone. Innervation of smooth muscle impacts on tone via the release of neurotransmitters such as NE. Thus, the regulation of vasomotor tone depends on multiple signals and responses to those signals [reproduced with permission from Brophy, C. M., Awolesi, M., and Sumpio, B. E. (1997). Regulation of vasomotor tone and vasospasm. In *The Basic Science of Vascular Disease* (A. N. Sidaway, B. E. Sumpio, and R. G. DePlama, Eds.), Futura, Armonk, NY.].

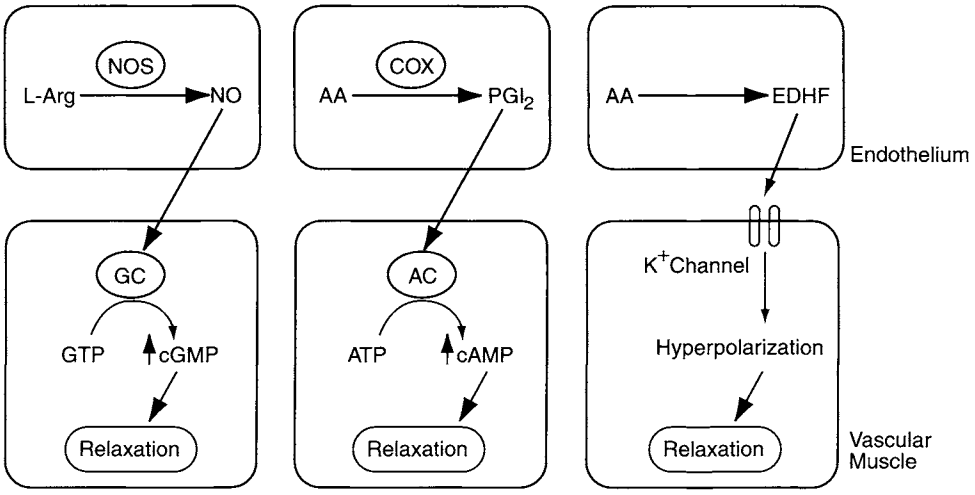


**FIGURE 6.14** Summary of mechanisms of endothelium-dependent relaxation of cerebrovascular muscle. NO is produced by NOS from the amino acid L- arginine (L-Arg). NO diffuses to vascular muscle, where it activates soluble guanylate cyclase (GC), causing increased production of cGMP that results in relaxation. $PGI_2$ is produced by cyclooxygenase (COX) from arachidonic acid. $PGI_2$ diffuses to vascular muscle, where it activates adenylate cyclase (AC), causing increased production of cAMP that results in relaxation. Endothelium-derived hyperpolarizing factor (EDHF) is produced from AA. EDHF diffuses to vascular muscle, where it activates $K^+$ channels. Increased activity of $K^+$ channels produces hyperpolarization and relaxation of vascular muscle [reproduced with permission from Faraci, F.M., and Heistad, D.D. (1997). Biology of cerebral vascular muscle. In *Primer on Cerebrovascular Diseases* (K.M.A. Welch, L. R., Caplan, D. J., Reis, B. K. Seisgö, and B. Weir, Eds.), Academic Press, San Diego].

measured using a bioassay. They wrote that EDRF was NO because the relaxation of bioassay tissues induced by EDRF was indistinguishable from that induced by NO. BK caused concentration-dependent release of NO from cells in amounts sufficient to account for the biological activity of EDRF. Hb inhibited the relaxations induced by EDRF and NO and SOD enhanced them to a similar degree. They concluded, "NO released from endothelial cells is indistinguishable from EDRF in terms of biological activity, stability, and susceptibility to an inhibitor and to a potentiator. We suggest that EDRF and NO are identical." (501). EDRF and NO are considered equivalent because their half-life in solution is similar (5 sec), they both elevate cGMP, pyrogallol inhibits them, Hb inhibits them, and SOD prolongs their activity (145). NO is secreted not only toward the underlying vascular smooth muscle cells but also into the blood vessel lumen. It has a physiological role at the interface between endothelium and blood. NO inhibits the adhesion of platelets and leukocytes to the endothelium (143). Others have agreed that EDRF appears to be NO or a labile nitrosocompound that is synthesized from endogenous L-arginine or an L-arginine-containing substance within vascular endothelial cells (501–504). Some investigators still claim differences between NO and EDRF and the debate is still active (505–507). Kontos suggested that in the cerebral circulation the activation of soluble guanylate cyclase by NO or nitrovasodilators is indirectly mediated-via the release of CGRP from sensory nerve fibers. He also suggested that the EDRF for cerebral arteries is not NO but a nitric oxide-containing compound, likely a nitrosothiol. Nitrosothiols activate soluble guanylate cyclase in cerebral arterioles by a direct action which is independent of CGRP (508). Freshly mounted, endothelium – denuded arterial rings were not relaxed by L-arginine but were by the diapeptide L-arginyl-L-alanine. Increases in smooth muscle levels of cGMP and nitrite accompany these relaxant responses. Elevation of cGMP was inhibited by oxyHb, methylene blue, and $N^G$-nitro-L-arginine. The latter also caused endothelium – independent contraction. It was concluded that a relaxing factor with the properties of NO can be generated from vascular smooth muscle (509).

Under physiological conditions NO plays a key role as a short-range messenger for maintaining vascular tone and intracellular communication and for modulating platelet aggregation and neutrophil adhesion (510–526). NO may also be written as NO• since it is a radical . SAH may divert normal metabolic function of the highly reactive NO radical (364,378,527–529). Nitrotyrosine can be formed after SAH, which is a pathological reaction of NO with tissue proteins (530). The influence of NO on the cerebral circulation is complicated by the fact that neurons and glia, in addition to endothelium, produce NO in

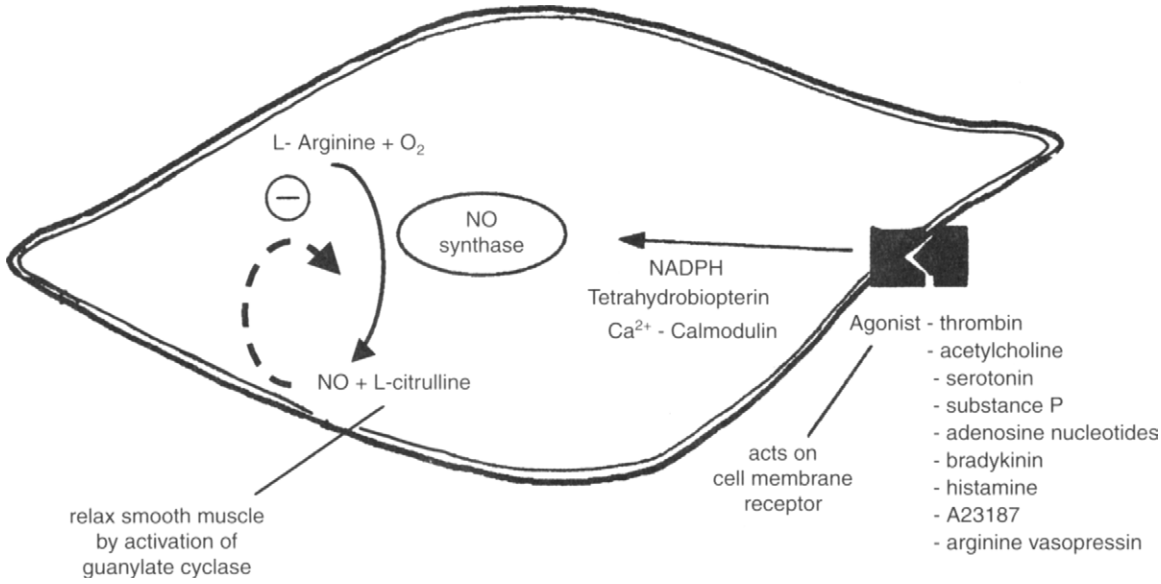


**FIGURE 6.15** Diagram of NO production [reproduced with permission from Weir, B., Stoodley, M., and Macdonald, R. L. (1999). Etiology of cerebral vasospasm. *Acta Neurochir.* **72,** 27–46. Copyright © Springer-Verlag GmbH & Co.].

response to certain stimuli. Neuronally derived NO may induce slow local vasodilation in response to increased neuronal activity. In addition to NO, cerebral endothelium produces prostacyclin, EDHF, and oxygen-derived free radicals, all of which can affect tone. Excessive production of endothelial–derived constricting factors such as the cyclooxygenase products of AA and ET may induce VSP (531).

NO is a small, gaseous paramagnetic molecule that reacts with iron-containing compounds to form paramagnetic iron-nitrosyl complexes. It has three redox forms. It can exist as a reactive free radical. The most widely studied NO interacting metalloproteins are the heme-containing proteins, guanylate cyclase and Hb. Affinity of $Fe^{2+}$ Hb for NO is several orders of magnitude greater than that for $O_2$. NO binds to both $Fe^{2+}$ heme and $Fe^{3+}$ heme, whereas $O_2$ and CO bind primarily to $Fe^{2+}$. At the point of binding, NO converts $Fe^{3+}$ heme to $Fe^{2+}$. NO, which has a half-life of only 2–5 sec, and reacts with $O_2$ to produce nitrate and nitrite ions. NO produced *in vivo* circulates as *S*-nitrosoalbumin, which has a relatively long half-life (532). NO is produced by NOS from the terminal guanidino moiety of L-arginine (533). NOS can exist as a constitutive or inducible form. The inducible form is usually absent in target cells and appears 4 hr after exposure to a stimulus. It is stable following expression. There is a tonic, basal level of NO production. Hypertension can be induced by the infusion of inhibitors of NO synthesis. A state of basal vasodilation is maintained by basal secretion of NO. L-Arginine analogs such as L-NMMA inhibit NO production and increase peripheral resistance, decrease tissue perfusion and $O_2$ delivery, and increase blood pressure. They also contribute to platelet adhesion and aggregation. The soluble enzyme guanylyl cyclase acts as receptor for the signaling molecule NO. The cyclase has a prosthetic heme group which is stimulated by NO.

In addition to being a potent endogenous vasodilator, NO has roles in inflammation, thrombosis, immunity, and neurotransmission. Some of its functions, such as vasodilatation and inhibition of platelet aggregation, are similar to those of other endothelial substances such as prostacyclin. It is formed in small amounts *in vivo* and is rapidly destroyed by interaction with $O_2$ which renders measurement difficult. NO synthesis is inhibited by arginine analog or agents that inactivate NO such as reduced Hb. None of the NO inhibitors are completely specific. Assays for NO involve the following: (i) NO is trapped by nitroso compounds or reduced Hb, forming a stable adduct which is detected by electron paramagnetic resonance; (ii) NO oxidizes reduced Hb to metHb, which is detected by spectrophotometry; and (iii) NO interacts with ozone-producing light—this chemiluminescence detection has a threshold of approximately 20 pmol compared to the 1 nmol threshold of the other methods. New amperometric microcelectrode assays may offer even more accurate methodologies in the future (510).

Human blood was incubated with NO (50–300 $\mu M$) and the formation of metHb, HbNO, and plasma nitrite and nitrate was measured. In plasma, NO is converted to

nitrite and nitrate in a 5:1 ratio. In arterial blood NO is quantitatively converted to nitrate and metHb. Nitrite is not produced, and HbNO formation is low. When $O_2$ saturation is less than 85% more HbNO is formed. It has been hypothesized that NO liberated from the endothelium of conductance and resistance vessels is taken up by RBCs and inactivated by $HbO_2$ via stoichiometric conversion to MetHb and nitrate (447). The metabolism and excretion of NO in humans was studied by the inhalation of NO in healthy subjects at 22 ppm in patients with severe heart failure in variable concentrations for 10-min periods. During inhalation of NO the plasma levels of nitrate increased progressively in healthy subjects and patients. MetHb also increased. No change in HbNO was detected. At $O_2$ saturations above 94%, nitrite was semiquantitatively converted to nitrate and metHb. At $O_2$ saturations below 85%, moderate amounts of HbNO were formed. Plasma and urinary clearance of nitrate in healthy subjects averaged 20 ml/min. Endogenously formed NO uses RBC uptake with subsequent conversion to nitrate and MetHb as its metabolic pathway. Nitrate then enters the plasma and is eliminated via the kidneys (534).

### B. Injury Induced by Nitric Oxide

NO may injure cells by direct injury, generation of highly reactive free radical species, lipid peroxidation, inhibition of mitochondrial enzymes, and disruption of gene transcription (535–544). NO induces $NO^{2-}$ (nitrite) and $NO^{3-}$ (nitrate) formation. In *in vitro* experiments NO combines with superoxide $O_2^{-\bullet}$ to produce peroxynitrite (535). Nitrites oxidize human deoxyHb. Nitrosoylhemoglobin (HbNO) is produced concurrently with metHb (545). The endothelial NOS in cerebral vessels is upregulated at 1 hr after induction of ischemia and progressively increases for up to 24 hr of ischemia. The NOS in the periphery of the area of necrosis in the cortex remained constant throughout the duration of ischemia (546).

Impaired endothelium-dependent relaxation may contribute to the pathophysiology of cerebral ischemia or stroke. NOS-containing neurons innervate large cerebral arteries on the brain surface. Parasympathetic fibers that innervate cerebral blood vessels produces NO-dependent increases in CBF on activation. NO may mediate the increases in CBF which follow hypercapnia. However, expression of inducible NOS in response to cytokine production may exert cytotoxic effects after ischemia. Under normal conditions, constitutively produced NO influences basal cerebral vascular tone and mediates the response of the blood vessels to a diverse group of stimuli (513). Reperfusion of cerebral ischemia was associated with increased NO generationin the brain which in turn led to an increase in nitrosylation of RBC Hb in cerebral circulating blood (547). In a cat model of MCA occlusion, inhibition of NOS decreased caudate injury volume. The beneficial effect was reversed by L-arginine. Because L-NAME was efficacious when administered at the time of reperfusion, it was assumed that NO generated during reperfusion contributed to caudate injury (548). Excessive production of NO can inhibit mitochondrial enzymes, disrupt gene transcription, increase oxidative injury and generate highly reactive free radicals species, and increase lipid peroxidation, all of which can cause cellular injury (379). Following severe head injury in humans, serum and CSF have been monitored for nitrite ($NO_2^-$) and nitrate ($NO_3^-$) levels. Levels were highest in the interval 30–42 postinjury. Levels ranged between 17 and 26 $\mu M$. At 30–42 hr the CSF nitrite and nitrate concentrations were 80% higher in patients who died than in survivors (549). Nitrite and nitrate levels in CSF post-SAH rose to peak levels in about 4–6 days, nitrate levels were higher than nitrite levels, and Fisher grade III patients had the highest levels (Fig. 6.16) (523).

There is evidence that the NO metabolite peroxynitrite injures endothelium associated with atherosclerosis by a peroxidative mechanism. In a rat model of SAH, vessels were perfusion fixed and the brains removed for immunohistochemical assessment of nitrotyrosine, the peroxidation product of peroxynitrite with tyrosine contained in tissue proteins. The brains of rats with angiographic VSP revealed nitrotyrosine predominantly located with a

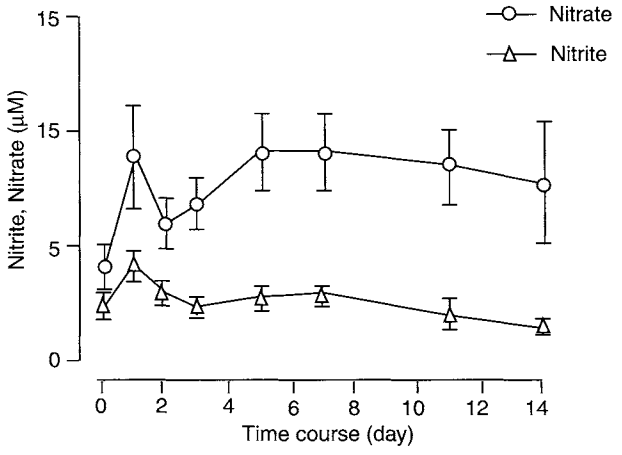


**FIGURE 6.16** Concentrations of nitrite and nitrate in the basal cisternal CSF of patients with SAH from Days 0 to 14. Each point represents the mean ± SE obtained from 31 patients [reproduced with permission from Suzuki, Y., Osuka, K., Noda, A., Tanazawa, T., Takaysu, M., Shibuya, M., and Yoshida, J. (1997). Nitric oxide metabolites in the cisternal cerebral spinal fluid of patients with subarachnoid hemorrhage. *Neurosurgery* **41**, 807–812].

perivascular distribution and in the pia. It was concluded that the peroxidation of membrane proteins by the NO metabolite peroxynitrite may contribute to the morphological damage associated with chronic VSP (530). Cultured bovine MCA endothelial cells were grown to confluency on plates. Factor VIII-related antigen staining and low-density lipoprotein uptake were assessed. The plates were exposed to hemolysate. Pretreatment with tirilazad mesylate (an inhibitor of iron-dependent lipid peroxidation) or *N*-nitro-L-arginine attenuated the significant hemolysate-induced changes in the endothelial cell barrier. The permeability of sucrose tracer across the layer of endothelial cells was examined. It was suggested that endothelial cells could provide a sufficient source of NO to damage their own cellular function (550).

### C. Nitric Oxide Synthase

Two major classes of NOS, constitutive and inducible, exist. Constitutive ones are found in endothelial and neuronal sites (Table 6.10). In endothelium, a constitutive isoform of NOS, regulated by $Ca^{2+}$/CaM, responds to agonist-induced receptor activation. The formation of NO from L-arginine by NOS can be inhibited by $N^G$-substituted analogs of L-arginine such as L-NMMA, $N^G$-nitro-L-arginine (L-NNA), and L-NAME. An inducible isoform of NO which is calcium independent can also be expressed in cerebral endothelium by endotoxins and cytokines. Relaxation in response to many agonists is dependent on the presence of endothelium in arteries from experimental animals and humans. Such agonists include ACh, BK, A23187, ADP, ATP, thrombin, histamine, SP, neurokinins A and B, neuropeptide A, AVP, and prostaglandin $PGF_{2\alpha}$. Inhibitors of NOS decrease the basal levels of cGMP production *in vitro*, which is dependent on endothelium and the basal release of NO. *In vivo* administration of L-NMMA, L-NNA, and L-NAME produces concentration-dependent constriction of cerebral blood vessels and inhibitors of NOS decrease CBF under basal conditions in several species. The influence of basal NO levels may be greater in larger arteries. NO may antagonize the effect of circulating long-acting vasoconstrictors such as 5-HT. NO may also modulate responses to activation of the sympathetic nerves. The substrate for NO, L-arginine, has little effect on vascular tone (145, 531). NO is not stored in vesicles like classical neurotransmitters but is produced by NOS upon stimulation. Activation of methyl-D-aspartate and kainate receptors can stimulate NOS to produce local NO. Arterial smooth muscle cells in the neointima formed after deendothelializing balloon injury in rat carotid artery expressed the cytokine-inducible form of NOS. NOS mRNA was demonstrated in neointimal cells, particularly on the surface of the lesion. This was associated with systemically detectable NO production as revealed by the electron paramagnetic resonance spectroscopic analysis of nitrosylated RBC Hb (551). Vascular smooth muscle cells respond to NO by relaxation and inhibition of mitochondrial respiration. NOS-dependent nitrite production demonstrated that the enzyme requires NADPH but not $Ca^{2+}$ as a cofactor. *De novo* NOS gene transcription and protein synthesis are required since actinomycin and cycloheximide abolish the cytokine effect (552). NADPH-diaphorase, a marker of NOS, indicates that there is innervation of cerebral vessels by NOS-containing nerve fibers. These fibers appear to originate in the pterygopalatine ganglion (520,553). There is a loss of vasodilator properties in the vessel wall in chronic VSP (554,555). This may be relatively greater than the preservation of vasoconstrictive ability (554). NOS activity is present in the nerve endings in the adventitia (520,556,557). Stimulation of the spenopalatine ganglion increases CBF by vasodilation (556,557). The nerve endings have been shown to disappear following variety of immunohistochemical tests

**TABLE 6.10 Types of Nitric Oxide Synthase**[a]

| Characteristic | Endothelial | Neuronal | Inducible |
|---|---|---|---|
| Location | Endothelial cells, membrane associated | Neurons, cytosolic | Macrophages, smooth muscle, endothelial cells, cardiac myocytes, astrocytes |
| Cofactors | Calcium – calmodulin | Calcium – calmodulin | Not calcium dependent |
| | Tertrahydrobiopterin | Tetrahydrobiopterin | ? Tetrahydrobiopterin |
| | NADPH | NADPH | ? NADPH |
| Activity | Constitutive | constitutive | Induced by cytokines and lipopolysaccharides; inhibited by steroids and some cytokines |
| | Picomolar levels of NO | Picomolar levels of NO | Nanomolar levels of NO |

[a]From Weir, B., Stoodley, M., and Macdonald, R. (1999). Etiology of cerebral vasospasm. *Acta Neurochir.* **72,** 27–42. Copyright © Springer-Verlag GmbH & Co.

(24,31,126). The loss of NOS immunoreactivity from the adventitial periarterial nerves can reasonably be attributed to a direct effect of Hb on the nerves. Hb accumulates adjacent to the nerves in the subarachnoid space (293, 315,407) as well as in the vessel wall (558). There may be a reduction in NO delivery (390,448,515,559) to smooth muscle guanylate cyclase (528,560,561) and a decrease of cGMP in the vessel wall (439,442,561,562) leading to vasocontriction (559,563).

Pluta and colleagues demonstrated the presence of NOS-containing nerves in adventitia. They demonstrated the disappearance of NOS activity from the adventitial nerve endings during chronic VSP and also showed preservation of endothelial NOS. They hypothesized that the loss of vasodilatory input from the adventitia in response to clot is a key factor in VSP rather than the loss of NO produced by endothelial cells. The primate clot model was used. Periadventitial blood clot resulted in the virtual absence of these nerve fibers around the spastic right MCA but persistent normal staining on the contralateral clot side. In monkeys in which VSP resolved by day 14 post-clot application the return to normal caliber of the MCA was not accompanied by a return of NOS immunoreactivity (564). Chronic VSP was also induced in the MCA using the primate clot application model. Vessels in spasm showed a significant decrease in endothelial NOS mRNA. There was an increase in the ipsilateral cortex in endothelial and NOS mRNA compared to the contralateral nonclot side cortex. There was a nonsignificant decreased in soluble guanylate cyclase in the MCA and in the cortex ipsilateral to the clot. The significant decrease in endothelial NOS mRNA a week after SAH is a contributory factor to VSP (565). In the primate model of chronic VSP, 3-min intracarotid infusions of L-arginine ($10^{-6}$ M) did not affect the degree of VSP but did increase the rCBF by 21%. Intravenous infusion over 14 days of L-arginine ($10^{-3}$ M) did not affect VSP, although L-arginine levels increased form 13 to 22 mg/ml on day 7 and to 19 mg/ml on day 14 (566).

In a rat double-hemorrhage SAH model angiographic VSP was demonstrated in 11 of 15 animals 7 days later. Animals were perfusion fixed and immunohistochemical assessment with staining for inducible NOS was quantified. The staining for iNOS was markedly more intense in animals with significant VSP. Control animals showed virtually no staining. The inducible NOS was mainly found in adventitial cells but was also present in endothelial cells, vascular smooth muscle cells, microglia, glia, and neurons. It has usually been hypothesized that a reduction in NO or a decrease in efficacy are substantial causes of VSP (96,274,440,515,564–569). This conclusion was based on the effect of NO and NO antagonists on experimental VSP.

NOS has important actions on the heart which would have to be considered in any systemic therapy seeking to modify its activity in the brain blood vessels. Endothelial NOS inhibits cardiac contractile tone and vascular smooth muscle proliferation, inhibits platelet aggregation and monocyte adhesion, promotes diastolic relaxation, and decreases $O_2$ consumption in cardiac muscle. It opposes the inotropic action of catecholamines after muscarinic cholinergic and β-adrenergic receptor stimulation. Inducible NOS participates in immune defense and may promote cell death through apotosis. In cardiac myocytes NO may regulate L-type $Ca^{2+}$ current and contraction through activation of cGMP-dependent protein kinase and cGMP-modulated phosphodiesterases. NO may elevate cGMP by interaction with heme proteins, non heme iron, or free thiol residues on target signaling proteins, enzymes, or ion channels. There appears to be tight molecular regulation of NOS expression and NO plays many roles in heart function (570).

Recent advances in recombinant DNA technology have made it possible to increase local NO production in the vascular wall of a double-hemorrhage canine model of SAH (571,572). Arteries were removed and exposed for 24 hr to replication-deficient recombinant adenovirus vectors encoding bovine endothelial NOS and the marker β-galactosidase genes. Twenty-four hours after gene transfer, expression and function of recombinant genes were evaluated by immunohistochemical staining, β-galactosidase protein measurement, and isometric tension recording. The expression of β-galactosidase protein was double in SAH arteries compared to normal ones. Endothelium-dependent relaxation caused by BK and SP was suppressed in SAH arteries. However, the relaxation to BK was significantly augmented in both normal and SAH arteries after gene transfer of the encoded bovine endothelial NOS to the adventitia of the canine rings. The relaxation to SP were augmented by endothelial NOS transduction only in normal arteries. BK and SP caused relaxation in endothelium-denuded arteries when the vessels were transduced with endothelial NOS. The expression of recombinant proteins after adenovirus-mediated gene transfer may be enhanced in arteries affected by SAH, and successful endothelial NOS gene transfer to spastic arteries may partly restore NO-mediated relaxation through adventitial production of NO (573).

Submicromolar concentrations of NO bind to the heme moiety of guanylate cyclase to evoke large increases (over 100-fold), in the activity of the enzyme that converts GTP to cGMP. Vascular smooth muscle cells do not normally produce their own NO since they rarely express NOS. In certain pathological circumstances, however, cytokines can induce NOS in vascular smooth muscle cells resulting

in large increases of NO and cGMP production. The effects of NO signaling in vascular cells are associated with low ($< 1\mu M$) concentrations of NO or NO-generating drugs. cGMP reduces the level of physiologically meaningful $[Ca^{2+}]i$ in vascular smooth muscle. Depolarization elevates $[Ca^{2+}]i$ through the opening of voltage-gated channels mainly, whereas G protein agonists such as vasopressin and angiotensin elevate $[Ca^{2+}]i$ through activation of PLC and the generation of $IP_3$. There are multiple sites of action of cGMP to reduce $[Ca^{2+}]i$ in vascular smooth muscle cells. NO and cGMP may bring about hyperpolarization (145).

### D. Nitric Oxide Synthase Inhibitors

Treatment with L-NMMA abolished the relaxant response of transmural electrical stimulation and this inhibition was reversed by L-arginine. The relaxant responses of monkey cerebral arteries to transmural electrical stimulation were suppressed by treatment with L-NMMA, a NO synthesis inhibitor. The inhibitory effect was prevented and reversed by L-arginine but not D-arginine (574). Observations were made on cats using cranial windows. L-NMMA abolished vasodilation from ACh and eliminated the production NO. L-Arginine reversed the effects of the inhibitors of NO synthesis. The inhibitors did not affect baseline vascular caliber or generate vasoconstrictor agents. The tested inhibitors of NO synthesis did not affect the response to SNP or adenosine, demonstrating that the effect on responses to ACh was specific. It was concluded that EDRF is either NO or a NO-containing substance. Inhibitors of NO synthesis abolishes the response of the ACh. There is no apparent involvement of radicals and no vasoconstrictor agent is generated (575). Secondary branches of the basilar arteries from dogs exposed to SAH for 7 days and control animals were studied using intraluminal perfusion. In non-SAH arteries vasopressin, BK, and A23187 all caused endothelium-dependent relaxations. L-NAME abolished relaxations due to vasopressin and reduced response to BK and A23187. SAH abolished relaxations to vasopressin but did not affect relaxations to BK or A23187. This suggested that smaller arteries might be relatively resistant to VSP after SAH (438). In dogs following SAH on days 4 and 7 post-SAH, after two intracisternal injections of autologous blood L-arginine produced transient vasodilation of the spastic basilar artery, but L-NMMA produced no significant vasoconstriction. The SAH reduced the vasodilatory effect of L-arginine more on day 7 than on day 4. Intracisternal injection of SOD, which is assumed to protect NO from oxidation by $O_2^{-\bullet}$, had no effect by itself but appeared to enhance the vasodilatory effect of L-arginine on both days 4 and 7 (515). In a rat model the effect of NOS inhibitors on focal cerebral ischemia was studied. Rats were subjected to 3 hr of combined left MCA and bilateral common carotid artery occlusion under anesthesia. A nonselective NOS inhibitor and two NO donors were administered intravenously 30 min before ischemia was induced. Infarct size was estimated 3 days later. Infarct size was reduced by pretreatment with the NO inhibitor compared to controls, treatment with NO donors did not significantly alter infarct volume. This suggested a role for NO in ischemic neurotoxicity and a therapeutic role for the inhibition of neuronal NOS (576).

In a rat endovascular suture model of acute SAH, the NOS inhibitor L-NAME given intravenously produced a decrease in resting CBF. Twenty minutes after SAH it did not add to the decrease in CBF from SAH. One hour later it decreased CBF recovery. In this model NO metabolites were decreased at 60 min post-SAH and the administration of an NO donor within this time increased CBF and decreased ischemic brain damage (577).

## XI. Nitrovasodilators

### A. Mechanisms of Action

The vasodilatory effect of amyl nitrite (a NO donor) on angina pectoris was first described by Bruton in 1863 (497). Organic nitrates had been used for 120 years prior to the clarification of their mechanism of action. Nitrovasodilators induce smooth muscle relaxation in association with an increase in guanosine 3′,5′-cyclic monophosphate (cGMP) content. The mode of action differs from that of other vasodilators such as papaverine and prostacyclin (Table 6.11). The action of nitrovasodilators may be mediated through the intracellular formation of NO. NO interacts with the ferrous heme moiety linked to soluble guanylate cyclase to stimulate the enzyme. This ferrous heme moiety may be regarded as the receptor for NO and possibly for endogenous stimulants of soluble guanylate cyclase. Competition between exogenous Hb and the ferrous heme moiety on soluble guanylate cyclase for binding of NO may explain the ability of Hb to inhibit the smooth muscle relaxation and concomitant increases in cGMP induced by NO and also the nitrovasodilators. Only ferrous heme proteins with ligand-binding properties inhibit the actions of NO and nitrovasodilators (373).The most commonly employed organic nitrates are nitroglycerine, isosorbide dinitrate, and isosorbide-5-mononitrate. These agents work by degrading to the free radical NO, which stimulates guanylate cyclase in vascular smooth muscle cells to produce cGMP. The latter induces vasodilation by reducing the availability of

$[Ca^{2+}]i$ for contractile proteins. Interestingly, organic nitrates cannot be given in a way which provides therapeutic effect throughout 24 hr each day. Nitrate tolerance cannot be modified by concurrent medications. The only available method is to give these agents intermittently and to provide a washout period. From the coronary vasodilation stand-point it is only possible to provide therapeutic nitrate effect for approximately 12 hr in any 24-hr period (578). The mechanism underlying this tolerance is thought to be depletion in the cells of thiones responsible for the conversion of glycerol nitrate to NO (500). Several vasodilators relax vascular smooth muscle by releasing EDRF, which diffuses to the vascular smooth muscle and activates guanylate cyclase inducing relaxation. The EDRF induced by ACh has a half-life of approximately 6 sec and is anionic and hydrophylic. Although most investigators have suggested that EDRF from ACh is NO, Marshall and colleagues provided evidence that the EDRF generated by ACh in the cerebral microcirculation is not NO (579). NO is a highly unstable substance which is rapidly converted to $NO_2^-$ and $NO_3^-$ in oxygenated solutions. Hb has a greater affinity for NO than for $O_2$. NO probably acts by direct cell-to-cell transfer, rapidly crossing cell membranes, thereby escaping inactivation by Hb. Vascular smooth muscle may also operate by spreading hyperpolarization between smooth muscle cells (580) or by direct cell-to-cell communication (581).

Nitrovasodilators react with Hb to form heme (III) and HbNO. Carbon monoxide binds to heme (II), completely blocking the reactions of SNP and GTN. The most $O_2$-sensitive step in the nitrosylation of Hb by SNP is probably the transfer of NO to heme (II) (582). In cultured vascular smooth muscle cells SNP lowered $[Ca^{2+}]i$ in cells in which it was elevated after depolarization. SNP decreased current through voltage-gated $Ca^{2+}$ channels but did not affect the release of $Ca^{2+}$ from intracellular stores. Hb reversed the effect of SNP on $[Ca^{2+}]i$, and 8-Br-GMP, a membrane permeant form of cGMP, mimicked the effect of SNP on $[Ca^{2+}]i$ and on $Ca^{2+}$ currents (583).

**TABLE 6.11 Vasodilators**

| | | |
|---|---|---|
| Papaverine | Cyclic nucleotide phosphodiesterase inhibitor | Prevents breakdown of cAMP and cGMP Increases cAMP |
| NO and NO Donors (GTN, SNP) | Guanylate cyclase activators | Increases intracellular cGMP |
| Prostacyclin | Interacts with cell surface receptors to activate adenylate cyclase | Increases cAMP |

### B. Animal Models of Subarachnoid Hemorrhage

Frazee *et al.* showed the efficacy of intravenous GTN in the prophylaxis of chronic VSP in a primate model (584). In the monkey clot placement model, cGMP and cAMP levels were measured in the cerebral arteries and bilateral parietal cortices 7 days after clot placement. Significant angiographic VSP occurred on both sides, but was more prominent on the side of clot placement. rCBF on the clot side was significantly decreased. In the clot MCA arteries, cGMP levels were significantly lower than in normal arteries. After the administration of nitroglycerin for 3 hr at 3 μg/kg/hr or saline in equivalent volumes, the rCBF was significantly increased on the clot side but not on the control side. Administration of nitroglycerin did not elevate the depressed cGMP levels on the clot side artery to normal, but there was a significant increase in cGMP levels in the basilar artery which was not directly surrounded by clot. In both parietal cortices cGMP levels were significantly decreased after SAH and unchanged after nitroglycerin treatment. Since the increase in CBF was due to vasodilation and there was not an apparent corresponding increase in cGMP, it was hypothesized that the effect of intravenous nitroglycerin might have been to cause hyperpolarization of the vascular smooth muscle cells. It was suggested that nitroglycerin might be therapeutic in the treatment of VSP (585).

Rat basilar arteries were studied using a perfusion system post-SAH. Intraluminal application of GTN had a stronger relaxing effect than extraluminal application, but the difference was not significant. The relaxing effects of GTN were more potent if the arteries were precontracted by ET than if they were precontracted by KCl. Extraluminal oxyHb significantly inhibited the relaxation induced by GTN. SAH did not attenuate the relaxing effects of GTN on the arteries precontracted by KCl. The ability of GTN to relax SAH arteries precontracted by ET was significantly diminished. Hb is such a large protein that it is likely confined to the extracellular space and as such cannot be expected to inhibit vascular relaxation induced by the intracellular actions of NO converted from GTN. It is possible that as NO crosses cell membranes, binding to Hb in the extracellular space occurs (586). A new class of NO-donating compounds with predictable kinetic behavior *in vivo* has recently been developed—NONOates. Pluta and colleagues investigated diethylamine-NO (half-life, 2.3 min), glucantime-NO (half-life, 0.8 min), and proli-NO (half-life, 1.8 sec) (587–589). Also, using the monkey clot placement model 21 animals were studied in two experimental paradigms. In

an acute infusion experiment, saline or NO-donating compounds (NONOates) were infused intracarotidly in four normal and four post-SAH animals. In chronic infusion experiments, saline or NONOate (diethylamine-NO or proli-NO) was infused intracarotidly until day 7 post-SAH. In the acute infusion experiment 3 min of intracarotid diethylamine-NO reversed angiographically confirmed VSP of the right MCA. The reversal of VSP was accompanied by an increase in rCBF and a decrease in mean systolic TCD velocity in the right MCA. In the long-term infusion experiments the area of the right MCA in control animals decreased 63%. After 7 days of infusion of glucantime-NO by intracarotid infusion the area of right MCA decreased by only 15%, and with 7 days proli-NO infusion the decrease was 11%. The glucantime-NO infusion reduced mean blood pressure (BP) from 75 to 57 mmHg, but BP was unchanged in animals undergoing proli-NO infusion. It was suggested that VSP could be both reversed and prevented by NO replacement. Only the infusion of NONOate, with its extremely short half-life, was able to dilate the vessel without a concomitant drop in BP. This appears to be an extremely promising approach, but the risk of continuous intracarotid infusion would be substantial (590).

The CBF in rats was studied 48 hr post-SAH. Laser Doppler flowmetry was employed in connection with intracarotid injection of NO donors or cortical superfusion. Phenylephrine had to be used to control hypotension. It was possible to increase CBF 129% in control animals and 112% in the SAH group. SAH did not affect the CBF increases produced by intracarotid 8–bromo-cGMP or papaverine (569). In another acute rat model of SAH, intracarotid injection of the NO donor *N*-nitroso glutathione caused vasodilation as evidenced by an increase in CBF, increased blood vessel diameter, decreased vessel wall thickness, and decreased extracellular glutamate levels. Blood pressure decreased transiently (591).

The intracellular NO donor hydroxylamine given intraperitoneally twice daily for 7 days post-experimental SAH reduced basilar arterial wall damage, attenuated neurological deficit, reduced SAH, and induced increases in hippocampal and cortical β-APP immunoreactivities and hippocampal NOS activity (592).

### C. Intrathecal Nitrovasodilators

In an animal model multiple fresh blood injections were injected into the cisterna magna. In the treated group, SNP was injected intrathecally beginning on day 4 post-fresh blood injection and continuing for 2 days at a dosage of 25 μg/kg/day. Arterial diameter was reduced 73% in the SAH and 29% in the SNP group. The injection of SNP alone without blood did not result in significant changes in ICP, BP, or ECG. Vascular responses were apparent with intravenous injection. It was concluded that intrathecal SNP is an effective treatment for VSP (593). In a canine model of SAH basilar artery diameter was measured on days 0 and 7. A group of animals received DETA/NO, a long-half-life diazeniumdiolate-class NO donor. Drugs were administered in a 2-ml intrathecal bolus via the cisterna magna. Arterial caliber was then monitored by serial angiography over the subsequent 4 hr. The drug DETA/NO produced reversal of VSP in a dose-dependent fashion that roughly followed a double exponential time course. DETA/NO (2 μ*M*) restored basilar artery diameter to pre-VSP size 1.5 hr posttreatment and this was sustained at 88% of baseline at 4 hr. A group receiving one-tenth the dose of this agent achieved only partial and transitory relaxation. No histopathologic damage attributable to the donor could be identified (594). The potent vasodilator and precursor of NO, SNAP, a stable *S* – nitrosothiol compound, reversed VSP in a rabbit model. Arterial narrowing was prevented without systemic hypotension (595). The use of intrathecal nitrovasodilators in humans is discussed elsewhere.

### D. Effect on Vascular Smooth Muscle Cells

Some vasoactive substances are also capable of mitogenic and smooth muscle proliferative influences (596–598). Smooth muscle mitogens such as PDGF and epidermal growth factor are also capable of inducing vasoconstriction (599,600). Vasorelaxants such as nitrovasodilators and prostacyclins can prevent vascular smooth muscle proliferation (601). Human vascular smooth muscle cells inhibit platelet aggregation when incubated with GTN. The inhibitory effect on platelet aggregation was markedly attenuated by Hb. This suggests that vascular smooth muscles can generate NO from GTN (602). NO-generating compounds such as SNP inhibit the relative rate of protein synthesis in cultured aortic smooth muscle cells. This inhibition of protein synthesis by SNP was prevented by Hb (10 μmol/liter), which suggests that the protein synthesis inhibition was due to NO release. NO may modulate vascular smooth muscle protein synthesis and extracellular matrix component production (603).

## XII. Free Radicals

### A. Oxygen and Free Radicals

$O_2$ is essential for aerobic life. $O_2$ is consumed during $O_2$ metabolism and reactive $O_2$ species are by-products.

The amount of $O_2$ consumed and diverted to reactive $O_2$ species is considered to be less than 2 or 3% of the total amount of $O_2$. The continuous production of reactive $O_2$ species places biological systems under a constant oxidative stress (604). The body has evolved a whole series of defense mechanisms consisting of enzymes and vitamins to scavenge free radicals and various biological systems impede the production of free radicals initially. Systems generating free radicals are numerous and include the most fundamental enzymatic systems of the mitochondria and cytosol. About 10% of cellular reactions involving molecular $O_2$ as the electron acceptor generate oxy radicals. Therefore, thousands of such radicals are produced in every cell everyday. Activated neutrophils, eosinophils, monocytes, and macrophages release free radicals with a respiratory burst to kill pathogens. Among various sources of $O_2$ radicals are peroxidases, which react with peroxide and ions such as chloride to produce hypohalous acids. Lipid peroxidation yields malondialdehyde. $Fe^{+3}$ in Hb reacts with $O_2$ to produce the superoxide radical. Lipids are perhaps most susceptible to free radical damage but proteins, RNA, DNA, and other molecules may also be damaged. $O_2$ free radicals are generated by intracellular production from mitochondria, auto oxidation of catecholamines, the conversion of xanthine dehydrogenase to xanthine oxidase, activated neutrophils, and the AA cascade (Fig. 6.17) (605, 606).

A free radical is any species capable of independent existence that contains one or more unpaired electrons. The presence of one or more unpaired electrons tends to make the species more active in a magnetic field and also to make it highly reactive. The $O_2$ molecule as it occurs naturally qualifies as a radical because it has two unpaired electrons, each located in a different antibonding orbital. $O_2$ is a good oxidizing agent. Oxidation is defined as the loss of electrons by an atom or molecule such as the conversion of Na atom to the ion $Na^+$. Reduction is a gain in electrons by an atom or molecule such as the conversion of a Cl atom to a $Cl^-$ ion. An oxidizing agent absorbs electrons from the molecule that it oxidizes, whereas a reducing agent donates electrons. Forms of oxygen more reactive than ground-state $O_2$ are known as singlet $O_2$ ($^1O_2$). They can be generated by an input of energy but strictly speaking are not radicals since they have no unpaired electrons. If a single electron is added to the ground-state $O_2$ molecule, the product is a $O_2^{\bullet}$. In biological systems the two-electron reduction product of $O_2$ is $H_2O_2$, when three react the hydroxyl radical ($OH^{\bullet}$) is formed, and the four-electron product is $H_2O$ (606,607).

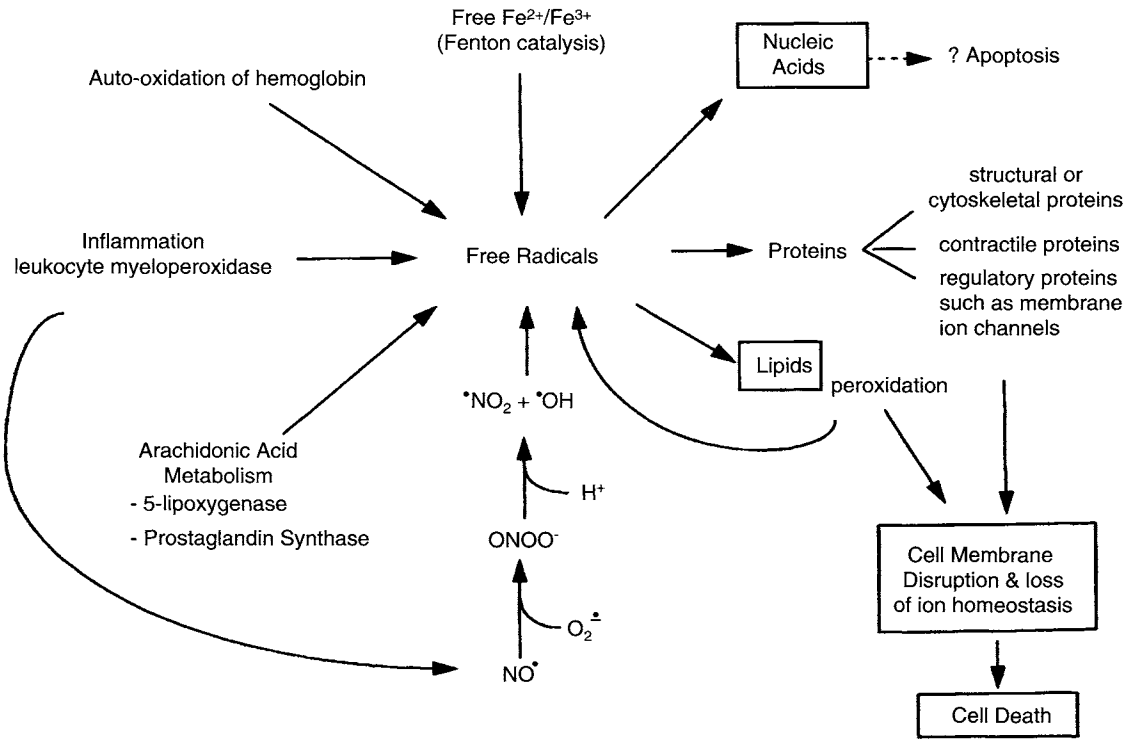


**FIGURE 6.17** Diagram of pathways for free radical generation after SAH [reproduced with permission from Weir, B., Stoodley, M., and Macdonald, R. L. (1999). Etiology of cerebral vasospasm. *Acta Neurochir.* **72,** 27–46. Copyright © Springer-Verlag GmbH & Co.].

The major free radicals that are of importance in neurobiology are of two classes; $O_2$ free radicals and nitric oxide. The existence of a free radical is an unusual occurrence in most biochemical reactions because the free radical state is very unstable so that free radicals have a strong tendency to donate or except extra electrons to complete their molecular orbitals. The reactivity of the original free radical can result in a chain reaction to form free radicals in molecules far removed from the original ones. Several different molecules can be affected in a series of reactions. These reactions can be harmful to the biological systems if the chain reactions occur within membranes (604).

### B. Superoxide Radical

The $O_2^{-\bullet}$ is generated by multiple pathways and often placed at the start of an oxidative stress cascade. The brain obtains its energy from oxidative respiration through the mitochondrial electron transport chain. During the production of ATP there is a small high-energy electron leak of perhaps 1–3% resulting in the generation of $O_2^{-\bullet}$. It is also produced by cells involved in the host immune response. Most likely, the $O_2^{-\bullet}$ generated in the cell undergoes catalytic conversion to $H_2O_2$ through the action of SOD. $O_2^{-\bullet}$ and $H_2O_2$ can also be produced by the action of several oxidase enzymes such as xanthine oxidase. $H_2O_2$ is scavenged by gluthathione peroxidase and catalase. $O_2^{-\bullet}$ can react as a reducing agent and donate its extra electron or an oxidizing agent, in which case it is reduced to $H_2O_2$. $O_2^{-\bullet}$ can also oxidize molecules such as ascorbic acid and catecholamines (608).

The oxidative damage potential is a summation of reactive $O_2$ species such as $O_2^{-\bullet}$, $H_2O_2$, and $^{\bullet}OH$, Fe and Cu ions, oxidized proteins, oxidized nucleic acids, lipid peroxides, and other peroxides. The antioxidant defense capacity is provided by SOD, catalase, glutathione peroxidase, peroxidases, glutathione tranferases, vitamins A, C, and E, and other repair enzymes and antioxidants. After the onset of ischemia the main free radical produced is $O_2^{-\bullet}$. Especially after circulation is restored, there is a significant production of free radicals. These can result from the arachidonic acid cascade, production by enzymes such as monoamine oxidase or autooxidation of amine neurotransmitters such as dopamine, mitochondrial leak, xanthine oxidase activity, and the oxidation of extravasated Hb. More $O_2^{-\bullet}$ may be produced by leukocytes which infiltrate the infarct. $O_2^{-\bullet}$ which is formed by the single electron reduction of $O_2$ can act as either an electron acceptor or a donor. Once it is formed it undergoes spontaneous dismutation to form $H_2O_2$ in a reaction which is accelerated by SOD. $O_2^{-\bullet}$ oxidizes oxyHb to metHb and reduces metHb to oxyHb. The reactions of $O_2^{-\bullet}$ and $H_2O_2$ with oxyHb or metHb and their inhibition by SOD or CAT were used to detect the formation of $O_2^{-\bullet}$ or $H_2O_2$ on autooxidation of oxyHb. The copper – catalyzed autooxidation of Hb involves $O_2^{-\bullet}$ production. The rate of hemichrome formation from metHb varies with the type of Hb (609,610).

Incubated whole blood or washed RBCs under conditions simulating SAH were able to generate $O_2^{-\bullet}$ and showed SOD activity over 8 days. Electron spin resonance showed changes from high to low spin corresponding to changes from oxyHb to metHb and hemichromes. $O_2^{-\bullet}$ was generated (427). During ischemia, ATP levels decrease in the brain and AMP levels rise. AMP is metabolized via the purine salvage pathway to adenosine, inosine, and hypoxanthine. The rising $[Ca^{2+}]i$ activates proteolytic and phospholipase enzymes. This can convert xanthine dehydrogenase to xanthine oxidase which uses molecular $O_2$ instead of the nucleotide radical as an electron receptor and catalyzes the production of $O_2^{-\bullet}$ at the moment of reperfusion. This newly produced $O_2^{-\bullet}$ can cause tissue damage and generate secondary radical species. $O_2^{-\bullet}$ promotes the release of iron from ferritin (611).

### C. Hydroxyl Radical

Molecular $O_2$ can be reduced in a series of univalent steps to generate three oxidant species: $O_2^{-\bullet}$, $H_2O_2$ and $^{\bullet}OH$. Univalent reduction of molecular $O_2$ generates $O_2^{-\bullet}$, which can then be converted to $H_2O_2$ either by spontaneous dismutation or by the action of the enzyme SOD. $H_2O_2$ can interact with $O_2^{-\bullet}$ in the Haber – Weiss reaction or with $Fe^2$ in the Fenton reaction to form the $^{\bullet}OH$ radical (604,607). $^{\bullet}OH$ is highly reactive with a variety of cellular components, including membrane lipids, nucleic acid, receptors, and enzymes. $^{\bullet}OH$ can alter membrane fluidity and lead to cell lysis. The central nervous system (CNS) is rich in peroxidizable polyunsaturated fatty acids and therefore susceptible to attack by reactive $O_2$ species. The CNS is relatively poor in catalase and glutathione peroxidase, which can inactivate $H_2O_2$ by converting it to $H_2O$. Another adverse factor is the presence of high concentrations of Fe in the nervous system. A reactive $O_2$ species can be generated in ischemic/perfused tissues by several pathways, including purine metabolism and AA (612). The hydroxyl radical ($^{\bullet}OH$) has an exceedingly short half-life but in that time can damage any biological molecule it contacts. For polyunsaturated fatty acids and membranes a spreading wave of peroxidation may be initiated which causes loss of membrane fluidity, secretory function, ionic gradients, and ultimately cell lysis. The peroxyl radical $LOO^{\bullet}$ (L stands for lipids) is a radical formed in lipid oxidation chain reactions. It has a relatively long half-life lasting seconds. It is an impor-

tant cause of membrane damage and it can also form by autooxidation of polyunsaturated fats. A major source of $OH^{\bullet}$ in biological systems is the reactive sequence of $O_2^{-\bullet}$ with $H_2O_2$ in the presence of the iron ion – the iron–Haber–Weiss reaction, which is thought to initiate lipid peroxidation (611). Although $^{\bullet}OH$ cannot be directly measured, if salicylate is present a combination of salicylate and $^{\bullet}OH$ is formed, dihydroxybenzoic acid, which can be obtained by microdialysis from the brain and measured (611).

### D. Nitric Oxide Radical

Nitric oxide radical ($NO^{\bullet}$) is synthesized by NOS from the amino acid L-arginine. It is a small, easily diffusible, and reactive free radical. It is becoming increasingly recognized as an important second messenger molecule in neuronal communication. An important oxidant involved in the generation of neurotoxicity is peroxynitrite ($ONOO^-$), which is formed when $NO^{\bullet}$ reacts with $O_2^{-\bullet}$. This ion is stable and does not have an unpaired electron. However, $ONOO^-$ can be protonated to form an acid with a short half-life. This in turn can attack proteins important for cellular respiration and signal transduction. $ONOO^-$ activates mitochondrial respiration and causes mitochondria to release $Ca^{2+}$. It also attacks tyrosine residues on proteins (613). For a neurotoxic cascade to be initiated $NO^{\bullet}$ radical has to be present in abnormally high concentrations. Hb combines with the $NO^{\bullet}$ radical and prevents it from damaging adjacent cells. $NO^{\bullet}$ and $O_2^{-\bullet}$ combine to form peroxynitrite. The concentrations of both these free radicals increase during ischemia and peroxynitrite may be a major mechanism of damage to important intracellular molecules of all types with the result of cell death. In physiological circumstances $NO^{\bullet}$ radical plays a key role in regulating physiological CBF. It has a chemical antioxidant effect and stabilizes membranes by decreasing membrane phospholipid fluidity. It scavenges both lipid peroxyl and hydroxyl radicals and spares endogenous vitamin E and ascorbic acid, enhancing endogenous antioxidant defenses. Lipid peroxidation can damage the functional and structural integrity of the cell membranes. Calcium pumps in the membranes can also be damaged. Its primary site of production is in the cerebral vascular endothelium and it penetrates the normal BBB very poorly even with intraarterial injection. Its penetration of the brain parenchyma is enhanced by injury with BBB damage.

$NO^{\bullet}$ has important roles in the CNS. Neurons express as much as 20 times more NOS activity than all the endothelium combined. The production of $NO^{\bullet}$ is linked to $Ca^{2+}$ influx through *N*-methyl-D-aspartate receptors on neurons. Inhibiting $NO^{\bullet}$ before inducing a stroke in experimental animals worsens the injury, whereas inhibiting $NO^{\bullet}$ during reperfusion generally improves it. NO is no more reactive than molecular $O_2$ and is unreactive with most biological molecules. Although not particularly toxic, it can be converted into more reactive species.

$NO^{\bullet}$-related species include other redox-related forms with one less electron ($NO^+$) or one additional electron ($NO^-$). All of these redox-related forms may be important biologically. Each of these $NO^{\bullet}$-related species can exist in singlet or triplet energy states and participate in completely different chemical reactions. $NO^+$ and $NO^-$ react with SH or thiol groups in different protein targets to modulate their function. SOD and catalase do not prevent the lethal action of $ONOO^-$ once it is formed. The actions of the $NO^{\bullet}$ are correlated with the redox state. The biological activity of other proteins containing cysteine residues can be regulated by S-nitrosylation and other redox reactions (614). OxyHb generates $O_2^{-\bullet}$ and binds with high affinity to $NO^{\bullet}$. This latter activity could be responsible for the inhibition of endothelium-dependent relaxation after SAH (435).

### E. Free Radicals and Stroke

$O_2$ free radicals may play an important role in secondary processes of brain injury following stroke. The nervous system may be particularly susceptible to tissue damage by $O_2$ free radicals because membrane lipids have a high concentration of cholesterol and polyunsaturated fatty acids as well as a relatively low concentration of catalase and only moderate amounts of SOD and glutathione peroxidase. The brain is also a rich source of iron, which can be an initiator of free radical reactions. The brain has high concentrations of ascorbic acid. It is an antioxidant only when present alone in high concentrations, but in the presence of copper and iron (present in extravasated blood) ascorbic acid can also produce large quantities of $O_2$ free radicals. Lysosomes released from dying neurons can be a source of hydrolytic enzymes and ultimately further free radical production (615–617). $O_2^{-\bullet}$ generation during reperfusion following ischemia was localized by histochemical techniques to the walls of the cerebral vessels (618). According to Kontos, $OH^{\bullet}$ can eliminate endothelium-dependent relaxation, increase vascular permeability to proteins, decrease $O_2$ consumption in the vessel wall, and injure the endothelium in smooth muscle cells. The cellular effects of $O_2$ free radicals are lipid peroxidation, increased membrane permeability, inhibition of enzymes, damage to DNA, elevation of $[Ca^{2+}]i$, mitochondrial damage, and cytoskeletal disruption (618). The free radical hypothesis for the production of VSP depends on the detection of free radicals post-SAH, the successful treatment of VSP by inhibition of

free radicals, a demonstration of the production of free radicals in the subarachnoid space, and the elaboration of mechanisms of free radical-induced VSP. Some available evidence supports this hypothesis. Lipid peroxides as measured by the thiobarbituric acid test and malondialdehyde are elevated in CSF post-SAH. Some free radical scavengers have shown efficacy in ameliorating experimental and human VSP. 21-Aminosteroids have been shown to reduce VSP in experimental animals, and in one study there was a reduction in lipid peroxidation. Systems which generate CSF free radicals have been shown to induce vasoconstriction in animals. The effect of free radical generation on endothelium-dependent response has varied. It has been suggested that free radicals can increase $[Ca^{2+}]i$ and lead to the generation of certain eicosanoids and affect lipid membrane structure and nucleic acid integrity. Free radicals can be detected by electron-spin resonance spectroscopy, chemiluminescence, or by reacting them with spin traps to form stable hydroxylation products (607,619). Less direct methods rely on detection of the products of free radical reaction such as lipid peroxides, malondialdehyde, conjugated dienes, and hydrocarbons such as ethane and pentane or on the consumption of scavengers. The more specific methods are spin trapping and electron-spin resonance spectroscopy, but these are not applicable to *in vivo* experiments. Thiobarbituric acid reactivity is a relatively imprecise method (607).

### F. Production of Vasospasm by Free Radicals

Injection of a mixture of xanthine, xanthine oxidase, ferric choride, metHb, and ethylenediaminetetraacetic acid–iron into the cisterna magna of cats produced acute vasocontriction lasting 30 min (383). In another experiment, when mixtures of red cell membranes, nicotinamide, adenine dinucleotide, adenosine diphosphate, and ferrous sulphate were injected into the cisterna magna of cats, VSP lasted for 2 days (620). When rose bengal was injected into the subarachnoid space of rats and subsequently photochemically illuminated to produce singlet oxygen there was a narrowing of the basilar artery for at least 90 min but less than 24 hr (621).

### G. Effect of Free Radicals on Vascular Smooth Muscle

$H_2O_2$ contracts some smooth muscle preparations and relaxes others (622–626).

$H_2O_2$ *in vitro* produced endothelium-dependent and -independent contraction of canine basilar arteries, which was not due to endogenous vasoconstrictors but rather was $Ca^{2+}$ dependent. Intracellular signal transduction systems such as PKC, protein tyrosine phosphorylation, $IP_3$, MAPK, and PI3 kinase seemed to be involved as judged by the effect of specific antagonists on the contractions and elevation of $[Ca^{2+}]i$ (627).

By activating guanylate cyclase, $H_2O_2$ relaxes canine coronary arteries (626). Relaxation or contraction in other vascular beds have been attributed to mediation by prostaglandins (625). Methylene blue was thought to block ACh-induced EDRF by generating the $OH^{\bullet}$ radical. The EDRF released by acetylcholine was not NO (579). Endothelium-dependent contraction can be induced in dog basilar artery by A23187, but this was prevented by removal of the endothelium or treatment with SOD or indomethacin. Cyclooxygenase metabolites were produced by treatment with A23187 but this was abolished by removal of the endothelium or treatment with indomethacin, but not SOD plus CAT. It was suggested that $O_2^{-\bullet}$ could be an endothelium-derived contracting factor (628). Experiments with free radical-generating systems suggest that $O_2^{-\bullet}$ and $OH^{\bullet}$ can inhibit endothelium-dependent relaxation by selectively destroying $NO^{\bullet}$ (579,618, 628–631).

### H. Lipid Peroxidation

The brain has a high iron content. In the blood iron is transported tightly bound to proteins in the $Fe^{3+}$ form. Intracellularly, it is stored with the protein ferritin in an acidic environment. Iron binding proteins readily part with iron. Once released from the binding proteins, iron catalyzes $O_2$ radical reactions. Hb is also a source of iron which is placed in contact with the brain as a result of SAH or ICH. Hb catalyzes $O_2$ radical formation and lipid peroxidation either directly or through the release of iron by $H_2O_2$, lipid peroxides, and the ischemia-induced acidosis. The autooxidation of $Fe^{2+}$ may provide an additional source of $O_2^{-\bullet}$. $Fe^{2+}$ combined with $H_2O_2$ forms $^{\bullet}OH$ or ferryl ion, both of which are extremely potent initiators of lipid peroxidation (611). $^{\bullet}OH$ may also be formed through the peroxynitrite pathway of leukocytes. Such cells can produce $O_2^{-\bullet}$ and $^{\bullet}NO$, which is produced by NOS. These two radicals can combine to form peroxynitrite anion, which in turn can decompose to yield two oxidizing radicals, $^{\bullet}OH$ and nitrogen dioxide ($^{\bullet}NO_2$). This mechanism may also operate in ischemic brain. Lipid peroxidation begins when a radical with a high oxidizing capability such as $^{\bullet}OH$ removes an allylic hydrogen from an unsaturated fatty acid to initiate a radical chain reaction. Once lipid peroxidation begins, iron may participate and drive the process as lipid hydroperoxides are decomposed by reacting with iron. Either alkoxyl or peroxyl radicals arising from lipid

hydroperoxide decomposition by iron can promote the lipid hydroperoxide-dependent lipid peroxidation, resulting in chain branching reactions. Not only does the brain have much iron but also the membrane phospholipids have a high proportion of polyunsaturated fatty acids, such as linoleic and arachidonic acid, which are susceptible to peroxidation (611). Heme compounds can accelerate as well as inhibit the oxidation of unsaturated fatty acids. Some colorless products of heme degradation have a marked antioxidant effect. Peroxidant activity of heme occurs when the peroxide to heme ratio is so high that the oxidation of the heme goes beyond the initial stages (632). Along with $Fe^{2+}$ in Hb, $O_2^{-\bullet}$ is known to initiate and propagate lipid peroxidation by the Haber–Weiss reaction and Fenton chemistry (297,365). The vasoactivity of blood incubated *in vivo* correlates with its concentration of oxyHb and with its content of lipid peroxides as judged by thiobarbituric acid-(TBA) reactive substances (297, 365,633).

Sano's group originally theorized that free radicals had a role in the genesis of VSP (634). They suggested that SAH generates lipid peroxides from $O_2$ free radical reaction catalyzed by iron and by Hb degradation products by the oxidative catabolism of arachidonic acid. They found that lipid hydroperoxide (15-hydroperoxy arachidonic acid), when injected intracisternally, could produce chronic VSP (171). Lipid peroxidation was assessed by the TBA method in canine clot incubated for 7 days. Daily changes in the peroxides and vasoconstrictor activity were assessed. The TBA value of the supernatant of incubated RBCs gradually increased to a maximum at 5 days of incubation, and there was a significant linear correlation between the TBA value and the vasocontractile capacity of the supernatant (171,297). Sasaki *et al.* suggested that lipid peroxidation coupled with insufficient antioxidant defense mechanisms in the arterial wall and in CSF could account for VSP and cerebral edema post-SAH. In a canine model the concentration of lipid peroxides in CSF increased markedly up to the eighth day following SAH and the concentration also rose in the arterial wall. Activities of SOD decreased significantly up to day 8. There was a gradual increase in glutathione peroxidase in the CSF. Arterial walls showed decreases in activity of SOD and glutathione peroxidase (635).

In four studies involving 90 patients, CSF levels of TBA reaction products were increased in patients with VSP compared to those without VSP. The highest levels occurred 1–3 days post-SAH (297,365,633), 5-Hydroxy eicosatetraenoic acid was detected in CSF 7 days post-SAH. This was taken as evidence of lipid peroxidation. Ten patients were studied (636). In a prospective randomized clinical trial of 211 patients with SAH, the free radical scavenger AVS 1,2-bis(nicotinamide) propane was administered continuously iv for 14 days. Treatment significantly reduced the incidence of infarction as judged by CT scanning and the incidence of hemiparesis 1 and 3 months post-SAH (634).

Free radicals scavenging enzymes have been reported to affect oxyHb-induced constriction (295,300,370,382, 389,396). For instance, in cat basilar artery SOD, catalase, and 1,4-diazobicyclo (2.2.2) were effective inhibitors of oxyHb-induced contraction (383). In arterial dog blood incubated at 37 °C for 14 days, the peroxide value was assessed using the TBA test. The peroxide value of the supernatant was initially low and gradually reached a plateau at 3–5 days. This high level was maintained thereafter. The conversion of oxyHb to metHb occurred in parallel with the increase in the peroxide value (637). OxyHb probably does not propagate lipid peroxidation after SAH, but it has not been shown *in vivo* that the process is essential for the development of VSP. Inhibitors of lipid peroxidation have not been shown to prevent VSP (638). Lipid peroxidation may be a result of VSP rather than its cause (639). In a canine single-injection model a thiocarbohydrazide and silver protein method was used to detect lipid peroxides in the walls of the spastic basilar artery. The wall lipoperoxide content increased with VSP (425). Hb injected into the spinal cord of anesthetized cats markedly inhibited $Na^+,K^+$-ATPase activity. Hb also catalyzed substantial peroxidation of CNS lipids. Desferroxamine blocked these adverse effects of Hb both *in vitro* and *in vivo* (640). Using mouse brain homogenates pure metHb and to a lesser extent oxyHb both increased the rate of formation of TBA-reactive substances. Hb-derived iron was apparently necessary since heme-free globin had no effect and desferrioxamine B blocked the action of Hb. In addition, free iron had the same effect as Hb. OxyHb and metHb released free iron in the homogenates (641). In a rat SAH model assays of different brain areas for TBA-reactive material and $Na^+,K^+$-ATPase activity showed no differences in lipid peroxide content between SAH and sham-operated animals (642). A Hb-free RBC membrane, NADPH, and ferrous sulfhate were injected into the cisterna magna of cats to stimulate lipid peroxidation. This resulted in significant angiographic VSP (620). The injection of oxyHb into monkeys caused VSP which was associated with higher levels of malondialdehyde in the CSF compared to injections of methHb or bilirubin (643).

## I. Amino Steroids

### *1. Animal Studies*

Derived from steroids, a new class of drugs was developed which potently inhibited lipid peroxidation–21-aminosteroids (644). One of these compounds is

tirilazad mesylate (U-74006F or Freedox). The antioxidant mechanism is considered to be the scavenging or decreased formation of $^{\bullet}OH$, the scavenging of lipid peroxyl radicals, the maintenance of endogenous antioxidant levels, membrane stabilization by decreasing phospholipid fluidity, and the maintenance of $Ca^{2+}$ homeostasis.

Several experimental studies in animals showed a tendency for the 21-aminosteroid tirilazad to reduce the severity of VSP (645–652). Angiographic VSP 3 days post-SAH in rabbits was ameliorated by tirilazad (646). In a rat model the same investigators found that SAH caused a sixfold increase in Evans blue extravasation; an even greater extravasation occurred with AA or $FeCl_2$. This excess capillary permeability was normalized by pretreatment with tirilazad (648). In a rabbit SAH model VSP was lessened by low but not high-dose intraperitoneal injection of tirilazad and pretreatment plus continuous iv dosing, but not solely with post-continuous iv dosing. The greater efficacy of continuous infusion over intraperitoneal injection or intermittent intravenous injection was attributed to more effective maintenance of therapeutic plasma concentrations. The greater protection with pretreatment suggested that post-SAH lipid peroxidation occurred rapidly in the rabbit SAH model (649). In a canine model of SAH intermittent intravenous injection of tirilazad post-SAH showed a beneficial effect on VSP, although the most effective dosage was the lowest used (0.5 vs 1.5 and 3.0 mg/kg) (650). The free radical scavenger U74006F (tirilazad) diminished but did not prevent VSP in a primate model (638,651). In a primate model of chronic SAH, in both saline and vehicle placebo treatment groups significant VSP occurred adjacent to clot application. After tirilazad treatment the VSP was significantly less in the MCA, which is most involved in this model. There was no significant difference between the 0.3 and the 3.0 mg/kg dosage groups (652). The content of malondialdehyde was measured by both the TBA test and high pressure liquid chromatography. The latter was thought to be a more accurate test for malondialdehyde. In the placebo-treated group the malondialdehyde content of the previous placed clot was significantly increased on day 7 postplacement, which contrasted with the content of freshly prepared clot which was very low. The results suggested that lipid peroxidation in subarachnoid clot played a role in the pathogenesis of VSP (651).

A second-generation nonsteroidal 21-aminosteroid (U78157G) was studied in a rat SAH model. This treatment improved brain hypometabolism which generally follows SAH. The antioxidant potency of this drug was 10 times that of tirilazad (653). The same 21-aminosteroid was also used in the rat SAH model. The TBA-reactive substance increase which followed SAH in untreated animals was not seen in animals treated with 21-aminosteroid. Similarily, the reduction in $Na^+K^+$ ATPase activity in cortical synaptosomes was apparently ameliorated by the 21-aminosteroid (654). A newer 21-aminosteroid, U88999E, was able to relax preconstricted arterial rings following exposure to elevated $K^+$, uridine triphosphate, or ET. VSP of basilar arteries in rabbits was also ameliorated. The drug was given intraperitonally. The effect did not achieve statistical significance, however (655). In a canine model of two blood injections there was significantly less VSP at 7 days from the first injection in animals treated with the 21-aminosteroid U74389G (25 vs 51% reduction). However, high pressure liquid chromatography assays of malondialdehyde and dihydroxybenzoic acids in CSF, subarachnoid clot, and basilar arteries showed no significant differences between groups (656).

### *2. Human Trials*

The various animal models of SAH were therefore encouraging but certainly not conclusive that 21-aminosteroids could inhibit the infarct-producing cascade resulting from aneurysmal rupture. The basic scientific rational was sufficiently persuasive that clinical trials were embarked upon. It was first demonstrated that tirilazad was safe in the setting of aneurysmal SAH in doses up to 15 mg/kg for periods of 8–10 days following SAH. Two major multicenter trials were embarked upon. The first to be analyzed involved many patients from Europe and Australasia. The results were very impressive, with an apparent dramatic reduction in mortality and particularly morbidity attributable to VSP. The effect was particularly pronounced in males. Given the large size of this patient population it was surprising that an similarly large North American study failed to provide equally strong evidence in favor of the use of tirilazad. It was noticed in both studies that the apparent benefit was most pronounced in males and in those in poor initial neurological condition following SAH. Females apparently metabolized the drug more quickly than males. The trend toward benefitting poor-grade males was evident even in the North American trial. Two subsequent vehicle-control trials were performed in females using 15 mg/kg, and again a beneficial effect on mortality was evident in the poor-grade patients. A meta-analysis of all four vehicle-control prospective randomized trials provided evidence that in grade 4 and 5 patients there was a protective effect of tirilazad but the U.S. Food and Drug Administration's advisory committee did not recommend approval of this drug. A conclusion was that the possible benefit might have depended on where the boundary between grade 3 and 4 patients was drawn. The only other drug released in the United States for the treatment of complications of SAH is the $Ca^{2+}$ antagonist nimodipine, which has not been released for use in grade 4 and 5 patients. Whether

similar drugs modified to achieve significant CSF levels (which tirilazad did not) would have greater efficacy is not known.

### J. Oxidation of Hemoglobin

OxyHb spontaneously autooxidizes to metHb-releasing superoxide ion anion radical (657,658). A polarographic oxygen analyzer was used to study the effects of heme compounds on oxidation rates of linoleate emulsion systems. Low levels of Hb effectively catalyzed the oxidation, whereas higher levels were less effective or ineffective (659). Autooxidation of oxyHb was followed at 430 nm and found to be inhibited by catalase and by SOD. It was concluded that oxyHb oxidation leads to generation of superoxide (657). Incubation of oxyHb with cytochrome c at neutral pH and low salt concentration led to slow cytochrome c reduction, corresponding to the slow spontaneous autooxidation of oxyHb. SOD inhibition of the reduction of cytochrome c indicated that superoxide is formed during oxyHb autoxidation (658). Blood was incubated *in vitro* for 8 days at body temperature. Electron spin resonance showed signals of ferric protein compound changing from high to low spin corresponding to changes from oxyHb to superoxide, metHb, and hemichrome (427).

Chromatographically pure human oxyHb mixed with linoleic acid was shown by electron spin resonance to generate free radicals. Superoxide radical was generated by adding $KO_2$ to the bath. Contraction resulted even in the absence of intracellular $Ca^{2+}$. OxyHb caused prolonged contraction which became resistant to papaverine relaxation. Catalase and α-tocopherol, which both remove hydroxyl radical and DABCO ($^1O_2$ scavenger), inhibited the contractile responses induced by oxyHb (660). The vasoconstrictor activity of incubates of fresh dog RBCs on dog basilar arteries studied *in vitro* increased with time of incubation, and this correlated with the TBA value of the sample and with the conversion of oxyHb to metHb assessed by spectrophotometry. $H_2O_2$ and linoleate hydroperoxide possessed significant vasocontractile capacity. 15-Hydroperoxyarachidonic acid injected into the subarachnoid space of dogs caused myonecrosis of the basilar artery by electron microscopy. Asano *et al.* found that the peroxide content of the CSF of SAH patients correlated with the presence of VSP (365).

### K. Free Radical Scavengers

$O_2$ free radical scavengers have been classified as enzymatic [SOD, catalase, allopurinol, and oxypurinol (xanthine oxidase inhibitor], nonenzymatic (dimethyl sulfoxide, dimethyl thiourea, deferoxamine, and mannitol) hydrophilic (ascorbic acid, glutathione, and L-methionine), and hydrophobic (vitamin E and barbiturates) (661). The majority of $O_2$ reduced by aerobic cells is carried out in a tetravalent manner by the cytochrome oxidase electron transport system, which results in the production of $H_2O$ and prevents the release of $O_2^{-\bullet}$, $H_2O_2$, and $OH^{\bullet}$. When univalent electron transfer results in the production of $O_2^{-\bullet}$, SOD converts the $O_2^{-\bullet}$ into $H_2O_2$ and $O_2$. The $H_2O_2$ produced by dismutation of $O_2^{-\bullet}$ is converted by the enzyme CAT into $H_2O$ and $O_2$ and by the enzyme GHS to produce reduced glutathione. A compound that removes free radical species is known as an $O_2$ free radical scavenger (611). Vitamin E is a lipid phase antioxidant which converts $O_2^{-\bullet}$, $OH^{\bullet}$, and lipid peroxyl radicals to less reactive forms. Vitamin C is an aqueous phase antioxidant, but in the presence of transition metals it may contribute to the formation of free radicals. The therapeutic utility of some antioxidants is limited because of their extremely rapid plasma clearance, pharmacological instability, and immunogenicity. Damage resulting from free radicals can be prevented by inhibiting enzymatic processes which generate free radicals; an example is using allopurinol to inhibit xanthine oxidase, but the efficacy of such maneuvers depends on the penetration of the therapeutic agent into the brain across the BBB or into the cerebral blood vessel wall. Specific enzymes such as SOD can also be employed. Considerations such as distribution and half-life are important. Iron can be scavenged, but the efficacy of this approach has been limited because of the inability to get agents such as deferoxamine into the brain in high concentrations. Nonspecific antioxidants such as vitamin E or the 21-aminosteroid tirilazad act therapeutically by inhibiting peroxidation, stabilizing membranes, inhibiting phospholipase activation, and directly scavenging the radicals (618).

When blood flow to the brain is initially cut off, it may not be the lack of oxygen but rather the return of it which initiates a deadly cascade of $O_2$ radical damage.

#### *1. Superoxide Dismutase*

If free radicals are generated by the Haber–Weiss reaction, then free radical antagonists such as catalase and SOD might ameliorate the spasmogenic effects of free radicals. It was claimed that SOD and catalase reduce the ability of Hb to produce contraction in feline blood vessels exposed to Hb *in situ* (383), but similar experiments in isolated canine basilar artery were unsuccessful (370). Gels of agarose containing oxyHb were placed in the subarachnoid space of monkeys. Some animals had intrathecal administration of SOD and catalase. This treatment was thought to attenuate angiographic VSP, although significant narrowing persisted in animals so

treated. Malondialdehyde was undetectable in CSF after subarachnoid placement of agarose gel alone, although it was present in similar amounts in all groups receiving subarachnoid placement of oxyHb. In the doses used in this experiment intrathecal SOD and catalase failed to protect against oxyHb-induced VSP (662). The SOD activities were studied in the CSF from 17 patients post-SAH. All patients were operated within 4 days and CSF was sampled from the lateral ventricles or the cisterns. There was a tendency for patients showing severe VSP and clinical deterioration to have a significant decrease in CSF SOD activity and increased lipid peroxides in the CSF (663). *In vitro* studies using SOD, catalase, AVS, and α-tocopherol inhibited lipid peroxidation of RBC membrane induced by oxyHb. Cisternal SOD and catalase in experimental two-hemorrhage canine models showed significant reduction in angiographic VSP following the use of AVS. SOD and catalase had no effect (664). In a canine model in which endothelial injury was produced by balloon catheters in coronary arteries, animals were followed for 2 hr postinjury until death. Control animals exhibited localized and persistent vasoconstriction. Treatment with SOD resulted in less severe constriction, whereas catalase, deferoxamine, or the $OH^{\bullet}$ scavenger 1,3-dimethyl-2-thiourea all failed to prevent the coronary artery vasoconstriction. The presence of mural thrombosis was similar in all groups (665). SOD production was measured as the SOD-inhibitable portion of nitro blue tetrazolium reduction after the cerebral ischemia – reperfusion phase in anesthetized cats equipped with cranial windows. Significant $O_2^{-\bullet}$ production was found in the early reperfusion in a complete ischemia model. $O_2^{-\bullet}$ and its derivatives were thought to be responsible, at least in part, for the vasodilation and abnormal reactivity following the ischemia as well as the increase in BBB permeability (666). Human recombinant copper–zinc SOD was effective in a rat SAH model in preventing angiographic VSP 2 days following the blood injection. SOD was also thought to prevent endothelial vacuolation, loss of endothelial tight junctions, and fragmentation of the internal elastic lamina (667).

### 2. *Other Free Radical Scavengers*

In a canine SAH model VSP was produced 3 days post-SAH injection. 1,2-Bis(nicatinamide)-propane (AVS; an antioxidant) injected intrathecally produced immediate relaxation lasting for 3 hr. Continuous intravenous injection was thought to significantly reduce angiographic VSP from 30 min to 4 days post-SAH (637). The seleno organic compound ebselen reduced angiographic VSP in the canine two-hemorrhage model. Basilar artery segments from the ebselen-treated group studied *in vitro* produced less 5-HETE than segments from the nontreated animals (668). Also, in a two-hemorrhage canine model the free radical scavenger MCI-186 was found to inhibit both non enzymatic peroxidation and lipoxygenase activity. There was significantly less angiographic VSP in treated animals and more endothelium-dependent relaxation to ATP and thrombin when *in vitro* testing was performed (669). VSP of the rat basilar artery was induced photochemically after illumination of intracisternal injected rose bengal. $^1O_2$ generated in the subarachnoid space elicited VSP shown angiographically 90 min and 24 hr after photosensitization (621). Treatment of oxyHb with ascorbic acid suppressed its ability to contract the cerebral arteries of monkeys, dogs, and cows. Indomethacin and aspirin reduced the contractions of isolated bovine cerebral arteries to oxyHb. Endothelium-dependent relaxation to substance P and nicotine was suppressed by treatment with oxyHb, although this inhibition was diminished by exposure of Hb to ascorbic acid (vitamin C). Ascorbic acid combined with Hb was much less effective in attenuating nitroglycerine-induced relaxations than Hb. These observations were thought to provide a rational for the use of ascorbic acid in cisternal irrigation. The ascorbic acid–Hb mixture was composed of $1.2 \times 10^{-4}$ M of potassium phosphate, 4 mg of oxyHb, and $9.0 \times 10^{-5}$ M of ascorbic acid in a total volume of 4 ml. It was adjusted to pH 7.4 (392).

Histidine is a scavenger of $^1O_2$ and was used in a rabbit model of SAH-induced VSP. L-Histidine injected intravenously attenuated the SAH-induced constriction of basilar arteries observed 2 days following SAH (670). OxyHb applied to the exposed cat basilar artery *in vivo* caused a 32% decrease in diameter. MetHb caused only a 15% decrease. The addition of SOD to oxyHb decreased its vasoconstricting effect to 15%, or about the same as that of metHb. After oxyHb had already constricted the artery, SOD did not reverse the constriction (365). Hb induced contraction of the dog basilar artery *in vitro* was not affected by SOD or catalase. The $O_2^{-\bullet}$-generating system of xanthine–xanthine oxidase was not a vasoconstrictor (370). SOD and catalase are poor antagonists of oxyHb-induced cerebral artery contraction. Both SOD and a $H_2O_2$ scavenger may be necessary to prevent the $O_2$-derived free radical since SOD alone could produce $H_2O$, which in turn can form $OH^{\bullet}$ radicals in the presence of iron or ferrous protein. Catalase or glutathione peroxidase could prevent this reaction by catabolizing $H_2O_2$ (671,672). Supportive evidence for the involvement of $O_2$ free radicals in the pathophysiology of SAH was provided by the observation that dietary supplementation with vitamin E prevented acute SAH-induced cerebral hypoperfusion (673).

It is assumed with RBC hemolysis post-SAH that increasing levels of oxyHb undergo spontaneous oxida-

tion to metHb and $O_2^{-\bullet}$. Heme-containing Fe is tightly bound to oxyHb but more readily released from MetHb. The release of heme or Fe from the globin chains may be important since both heme and iron might penetrate the arterial wall from the adventitial side with greater ease than the whole oxyHb molecule. Heme and Fe might penetrate the arterial wall more easily than SOD and other high-molecular-weight free radical scavengers. SOD and catalase are found in large amounts in RBCs and would be released into the subarachnoid space along with the Hb. CSF is deficient in Fe scavenging proteins such as transferrin and ferritin. CSF is also deficient in naturally occurring free radical scavengers and antioxidants. Levels of heme oxygenase are initially low in CSF and in vessel wall (674). OxyHb contracted isolated rat cerebral arterial smooth muscle cells. Catalase (which removes $H_2O_2$ and $OH^{\bullet}$, leaving only $O_2^{-\bullet}$) prevented the effect of oxyHb. SOD, which removes only $O_2^{-\bullet}$, did not protect against the effect of oxyHb and the xanthine oxidase–xanthine system had no effect. Dimethylsulfoxide ($OH^{\bullet}$ scavenger) protected cells from oxyHb. The generation of $OH^{\bullet}$ from copper and $H_2O_2$ produced significant cell damage (396). The antioxidant ebselen has been used to reduce the contractile response of rabbit basilar arteries to ET-1. Ebselen as well as a combination of catalase and SOD inhibited the potentiating effect of oxyHb ($10^{-5}$ M/liter) on ET-1-induced contraction (675).

Glycosylated oxyHb induced contraction and impaired endothelium-dependent relaxation in rat aortic segments. The vascular effects induced by glycosylated Hb were prevented by SOD. Glycosylated oxyHb produced higher amounts of $O_2^{-\bullet}$ anions than did other Hb derivatives. It was hypothesized that glycosylated Hb requires the assistance of a functioning heme group containing iron in the $Fe^{2+}$ state to interfere with the endothelial functions at nanomolar concentrations. The effect is presumed to be mediated by the generation of $O_2^{-\bullet}$ anions (676). AVS ameliorated chronic VSP in a canine model. This compound scavenges $O_2$ free radicals and inhibits production of lipoxygenase products induced by 15-hydroperoxyeicosatetraenoic acid. In a single-hemorrhage model single intracisternal doses of AVS were effective in reversing VSP (637). In the rat, SAH induced a decrease in CBF and cerebral glucose utilization as judged by positron emisson tomography scan. AVS given intravenously significantly improved both parameters (677). SAH in canine models caused a marked elevation in uric acid, which is the product of xanthine oxidase in the CSF. Parental administration of allopurinal prevented this elevation in uric acid but did not affect VSP or vascular damage (679).

## XIII. Recent Novel Pharmacological Approaches

Fasudil HCl is an intracellular $Ca^{2+}$ antagonist which differs from other $Ca^{2+}$ channel blockers. Studies from Japan suggest it may improve symptomatic VSP (680). The nontoxic endotoxin analog, monophosphoryl lipid A (which is presumed to stimulate the immune system), blocked the loss of SOD in the basilar artery of rabbits which accompanies VSP (681). The angiotensin-converting enzyme alacepril prevented VSP as well as the suppression of ACh-induced relaxation by SAH (682). In rabbits, after SAH the vasodilator peptide maxadilan, which is isolated from the sand fly, dilated spastic arteries and prevented VSP when given intrathecally in tablet form (683). In dogs nicardipine administered in a lactic/glycolic acid prolonged-release pellet (62% released by day 4) appeared to prevent significant VSP compared to controls (684). Thromboxane $A_2$ synthetase inhibitor has been reported to ameliorate VSP in dogs (685), but a similar drug was found to be ineffective in the same model (686). Dactinomycin, an RNA synthesis inhibitor, given in the hope of suppressing the induction of ET, was thought to suppress VSP if given on day 2 but not day 3 post-second SAH in the dog model (687). In precontracted canine basilar artery ring segments selective inhibitors of the phosphodiesterase type IV isozyme produce potent and complete relaxation. Basilar VSP in the dog was reversed by three different inhibitors of this phosphodiesterase. It seems that this isozyme is the predominant regulator of vascular tone by cAMP hydrolysis. Vasorelaxation modulated by phosphodiesterase IV is compromised in chronic VSP (688). 1-(5-Isoquinolinesulfonyl)-homopiperazine is a serine/threonine protein kinase inhibitor; it may target Rho-kinase, which regulates phosphorylation of myosin light chains and vasoconstriction (689).

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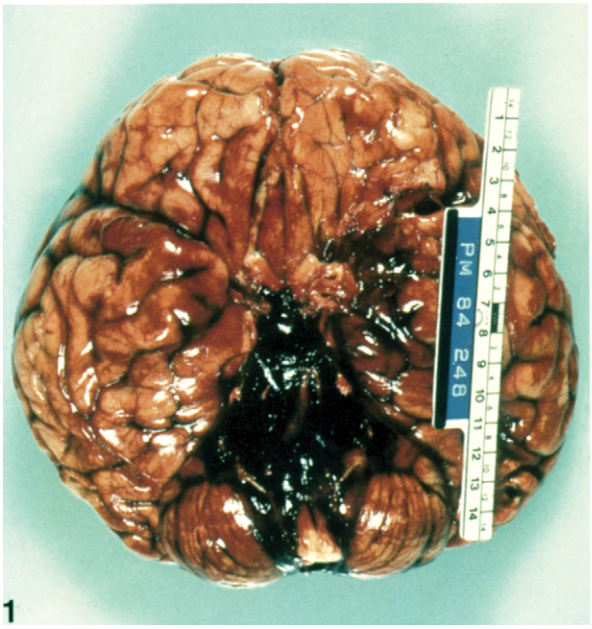


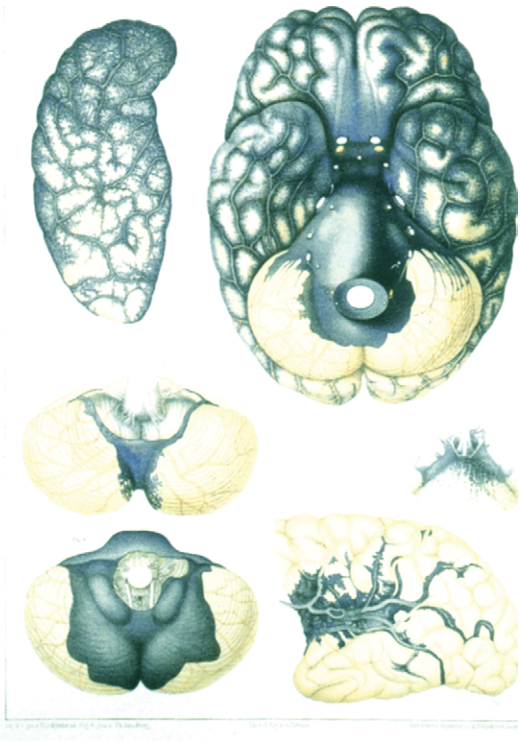
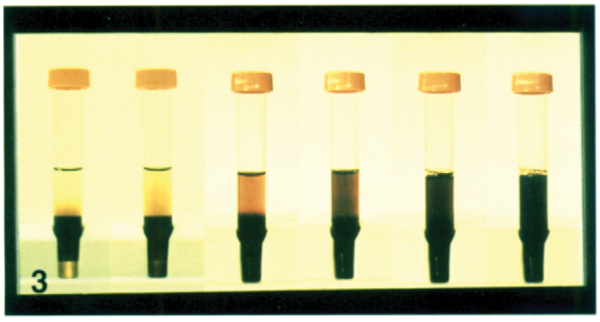




**PLATE 1** Thick basal clot and subarachnoid hemorrhage following early death from ruptured aneurysm.

**PLATE 2** Demonstration of subarachnoid space and basal cisterns using injected dye [from Key, A., and Retzius G. (1875). *Studien in der anatomie des nervensystems und des binde gewebes.* PA Norstedt & Söner, Stockholm].



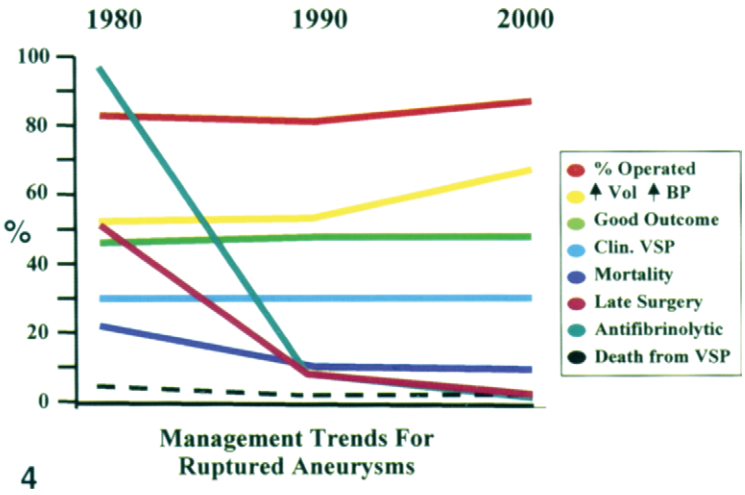
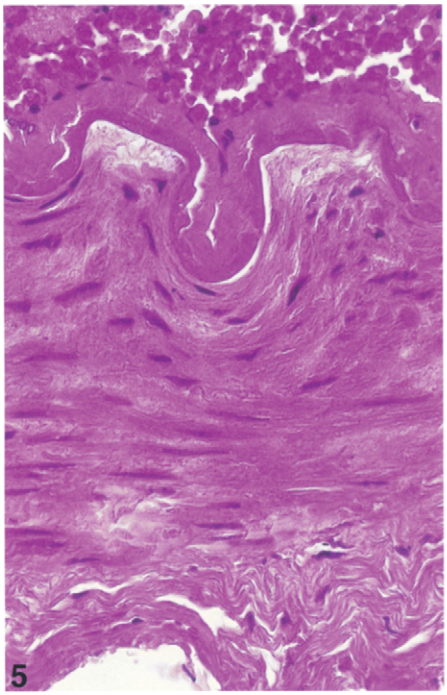




**PLATE 3** Progressive hemolysis of incubated RBCs in artificial CSF. The specimen at left was incubated for 1 day; the one on the right was incubated for 6 days.

**PLATE 4** Our impression of management trends for ruptured aneurysms based on analysis of selected large series. In the past two decades, deaths from VSP alone have fallen slightly to <5%. The major changes are the abandonment of antifibrinolytics and the widespread adoption of early definitive treatment. The use of hypertension/hypervolemia has increased slightly. Overall surgical mortality has decreased by half.



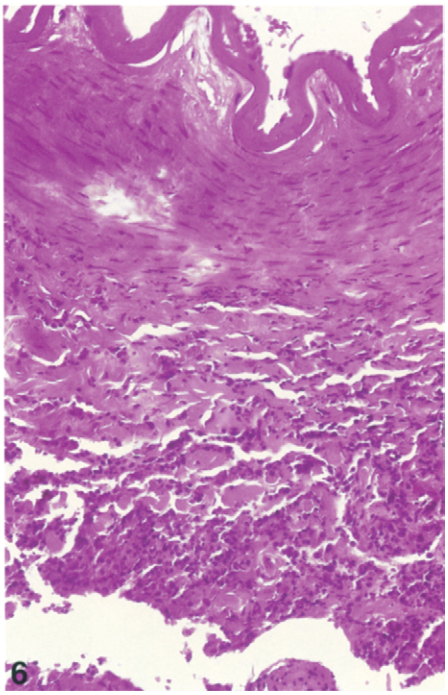
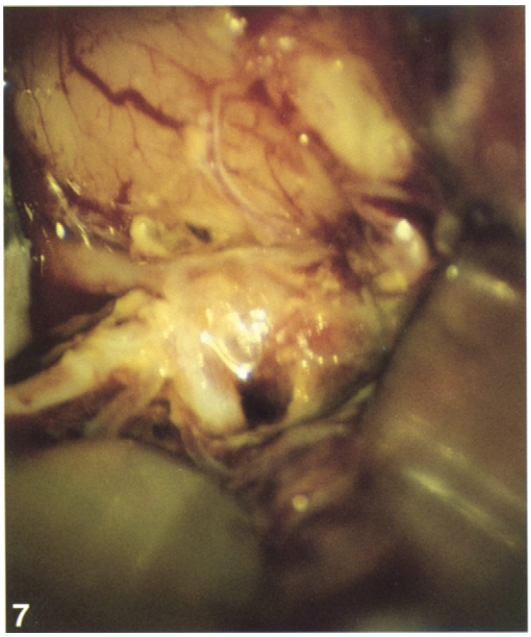




**PLATE 5** MCA from patient dying from VSP. Note corrugation of lumenal surface, absence of smooth endothelium, edematous regions under the elastica at the tip of the corrugations, pyknotic nuclei in the media with pale areas in the media, and thickening of the adventitia with fibrin, collagen, and cells. The patient died on day 14.

**PLATE 6** Anterior cerebral artery showing changes similar to those shown in Plate 5.



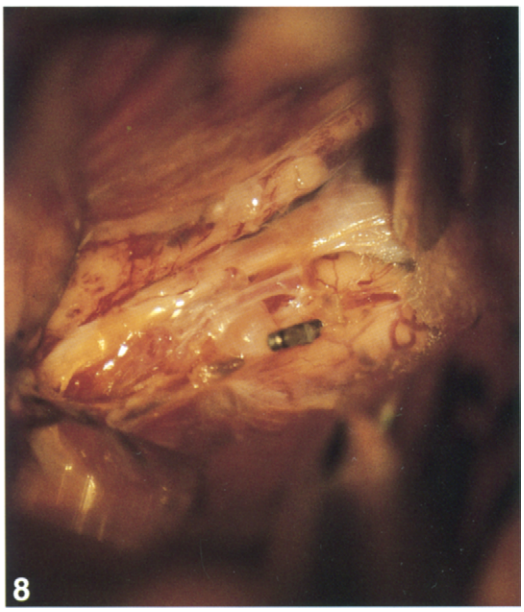




**PLATE 7** View of anterior communicating artery complex with established VSP. Vessel walls are whitish-gray, and normal red blood columns are absent. Orange-yellow clot remnants have been suctioned off the arteries.

**PLATE 8** Changes in a MCA similar to those shown in Plate 7. Both patients were operated on after day 10.

CHAPTER 7

# STRUCTURE, PHYSIOLOGY, AND BIOCHEMISTRY OF VASCULAR SMOOTH MUSCLE



## I. Introduction

Vascular smooth muscle is capable of extremely prolonged contraction with minimal metabolic expenditure. The type of contraction is described as tonic as opposed to more rapid phasic contraction in other smooth muscle such as in a hollow viscus. Tonic muscle does not show spontaneous activity or rapid contraction kinetics. Each muscle type has unique membrane properties, signaling mechanisms, and contractile systems (1).

Two fundamental proteins, actin and myosin, make up about 80% of the structural proteins in muscle cells. The organelle within the muscle cell responsible for contraction is the myofibril. Actin and myosin are ubiquitous within eukaryotic cells. Actomyosin utilizes adenosine 5′-triphosphate (ATP) in energy transduction. The meta-

bolic activity of muscle cells can change by several orders of magnitude within a fraction of a second and this relates to changes in intracellular $Ca^{2+}$ concentrations ($[Ca^{2+}]_i$). All muscles involving interactions between actin- and myosin-containing filaments are fueled by ATP hydrolysis. The contractile filaments of the various kinds of muscle differ in the arrangement and protein isoform content. Muscle types also vary in the mechanism employed to generate ATP. Smooth muscles appear almost structureless in comparison to striated muscle at certain resolutions. Smooth muscles are concerned with the constriction of certain internal organs and vessels. They are generally under involuntary control and contract slowly. Molluskan smooth muscles have developed a mechanism for holding very high tensions with little energy expenditure, which is called the "catch" state. Recently, a similar property was observed in vertebrate smooth muscle which was termed the "latch" state. Muscle types of different species have characteristic myosin isoforms which differ in amino acid composition and ATPase activity.

Vascular smooth muscle is capable of sustained and tonic contraction to maintain arterial dimensions against the imposed load of the blood pressure. Since the vascular muscle cells are mechanically coupled like links in a chain, all the cells must respond in a highly coordinated fashion. This requires complex neural and hormonal control systems and extensive intercommunication between the smooth muscle cells. The fibrillar contractile apparatus and its force-transmitting cytoskeleton operate in nonlinear configurations. Like all smooth muscle cells, vascular smooth muscle cells are capable of both phasic and tonic behavior. The cells are arranged circumferentially around the lumen of the blood vessel so that contraction increases the resistance to the flow of blood. Vascular smooth muscle cells are linked anatomically to contiguous cells not only for apparent mechanical reasons but also to permit simultaneous activation (2).

When the plasmalemma or muscle cell wall is permeablized the addition of ATP to the surrounding medium will contract or relax the muscle depending on the $Ca^{2+}$ concentration. There is an electric potential of $-60$ to $-90$ mV inside a muscle cell in relation to an external bathing medium. The voltage is generated by the differential concentration of certain ions on either side of the relatively impermeable membrane. When muscle membrane is permeabilized the muscle becomes equilibrated with ions in the bathing medium. Since exquisitely small concentrations of $Ca^{2+}$ can result in muscle contraction, the bathing medium in experimental setups usually contains a $Ca^{2+}$ chelator to reduce the free $Ca^{2+}$ to $<1\mu M$ to achieve relaxation. A high free $Mg^{2+}$ is required for both contraction and relaxation since the actual substrate for myosin is Mg–ATP (3).

The synchronous contraction of smooth muscles is probably not solely achieved by electrical coupling via junctions or fired action potentials but also by diffusion of transmitter substances through the tissue or neural control. Neurotransmitters, hormones, or drugs with specific receptors can activate contraction of vascular smooth muscle cells by increasing the intracellular $[Ca^{2+}]i$. The relative importance of the electrical coupling and neural regulation in vascular smooth muscle probably varies with the type and its state. Elaborate neuromuscular contacts at axon terminals are not found in vascular smooth muscle, although there is usually a well-developed periadventitial nerve plexus (2). Electromechanical and pharmacomechanical coupling mechanisms are inextricably mixed in vascular smooth muscle. Contraction and relaxation are regulated by a wide diversity of mechanisms (4). Vasomotor tone reflects a balance between contracting and relaxing stimuli. Vasospasm (VSP) has been defined as an alteration in tone in which sustained contraction predominates (5). True VSP represents a state of smooth muscle contraction which is refractory to active relaxation by the addition of potent vasorelaxants and/or refractory to passive relaxation by removal of vasoconstrictors. It can be viewed as a state inherent to the vascular smooth muscle. VSP may be caused by primary alterations in smooth muscle cell signaling events which modulate normal states of contraction and relaxation (6).

Vascular smooth muscle cells can be induced to contract by the contact of molecules interacting with specific cell surface receptors and changes in extracellular ions which alter the polarization of the cell membrane. The smooth muscle cells can respond to these stimuli by initiating a cascade of intracellular biochemical events culminating in muscle contraction (Fig. 7.1). It is generally agreed that for the initiation of contraction there must be an increase in intracellular $[Ca^{2+}]i$. This results in the formation of $Ca^{2+}$–calmodulin (CaM) complexes that bind to and activate myosin light chain kinase (MLCK). MLCK phosphorylates myosin light chains ($LC_{20}$) and this in turn activates actomysin ATPase, causing cross-bridge formation and contraction (6). Covalent modification of myosin by phosphorylation of the $LC_{20}$ is the significant mode of regulation of contraction in smooth muscle, especially in the generation of vasocontraction in the initial development of tonic contractions. It is much more important in smooth muscle than in cardiac or skeletal muscle (7).

Arterial smooth muscle cell contraction is brought about by multiple mechanisms: constrictors, high $(K^+)_e$, depolarization of the smooth muscle membrane, and increase in the $Ca^{2+}$ influx through voltage-dependent

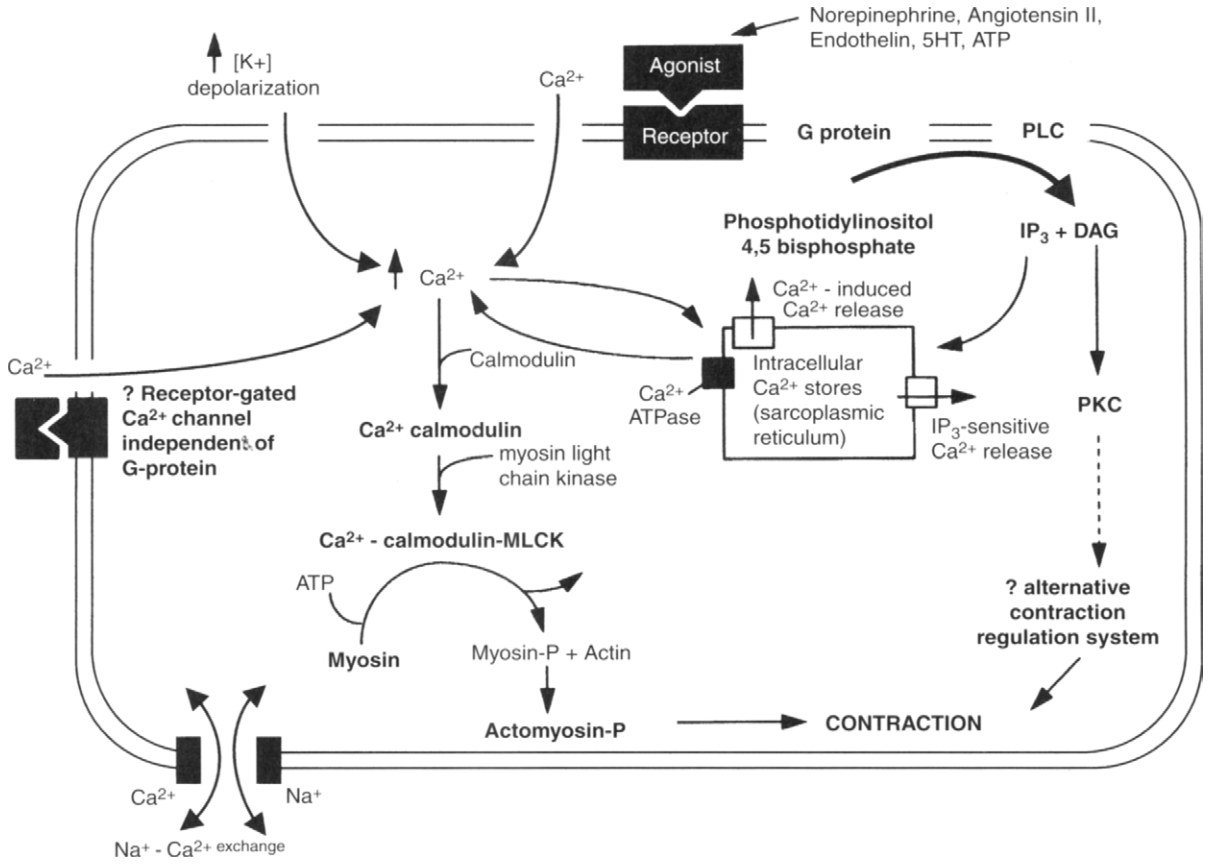



**FIGURE 7.1** Diagram of pathways of smooth muscle contraction. PLC, phospholipase C; DAG, diacylglycerol; PKC, protein kinase; MLCK, myosin light chain kinase; ADP, adenosine diphosphate; ATP, adenosine triphosphate; $IP_3$, inositol 1,4,5-triphosphate [reproduced with permission from Weir, B., Stoodley, M., and Macdonald, R. L. (1999). Etiology of cerebral vasospasm. *Acta Neurochir.* **72,** 27–46. Copyright © Springer-Verlag GmbH & Co.].

$Ca^{2+}$ channels. Electromechanical coupling involves a change in the membrane potential ($E_m$). In the resting state the interior of the cell membrane is negative. With contraction the $E_m$ increases. Changes in $E_m$ can be induced by increases in $(K^+)_e$ or by vasoconstrictor agonists binding to their membrane receptors. Depolarization causes a $Ca^{2+}$ influx through L-type $Ca^{2+}$ channels. As $[Ca^{2+}]i$ elevates, it binds to CaM, thereby activating MLCK, which then phosphorylates the 20–kDa light chain of myosin. Other mechanisms of contraction include agonist-induced release of $Ca^{2+}$ from intracellular stores, agonist activation of both voltage- and receptor-operated $Ca^{2+}$ channels to increase the $Ca^{2+}$ influx above that expected for a given degree of depolarization, and agonist-induced increase in $[Ca^{2+}]i$ sensitivity which increases the force for a given $[Ca^{2+}]i$. The latter mechanism can produce contraction without changing $[Ca^{2+}]i$. High $(K^+)_e$ appears to operate solely by electromechanical coupling. Vasoconstrictors appear to involve some or all of these mechanisms to varying degrees (4).

Responses of vascular smooth muscle cells (VSMCs) which might result from SAH are summarized in (Table 7.1).

**TABLE 7.1 Responses of Cerebral Arteries to SAH[a]**

| |
|---|
| SAH → endothelial damage → ↓ NO production |
| RBC → oxyHb → ↓ NO production or NO inactivation |
| SAH → ↓ NO → ↓ cGMP |
| SAH → ↓ NO + ↓ cGMP → ↑ ET |
| SAH → ↓ NO + ↓ cGMP → ↑ PKC |
| SAH → ↓ $K^+$ channel activity → ↑ depolarization (↑ tone) |

[a]Modified from Sobey, C. G., and Faraci, F. M. (1998). Subarachnoid hemorrhage: What happens to the cerebral arteries? *Clin. Exp. Pharmacol. Physiol.* **25,** 867–876.

## II. Tension, Tone, and Work

Relaxed muscle is readily extensible. In this condition actin and myosin filaments do not interact strongly and the elasticity is provided by other cytoskeletal proteins, associated connective tissue, and membranes. Muscle held at a fixed length will develop tension, this type of contraction is termed isometric despite the fact that the muscle does not actually shorten or contract. Only if the load attached to the muscle is less than the isometric tension will the muscle shorten. The steady velocity of the constant load (isotonic) contraction reaches a maximum velocity with zero external load. The muscle is extended if the force is greater than the isometric tension. Normally functioning muscle can experience all of these conditions (3).

The regulation of resistance by shortening is the primary function of most smooth muscle. Contractility is the relationship between maximum values of force, shortening, and velocity, with time-dependent effects being held constant. Usually, muscle can shorten by as much as one-third of its original length. Smooth muscles can develop similar maximum forces to skeletal muscle, although the velocity of contraction is 50 times less.

When the energy source of the muscle contraction (the ATP) is not renewed a muscle becomes stiff (the rigor state). Permanent damage is done to muscle fibers when a muscle in rigor is stretched by more than a tiny amount. In the rigor state the actin and myosin filaments are strongly interactive. Smooth muscle generally operates in a contracted state with maintained tone from which it can contract further or relax depending on the physiological responses. The tone is attributable to intrinsic mechanisms which can be modulated by mechanical, excitatory, or inhibitory neurohormonal stimuli. Muscle with intrinsic tone can respond to graded changes in membrane potential. Myogenic tone is the partially constricted state resulting from elevation of intravascular blood pressure which depolarizes smooth muscle cells in resistance arteries, raising $[Ca^{2+}]i$ and causing vasoconstriction. Pressure-induced membrane depolarization presumably activates $K_{Ca}$ channels (8). Smooth muscle is capable of generating more force per cross-sectional than striated muscle despite the fact that it has only one fifth the myosin content. This is possible because the longer myosin filaments in smooth muscle compared to skeletal muscle result in a greater numbers of cross-bridges arranged in parallel, and insertion of multiple filaments along the sarcolemma produces more total contractile units arranged in parallel (7).

The velocity of muscle contraction depends on the load; it is maximum when the load is zero and declines to zero when the load equals the isometric tension. A muscle performs work when the tension operates over distance. It requires the utilization of chemical energy even when muscle is at its isometric tension (not shortening) or contracting at its maximum velocity and doing no external work (a force acting over a distance). The economy of a muscle is the cost of maintaining a steady tension such as bearing a load. The tension or force generated by muscle can be defined as mass times acceleration, expressed as $kg/m/sec^{-2}$. Tension can also described as tension per unit area, which normalizes for cross-sectional area. Muscle stiffness is tension divided by length. The elastic modulus is the tension per unit area per unit length. The sliding filament theory proposes that contraction occurs solely by interdigitation of thick and thin filaments. In skeletal muscle the sarcomere length depends on the degree of overlap, whereas the filaments remain constant in length. The thick filament is composed mainly of myosin and the thin filament mainly of actin.

The shortening velocities and ATP consumption of vascular smooth muscle are very low compared to those of skeletal muscle. The velocity–stress relationship in smooth muscle is generally variable, which reflects regulation of the number of cross-bridges which determine force as well as their average cycling rates which for a given constant load determine velocity of contraction. The sustained stimulation of blood pressure results in a sustained force maintenance by the vascular smooth muscle cells; this reduces cross-bridge cycling rates and lowers ATP consumption. In vascular smooth muscle, phosphorylation of cross-bridges by $Ca^{2+}$-dependent myosin kinase is necessary for the thin filaments to attach to the thick ones. Dephosphorylation of an attached cross-bridge slows down its rate of detachment. Cross-bridge states may be free or attached and phosphorylated or dephosphorylated. There are two cross-bridge cycles that differ in their turnover rate. Phosphorylation accounts for the majority of ATP consumption by smooth muscle and results in a low efficiency. This relative disadvantage is overcome by the low rate of consumption of ATP during tonic contraction. Smooth muscle can maintain the same force as striated muscle with a 300 times less rate of ATP consumption (3). Tonic smooth muscles do not display action potentials or regenerative activity under physiological conditions. They have slow shortening velocities but are very effective in maintaining tone. The contractile apparatus of tonic smooth muscles is more sensitive to $[Ca^{2+}]i$ and to the 20-kDa regulatory light chain ($LC_{20}$) phosphatase inhibitors than are phasic smooth muscles. The speed of contraction reflects the velocity of cross-bridge cycling, which is determined by the $Mg^{2+}$–ATPase activity of the myosin head and other factors (7).

## III. Structural Components

### A. Blood Vessel Walls

Blood vessel walls contain extensible elastin and inextensible collagen fibrils surrounding and bridging the smooth muscle cells. This structural matrix determines the passive force–length curve measurable in relaxed vessels and withstands the distending force of the blood pressure. Vascular smooth muscle is normally under some degree of active tone which can be accentuated with an appropriate stimulus. Modest degrees of skeletal smooth muscle contraction can result in a disproportionately large change in the arterial wall volume (2). The long axis of the nuclei of the endothelial cells (which are in parallel with the line of flow of blood) tend to be at right angles to the long axis of the nuclei of the VSMCs. Vessels constricted by immersion in buffered saline containing norepinephrine show highly corrugated surfaces. Systematic measurement of VSMCs has demonstrated that a reduction in the diameter of the small rat cerebral arteries from 90 to 60 μm is associated with shortening of individual smooth muscle cells by about 40%. The circular orientation remains unaltered irrespective of the functional states of the vessels (9).

### B. Vascular Endothelium

VSMCs have a close structural and humoral relationship with vascular endothelium. Vascular smooth muscle is separated from the endothelial layer by the internal elastic lamina, but there are defects in the lamina which permit junctional contacts between endothelial cells and the innermost smooth muscle cells (2). The endothelium responds to a myriad of influences, including hormones and chemical and hydraulic factors. As in smooth muscle cells, much of the signaling in endothelial cells occurs through increases in cytosolic $Ca^{2+}$ and activation of $Ca^{2+}$-dependent enzymes, such as nitric oxide synthase and phospholipase $A_2$. The most important trigger for intracellular $Ca^{2+}$ release is $IP_3$ generated by phospholipase C (PLC), which is activated in turn by the receptor–G protein cascade. The cell membrane's electrochemical gradient controls $Ca^{2+}$ influx. A large inward rectifier $K^+$ current dominates the membrane potential under basal conditions. Hyperpolarization of the membrane increases the electrochemical gradient for $Ca^{2+}$ which is modulated by activation of $Ca^{2+}$-dependent $K^+$ and $Cl^-$ currents (10).

### C. Vascular Smooth Muscle Cells

Smooth muscle cells are spindle shaped with single nuclei and no obvious cross-striations. Longitudinally running actin filaments can be seen by electron microscopy. Smooth muscle contracts because actin filaments are drawn together by myosin filaments. The actin filaments are fixed at opposite ends of the cell to structures called dense bodies (3).

Vascular cells are typically much smaller than skeletal muscle cells. They have long, tapering shapes. Individual VSMCs are 4 or 5 μm in their widest diameters and range from 50 to 70 μm in length. They are generally circularly oriented at right angles to the long axis of the blood vessel. The arrangement tends to more disorderly at branch points (11). The cross section may appear irregular during contraction as a result of the force exerted on the cell by its attachment to other cells or the extracellular matrix. Smooth muscle cells have a sarcoplasmic reticulum which connects to the cell surface and contains an intracellular calcium pool. $Ca^{2+}$ can be mobilized when stimulatory neurotransmitters, hormones, or drugs bind to sarcolemma receptors. The volume of the sarcoplasmic reticulum varies between 2 and 6% of cell volume. VSMCs contain a prominent endoplasmic reticulum and Golgi apparatus which are located centrally at each end of the nucleus. The mitochondria are scattered throughout the cell (2). Junctions between smooth muscle cells consists of various types: simple apposition, intermediate contacts, desmosome, and gap junctions (2). Gap junctions between adjacent plasma membranes are only 2 or 3 nm. These form low-resistance pathways. They also permit diffusion of low-molecular-weight compounds. Action potentials are readily propagated from cell to cell through these junctions (11). The wall of the VSMC or sarcolemma has longitudinal rows of small, sac-like in-pocketings called caveoli. These increase the surface to volume ratio of the cells (2). The components of smooth muscle include contractile proteins, regulatory proteins, force-transmission systems (cellular cytoskeleton and linkages between cells and to the extracellular matrix), and membrane systems that transduce extracellular signals into changes in myoplasmic $Ca^{2+}$ concentration. Since smooth muscle cells are anatomically arranged in series, they must be activated simultaneously to the same degree, as well as be mechanically linked. The mechanical connections result from attachments to sheaths of connective tissues and specific junctions between muscle cells (11).

#### *1. Sarcoplasm and Muscle Filaments*

Smooth muscle has series and parallel elastic elements (12). Thick (myosin, 15 nm) and thin (actin, 7 nm) filaments are 10,000 times longer than they are wide and are densely packed in smooth muscle sarcoplasm (2). They are roughly aligned in the long axis of the cell. Filaments attach to the cytoskeleton in an orderly fashion and

connect with centrally located ellipsoidal dense bodies and dense areas along the sarcolemma. The central dense bodies, so named because their diameters are in between those of thick and thin filaments, are linked by intermediate filaments. Cytoskeletal proteins include intermediate filaments (100 Å diameter) which are composed of acidic keratin, neutral basic keratin, vimentin, desmin, glial fibrillary acidic protein, peripherin; neurofilaments and nuclear laminin. A chain-like arrangement of cytoplasmic dense bodies and intermediate filaments is seen in VSMCs. Vascular tissue chiefly contains desmin (MW = 53 kDa) and vimentin (MW = 54 kDa). Thick filaments are surrounded by many thin ones. The dense bodies of the sarcolemma of adjacent smooth muscle cells are interconnected to mechanically couple the functionally dependent contractile units (12). A schema for the cytoskeleton is given in Fig. 7.2. The cytoskeletal and contractile elements in vascular smooth muscle are not aligned precisely transversely and therefore their three-dimensional packing is not obvious from two-dimensional images (2). Various proposals have made (Fig. 7.3). The thick myosin and thin actin filaments do not change their length during contraction but slide past each other to overlap to a greater extent than in the resting state (3).

### *2. Cytoskeletal Proteins*

Smooth muscle cells contain a large number of cytoskeletal proteins, some of which undergo phosphorylation on stimulation. Such proteins may crosslink the actin filaments with the dense bodies (2). Actin-binding skeletal proteins may participate in contraction by tethering together the thick and thin filaments after force development, thereby maintaining force in a noncycling crossbridge latch state in which minimal energy is expended (7). The cytoskeleton may play an active role in organizing the contractile machinery. The intermediate filamentous component transmits contractile force. Regulatory proteins such as calponin (CaP) and nonmuscle actin are present in the cytoskeleton. The numerous proteins known to be present in the cytoskeleton are shown in Table 7.2 (13). Mediators in the contractile–cytoskeletal interactions may include the rho family of small G proteins (13). The cytoskeleton is the point of attachment for the thin filaments and permits force transmission to the

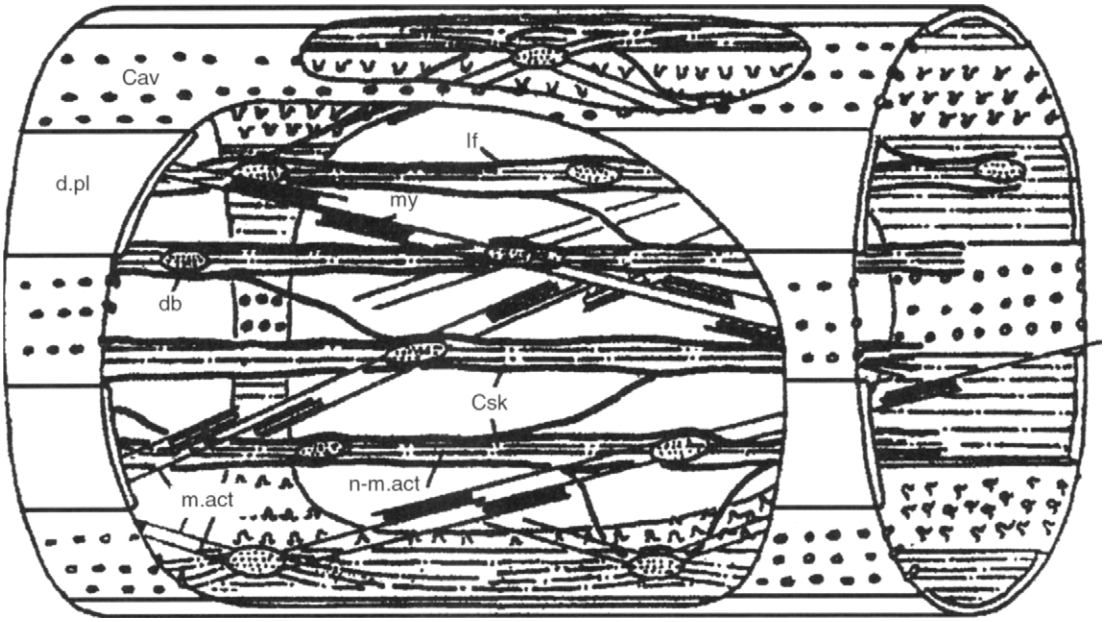



**FIGURE 7.2** Schematic representation of the organization of the cytoskeleton and contractile apparatus of the smooth muscle cell. For clarity, only a representative fraction of the filamentous elements are shown and the angles subtended by the contractile apparatus to the cell axis are exaggerated. The cytoskeleton (Csk) is composed of intermediate filaments (if) and nonmuscle actin filaments (n-m.act) that pass through the ovoid dense bodies (db). The dense bodies are presumed to also couple to the muscle actin filaments (m.act) of the contractile apparatus, which interacts with myosin (my). The plasmalemma exhibits alternating, longitudinal channels of two types, one containing the dense plaques (d.pl) corresponding to the adherens junctions that anchor the cytoskeleton and contractile apparatus and the other channel with numerous caveolae (Cav) [reproduced with permission from Small, J. V., and Gimona, M. (1998). The cytoskeleton of the vertebrate smooth muscle cell. *Acta Physiol. Scand.* **164,** 341–348].

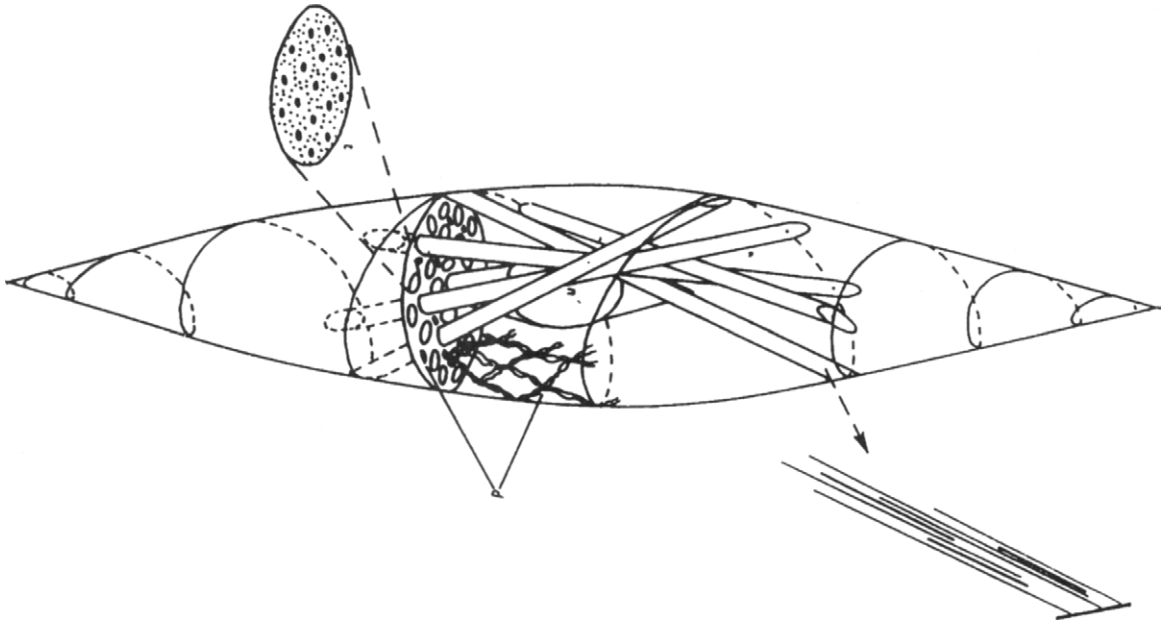


**FIGURE 7.3** Schematic diagram indicating the general functions or the proposed organization of the contractile apparatus of the smooth muscle cell. For clarity, the cell proportions have been chosen for a cell at about its shortest length. One family of contractile units is here envisioned as having membrane attachment sites located on a common spiral along the cell surface. Several families of such units on similar spirals translated along the cell axis would be required to occupy the entire cell volume. The dense body, 10-nm filament network (d) forms a structural framework between the contractile units and is also attached to the cell surface. The number of filaments in a cross section of an individual unit (t) corresponds approximately to the average number noted in the myofilament groups in rigor; *n* corresponds to the cell nucleus with the associated organelle-containing regions at the nuclear poles [reproduced with permission from Small, J. V. (1977). Studies on isolated smooth muscle cells: The contractile apparatus. *J Cell Sci.* **24,** 327–340].

**TABLE 7.2 Components of the Contractile Apparatus and Cytoskeleton of the Vertebrate Smooth Muscle Cell**[a]

| | Cytoskeleton | | Membrane skeleton | |
|---|---|---|---|---|
| **Contractile apparatus** | **Dense bodies** | **Cytoskeletal domain** | **Adherens junctions** | **Caveolar domain** |
| Actin (smooth muscle) | Actin (nonmuscle) | Actin (nonmuscle) | Actin (nonmuscle) | Actin (nonmusle) |
| Myosin (muscle type II) | α-Actinin | Desmin (or vimentin) | Filamin | Dystrophin |
| Tropomyosin | Calponin | Filamin | Calponin | Caveolin |
| Caldesmon | | Calponin | Vinculin | $IP_3$ receptor |
| Calponin | | Smoothelin | Metavinculin | $Na^+/Ca^{2+}$ exchanger |
| Myosin light chain kinase | | Synemin | Talin | $Na^+/K^+$ pump |
| Myosin light chain phosphatase | | Paranemin | Paxillin | |
| Calmodulin | | | Tensin | |
| | | | α-Actinin | |
| | | | Integrins | |
| | | | Plectin | |

[a]Reproduced with permission from Small, J. V., and Gimona, M. (1998). The cytoskeleton of the vertebrate smooth muscle cell. *Acta Physiol. Scand.* **164,** 341–348.

ends of the cell. Vascular smooth muscle plays a significant role in forming the extracellular matrix which surrounds it and produces collagen, elastin, and proteoglycans. Vascular smooth muscle contains extensible elastin fibrils and inextensible collagen ones. This matrix results in a characteristic passive force–length curve for relaxed tissues. Highly distending blood pressure would be resisted by this extracellular matrix.

It has not definitely been established if fibrosis or smooth muscle phenotype changes might contribute to VSP. Immunohistochemical studies of primate vessels known to have been in chronic VSP showed no increased content of collagen, α-actin, myosin, vimentin, desmin, fibronectin, or laminin (14).

## IV. Actin and Myosin

### A. Sliding Filament Theory

The investigations of A. F. Huxley and Niedergerke and H. E. Huxley and Hanson led to the sliding filament theory in which contraction is considered to occur solely by interdigitation of thick myosin and thin actin filaments (15,16). The most widely accept theory for an explanation of what makes filaments slide along one another is that of independent force generators, which have become synonymous with the cross-bridges observed between actin and myosin filaments. The sliding of filaments is explained by the action of an array of individual elements within the filaments which produce a force in the direction of shortening. Cross-bridges have been identified as physical entities in electron micrographs. Side pieces elastically connected to one filament presumably attached to the other filament in a strained state with a moderate rate constant in order to detach rapidly under the influence of ATP when in an unstrained or compressed state. Muscle shortening and ATP hydrolysis are coupled by a variable rate constant for attachment. If filaments are fixed an isometric tension is generated by attached strained side pieces which accumulate because of an unfavorable dissociation rate. The cycling rate and the ATPase rate are less than those of an isotonic contraction when the side pieces are moved into an unstrained position. Cross-bridges originate on the thick filament. These entities are presumed to correlate with the head of the myosin molecule and were identified as the site of ATP hydrolysis. Net isometric tension presumably depends on the number attached and is proportional to the degree of overlap between thick and thin filaments. Cross-bridges move relative to the actin filament. The myosin head length is about as long as the distance between the surfaces of the thick and thin filaments, and only slight movement is required for the formation of a cross-bridge. An analogy has been drawn between cross-bridges and members of a tug-of-war team (3).

### B. Structure and Interactions

#### *1. Myosin*

Myosin forms thick filaments, hydrolyses ATP, and binds actin. It is an ATPase. ATP and $H_2O$ reversibly change to adenosine diphosphate ADP + Pi $+H^+$. This reaction is the immediate source of free energy that drives muscle contraction. Myosin is a very large molecule, with a molecular weight of 470,000; it contains two identical heavy chains (above 200 kDa each) and four light chains (about 20 kDa each). Myosin consists of a double-headed globular region joined to a rod. The rod is a double-stranded α-helical cable that is 1340 Å long and the globules have a diameter of 90 Å (7). The myosin molecule contains two types of light chains, regulatory light chains (RLCs) and essential light chains (ELCs), with each myosin head having one RLC and one ELC. The molecular mass of RLC is 20 kDa and that of ELC is 17 kDa. $LC_{20}$ is phosphorylatable, and its phosphorylation is the key event in regulation of smooth muscle contraction. The functional role of $LC_{17}$ is unknown. The phosphorylation of $LC_{20}$ is a prerequisite for the activation of the Mg-ATPase activity of smooth muscle myosin by actin and consequently for smooth muscle contraction (17).

Smooth muscle myosin is a bifunctional molecule composed of six polypeptide chains. The two most outstanding properties are its ability to convert the chemical energy of Mg-ATP into mechanical work, the enzymatic property of which resides in the amino-terminal globule head domain, and its ability to organize itself into polar filaments which reside on the carboxyl-terminal, rod-like end of the molecule. The importance of this molecular motor molecule lies in its ability to hydrolyze ATP and then convert this chemical step into mechanical motion which involves the propelling of actin filaments (18).

#### *2. Actin*

Actin is the major constituent of thin filaments. In low-ionic-strength solutions it is a 42-kDa monomer with a globular shape (G-actin). At physiological pH it polymerizes into a fibrous form (F-actin) which closely resemble thin filaments. It resembles two strings of beads wrapped around each other. Actin exists in three (α, β and γ) isoforms. α-Actin is predominant in vascular smooth muscle (12). It plays a vital role in numerous cytoskeletal processes. There is a huge diversity of

actin isoforms in different muscle types. Actin monomers in their association with F-actin are flexible, which permits interaction with different binding proteins. Actin accounts for between 30 and 50% of the total noncollagenous protein in smooth muscle. The structural data suggest that both caldesmon (CaD) and tropomyosin regulate thin-filament (actin) interaction with myosin by a unique steric effect on the tropomyosin position which subsequently influences actomyosin ATPase. Low actomyosin ATPase activity is associated with stable actin–myosin binding, which may be the basis for the latch state of tension maintenance, despite tonic smooth muscles having low $Ca^{2+}$ concentrations (19). The ATPase activity in myosin is markedly increased by adding F-actin. Actin increases the turnover rate of myosin by binding to the myosin–ATP–Pi complex and accelerating the release of ADP and Pi. Actomyosin then binds ATP, which leads to the dissociation of actin and myosin. The resulting ATP–myosin complex is then ready for another round of catalysis (2,3). The active myosin kinase (MLCK) calmodulin–$Ca^{2+}$ complex transfers Pi from ATP to the regulatory light chain of myosin. Phosphorylated myosin then reacts with actin. Myosin kinase is inactivated by the removal of $Ca^{2+}$ and myosin is dephosphorylated by myosin phosphatase. When ATP is absent, actin and myosin form a high-affinity complex which is associated with muscle rigor.

### *3. Actomyosin*

Actin and myosin may be involved with locomotion, shape changes, and movement of intracellular organelles as well as contraction. Within smooth muscle the myofibrils are relatively stable structures (3). In muscle contraction chemical and mechanical events are related. These processes are quantitatively coupled in extent and time course. Myosin is an enzyme and actin is an activator. Myosin–ATPase is activated by F-actin filaments but not by G-actin (3). Vascular smooth muscle contracts in response to a stimulus-induced increase in $[Ca^{2+}]i$ with activation of MLCK by the $Ca^{2+}$–calmodulin complex, phosphorylation of $LC_{20}$ with subsequent cross-bridge cycling, and the development of force. The relationship between $[Ca^{2+}]i$ and myosin phosphorylation (the calcium sensitivity of phosphorylation) is regulated. For a given physiological stimulus the relationship between myosin phosphorylation and stress is invariant (20). In the late 1970s it was shown that $LC_{20}$ became phosphorylated during arterial contraction induced by $K^+$ or NE. Shortly thereafter, reversible phosphorylation and dephosphorylation of $LC_{20}$ during the contraction–relaxation cycle was demonstrated. In the early 1980s it was found that concurrent changes in stress, shortening velocity, and $LC_{20}$ phosphorylation accompanied the initial phase of arterial contraction. It was not anticipated that when stress was maintained, $LC_{20}$ phosphorylation would decline. This observation led to the concept of latch state or a slow cycling of dephosphorylated cross-bridges during stress maintenance (17). By the late 1980s caldesmon, another smooth muscle protein, was also shown to be capable of phosphorylation. Beginning in 1983 it was possible to observe the movement of polymer beads coated with myosin filaments along actin cables. These *in vitro* motility assays enabled biochemists to quantitate myosin–actin interactions (21). Exposing smooth muscle to glycerin results in the plasmalemma being permeabilized. The resulting preparation is intermediate between studies of actomyosin in solution and the intact smooth muscle system. Intracellular signaling cascades can be investigated without the influence of transmembrane ion currents. Force, ATPase, stiffness, and shortening velocity can still be measured in permeabilized preparations (22). Regulatory proteins such as tropomyosin are associated with the thin actin filament. Pure actin can be extracted from muscle in its monomeric globular form (G-actin). In physiological salt solutions the actin polymerizes to produce fibrous F-actin, which has a double-helical structure. The G-actin binds $Mg^{2+}$ or $Ca^{2+}$ and ATP reversibly. Myosin forms the thick filaments and can hydrolyze ATP and interact with actin to produce movement. These interactions are affected by $Ca^{2+}$ concentration. In the absence of ATP, actin and myosin form a complex which corresponds to muscle in the rigor state. Myosin ATPase is activated by F-actin filaments but not G-actin. The globular heads of the thick myosin filaments attach to the thin actin filaments at an angle of approximately 90° and then move to 45° thereby causing the filaments to slide past each other. Cross-bridges are central to the molecular basis of muscle contraction because they are the location of the enzymatic activity responsible for the hydrolysis of ATP. It is uncertain whether cross-bridges operate like a row of oars. Myosin light chain phosphorylation is definitely involved in the activation of smooth muscle contraction. Regulation of the actin (thin) filament is also present. It has been suggested that the light chains are dephosphorylated while their heads are attached. In the long-lived latch state tension is maintained, but the decreasing velocity and ATPase activity are reduced several-fold (3).

### *4. Phosphorylation and Cross-Bridge Cycling*

Phosphorylation of $LC_{20}$ facilitates the ability of myosin monomers to assemble into filaments and increases the ATPase activity of myosin 100-fold compared to unphosphorylated filaments. Dephosphorylation of myosin in the presence of Mg–ATP results in the disassembly of filaments. Filaments containing dephosphorylated myosin

exist in relaxed smooth muscle cells. Phosphorylation of $LC_{20}$ increases the $Mg^{2+}$–ATPase activity of smooth muscle myosin (23). The covalent modification of myosin by phosphorylation of the 20-kDa myosin light chains is a significant mode in regulation of contractile activity of smooth muscle, particularly in the initial development of tonic contraction and the generation of phasic contractions. Protein kinase C(PKC) has an important role in the regulation of smooth muscle tone maintenance, especially in vascular smooth muscle. There may be a link between PKC and actin-based regulatory mechanisms. $[Ca^{2+}]i$ is a major determinant of smooth muscle contractility. As levels of this ion in sarcoplasm increase, there is activation of CaM, MLCK, and phosphorylation of the $LC_{20}$, resulting in an increase in myosin ATPase activity and cross-bridge cycling. The largest elevation in $LC_{20}$ phosphorylation is seen in the first 30–60 sec while tone is developing, but subsequently phosphorylation decreases to much lower levels suggesting that the elevated $LC_{20}$ phosphorylation does not play a role in sustained contraction (7). The extent of myosin light chain (MLC) phosphorylation rather than $[Ca^{2+}]i$ levels determines cross-bridge kinetics. Cross-bridge turnover slows at low levels of activation. The amount of latch bridges formed may depend on a balance of MLCK and phosphatase activity. The binding of ATP dissociates rigor cross-bridges. Both force and shortening velocity are modulated by the extent of MLC phosphorylation (1).

In mammalian smooth muscle under resting isometric conditions when the MLC is not phophorylated, myosin cycles very slowly. Phosphorylation of MLC increases the cycling rate 50-fold to 0.2/sec. The turnover rate of light chain phosphate can increase to 0.3 or 0.4/sec at suprabasal $Ca^{2+}$ concentrations. Some myosin may remain phosphorylated for a long time instead of it all cycling through the phosphorylated state in a short time (24). The overall rate-limiting step for cross-bridge cycling is the phophorylation of Ser 19 of the $MLC_{20}$ by $Ca^{2+}$–CaM–MLCK. Myosin phosphatase dephosphorylates $MLC_{20}$. Cross-bridge cycling and force generation are not entirely dependent on $[Ca^{2+}]i$. The myosin motor may also be regulated by Mg–ADP in force maintenance, MLC isoform ($LC_{17}$) in velocity changes, and cGMP in phosphatase activation. Tonic smooth muscles have a higher affinity for Mg–ADP than Mg–ATP, so there is a larger population of dephosphorylated cross-bridges. Such cross-bridges may contribute to force maintenance at low levels of $MLC_{20}$ phosphorylation. Telokin is the independently expressed C terminus of MLCK and is extensively phosphorylated during certain types of induced relaxation. It accelerates the dephosphorylation of regulatory MLC, thereby contributing to cAMP/cGMP kinase-mediated $Ca^{2+}$ desensitization (25).

#### *5. Unanswered Questions*

Sustained phosphorylation of $LC_{20}$ during tonic contractions is probably exceptional. It is still a mystery exactly how vascular smooth muscle maintains contractile tone for indefinite periods without depleting energy stores (7). The smooth muscle motor system is therefore incompletely understood. The mechanism of slow cross-bridge cycling, the role of the thin filament-associated proteins, and the structural organization of smooth muscle (as opposed to skeletal) contractile units and filaments remain to be elucidated. Since most agonist-mediated VSMC contractions involve $Ca^{2+}$–CaM-dependent phosphorylation of MLCK, which in turn promotes contractile force generation through actin-myosin ATPase, Harada *et al.* measured non-, mono-, and diphosphorylated forms of $MLC_{20}$ using immunoblotting and polyacrylamide gel electrophoresis. Using the rat femoral artery model of VSP, they found at 7 and 10 days post-clot application that phosphorylated $MLC_{20}$ was undetectable; up to 5 days post-blood application the levels were comparable to controls (25a). It seems likely that VSP results from some form of initial dynamic contraction with associated MLC phosphorylation which enters a prolonged phase of rigor or catch in which active phosphorylation ceases but cross-bridges are maintained.

### C. Rigor and Latch States

In metabolically deranged muscle in which ATP cannot be replenished the muscle becomes stiff and is described as being in rigor. During rigor, actin and myosin filaments strongly interact. If the membrane of the nonreacting muscle in rigor state is rendered permeable to ATP, the muscle will again become reactive; whether it contracts or relax will be a function of the $Ca^{2+}$ concentration (3). Some invertebrate muscles have very long thick and thin filaments and can maintain previously developed high forces with only basal energy consumption. This is termed catch and is a lock-up of previously developed force. If such invertebrate muscles are subjected to a step shortening the force falls to zero and is not redeveloped. In this respect it differs from latch, in which vertebrate smooth muscle would slowly redevelop full force. The regulatory mechanisms involved in catch remain uncertain. The relaxation of catch is independent of $[Ca^{2+}]$ and is probably caused by the phosphorylation of contractile proteins (26). In the presence of many agonists, and to a lesser degree in the presence of high levels of KCl-induced membrane depolarization, smooth muscles are able to maintain tone despite low or basal levels of $[Ca^{2+}]i$ and $MLC_{20}$ phosphorylation. The phenomenon of $Ca^{2+}$-dependent force maintenance without detectable

elevations of phosphorylation is thought to indicate mechanical similarities with molluskan catch muscles (27). Molluskan catch muscles can maintain closure of the shell with negligible energy consumption. By analogy, latch is a state of reduced or noncycling cross-bridge cycling rates dependent on low but significant levels of $Ca^{2+}$-dependent $MLC_{20}$ phosphorylation. It is generally held that the only regulatory mechanism that needs to be considered in smooth muscle is phosphorylation and dephosphorylation of $LC_{20}$ (7). Vascular smooth muscle contraction can be maintained in the absence of maintained $MLC_{20}$ phosphorylation. In some fully contracted muscle levels of $[Ca^{2+}]i$ can return to nearly baseline. To explain these findings, a latch state has been hypothesized which assumes that the rapidly cycling cross-bridges which form between myosin and actin undergo dephosphorylation in such a way that kinetically stable latch cross-bridges form. This new kinetic state is one in which there is slow dissociation of the high-affinity actinomyosin complex. The postulated latch bridges account for the tonic phase of contraction (6). The term latch was originally used to describe near-maximal force maintenance despite decreases in both cross-bridge cycling rates and phosphorylation of the regulatory light chain in the tonic swine carotid media. According to Murphy's group (28,29), it is not a lock-up of length under the control of an unknown calcium-dependent, phosphorylation-independent mechanism. The latch state is due to specific dephosphorylated cross-bridge states which are formed by dephosphorylation of attached cross-bridges (dephosphorylation without detachment). This model requires only a single regulatory system—the phosphorylation and dephosphorylation of MLC by the action of MLCK and phosphatase. The role of CaD and CaP, if any, in latch is unknown (1). When smooth muscle is initially stimulated the rate of muscle shortening rapidly increases as the contraction builds up but then slows, although tension is maintained. Myosin phosphorylation and myoplasmic $Ca^{2+}$ levels change in parallel with velocity. The latch state occurs when tension is maintained while shortening velocity, phosphorylation, and $Ca^{2+}$ levels are low. This is neither rigor nor relaxed, and no comparable state exists in striated muscles. After chemical removal of the cell membrane smooth muscle cells can still show latch state, and ATPase activity is extremely low so that this unusual contraction is probably a property of the contractile apparatus.

Others have proposed a second regulatory system to explain the reversible equilibrium between attached and detached unphosphorylated myosin controlled by $Ca^{2+}$. This thin filament regulatory system is made up of actin, tropomyosin, CaD, an inhibitory protein, and a calcium-binding protein which reverses CaD inhibition at physiological $Ca^{2+}$ concentrations. Caldesmon acts in slowing the rate of product release from actomyosin–ADP–Pi; it has the same point of action as troponin and myosin phosphorylation mechanisms. When smooth muscle CaD is added to actin, ATPase is inhibited while myosin strongly binds to actin. This suggests a new form of thin filament–myosin interaction (30).

There is general acceptance that tonic contractions involve a combination of phosphorylated cross-bridges and dephosphorylated latch bridges. How the latter are formed and regulated is currently being vigorously investigated. There may be a thin filament regulatory mechanism operating in parallel with phosphorylation. One complicating factor in interpreting *in vitro* studies is that most have been done at room temperature, which blocks spontaneous contractions. There is strong evidence that latch is due to reduced detachment rates of cross-bridges (28).

## V. Modulating Proteins

### A. Calmodulin

Like troponin in striated muscle, CaM can bind four $Ca^{2+}$ molecules cooperatively. A single polypeptide chain of this highly conserved molecule consists of about 150 amino acid residues. It makes up about 1% of the protein of the cell (12). CaM is a ubiquitous $Ca^{2+}$-binding protein with many functions. It regulates smooth muscle contraction in response to changes in $[Ca^{2+}]i$. CaM activates cross-bridge cycling and the development of force in response to $[Ca^{2+}]i$ transients by activating MLCK and phosphorylating myosin. A second kinase, $Ca^{2+}$/CaM-dependent protein kinase II, also has a role in smooth muscle contraction. This kinase also phosphorylates MLCK and increases the CaM concentration required for activation of MLCK. This is the possible explanation for the desensitization of contractile response to $Ca^{2+}$. CaM may also regulate the myosin-associated proteins, CaD and CaP, which inhibit the actin-activated $Mg^{2+}$–ATPase activity of smooth muscle myosin (the cross-bridge cycling rate). CaM may also control the movement of $Ca^{2+}$ across the sarcolemmal and sarcoplasmic reticulum membranes. CaM perhaps regulates other proteins which indirectly regulate $[Ca^{2+}]i$ as well as cAMP and cGMP synthesis and breakdown (31).

### B. Caldesmon

The CaD runs longitudinally along the length of the thin filament (7). It is a ubiquitous protein in smooth muscle cells. Its molecular mass is 89–93 kDa. It is

localized within the contractile apparatus and is tightly bound. It may function to regulate thin filament activity and affect the assembly and stabilization of the thick and thin filaments. It plays a central role in the $Ca^{2+}$-dependent regulation of thin filament function. The physiological role in vascular smooth muscle is currently unknown (32). Smooth muscle thin filaments contain actin–tropomyosin and CaD in molar ratios of 14:2:1 (33). When it is purified, CaD binds to actin–tropomyosin and inhibits actomyosin ATPase activity; this inhibition is not $Ca^{2+}$ sensitive. It is possible that native thin filaments are regulated by calcium-binding proteins other than CaM, and several such proteins have been identified from smooth muscle (33). Smooth muscle CaD is an elongated protein consisting of a single polypeptide chain. CaD binds to F-actin, tropomyosin, and $Ca^{2+}$/CaM. In addition to thin filament proteins, CaD also binds myosin. This binding is enhanced in the presence of ATP. Because of these binding properties, CaD can crosslink actin and myosin (7). A CaD-to-actin ratio of 1:32 has been reported for vascular smooth muscle (34). It can inhibit myosin ATPase without binding to the thick filaments. It has been suggested that in the relaxed state thin filaments are "switched off" by CaD, which simultaneously stabilizes the $LC_{20}$ dephosphorylated thick filaments. Conformational changes in CaD may make thin filaments available to the myosin heads. There may be direct regulation of cross-bridge cycling by the thin filament-associated proteins CaD and CaP (35). CaD can be phosphorylated *in vitro* by a wide variety of protein kinases (7).

CaD inhibits the actin activation of myosin ATPase activity, possible by inhibiting the binding of myosin to actin, the transition between any two actin–myosin states, and the distribution between the inactive and active states of actin. CaD binds to both actin and myosin, and each CaD molecule binds to several actin monomers. CaD has been observed to decorate actin filaments in muscle fibers. It is localized to the fraction of actin that associates with myosin. CaD inhibits the actin-activated ATPase activity of myosin in solution (36). CaD may be an auxillary regulator of cross-bridge cycling in addition to myosin phosphorylation. It is a CaM-binding protein which is $Ca^{2+}$ dependent and also binds actin and myosin. CaD inhibits actin-activated $Mg^{2+}$–ATPase activity of phosphorylated myosin and its inhibiting activity is reversed by $Ca^{2+}$/CaM. There is no definite evidence regarding its role *in vivo* in muscle contractions. CaD may be involved in actin filament structure regulation, it is associated with myosin dephosphorylation in a way that force is maintained in the formation of latch bridges, and it organizes actin and myosin filaments into a coordinated contractile network. Elevated levels of CaD phosphorylation have been reported during prolonged contraction of porcine carotid arteries in response to KCl and phorbol esters (37).

### C. Tropomyosin

Currently, the role of tropomyosin remains to be clarified. Tropomyosin lies on a groove on thin filaments. It reversibly exposes myosin binding sites on actin. Tropomyosins are found in virtually all eukaryotic cells. Together with actin, myosin, and ancillary proteins, they function as part of the contractile apparatus and thin filament assemblies. They are arranged like a coiled coil. Their polypeptide chains consist of 284 amino acid residues. Although in striated muscles tropomyosin and troponin (absent in vascular smooth muscle) provide the calcium switch to turn actomyosin on and off, their roles in smooth muscle are not as clearly known. The concentrations in arterial smooth muscle are as follows: myosin, 56 $\mu M$; actin, 1.6 m$M$; and tropomyosin, 0.27 m$M$ (38).

### D. Calponin

Calponin is a basic protein with molecular weight of 34,000. It is expressed in four isoforms and binds strongly to actin in a $Ca^{2+}$-independent manner. It is localized to thin filaments in smooth muscles and is present in a ratio of 1 mol CaP/7 mol actin. CaP interacts with actin to inhibit actomyosin Mg–ATPase (cross-bridge cycling rate) without altering myosin phosphorylation. The activity of calponin can be reversed by phophorylation with either protein kinase C (PKC) or CaM kinase II (CaMK II). CaP is phosphorylated in intact smooth muscle in response to contractile stimuli. It is highly likely that calponin phosphorylation–dephosphorylation is a thin filament-linked regulatory system in vascular smooth muscle (39).

Smooth muscle CaP is an elongated molecule that can reach 18 nm in length. It consists of a single polypeptide chain. CaP binds F-actin with a higher affinity for the smooth muscle than the skeletal muscle actin isoform. CaP binds both monomeric and filamentous dephosphorylated smooth muscle myosin. It inhibits myosin ATPase in a dose-dependent manner. Upon phosphorylation *in vitro* by either CaMK II or PKC, calponin's inhibitory action is reversed. CaP is distributed in smooth muscle cells on filamentous structures along the whole length of the cell. It colocalizes with desmin at the periphery of the cytoskeletal domain distinct from the restricted localization of CaD to the contractile domain. It may interact with the phospholipids of the sarcolemma. CaP may be involved in thin filament regulation since it can be shown *in vitro* to bind actin and inhibit actomyosin ATPase. CaP is capable of inhibiting both force and shortening velocity to varying degrees. Transient CaP

phosphorylation probably will not explain the major riddle of the mechanism of tone maintenance in smooth muscle. Agonist-induced redistribution of CaP requires PKC activation (40,41). Calponin's properties are very similar to those of caldesmon (42). CaP is a marker of differentiated smooth muscle, and phosphorylation of CaP in porcine carotid artery is mediated by PKC (7). Phenylephrine, which activates PKC, caused a cellular redistribution of CaP from a primarily cytosolic to a primarily surface site. This movement was partially inhibited by the PKC inhibitor calphostin and coincided temporarily with PKC translocation, both of which preceded cellular contraction. CaP might mediate agonist-activated contraction via a PKC-dependent pathway (43). It probably binds with CaM, S100 proteins, tropomyosin, tubulins, myosin, and CaD. A direct reaction with phospholipids is also possible (44). CaP was found to be significantly reduced in spastic arteries on days 1 and 3 in a rat model (42).

## VI. Relaxation

### A. General

Smooth muscle relaxation is induced by agonists which interfere with one of the contractile mechanisms or which operate by additional mechanisms such as stimulation of $Ca^{2+}$ efflux or sequestration. The electromechanical mechanism for relaxation is hyperpolarization. NO activates soluble guanylyl cyclase (G cyclase). Vasodilators activate G cyclase or adenyl cyclase (A cyclase) through the mediation of a G protein. As a result, cGMP and cAMP are formed. In turn, these compounds can activate the cGMP-dependent protein kinase (G kinase), resulting in activation of plasma membrane $K^+$ channels and hyperpolarization and relaxation probably by other mechanisms. The hyperpolarization decreases $Ca^{2+}$ influx through L-type $Ca^{2+}$ channels. The net effect is to decrease $[Ca^{2+}]i$, myosin phosphorylation, and contraction. Relaxation may also be brought about by G kinase-dependent increases in the activity of sarcoplasmic reticulum $Ca^{2+}$ pumps which can lower $[Ca^{2+}]i$. $[Ca^{2+}]i$ may also be lowered by plasmalemmal pumps increasing the extrusion of $Ca^{2+}$. Vasodilators may also inhibit the formation of 1,4,5-$IP_3$, which decreases $Ca^{2+}$ release from endoplasmic stores. Also, increases in cAMP may activate A kinase, which is capable of phosphorylating MLCK and decreasing its sensitivity to $Ca^{2+}$ (4). Numerous drugs and hormones can relax smooth muscles by increasing the sarcoplasmic concentration of cAMP and cGMP. These nucleotides can activate second messengers by phosphorylating them. Relaxation is associated with increased $Ca^{2+}$ extrusion from the cell or sequestration within the sarcoplasmic reticulum. Electrically induced hyperpolarization reduces $Ca^{2+}$ influx and slows cross-bridge phosphorylation, thereby causing a decline in force generation (2,20).

The release of molluskan "catch" state (possibly analogous to VSP) can be rapidly initiated by 5-HT, which increases cAMP and activates PKA. The time course of change in phosphorylation of a recently described protein corresponds to the release of catch in molluskan muscle preparation. This protein had a molecular weight of 600 kDa and molar concentration 30 times lower than that of myosin heavy chain. A reversible thiophosphorylation of this protein prevented the development of catch (45).

In striated muscle, relaxation follows sequestration of $Ca^{2+}$ mobilized by an excitatory stimulus. Removal of the excitatory stimuli also relaxes smooth muscle *in vitro* after reductions in myoplasmic $Ca^{2+}$ and dephosphorylation of regulatory MLC. Experimentally, relaxation can be induced despite the presence of excitatory stimuli and $Ca^{2+}$-dependent cross-bridge phosphorylation. NO can initiate an increase in cGMP which may lower the $Ca^{2+}$ sensitivity of cross-bridge phosphorylation. Relaxation may be induced by activation of phosphatases. Relaxation involves a decrease in the attachment and cycling of phosphorylated cross-bridges or the reduction in the formation of force-generating dephosphorylated cross-bridges. Thin filament proteins may block cross-bridge attachments or block proteins which inhibit phosphatases, thereby leading to relaxation (46). Cytosolic $Ca^{2+}$ levels are reduced by the plasmalemmal $Ca^{2+}$ eflux and $2H^+$ influx using ATP. The plasmalemma also has a $Na^+/Ca^{2+}$ exchanger driven by the transmembrane $Na^+$ gradient and sustained by the $Na^+$ pump. One $Ca^{2+}$ is exchanged for three $Na^+$. The sarcoplasmic reticulum $Ca^{2+}$ pump can also sequester $Ca^{2+}$, thereby lowering cytosolic $Ca^{2+}$ (47).

### B. Phosphatases

In relaxed muscles the myosin is dephosphorylated. A balance between MLCK and myosin light chain phosphatase (MLCP) determines the level of myosin phosphorylation and the state of contraction of vascular smooth muscle. Force: $Ca^{2+}$ ratios vary according to the method of stimulation. Protein phosphatases are divided into phosphoserine/threonine-specific and phosphotyrosine-specific enzymes based on the distinct specificity toward the phosphorylated residues. Smooth muscle myosin is phosphorylated at serine 19 at the N terminal of $LC_{20}$. There are many protein phosphatases in eukaryotic cells and several of these use phosphorylated myosin as a

substrate. It may be that more than one phosphatase acts on myosin (48). In physiological conditions smooth muscle $LC_{20}$ phosphatase is tightly bound with myosin and is not dissociated under physiological ionic conditions. At body temperature in the presence of most agonists the elevations of $[Ca^{2+}]i$ and MLC phosphorylation are transient and during tone maintenance both are low or basal (7). Generally, with increased $Ca^{2+}$ sensitivity (increased force for a given $[Ca^{2+}]i$ such as follows agonist stimulation), the key factor seems to be inhibition of phosphatase activity (49).

Myosin phosphatase was only discovered in the late 1970s. The primary mechanism of relaxation is the dephosphorylation of the regulatory 20-kDa MLC at Ser 19 by $MLC_{20}$ phosphatase. The ratio of kinase to phosphatase activity and consequently the degree of contractile force depends on the amount of $Ca^{2+}$–CaM, which in turn relates to $[Ca^{2+}]i$. The sensitivity of force to $[Ca^{2+}]i$ can be altered by additional mechanisms such as actin disinhibition and modulation of the $[Ca^{2+}]i$ dependence of $MLC_{20}$ phosphorylation (50).

Pharmacomechanical control of $[Ca^{2+}]i$ principally depends on the phosphatidylinositol cascade and $[Ca^{2+}]i$ pumps of the plasmalemma and sarcoplasmic reticulum. The monomeric GTPase, Rho A, is an upstream component which can affect $Ca^{2+}$ sensitization. Inhibition of Rho A depresses the tonic component of agonist-induced contraction. Inhibition of smooth muscle myosin phosphatase is the major mechanism of G protein-coupled $Ca^{2+}$ sensitization; PKC is not involved. Activation of phosphatase with resultant $Ca^{2+}$ desensitization is at least partly due to a cyclic nucleotide-dependent kinase and its major substrate telokin (25). Vasoconstrictors activate PLC and $Ca^{2+}$ channels as well as downregulate MLCP by a distinct mechanism involving Rho p21 and PKC (51). A novel protein, CPI-17, has recently been identified in vascular smooth muscle. Its phosphorylation and thiophosphorylation by PKC inhibit certain protein phosphatases including MLCP. Phosphorylation of CPI-17 potentiates contractions at constant $[Ca^{2+}]i$ in certain arterial smooth muscle preparations. Thiophosphorylated CPI-17 can induce large contractions even under $Ca^{2+}$-free conditions. The major physiologic effect of CPI-17 appears to be the inhibition of MLC phosphatase (52).

## VII. Calcium

### A. Regulation of Calcium in Vascular Smooth Muscle

Vascular smooth muscle contraction depends on an increase in the sarcoplasmic $[Ca^{2+}]i$. Since there is no troponin, the $Ca^{2+}$ precisely but indirectly regulates cross-bridge attachment and cycling. $Ca^{2+}$ controls MLCK activity by covalent regulation. A smooth muscle cell does not have an elaborate sarcoplasmic reticulum surrounding the myofilaments as is found in striated muscle. $Ca^{2+}$ enters the sarcoplasm through the sarcolemma during activation. Chemical signals link sarcolemma and the sarcoplasmic reticulum.

$Ca^{2+}$ homeostasis is dependent on multiple mechanisms (Fig. 7.4; Table 7.3). There are two main $[Ca^{2+}]i$ integrating systems. The first resides in the sarcolemma or cell wall and is under the control of membrane electrical potential and chemical agonists such as hormones and autocoids. The plasmalemmal voltage-gated and receptor-operated channels permit $Ca^{2+}$ entry in certain circumstances. The membrane potential is determined by ionic concentration gradients and permeability. The voltage-gated channels may be activated by the electrical depolarization transmitted via gap junctions from adjacent cells. The occasional spread of VSP to the cervical internal carotid may reflect such a mechanism. In the cell wall there are voltage-dependent $Ca^{2+}$ channels, receptor-operated $Ca^{2+}$ channels, $Ca^{2+}$ pumps, electrogenic $Na^+$–$K^+$ pumps, and $Na^+$–$Ca^{2+}$ exchangers.

$Ca^{2+}$ pumps which extrude $Ca^{2+}$ from the cell, the $Na^+/Ca^{2+}$ exchanger, and different $Ca^{2+}$ channels are situated in the plasmalemma. The plasmalemma's inner and outer surfaces may bind $Ca^{2+}$ and release it in response to different agonists. The sarcoplasmic reticulum membrane also possesses $Ca^{2+}$ pumps to facilitate $Ca^{2+}$ entry into its lumen. This pump can be inhibited by several agents. Physiologically, it is regulated by the protein phospholamban, which inhibits the pump if it is in its unphosphorylated state. The sarcoplasmic reticulum membrane also possesses $IP_3$ and ryanodine receptors, which release $Ca^{2+}$ from the stores when stimulated. Although the plasmalemma and sarcoplasmic reticulum are the main buffers for $Ca^{2+}$ movement, the cytosol also has some buffering capacity (47).

$[Ca^{2+}]i$ is a central second messenger in smooth muscle contraction (Table 7.4). It is present in submicromolar concentrations despite a $[Ca^{2+}]e$ of 1 or 2 m*M* and total cellular $Ca^{2+}$ of several m*M*. $[Ca^{2+}]i$ increases during contraction as a consequence of influx of $Ca^{2+}$ from the extracellular space or its release from intracellular stores. $Ca^{2+}$ crosses the sarcolemma through ion channels or via ion exchangers. Relaxation is usually accompanied by a decrease in $[Ca^{2+}]i$ to resting values. This is accomplished by its extrusion from the cell by the sarcolemma $Ca^{2+}$–ATPase or the $Na^+/Ca^{2+}$ exchanger. $[Ca^{2+}]i$ can also be taken up by intracellular stores by the action of $Ca^{2+}$–ATPase pumps on the sarcoplasmic reticulum membrane. Binding molecules for $Ca^{2+}$ in the myoplasm and in the sarcoplasmic reticulum act as cell buffers and therefore

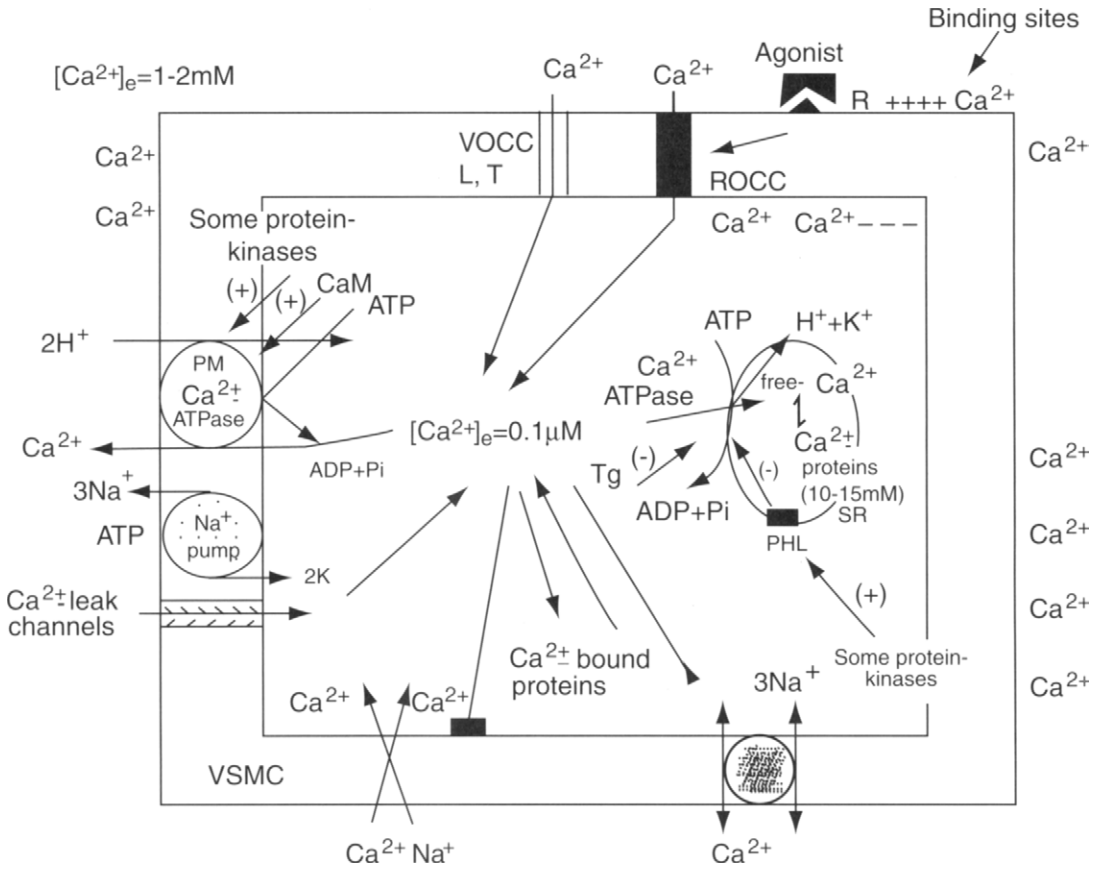



**FIGURE 7.4** Scheme of the main mechanisms involved in the maintenance of the cytosolic (intracellular) free $Ca^{2+}$ concentration ($[Ca^{2+}]c = [Ca^{2+}]i$) in a vascular smooth muscle cell (VSMC). $Ca^{2+}$ entry is produced by activation of receptor-operated $Ca^{2+}$ channels (ROCCs), voltage-operated channels (VOCCs) of L and T type, and $Ca^{2+}$-leak channels. The $Na^+/Ca^{2+}$+ exchanger, $Na^+$ pump, plasma membrane (PM), and sarcoplasmic reticulum (SR) $Ca^{2+}$-ATPase participate in the cytosolic $Ca^{2+}$ homeostasis. Pi, inorganic phosphate; Tg, thapsigargin; CaM, calmodulin; $[Ca^{2+}]e$, extracellular free $Ca^{2+}$ concentration; R, receptor; PHL, phospholamban [reprinted from *Life Sci.* **64,** Marin, J., Encabo, A., Briones, A., Garcia-Cohen, E. C., and Alonso, M. J. Mechanisms involved in the cellular calcium homeostasis in vascular smooth muscle, 279–303, copyright © 1999, with permission from Elsevier Science].

**TABLE 7.3 Mechanisms to Maintain $Ca^{2+}$ Homeostasis at Rest**[a]

| |
|---|
| Active transport across the plasmalemma by $Ca^{2+}$–ATPase, 1 $Ca^{2+}$ out: 2 $H^+$ in |
| $Na^+$–$Ca^{2+}$ plasmalemma exchange (antiporter) system, 1 $Ca^{2+}$ out: 3 $Na^+$ in |
| Passive transport of $Ca^{2+}$ down electrical gradient into mitochondrial lumen (possibly only when very high $[Ca^{2+}]i$) |
| Transport into nucleus |
| Transport (active) into sacroplasmic reticulum using $Ca^{2+}$–ATPase |
| Possible sequestration within cytoplasmic compartment |

[a]Modified from Orallo, F. (1996). Regulation of cystolic calcium levels in vascular smooth muscle. *Pharmacol. Ther.* **69,** 153–171.

reservoirs of bound $Ca^{2+}$. Cells have a relatively large capacity for $Ca^{2+}$ storage in relationship to the low free concentrations in the cell (7).

Contraction in vascular smooth muscle depends on an increase in myoplasmic $[Ca^{2+}]i$ concentration. Since troponin is absent the $Ca^{2+}$ regulates cross-bridge attachments and cycling indirectly. The state of the cross-bridges which connect and move actin on myosin is determined by phosphorylation carried out by the enzyme myosin kinase and dephosphorylation performed by myosin phosphatase. Sustained contraction of smooth muscle depends on the extracellular $Ca^{2+}$ pool. Steady-state myoplasmic $Ca^{2+}$ and cross-bridge phosphorylation depend on the sum of the stimulus-dependent processes

**TABLE 7.4 Calcium, Contraction and Relaxation**[a]

| |
|---|
| Mechanisms to raise $Ca^{2+}$ to develop tone by graded contraction |
| Electromechanical coupling |
| Electrical depolarization by $Ca^{2+}$ entry through voltage-dependent transmembrane channels; VSMC have transient (T-type) and long-lasting (L-type) channels |
| Increased intramural pressure and stretch may open $Ca^{2+}$ channels |
| Pharmacomechanical coupling; receptor binding of contractile agonist can cause $[Ca^{2+}]i$ increase from extracellular or endoplasmic reticulum stores. $Ca^{2+}$ receptors can be activated by neurotransmitters, endothelium-derived agents, humoral agents, blood physiochemical factors, and myogenic factors; G protein-coupled membrane receptors activate phospholipase C, which splits $PIP_2$ into $IP_3$ and DAG; $IP_3$ induces release of $Ca^{2+}$ from sarcoplasmic reticulum; Increased $[Ca^{2+}]i$ stimulates PKC to migrate to plasmalemma where it is activated by DAG; PKC causes contraction by |
| Voltage-dependent opening of plasmalemmal $Ca^{2+}$ channels |
| Increasing sensitivity of myofilaments to $Ca^{2+}$ |
| Inducing intracellular alkalosis |
| Transmembrane influx of $Ca^{2+}$ due to excitatory agonist in tonic phase of contraction |
| Independent of membrane polarization |
| Dependent on agonist-induced, direct membrane depolarization |
| Dependent on membrane depolarization resulting from $Ca^{2+}$ release from intracellular stores ($Ca^{2+}$-induced $Ca^{2+}$ release) or via $Ca^{2+}$-dependent nonspecific cation opening |
| Activation of non-phospholipase C-coupled membrane receptors coupled to pertussis-sensitive G protein |
| Direct vasoconstrictor activation of normally voltage-operated $Ca^{2+}$ channels |
| Mechanisms to lower $Ca^{2+}$ to induce relaxation |
| Increase in cyclic nucleotides (mainly cGMP) in cytosol by inhibition of various phosphodiesterases by antagonists; activation of soluble guanylate cyclase by NO and other nitrocompounds; activation of membrane guanylate cyclase by ANF |
| cGMP activates cGMP-dependent protein kinase, which may |
| Reduce $Ca^{2+}$ sensitivity of actomyosin by increasing $MLC_{20}$ dephosphorylation |
| Activate $Ca^{2+}$ uptake by intracellular stores via phosphorylation of phospholamban and activation of SR $Ca^{2+}$-ATPases |
| Increase $Ca^{2+}$ efflux by stimulation of plasmalemmal $Ca^{2+}$-ATPase and $Na^+$-$Ca^{2+}$ exchanger |
| Inhibit SR $Ca^{2+}$ release by phosphorylation of $IP_3$ SR receptor |
| Hyperpolarize plasmalemma by opening of $K^+$ channels |
| Inhibit transmembrane current by inducing dephosphorylation of voltage-operated channels |
| Interference with mechanisms of contractions such as voltage channel blockage by calcium antagonists, selective antagonists of vasoconstrictor receptors, and $K^+$ channel agonists to induce hyperpolarization |

[a]Modified from Orallo, F. (1996). Regulation of cystolic calcium levels in vascular smooth muscle. *Pharmacol. Ther.* **69,** 153–171.

that govern $Ca^{2+}$ exchange with the extracellular pool. The differential permeabilities of $Na^+$ and $K^+$ ions through the sacrolemma determine the membrane potential of faster smooth muscle cells. The electrogenic ion pump expels the three $Na^+$ ions in exchange for two $K^+$ ions, so one positive charge is removed from the cell by each cycle with a resulting increased negativity of the membrane potential. The conductions of $Ca^{2+}$ through sarcolemmal potential-dependent channels increase with depolarization. $Ca^{2+}$ entry into the vascular smooth muscle cell increases force generation. $Ca^{2+}$ influx is affected by action potentials, slowing of the $Na^+ - K^+$ exchange, and depolarization of the sarcolemma by depolarization propagated via gap junctions from adjacent cells (2).

### B. Force and Sarcoplasmic Calcium

$Ca^{2+}$ is a key second messenger involved in signaling pathways for contraction in VSMCs. Increases in $[Ca^{2+}]i$ occur principally by influx through plasma membrane channels or through release from internal storage compartments. When $Ca^{2+}$ binds to CaM, there is a conformational change rendering it capable of binding to inactive MLCK. MLCK then isomerizes into its active form, which subsequently phosphorylates serine 19 of the

myosin regulatory light chain (RLC). The phosphorylation of RLC greatly increases actin-activated myosin Mg–ATPase activity. This initiates smooth muscle contraction (53).

In the reverse of this process a decrease in $[Ca^{2+}]i$ results in the dissociation of CaM from MLCK and conversion of the kinase to its inactive form. As the kinase is inactivated, RLC is dephosphorylated by MLCP, which is also found on the contractile elements. The opposing role of these myosin kinases and phosphatases in controlling RLC phosphorylation is influenced by $Ca^{2+}$ to a great extent but there other modulatory influences (53).

Pathways that modulate $Ca^{2+}$ dependence of force alter the dependence of $LC_{20}$ phosphorylation on $[Ca^{2+}]i$ or the dependence of force on the degree of $LC_{20}$ phosphorylation. $[Ca^{2+}]i$ activates myosin Mg–ATPase via $Ca^{2+}$/CaM-dependent phosphorylation of RLC by MLCK. Steady-state values of myosin $LC_{20}$ phosphorylation result from relative activities of MLCK and MLCP on myosin because altered activities of these two enzymes have opposite effects. Both of these antagonistic enzymes can be inhibited by second-messenger pathways. Agonists usually result in a greater degree of myosin RLC phosphorylation at a given $[Ca^{2+}]i$ than depolarization, thereby sensitizing force to $Ca^{2+}$. Most stimulus conditions have a unique relationship between phosphorylation and force in the steady state. Phorbol dibutyrate will increase the amount of force for a given degree of phosphorylation and sodium nitoprusside will decrease it. Thin filament regulatory proteins such CaD and CaP are also regulatable by $Ca^{2+}$/CaM and/or phosphorylation (53).

Sustained smooth muscle contraction depends on extracellular $Ca^{2+}$, without which agonist-induced contractions relax as the intracellular $Ca^{2+}$ pool is exhausted. Sustained agonist-induced contractions are dependent on sustained increases in $Ca^{2+}$ influx. Most $Ca^{2+}$ influx depends on agonist-dependent depolarization. There may be some directly receptor-operated $Ca^{2+}$-permeable channels (4).

It has been suggested that there is an increase in basal $Ca^{2+}$ sensitivity of the contractile elements in vasospastic vessels. In a two-hemorrhage dog model basilar artery strips were studied. Fura-2 was used to record $[Ca^{2+}]i$ at the same time as tension was recorded. High $K^+$ and a thromboxane $A_2$ analog were used as stimulants. The increase in contractile tension per $[Ca^{2+}]i$ per unit cross-sectional area was reduced in arteries which were exposed to SAH compared to controls (54).

### C. Plasmalemma and Calcium Control

Vascular smooth muscle is controlled and coordinated by coupling between groups of cells by junctions and by locally produced activators or inhibitors in the endothelium and smooth muscle cells as well as neurotransmitters (2). The sarcolemma is a barrier across which a 10,000-fold concentration gradient exists between $[Ca^{2+}]e$ and $[Ca^{2+}]i$. $Ca^{2+}$ enters the sarcoplasm via voltage-dependent channels (L type), receptor-operated channels (ROCs), and the $Na^+/Ca^{2+}$ exchanger. $Ca^{2+}$ is removed from the sarcoplasm by a plasma membrane ATPase as well as the $Na^+/Ca^{2+}$ exchanger. A normal smooth muscle cell has a transmembrane potential of $-40$ to $-55$ mV. High extracellular $K^+$ results in a rapid increase in intracellular $Ca^{2+}$, depolarization of the smooth muscle membrane, and muscle contraction (6).

The plasmalemma of most eukaryotic cells consists primarily of phospholipids and proteins which separate the cytoplasm from the extracellular fluids. Some lipid solutes and very small molecules can diffuse across the membrane. Larger molecules require the mediation of transport proteins. Water moves across the membrane by osmosis. The transport of $Na^+$ out of the cell by the $Na^+,K^+$–ATPase plays an important role in maintaining osmotic balance. Facilitated transport can transport a substance down its concentration gradient. Active transport requires expenditure of metabolic energy and can transport a solute against its concentration gradient. The usual energy source is phosphorylation by ATP. There are $Na^+Ca^{2+}$ exchangers and $Na^+H^+$ and anion exchangers (55). The sarcolemmal of VSMCs contains channels which are selective for $Ca^{2+}$, $K^+$, and $Cl^-$. The distribution and properties of these channels vary with the different types of vessels. Ion channels may open or close depending on membrane potential, in which case they are voltage dependent, or through the action of hormones and neurotransmitters either directly (ligand gated) or indirectly through second messenger pathways as a result of agonist receptor binding (8). The sarcolemma has both receptor-activated and potential dependent $Ca^{2+}$ channels. The conductance of the former depends on the receptor sites being occupied. Binding of neurotransmitters or hormones to these sites can induce contraction or relaxation with little change in membrane potential. This process is called pharmacomechanical coupling. The channels are usually linked by G proteins to receptors that bind inhibitory neurotransmitters or hormones (2). In pharmacomechanical coupling the regulation of force is independent of changes of $E_m$. This coupling involves changes in $[Ca^{2+}]i$ or change in the response of the cell to $[Ca^{2+}]i$ independent of changes in $E_m$ (4). The plasmalemma of two opposed VSMCs may have common gap junction channels. The major type in the VSMCs is connexin 43, which has a short half-life of only 1.5–3.5 hr. Recent electrical evidence suggests that the dynamical regulation of cell-to-cell coupling may serve

to reduce cell-to-cell coupling rather than enhance it as previously thought. Each gap junction channel is composed of 12 connexin subunits. The MW of connexins varies from 26 to 70 kDa. Gap junctions are very poorly selective for ions. There are about 100 channels in each typical junctional plaque between VSMCs. Gap junctions are critical to normal α agonist contractions of VSMCs (56).

Regulatory molecules can bind to their plasma membrane receptors and the ligand-bearing receptors subsequently interact with G proteins to activate them. There are many different classes of heterotrimeric G proteins (57). Most external molecules which alter cellular processes do so by a signal transduction pathway that involves GTP-binding proteins (G proteins). Such proteins exist in an activated state, which has a high affinity for GTP, or an inactivated state, which preferentially binds GDP over GTP. The activated G protein interacts with many effector proteins, usually enzymes or ion channels. The activated G protein has GTPase activity. Activated G proteins alter cellular concentrations of cAMP, cGMP, and $Ca^{2+}$. Adenylyl cyclase and cGMP phosphodiesterase synthesize cAMP and break down cGMP. $Ca^{2+}$ channels can be modulated directly by G proteins or indirectly by second messenger-dependent protein kinases. When a ligand binds to its receptor, inactive G protein can be activated. This in turn activates effector enzymes or ion channels. Examples include adenylyl cyclase, cGMP phosphodiesterase, PLC, phospholipase $A_2$ ($PLA_2$), and $Ca^{2+}$ or $K^+$ ion channels. Such activated enzymes can increase the concentration of second messengers such as cAMP, cGMP, $IP_3$, DAG, and $Ca^{2+}$, which can activate other enzymes or ion channels such as cAMP or cGMP kinases, calmodulin-dependent kinases, and PKC. $Ca^{2+}$–calmodulin is an activator for calmodulin-dependent protein kinase. $Ca^{2+}$–DAG is an activator for PKC. Second messenger-dependent kinases frequently phosphorylate proteins, thereby modifying their activity in the final steps of the signal transduction pathway (57). G proteins are transducing proteins that couple a large number of membrane receptors to intracellular effector systems. Pertussis toxin can inhibit certain G proteins. In porcine coronary artery endothelium-dependent relaxation to $\alpha_2$-adrenergic or serotonergic receptor stimulation are inhibited by pertussis toxin, but endothelium-dependent relaxation to NO, bradykinin, and ADP is uneffected (58). $PLA_2$ is activated by some agonists via a G protein-dependent pathway. It can cleave the number 2 fatty acid from membrane phospholipids. Arachidonic acid is produced which is a precursor for the cellular synthesis of prostaglandins and leukotrienes (57). Not all agonists activate G proteins or release $Ca^{2+}$ from intracellular stores. ATP acting on the $P_{2x}$ receptors can contract vascular smooth muscle without apparently releasing $Ca^{2+}$ from intracellular stores (4).

Some vasoconstrictors can change $E_m$ and thereby regulate contractile force. NE, 5-HT, histamine, and ET can all depolarize intact VSMCs. The membrane depolarization increases $[Ca^{2+}]$ influx through activated L channels. ATP acting on the $P_{2x}$ receptor and carbachol can activate L channels by causing $Na^+$ influx through nonspecific anion channels. Angiotensin II and ACh may increase $Ca^{2+}$ influx by causing depolarization through the inhibition of $K^+$ channels. Agonist-dependent depolarization is independent of $Ca^{2+}$ influx, activation PKC or PLC. At any given $E_m$ agonists produce a larger contraction than that observed in response to high $(K^+)_o$ (4).

Epinephrine and NE can act to open ligand-gated $Ca^{2+}$ channels, thereby increasing $[Ca^{2+}]i$. NE is also thought to activate membrane-bound G protein, which in turn can activate PLC which converts intracellular phosphatidylinositol biphosphate ($PIP_2$) to inositol 1,4,5-triphosphate ($IP_3$) plus DAG. $IP_3$ can then attach to ligand-gated $Ca^{2+}$ channels on the sacroplasmic reticulum, causing a further increase in $[Ca^{2+}]i$ (59). Following linkage to sarcolemmal receptors, vasodilator substances such as calcitonin gene-related peptide (CGRP), adenosine, prostacyclin, and β agonists can activate adenylyl cyclase and increase cAMP and ultimately PKA, which can open $K_{ATP}$ channels with a resultant hyperpolarization of the membrane potential and vasodilation. The $K_{ATP}$ channels are involved in the metabolic regulation of blood flow and activated during hypoxia (60). NO and other nitrovasodilators can cause glibenclamide-sensitive membrane potential hyperpolarization. NO can activate $K_{ATP}$ channels (60).

ATPase activity in plasma membrane of basilar artery VSMCs was examined using electron microscopic histochemistry and bioassay. The $Ca^{2+}$–ATPase activity increased significantly in response to the application of vasoconstrictors such as $PGF_{2\alpha}$ and a phorbol ester. The activity decreased significantly 24 hr after experimental SAH at a time when basilar artery contraction was developing. Double-SAH in the canine model exhibited a further decrease in $Ca^{2+}$–ATPase activity (61).

Embedded within the sarcolemma are $Na^+/Ca^{2+}$ exchangers and a $Ca^{2+}$–ATPase which can exchange $Ca^{2+}$ for $H^+$. Vasoconstrictors which activate phospholipases and PKC, as well as vasodilators which increase cyclic nucleotide levels, appear to activate the $Ca^{2+}$ pump (7). To lower the $Ca^{2+}$ concentration the sarcolemma pumps $Ca^{2+}$ out of the cell by active transport and by passive exchange coupled to the influx of three $Na^+$ ions for each $Ca^{2+}$ ion. The extracellular $Ca^{2+}$ pool determines sustained contraction of smooth muscle. The

stimulus-dependent processes combine to determine the steady-state myoplasmic $Ca^{2+}$ and thereby cross-bridge phosphorylation. Some stimuli can act on the sarcolemma to lower $Ca^{2+}$ and induce relaxation (2).

Channel-mediated extracellular $Ca^{2+}$ influx occurs through $Ca^{2+}$-selective channels or nonselective cation channels which are either voltage dependent or voltage independent. Smooth muscle cells have T- (transient) and L- (long-lasting) type voltage-dependent $Ca^{2+}$ channels. Sarcolemmal depolarization activates L-type channels, converting them from a closed to an open state. Repolarization and hyperpolarization favor deactivation of the channels. $[Ca^{2+}]i$ can reversibly inhibit L-type channel-mediated $Ca^{2+}$ influx. Phorbol esters promote L-type channel-mediated $Ca^{2+}$ influx, which suggests possible phosphorylation by PKC. The status of L-type channels may be modulated by receptor stimulation as well (7,8). Voltage-independent $Ca^{2+}$ channels have also been shown to exist in different types of smooth muscle. The $P_{2x}$ purinergic receptor for ATP is such a voltage-independent channel. It is insensitive to nifedipine and cadmium and can open at very negative potentials (7). Voltage-dependent $Ca^{2+}$ channels respond to depolarization by increasing their open-state probability. The influx of extracellular $Ca^{2+}$ may lead to contraction or may release additional $Ca^{2+}$ from the sarcoplasmic reticulum, so-called calcium-induced calcium release.

### D. Sarcoplasmic Reticulum and Calcium Control

The second $Ca^{2+}$-integrating system is in the sarcoplasmic reticulum (SR) under the control of chemical second messengers. Both membranes tend to resist a 10,000-fold concentration gradient to keep $[Ca^{2+}]i$ low. The SR contains an intracellular $Ca^{2+}$ pool which can be mobilized to increase $[Ca^{2+}]i$. Neurotransmitters or hormones affect the various receptor operated channels in the sarcolemma and activate a G protein which in turn induces PLC to hydrolyze membrane phospholipid (phosphatidylinositol 4,5-bisphosphate), with resultant formation of $IP_3$ and DAG. $IP_3$ stimulates release of $Ca^{2+}$ from intracellular stores. The $Ca^{2+}$-releasing activities of $IP_3$ are terminated by dephosphorylation to inositol 1,4-bisphosphate or phosphorylation to inositol 1,3,4,5-terakisphosphate by 3-kinase (62). $Ca^{2+}$ released from the SR can be carried out very rapidly. Various stimuli can alter the sarcolemmal channels controlling inflow or outflow of $Ca^{2+}$. The sarcolemma integrates many simultaneous excitatory and inhibitory inputs. Various pumps, exchangers, and enzymes are involved in the $Ca^{2+}$ flux.The efflux of $Ca^{2+}$ from the endoplasmic reticulum pool is linked to the binding of the second messenger $IP_3$ to receptors on the SR and not to voltage sensors. $IP_3$ results from a stimulus acting on sarcolemmal receptors that are coupled via a guanine nucleotide-binding protein (G protein) to activate PLC. PLC hydrolyzes $PIP_2$, and $IP_3$ is one by-product. $Ca^{2+}$ is released in a graded fashion from the SR. Refilling of the SR $Ca^{2+}$ pool is dependent on the extracellular concentration of $Ca^{2+}$ (2). Smooth muscle myoplasm contains many $Ca^{2+}$-binding proteins such as calmodulin which could bind a significant fraction of $Ca^{2+}$ (7). Nitrovasodilators may relax arterial smooth muscle by decreasing $[Ca^{2+}]i$ and uncoupling stress from myosin phosphorylation (63).

SR tends to be voluminous in the central region with a scarce peripheral distribution in tonically active smooth muscles such as vascular smooth muscle. Peripheral SR is excluded from those subsarcolemma areas around attachment plaques. Caveolae are flask-shaped sarcolemma invaginations into the sarcoplasm. These are arranged in rows of two to four caveolae wide parallel to the longitudinal axis of the cell. In contrast to pinocytic vessels, they are permanently connected to the cell surface. They are often closely associated with the SR. It may be that intracellular $Ca^{2+}$ stores can be filled by a sarcolemmal $Ca^{2+}$ influx pathway which communicates directly with the SR (7).

SR comprises 1.5–7.5% of smooth muscle cell volume. It stores $Ca^{2+}$ used by different agonists. Smooth SR synthesizes fatty acids and phospholipids and is involved in glycogenolysis. Rough SR has ribosomes and can synthesize elastin and collagen. SR is a network of interconnected tubules with the superficial portion close to the inside of the plasmalemma to which it may fuse. It regulates basal $[Ca^{2+}]i$. The rough endoplasmic reticulum closer to the nucleus may remove $[Ca^{2+}]i$ from the sarcoplasm following deactivation of the myofilaments in relaxation (47). In the SR lumen $Ca^{2+}$ is bound by the proteins calreticulin and calsequestrin. The drop in lumenal free $Ca^{2+}$ by this binding makes it easier for $Ca^{2+}$ to be pumped into the SR against a lesser concentration gradient by the sarcolemmal $Ca^{2+}$ ATPase. $Ca^{2+}$ in the SR is transported to the subplasmalemmal space, where it is more readily extruded extracellularly by a plasmalemmal $Ca^{2+}$ pump and $Na^+/Ca^{2+}$ exchanger. The pump which pumps $Ca^{2+}$ into the SR is controlled by the 6-kDa protein phospholamban. Phospholamban is phosphorylated by cAMP- and cGMP-dependent protein kinases and PKC which tends to lower sarcoplasmic $Ca^{2+}$, thereby inducing relaxation. On the other hand, inhibition of this pump by agents such as thapsigargin and cyclopiazonic acid reduces the relaxation caused by cGMP or cAMP. The SR $Ca^{2+}$ stores have more than 10 times the $Ca^{2+}$ needed to trigger a single contraction. At least two types of $Ca^{2+}$ channels are present in SR which are voltage independent and unlike the $Ca^{2+}$ channels of

the plasmalemma. These are involved with $Ca^{2+}$ release from the SR. One type is sensitive to $IP_3$ and the other to ryanodine and caffeine. The reduction in store $Ca^{2+}$ stimulates the release of a $Ca^{2+}$ influx factor which facilitates the entry of $Ca^{2+}$ through the plasmalemma via depletion-operated $Ca^{2+}$ channels. Since ryanodine blocks the $Ca^{2+}$ buffering capacity of the SR, it may result in contraction of smooth muscle since the $Ca^{2+}$ depletion from the SR induces a $Ca^{2+}$ entry into sarcoplasm from the exterior. $IP_3$ releases $Ca^{2+}$ from the SR. $IP_3$ diffuses from the plasmalemma to the SR. Up to a cytosolic concentration of 300 $\mu M$ $Ca^{2+}$, the $IP_3$ becomes more effective in releasing $Ca^{2+}$ from the SR, but above this level it is less efficient. The $IP_3$-sensitive $Ca^{2+}$ store in the SR may be more deeply placed than the ryanodine-sensitive store. $Ca^{2+}$ release from SR is also induced by elevated $Ca^{2+}$ in the cytoplasm ($Ca^{2+}$-induced $Ca^{2+}$ release).

### E. Calcium and Hemolysate

Freshly prepared rat VSMCs studied by calcium fluorimetry showed that hemolysate produced a transient $[Ca^{2+}]i$ peak followed by a slowly decaying plateau which was absent in $Ca^{2+}$-free solution. The effect of hemolysate was reduced by thapsigargin and cyclopiazonic acid, which block out the sarcoplasmic reticulum pumps; ryanodine and dantrolene, which block $Ca^{2+}$ release; econazole, a cytochrome *P*450 inhibitor; lanthanum, an inorganic $Ca^{2+}$ blocker; SKF-96365, a receptor-regulated $Ca^{2+}$ channel blocker; and nimodipine, a voltage-dependent $Ca^{2+}$ channel blocker. Membrane fractionation of hemolysate showed that the component >0.5 but < 1 kDa produced a $[Ca^{2+}]i$ peak and plateau similar to those of whole hemolysate. $P_2$-purinoceptor antagonists significantly attenuated the effect of ATP, hemolysate, and the fractions >1 and <12–14 kDa. Hemolysate apparently elevated $[Ca^{2+}]i$ by both releasing $Ca^{2+}$ from internal storage and triggering $Ca^{2+}$ entry (64).

### F. Calcium and Oxyhemoglobin

Rat VSMCs studied *in vitro* show a significant elevation of $[Ca^{2+}]i$ in response to oxyHb in the bathing medium. In one study, verapamil, a voltage-gated $Ca^{2+}$ channel blocker, did not prevent this increase (65). In monkey VSMCs, oxyHb produced a similar increase within 2 min of application. When daily applications of oxyHb were carried out the increase in $[Ca^{2+}]i$ on day 3 was larger than the initial response to a single application. Levels were estimated to change from 75 to 240 nmol/liter (66).

## VIII. Enzymes, Receptors, and Messenger Systems

### A. Myosin Light Chain Kinase

A major factor in the regulation of many cell processes is the phosphorylation of proteins. Most of the kinases which do this phosphorylate either Ser/Thr or Tyr and are associated with respective phosphatases that reverse the process. The major regulatory process in smooth muscle is the phosphorylation of myosin. The degree of myosin phosphorylation depends on the relative activity of MLCK and MLCP (myosin phosphatase) (67). MLCK is a $Ca^{2+}$/CaD-dependent protein kinase that phophorylates a serine residue in the N terminus of the RLC of myosin II. This is its only substrate. MLCK may initiate smooth muscle contraction (Fig. 7.5). MLCK (105 kDa) has four domains: calmodulin-binding, pseudosubstrate, constitutive, and ATP-binding.

When the regulatory light chain of smooth muscle myosin is phosphorylated, it enables actin to activate myosin ATPase and also makes monomeric myosin less soluble. It is possible that in some smooth muscles solubilization of thick filaments can occur during prolonged relaxation. Myosin light chain phosphorylation is certainly involved in activation of smooth muscle contraction, but this is not the entire explanation. Some degree of regulation may also reside in the actin filament. Dephosphorylation of the myosin light chains while the heads are attached is considered to result in the long-lived latch state in which tension is maintained but the shortening and ATPase activity are reduced several-fold (3). MLCK activity is sufficient and necessary for contraction. Thin actin filament regulation is one of several mechanisms thought to explain the ability of smooth muscle to maintain tone at low myosin $LC_{20}$ phosphorylation levels. Latch mechanism probably cannot explain all the contractile responses of smooth muscle (7). MLCK concentrations in smooth muscle are greater than those in cardiac muscle or nonmuscle tissue. MLCK averages 3 or 4 $\mu M$, which is less than the 70–80 $\mu M$ of myosin RLC, its only physiological substrate (53). MLCK phosphorylates the 20-kDa light chain of myosin on serine 19. This phosphorylation is associated with an increase in actin-activated myosin ATPase activity. Contractile force usually depends on increases in myosin phosphorylation, although there are examples of decreasing force without proportional decreases in myosin phosphorylation. This can be brought about by extracellular $[Mg^{2+}]$, nitrovasodilators, and $Ca^{2+}$ depletion (4). MLCK is expressed in almost all tissues, and its primary regulator is $Ca^{2+}$/CaM. The CaM affinity of MLCK may be decreased by phosphorylation of the kinase (7). $Ca^{2+}$/CaM-dependent

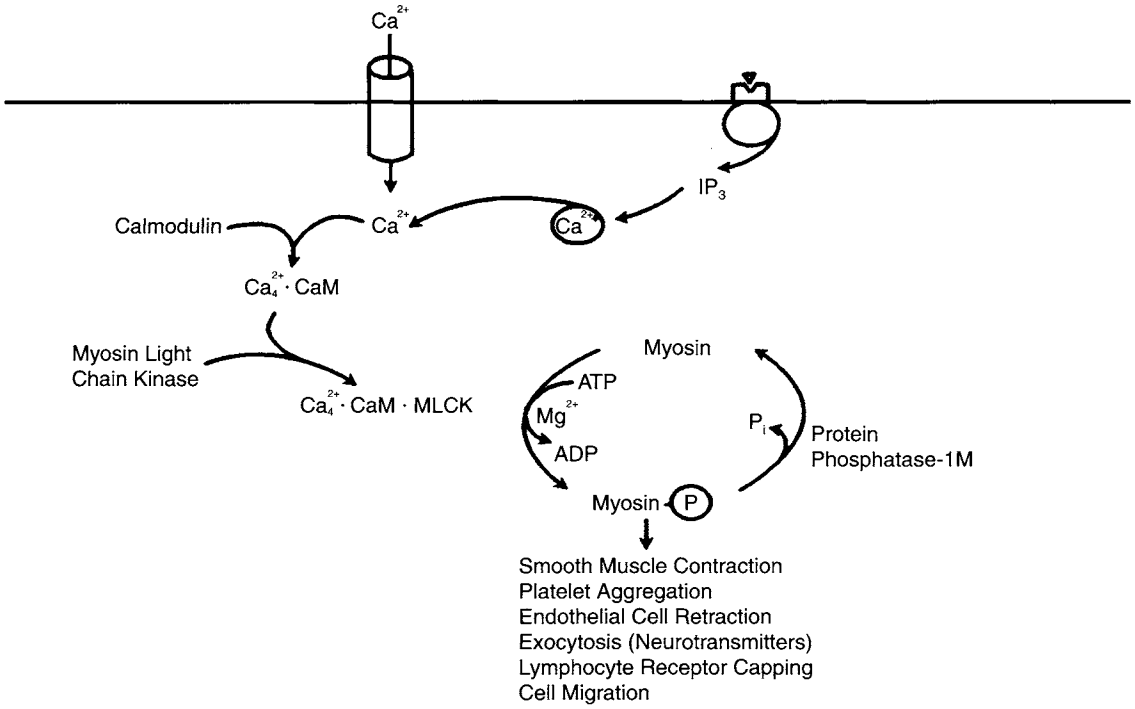



**FIGURE 7.5** Scheme for activation of MLCK in cells leading to myosin phosphorylation and cellular effects [reproduced with permission from Stull, J. T., Kraeger, J. K., Kamm, K. E., Gao, Z. H., Zhi, G., and Padre, R. (1986). Myosin light chain kinase. In *Biochemistry of Smooth Muscle Contraction* (M. Barany, Ed.). Academic Press, San Diego].

protein kinases mediate signal transduction events triggered by stimuli which increases $[Ca^{2+}]i$. Examples of $Ca^{2+}$/CaM-dependent kinases are phosphorylase kinase and MLCK. Multifunctional kinases include cyclic nucleotide-dependent protein kinases, PKC, and $Ca^{2+}$/CaM-dependent protein kinase II. The role of CaM-kinase II in smooth muscle has not been elucidated. It may have a role in controlling contraction by modulating availability of activator $Ca^{2+}$ and by phosphorylating and desensitizing MLCK (68). Myosin kinase phosphorylates and myosin phosphatase dephosphorylates specific sites on the myosin RLCs which form part of the cross-bridge. Phosphorylation of cross-bridges results from the covalent linkage of $PO_4$ derived from the hydrolysis of ATP to serine residues. Myosin light chain phosphorylation can be examined using two-dimensional gel electrophoresis. Smooth muscle is prelabeled with[$^{32}$P]-orthophosphate ATP and stimulated by various agonists. Tissues are homogenized and the protein is separated by charge using isoelectric focusing. The proteins may be further separated with SDS polyacrylamide electrophoresis. The various isoforms of phosphorylated myosin light chain are present at 20 kDa. In addition to $MLC_{20}$ other low-molecular-weight phosphoproteins are phosphorylated in a temporal manner concurrent with vascular smooth muscle contraction (69). The specific roles of these different phosphoproteins are under investigation. Stimulation by ACh release results in increased $[Ca^{2+}]i$ within 100 msec. About 500 msec following nerve stimulation there is a significant increase in RLC phosphorylation, which is the same latency as for force development. After this, both myosin RLC phosphorylation and stiffness increase faster than either force or $[Ca^{2+}]i$. Once begun, the phosphorylation of RLC is carried out rapidly by MLCK, which leads to attachment of cross-bridges and force development (53).

A selective antagonist of MLCK, ML-9, reversed chronic VSP in a canine model after direct application or cisternal injection but not after intraarterial injection (70). Anterior spinal arteries were obtained from animals exposed to SAH. Segments were suspended in a force transducer and stretched to optimal length for contraction. Developed tensions were compared at 37 and 0°C. The difference in tension between these two temperatures was defined as intrinsic tone and the residual tension was defined as the passive tension. Dogs with VSP had greater intrinsic tone and passive tension. The passive component accounted for almost 80% of the increased tension in vasospastic arterial segments. The vasospastic segments had $[Ca^{2+}]i$ of 398 vs 258 nmol/liter for nonvasospastic segments. The percentage myosin light chain

phosphorylation in vasospastic vessels was 37 vs only 2% for nonspastic ones. It was concluded on the basis of increased $[Ca^{2+}]i$ and increased myosin light chain phosphorylation that a $[Ca^{2+}]i$ – dependent pathway is involved in generation of VSP (71).

### B. Protein Kinase C

#### *1. Actions and Antagonists*

Although phosphorylation of 20-kDa myosin light chain is the main regulator of smooth muscle contraction, particularly in the generation of phasic contractions and the initial phase of tonic ones, there may be an important role for PKC in the regulation of smooth muscle tone maintenance, especially in vascular smooth muscle (7).

In unstimulated cells most PKC is cytosolic and inactive. When cytosolic $Ca^{2+}$ levels rise there is a resulting binding of PKC to the inner surface of these sarcolemma. Here, it can be activated by DAG produced by the hydrolysis of $PIP_2$. Membrane-bound PKC is also potently activated by phosphatidylserine. Phorbol esters directly activate PKC (they are lipophilic tumor-promoting substances) (57). PKC may have a uniquely important role in the regulation of smooth muscle contraction and tone maintenance, especially vascular smooth muscle tone, compared to other types of muscle. Several possible signal transduction cascades exist for the action of this kinase. It is probable that different signaling cascades are involved in generating transient contractions compared to tonic sustained contractions (7). PKC was identified in the late 1970s as a proteolytically activated protein kinase (72) and has since been shown to be ubiquitous in many tissues. The enzyme is recovered mainly from the soluble fraction as an inactive form, and it translocates to cell membranes in a $Ca^{2+}$-dependent fashion when cells are stimulated. More than one gene exists for this protein kinase. PKC requires $Ca^{2+}$ and phospholipid, especially phosphatidylserine, to be activated. DAG dramatically increases the affinity of PKC for $Ca^{2+}$. PKC is activated by limited proteolysis with calpain. Phosphatidylinositol-4-phosphate (PIP) and $PIP_2$ are minor phospholipid components which are produced by sequential phosphorylation. The primary products of $PIP_2$ hydrolysis are $IP_3$ and DAG. Inositol 1,4,5-$P_3$ acts on stored $Ca^{2+}$ through its own receptor. This receptor is removed by a specific phosphatase that has a short half-life. The appearance of DAG in cell membranes is transient and it usually disappears within seconds to minutes of its formation. It is mainly converted back to inositol phospholipids or may further be hydrolyzed, thereby producing arachidonic acid (AA), which can generate other prostaglandin messengers. PKC participates in a myriad of cellular responses and is important within endocrine and exocrine systems, the nervous, immune, and muscular systems, and the metabolism of the organism (73). The cascade of cellular phosphorylation initiated by activation of PKC includes cellular differentiation, long-term potentiation, early gene expression, apoptosis, and smooth muscle contraction (74). In bovine carotid artery muscle strips, PKC transiently migrates from cytosol to the membrane in response to agonists which induce transient contractile responses, whereas sustained PKC translocation is observed in response to agonists that induce sustained contractile responses. It is not certain whether the translocation of PKC is obligatory to its activation. PKC activation can induce slowly developing sustained contractions in vascular smooth muscle strips (7).

Several agonists of contraction stimulate phosphatidylinositol hydrolysis, resulting in a rapid and short-lived rise in $IP_3$. $[Ca^{2+}]i$ is subsequently released from endoplasmic reticulum. Phosphatidylinositol hydrolysis leads to an increase in membrane DAG. DAG activates PKC, which results in translocation or movement of PKC from sarcoplasm to sarcolemma (75). PKC is a multifunctional serine/threnonine protein kinase. Physiological concentrations of $Ca^{2+}$ and the membrane phospholipid DAG and phosphatidyl-serine synergistically activate the kinase independently of any proteolytic events. A variety of external stimuli can activate PLC, which subsequently hydrolyses membrane phosphoinositides and phosphatidylcholine to produce 1,3,5-trisphosphate, a soluble second messenger, that releases intracellular $Ca^{2+}$ and membrane DAG, the endogenous lipid activator of PKC. PKC isozymes are all single polypeptides of 67–115 kDa. PKC may be important not only in controlling vascular smooth muscle contraction but also in the feedback regulation of signal transduction controlling gene expression and cell growth. Activation of cytosolic kinases such as MAP-kinases might be one of the phosphorylation events triggered by PKC activators. PKC may also be involved in the phosphorylation of CaD (76). After PKC is activated by receptor-mediated hydrolysis of inositol phopholipids, it relays signals from membrane receptors and regulates many $Ca^{2+}$-dependent processes. In the early phase of cellular responses PKC can provide both forward and backward positive and negative feedback controls to its own signaling pathway as well as parallel ones (73).

PKC is a family of at least 11 different closely related serine–threonine kinases. These isozymes are involved in a huge variety of cellular processes in addition to smooth muscle contraction. The PKC family is divided into four major groups. Groups A and B show translocation of PKC from cytosol to membranous sites upon activation from DAG and certain phorbol esters. The groups also

differ in calcium and $Ca^{2+}$ sensitivity (77). PKC probably does not directly phosphorylate $MLC_{20}$ *in vivo*. It phosphorylates $MLC_{20}$ at regions separate from those phosphorylated by MLCK. PKC may modulate sustained contraction through a kinase cascade leading to the phosphorylation of intermediate and thin filament-associated proteins such as CaD and CaP. PKC may activate MAPK, which in turn can phosphorylate CaD. In its dephosphorylated state CaD interferes with the interaction of actinomyosin by inhibiting actinomyosin ATPase. CaD may regulate many other nonkinase intracellular proteins as well as CaD-dependent kinases (57). The phosphorylated CaD cannot block this enzyme and prevent crosslinkage between actinomyosin (78). In some circumstances of persistent high $Ca^{2+}$, the $Ca^{2+}$-dependent neutral protease calpain becomes activated. Calpain hydrolyzes the peptide bonds linking the regulatory and catalytic domains of PKC. The result is the production of a constitutively activated catalytic domain, protein kinase M (79).

Phorbol esters have an analogous structure to DAG, and this may be the reason they activate PKC. Phorbol esters can cause sustained contraction of a variety of vascular smooth muscle in both the presence and the absence of increased $[Ca^{2+}]i$. Certain activators of PKC can cause a slowly developing sustained contraction without the early phosphorylation events commonly associated with histamine (69). In the absence of $[Ca^{2+}]i$, phobol esters can still produce contraction in some smooth muscles associated with an increase in MLC phosphorylation. One phosphoprotein that is widely distributed, myristoylated alanine-rich C-kinase substrate, is exclusively phosphorylated by PKC (7).

Vasoconstrictors bind to the cell surface receptors and activate PLC and $Ca^{2+}$ channels resulting in mobilization of $Ca^{2+}$ from intracellular pools and activation of PKC. Vasoconstrictors also activate the mechanism to downregulate $MLC_{20}$ phosphatase activity which involves Rho $p_{21}$ and PKC, resulting in an increase in $Ca^{2+}$ sensitivity of $MLC_{20}$ phosphorylation. PKC also activates $MLC_{20}$ phosphorylation-independent mechanism for contraction. Vasorelaxants inhibit activation of PLC and gating of $Ca^{2+}$ channels or stimulate $Ca^{2+}$ extrusion across the plasma membrane, resulting in a decrease in $[Ca^{2+}]i$ (80).

PKC was first implicated in smooth muscle contractile regulation with the observation that phorbol esters induced slowly developing contractions in certain arteries. These contractions occurred without an increase in sarcoplasmic free-$Ca^{2+}$ concentration or MLC phosphorylation. The response in vascular smooth muscle is thought to be mediated mainly by PKC-ε. PKC triggers a cascade of phosphorylation reactions which activate MAPK and phosphorylation of the thin filament-associated protein CaD. Alternatively, or additionally, PKC may directly phosphorylate the other thin filament-associated protein CaP. These PKC-induced phosphorylations may reduce the inhibitory effect of these thin filament proteins on cross-bridge cycling rates (81–83).

Tumor-promoting phorbol esters differ from DAG in that they are resistant to degradation. The role of PKC in stimulus-response coupling was first demonstrated by the release of 5-HT from platelets. The two signaling pathways, PKC activation and $Ca^{2+}$ mobilization, can be induced selectively and independently. PKC can modulate ion conductance by phosphorylating membrane proteins such as ion channels. Substrate proteins for PKC include a wide variety of receptor, membrane, contractile, and other proteins. PKC may phosphorylate both seryl and threonyl residues in a single protein molecule (73). H-7 and staurosporine are inhibitors of PKC. Agonist-induced contractions, but not $K^+$ contractions, are blocked by PKC inhibitors (7).

Ferret aortic smooth muscle expresses two $Ca^{2+}$-independent PKC isozymes, ε and ζ. The ε isozyme induces contraction in vascular smooth muscle. This contraction is not affected by MLCK inhibitors, which suggests that PKC-ε induces contraction solely via thin filament disinhibition (84). Of the two $Ca^{2+}$-independent PKC isozymes found in ferret aorta, only PKC-ε translocates from the sarcoplasm to the sarcolemma upon stimulation of the tissue by phenylephrine (85). PKC can phosphorylate substrates such as phospholipase D. This enzyme converts phosphatidylcholine to phosphatidic acid, which can provide a source of eicosanoids via AA. Small GTP-binding proteins are involved both proximal and distal to PKC activation. There are approximately 50 of these low-molecular-weight GTPases (7). The vasoconstrictor effects of α-adrenoceptor activation, thrombin, NE, and angiotensin II were reversed by pretreatment of the femoral arteries by staurosporine, an inhibitor of PKC. This suggests an upstream modification in vascular sensitivity by tonic alterations in postsynaptic modulation by enzyme systems known to regulate $Ca^{2+}$-dependent phenomena (86). Similarly, the contraction of arteries to thrombin is sensitized by freshly obtained human platelets. The PKC inhibitor staurosporine blunted the platelet-induced augmentation to the response to thrombin but did not alter the contraction elicited by $K^+$ (87).

### *2. Experimental Studies in Vasospasm*

Using the two hemorrhage dog model, basilar arteries were exposed transclivally on day 7 post-SAH. The PKC inhibitor H-7 induced significant and dose-dependent dilation of the spastic basilar artery. The DAG content of canine arteries was studied post-SAH; it was significantly increased by day 2 and reached its highest level

on day 6 post-SAH. Compared to controls, [$^3$H]-inositol incorporation to PI, PIP, and $PIP_2$ was unchanged in the spastic basilar artery segment obtained on day 7 (88). In the two-hemorrhage dog model, severe VSP and a significant decrease in arterial wall cGMP levels were observed on day 5 and persisted until day 7 post-SAH. PKC activity was enhanced from day 5 until day 7. The changes resulting from a single blood injection were much milder. The increased PKC activity was the opposite of that observed by Nishizawa *et al.* (89).

In a canine SAH model the incorporation of radioactivity from [$\gamma$-$^{32}$P]ATP into cytosolic and membrane-bound fractions in control and treated animals was compared. The membrane activity of PKC was remarkably enhanced in treated animals compared to the control group. The percentage of activity in the membranes compared to total activity was greater in SAH vessels and the percentage of cytosolic activity in the SAH group was decreased. This was considered to be direct evidence for a role of PKC in the development of VSP (90). Also in a two-hemorrhage dog model, basilar arteries were exposed by the transclival route on day 7 post-SAH. H-7, the inhibitor of PKC which acts at the catalytic domain, calphostin C, which acts at the regulatory domain, or calpeptin, a selective inhibitor of calpain, were topically applied. The responses of arteries from the SAH dogs were compared to those from control animals whose arteries were acutely constricted by topical application of KCl or 5-HT. H-7 produced marked dilation in both the post-SAH and acutely contracted arteries. Calpeptin, the selective inhibitor of calpain, which cleaves PKC into catalytic and regulatory domains, significantly increased the ability of calphostin C to dilate spastic basilar arteries. It was hypothesized that the catalytic domain of PKC is markedly activated in VSP by being dissociated from the regulatory domain, possibly by limited proteolysis with calpain. Calpain is a cytosolic cysteine protease activated at the plasma membrane in the presence of $Ca^{2+}$ and phospholipid. It regulates the function of membrane-associated proteins by limited or nonlimited proteolysis (79). In a canine two-hemorrhage model, spastic arteries days 4 and 7 post-SAH showed decreases in cytosolic PKC activity of 40–45% with no significant changes in membrane PKC activity compared with nonspastic control arteries. Cytosolic PKC activity on day 14 returned toward normal control levels with the remission of VSP. Western blot analysis of PKC isoforms showed that the amounts of PKC-$\alpha$ and PKC-$\varepsilon$ were decreased in spastic arteries. Spastic arteries showed high rates of incorporation of [$^3$H] choline into phosphatidylcholine (PC) and [$^{14}$C]ethanolamine into phosphatidylethanolamine (PE) but not of [$^3$H]myoinositol into phosphoinositides. The turnover of PC and PE appeared to be stimulated. The extent of 20-kDa MLC phosphorylation was not increased in spastic arteries on days 4 or 7 compared with nonspastic controls (91).

PKC activity in cultured rabbit basilar artery smooth muscle cells was assessed by Western blot analysis. PKC of the cells did not increase for up to 6 days after exposure to oxyHb (92).

The PKC inhibitors H-7 and chelerythrine were found to reduce the contraction to oxyHb by about 60% and also produced a dose-dependent reduction in the responses to oxyHb when given at the peak of contraction in canine cerebral artery rings studied *in vitro* (93). In the canine SAH model, membrane-bound PKC activity increased on day 4 and returned gradually to control levels by day 14. Cytosolic PKC decreased from 4 hr to 14 days after SAH. The total PKC (cytosolic + membrane-bound) activity decreased significantly on day 2 and from days 7 to 14. A single intracisternal injection of a phorbol ester induced sustained contraction lasting over 3 days with a parallel change in membrane-bound PKC activity in the basilar arterial wall. This was in the single intracisternal model. Multiple intracisternal injections of phorbol esters produced 30–40% sustained contraction of the basilar artery which lasted for more than 10 days along with sustained activation of PKC to levels comparable to those seen in the SAH model. The histological changes resulting from multiple phorbol ester injections were much less than those seen post-SAH (94). Phorbol 12,13-diacetate can induce sustained contraction of the canine basilar artery presumably due to activation of PKC. The DAG content of these arteries increases during the chronic VSP phase. H-7 dilated chronically spastic canine basilar artery, whereas $Ca^{2+}$ channel blocking agents and CaD antagonist did not. The extent of 20-kDa MLC phosphorylation was not significantly different between spastic and normal control canine basilar arteries in this two-hemorrhage dog model. PKC activity in the cytosol of normal basilar artery was 29 pmol/min/mg wet weight. PKC-$\alpha$ was the predominant isoform. The cytosolic PKC was downregulated on days 4 and 7, whereas the membrane PKC activity was unchanged (95). The brains and basilar arteries of dogs were studied for immunoreactivity to PKC post-SAH. Following SAH, reactive astrocytes with PKC-$\alpha$ staining were found on the surface of the pons and hippocampus. The control basilar artery showed PKC-$\alpha$ staining which was decreased on day 7. The PKC responses in brain and artery appear to be opposite (96).

In the canine two-hemorrhage SAH model the basilar artery was exposed transclivally at various time intervals following the SAH. Progressive topical applications of nicardipine, H-7, and papaverine were administered in an accumulative fashion. The progressive vasodilation

was recorded angiographically. Between days 2 and 7 the dilatation to nicardipine or papaverine progressively decreased, whereas that induced by H-7 increased. When studied *in vitro* the arterial segments showed a progressive increase in stiffness from day 2 to day 7. The initial half-circumference of the arterial segment also significantly decreased, reaching a nadir on days 4 and 7. The initial half-circumference was considerable less than the angiographic diameter following SAH. Matsui and colleagues interpreted this to indicate that the augmented spontaneous tone of vascular smooth muscle is the predominant factor in chronic VSP and argued for involvement of the PKC contractile system (97). Small increases of membrane PKC were associated with significant contractions *in vitro* in normal dog arteries. Increases in membrane PKC of similar small magnitude were found *in vitro* when control arterial segments were exposed to hemolysate if the arterial smooth muscle cells were initially depolarized by increasing extracellular $K^+$ to values of membrane potential similar to those observed in arteries during chronic VSP. These increases in membrane PKC (6–8% of total PKC content) coincided with a significantly increased contraction to hemolysate (98).

### C. Tyrosine Kinase

#### *1. Tyrosine Kinase and Phosphatase*

Tyrosine phosphorylation may be important in the regulation of $[Ca^{2+}]i$ sensitivity. Vascular smooth muscle contains large quantities of tyrosine kinases. Agonist-induced constriction of intact smooth muscle can be inhibited by putative tyrosine kinase inhibitors. Inhibition of tyrosine kinases may specifically interfere with vasoconstrictor-induced increases in the $Ca^{2+}$ sensitivity of force. Tyrosine kinase inhibitors may also induce relaxation by interfering with agonist-induced increased $[Ca^{2+}]i$ (4,99,100). Tyrosine kinase and phosphatases are present in large amounts in contractile VSMCs. They influence contraction, ion channel gating, $Ca^{2+}$ homeostasis, and sensitization of the contractile process to $[Ca^{2+}]i$ (101). Tyrosine kinase pathways may acutely regulate smooth muscle contractility by receptor kinase-activating growth factors such as epidermal growth factor and G protein-coupled agonists such as angiotensin II. There may be a dynamic interplay between tyrosine kinase and tyrosine phosphatases (102). Tyrosine kinase inhibitors greatly diminish the contractile responses to muscarinic or a-adrenergic receptors (7). An increase in $[Ca^{2+}]i$ may exert a negative feedback inhibition of tyrosine kinase and activation of tyrosine phosphatase. Tyrosine kinase inhibitors include genistein, lavendustin, herbimycin, and tyrphostins (47).

Phosphorylation of proteins is a significant means of cell regulation. The degree to which a regulatory protein is phosphorylated at a given time results from the opposing activities of kinase that phosphorylate and phosphatases that dephosphorylate the protein. As with protein kinases, the phosphatases are classified as serine–threonine protein phosphatases and tyrosine protein phosphatases. Depending on the specific type of phosphatase, there is an absolute requirement for $Ca^{2+}$–calmodulin or $Mg^{2+}$. Tyrosine phosphorylation was originally identified in studies of oncogenesis and growth. The majority of receptors for growth factors are tyrosine kinases. Some tyrosine kinases are activated by an increase in $[Ca^{2+}]i$. Smooth muscle has high levels of tyrosine kinase activity even when it is unstimulated, indicating that such activity does not just occur in receptors. In this respect it differs from cardiac and skeletal muscle. Vasoconstrictors which act via the $R_7$ G protein receptor (NE, 5-HT, ET, histamine, and thrombin) increase tyrosine phosphorylation in arterial smooth muscle. Selective tyrosine kinase inhibitors inhibit contraction in response to contractile agents. Some tyrosine kinase inhibitors inhibit depolarization-induced contraction and some L-type voltage-gated $Ca^{2+}$ channels. Src kinase may influence $K^+$ channels (101).

#### *2. Experimental Studies in Vasospasm*

OxyHb produced a contraction of a basilar artery preparation which was reversed by genistein, an inhibitor of tyrosine kinases, and PD098059, an inhibitor of mitogen-activated protein kinase (MAPK). In cerebrovascular smooth muscle cells, oxyHb induced tyrosine phosphorylation of various proteins with a time course parallel with that of the contractile action of oxyHb. The 42- and 60-kD proteins were immunologically related to the MAPK, extracellular signal-regulated protein kinase (ERK2), and to p60c-Src (c-Src), respectively. The increase in protein tyrosine phosphorylation was attenuated by genistein and the phosphorylation of the 42-kDa protein was inhibited by PD098059. The result suggested that oxyHb-mediated signaling utilizes a protein tyrosine kinase-based mechanism (103). Rabbit basilar artery rings were studied by isometric tension in response to erythrocyte lysate, 5-HT, and KCl in the absence or presence of tyrosine kinase inhibitors. Preincubation with tyrosine kinase inhibitor tyrphostin A23 and genistein significantly attenuated the contraction induced by erythrocyte lysate. All the tyrosine kinase inhibitors failed to reduce the contraction caused by 5-HT. Genistein significantly attenuated the contraction induced by KCl (104). Tyrosine kinase phosphorylation and $[Ca^{2+}]i$ were measured in rat aorta and basilar artery smooth muscle cells using Western blot and microfluorimetry.

Erythrocyte lysate enhanced tyrosine phosphorylation, inducing a rapid $[Ca^{2+}]i$ transient followed by a prolonged plateau phase (105).

### D. Mitogen-Activated Protein Kinase

MAPK family members are expressed in response to a wide variety of stimuli by vascular smooth muscle. To date, they have mainly been studied with respect to growth, differentiation, and apoptosis but are potently activated by some vasoactive agents. Cross talk among MAPKs directly modulates signal transduction. MAPKs are components of specific kinase cascades. They are inactivated by specific phosphatases. The role in contractile mechanisms remains to be elucidated (106) but is likely significant (Fig. 7.6). Zhang *et al.* demonstrated involvement of MAPK in ET-1-induced contraction of rabbit basilar artery, suggesting it is downstream from PTK, Src, and Janus tyrosine kinase pathways but not the phosphatidylinositiol-3 kinase pathway (107).

### E. G Proteins

G proteins are small GTPases and GTP-binding proteins, an example of which is Ras. Ras includes six families including the Rho family, which has at least three members. Rho appears to be involved in smooth muscle contraction because it is a major GTP-binding protein in cytosol; the GTP-γS effect on $Ca^{2+}$ sensitivity is blocked by ADP ribosylation; a form of Rho induces $Ca^{2+}$ sensitization of permeabilized smooth muscle; toxins which block Rho function block increased $Ca^{2+}$ sensitiv-

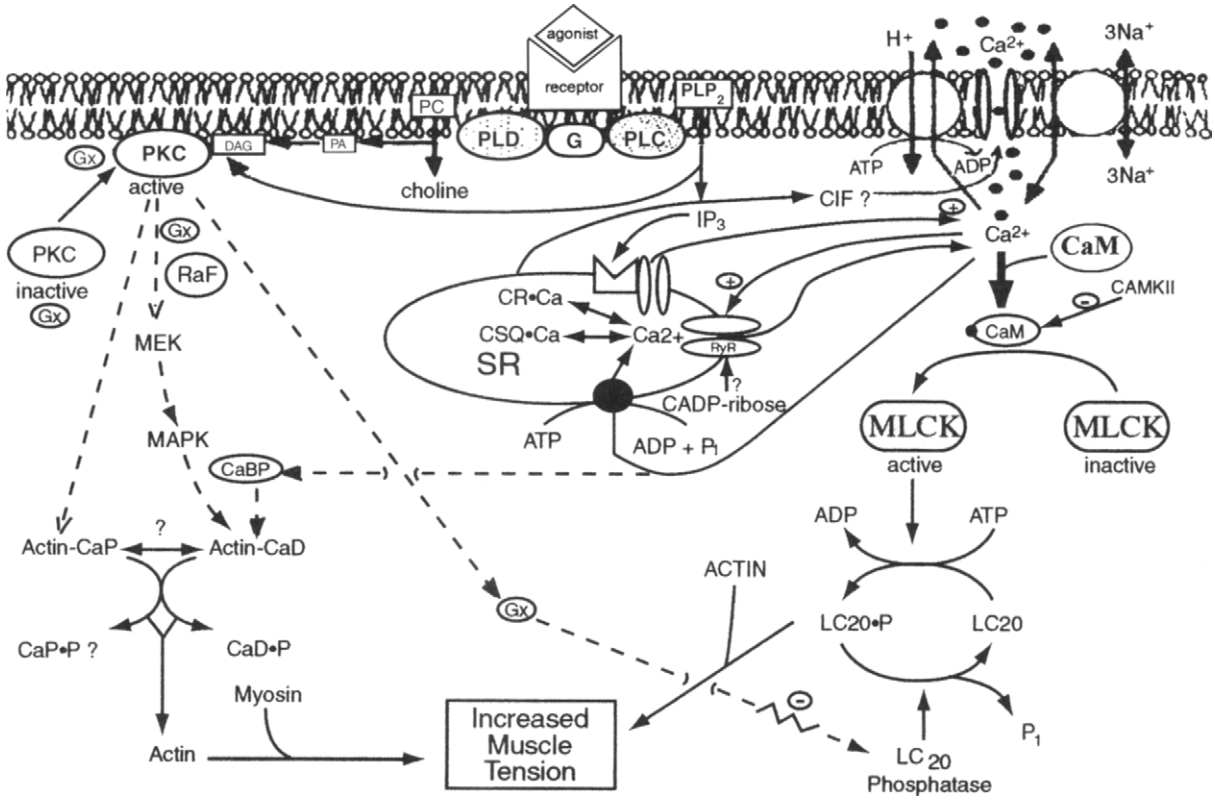



**FIGURE 7.6** Signal transduction mechanisms in vascular smooth muscle. G, heterotrimeric GTP – binding protein; PLC, phospholipase C; $PIP_2$, phosphatidylinositol 4,5-bisphosphate; PC, phosphatidylcholine; $IP_3$, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; SR, sacroplasmic reticulum; MLCK, myosin light chain kinase; PKC, protein kinase C; CaD, caldesmon; CaP, calponin; MAPK, mitogen-activated protein kinase; MEK, MAP/ERK kinase; Gx, small GTP – binding protein; CaBP, calcium-binding protein; CR, calreticulin; CSQ, calsequestrin; PA, phosphatidic acid; CaM, camodulin; RyR, ryanodine receptor; $LC_{20}$, 20 – kDa myosin light chain; CaMKII, $Ca^{2+}$/calmodulin protein kinase II. Dashed lines indicate pathways that may require kinases or cofactors not yet identified [reproduced with permission from Horowitz, A., Menice, C. B., Laporte, R., and Morgan, K. G. (1996). Mechanisms of smooth muscle contraction. *Physiol. Rev.* **76,** 967–1003].

ity to agonists; and translocation of Rho A from cytosol to plasmalemma causes $Ca^{2+}$ sensitization. Rho increases $Ca^{2+}$ sensitivity to agonists (49). Inhibition of MLCP may be the primary mechanism of G protein-coupled $Ca^{2+}$ sensitization (108,109).

### F. Rho A

The $Ca^{2+}$ sensitivity of MLC phosphorylation and thereby contraction varies over a wide range because MLCK and MLCP are subject to functional alterations at constant $Ca^{2+}$ by other signaling molecules such as cGMP-dependent protein kinase and Rho-associated kinase (110). There are two major families of GTP-binding proteins: the heterotrimeric G proteins, which are transducers for serpentine membrane receptors, and a superfamily of Ras-related monomeric, low-molecular-mass GTPases. Rho associates with Rho-kinase, which phophorylates MLCP. Rho-kinase can also directly phosphorylate MLC. In resting cells, the Rho proteins are in the cytosol as Rho GDP dissociation inhibitor complexes which translocate to the cell membrane on stimulation. This may be essential to $Ca^{2+}$ sensitization. It is unclear whether tyrosine kinase and phosphorylation are essential elements in the increased $Ca^{2+}$ sensitivity. Receptors coupled to G proteins activate the release of intracellular $Ca^{2+}$. Many of the receptors that activate the phosphatidylinositol cascade also activate Rho A. Guanine nucleotide exchange factor and guanine nucleotide dissociation inhibitor interact to dissociate cytosolic Rho A. GTP is exchanged for GDP in Rho A. The active Rho A GTP activates Rho kinase, which phosphorylates and thereby inhibits myosin phosphatase, whose action is to dephosphorylate myosin and induce relaxation. Rho A therefore acts to facilitate contraction at a constant $Ca^{2+}$. Although $Ca^{2+}$ is the principal activator of VSM contraction, the level of contraction can be modulated by other factors (25).

### G. Phosphatidylinositol Cascade and Diacylglycerol

Inositol-containing phospholipids comprise 3–5% of total membrane phopholipids in eukaryotic cells. Almost 20% of phosphatidylinositol (PI) is phosphorylated at positions 4 and 5 of the inositol moiety to yield $PIP_2$. Hormones, neurotransmitters, and growth factors can all activate isozymes of PLC which can hydrolyze PI to produce messenger molecules from $PIP_2$, $IP_3$ and DAG. $IP_3$ can mobilize $Ca^{2+}$ from SR stores and plays a central role in smooth muscle contraction. An increase in $IP_3$ parallels smooth muscle contraction. G proteins are involved in the regulation of PI–PLC activity (111). $IP_3$ is the intracellular $Ca^{2+}$ mobilizing molecule in smooth muscle. Its action is terminated by phosphorylation by a 3-kinase or dephosphorylation by 5-phosphatase. DAG is the physiological activator of PKC(111). Receptor–mediator hydrolysis of inositol phospholipids is a common mechanism for transducing various extracellular signals into the cell from many biologically active substances. In the early 1950s, the response of inositol phospholipids to the stimulation of cell surface receptors was recognized by the Hokins, who showed that acetylcholine induces a rapid incorporation of $^{32}P$ into phospholipids (112). It was later demonstrated that $IP_3$ serves as a mediator of $Ca^{2+}$ mobilization from endoplasmic reticulum. DAG, the other product of $PIP_2$ hydrolysis, remains in the membrane and initiates the activation of PKC. PKC is involved in a protein phosphorylation pattern which is separate but interrelated to the $Ca^{2+}$ signaling pathway (4,113). The correlation between smooth muscle stimulation and metabolism of inositol phosphates has been known since the 1970s. Time-course studies on changes in concentrations of inositol phosphates in stimulated smooth muscle showed rapid accumulations of $IP_3$ which were dose dependent and could occur in the absence of extracellular $Ca^{2+}$. The effects were blocked by atropine. Good correlations have been shown between hydrolysis of $PIP_2$, MLC phosphorylation, the appearance of $IP_3$, elevation of $[Ca^{2+}]i$, and contraction (111).

Several agonists are known to activate receptor-mediated PI turnover in smooth muscle through activation of PLC to produce DAG. DAG is the only well-characterized activator of PKC. DAG is also generated by PLC-mediated hydrolysis of phosphatidylcholine. Sustained increases in DAG occur in response to certain agonists which are known to cause sustained contraction (7). $IP_3$ controls many cellular processes by generating internal calcium $Ca^{2+}$ signals. The $IP_3$ receptors resemble the $Ca^{2+}$-mobilizing ryanodine receptors in muscle. Intracellular $Ca^{2+}$ channels display the regenerative process of $Ca^{2+}$-induced $Ca^{2+}$ release responsible for the complex spatiotemporal patterns of $Ca^{2+}$ waves and oscillations. Numerous cellular processes are controlled in this manner, including smooth muscle contraction. $IP_3$ is the focal point of two pathways, one initiated by the G protein-linked receptors and the other by receptors linked to tyrosine kinase. These mechanisms require energy from GTP or ATP, which activate PLC to hydrolyze $PIP_2$ to give $IP_3$ and DAG. $IP_3$ and ryanodine receptors are the principal intracellular $Ca^{2+}$ channels responsible for mobilizing stored $Ca^{2+}$. The endoplasmic reticulum from which $Ca^{2+}$ is released contains pumps to sequester $Ca^{2+}$ and $Ca^{2+}$-binding proteins, such as calsequestrin and calreticulin, and specific $IP_3$ or ryanodine channels to release $Ca^{2+}$ back into the cytosol. $Ca^{2+}$ contained within the intracellular stores is released to the cytosol when $IP_3$

binds to its receptor. $Ca^{2+}$ waves can spread from cell to cell through gap junctions or by means of a secreted intermediate such as ATP. The exact mechanism of how waves cross the gap junction is unknown but may depend on the diffusion of either $Ca^{2+}$ or $IP_3$ (114). Some neurotransmitters are thought to generate $IP_3$ release of $Ca^{2+}$ from SR stores. The emptied stores are thought to send a signal to ion channels, particularly the nonspecific cation channels, to promote calcium entry. On the other hand, elevation of $[Ca^{2+}]i$ activates calcium-dependent $K^+$ channels which hyperpolarize endothelial cells and increase the driving force for calcium entry (115). The principal means of pharmacomechanical coupling is therefore the activation of the phosphatidylinositol cascade by agonists coupling to G proteins to activate PLC-β or tyrosine kinases to activate PLC-γ, causing the release of $IP_3$ from $PIP_2$. $IP_3$-induced release is independent of but may be modulated by resting membrane potential as well as PKC (116).

### H. Inositol Phosphates and Hemoglobin

The intracellular concentrations of inositol phosphates, the second messengers of some types of smooth muscle contraction, are acutely increased by exposing vascular smooth muscle cells to oxyHb (117). Primate cerebrovascular arterial rings were studied. OxyHb produced a transient but significant increase in cellular levels of $IP_3$. $[Ca^{2+}]i$ levels in vascular smooth muscle cells were also increased by thrombin and oxyHb. Removal of oxyHb after as long as 48 hr of incubation with this compound allowed cells to rapidly reduce their $[Ca^{2+}]i$ to near normal. OxyHb produced contractions of isolated rings of both normal and spastic cerebral arteries, although the response in the latter was significantly smaller. The PLC inhibitor neomycin prevented the sustained elevation of $[Ca^{2+}]i$ and relaxed arteries contracted by oxyHb, 5-HT, or KCl. PLC might be involved in the generation of VSP. PLC mediates hydrolysis of the membrane inositol lipid in response to a variety of receptor agonists, including leukotrienes, prostaglandins, 5-HT, and ET (118).

### I. cGMP

#### *1. Mechanisms of Relaxation*

cGMP was first described as a biological product in the 1960s. It gradually came to be realized that it was an important second messenger. cGMP is present in low concentrations in tissues. Its emergence as one of the few fundamental second messengers was realized after it was shown that cGMP levels were elevated by nitrovasodilators, NO, and other free radicals, endothelial-derived relaxant factor (EDRF) and atrial natriuretic factor (ANF). The enzyme guanylate cyclase synthesizes cGMP and is present in virtually all cell types and phyla. The enzyme exists in a soluble cytosolic form and a membrane-associated particulate form. Soluble guanylate is the only known receptor for signal transduction involving NO. Soluble guanylate cyclase possesses a heme prosthetic group that may mediate many of the activation phenomena. This porphyrin moeity is easily dissociated from the protein. The particulate guanylate cyclase can be activated by a variety of proteases. It is also activated by a variety of agents that lead to the formation of NO. The term "nitrovasodilators" was coined by Murad to identify these agents, which include azide, nitrite, hydroxylamine, nitroglycerin, nitroprusside, nitrosamines, and nitrosoureas. The many compounds which activate guanylate cyclase are all potential sources of NO, but NO has been proposed as the proximal activator of guanylate cyclase and its formation may represent the common pathway by which most of these agents influence enzyme activity. There are specific inhibitors of guanylate cyclase. $Fe^{2+}$ metalloproteins but not $Fe^{3+}$ (such a metHb) inhibit NO activation. It is presumed that these heme-containing compounds in their $Fe^{2+}$ form inhibit the production of or scavenge the NO. The activation of soluble guanylate cyclase by nitrocompounds involves the interaction of the free radical NO with a heme moiety associated with the enzyme. This nitrosyl–porphyrin complex directly mediates the activation of guanylate cyclase. Many hormones, autocoids, and toxins elevate cGMP in their target tissue in a calcium-dependent fashion. Agents that inhibit activation of guanylate cyclase by nitrovasodilators such as Hb also inhibit smooth muscle relaxation and/or accumulation of cGMP. Nitrovasodilators relax blood vessels in the presence or absence of endothelium. In this respect, they differ from agents such as ACh, BK, A23187, ATP, and thrombin, which require an intact endothelium to relax blood vessels. Some of these agents actually become vasoconstrictors in the absence of the endothelium (119). Adjacent endothelium, certain endogenous circulating hormones, and administered nitrovasodilators cause vascular relaxation by stimulating guanylyl cyclase to produce cGMP.

NO and NO donors such as sodium nitroprusside (SNP) increase cGMP by activating guanylyl cyclase, whereas papaverine vasodilates by preventing the breakdown of cGMP and cAMP by phosphodiesterases.

cGMP lowers $[Ca^{2+}]i$ and desensitizes the contractile mechanism to $[Ca^{2+}]i$ (50). The specific mechanisms by which cyclic nucleotides relax smooth muscle are not completely characterized (Fig. 7.7). cAMP modulates many intracellular signaling processes by activating cAMP-dependent protein kinase (A kinase). This enzyme

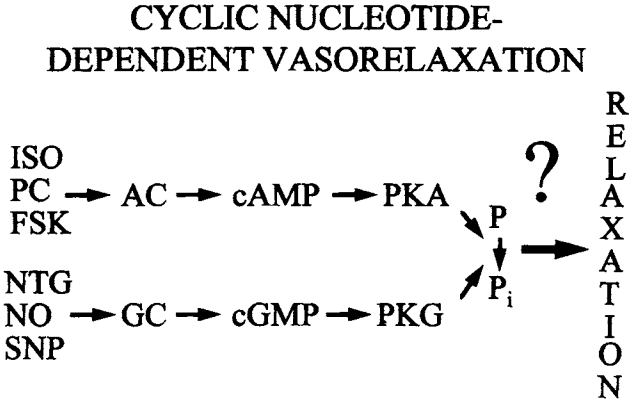



**FIGURE 7.7** Two pathways have been implicated in modulating vascular smooth muscle relaxation. Adenylate cyclase (AC) activation by agents such as prostacylcin (PC), isoproterenol (ISO), and FSK leads to increases in cAMP; cAMP activates cAMP – dependent kinase (PKA). In addition, NO and NO donors, such as nitroglycerine (NTG) and sodium nitroprusside (SNP), activate guanylate cyclase (CG), leading to increases in cGMP; cGMP activates cGMP-dependent protein kinase (PKG). Protein kinases such as PKA and PKG modulate cellular physiologic mechanisms through the phosphorylation of specific proteins. Many substrate proteins have been suggested to mediate vasorelaxation, including the $IP_3$ receptor, the plasma membrane $Ca^{2+}$ pump, phospholamban, and two unidentified 20-kDa proteins. The subsequent events that lead to dissociation of actin and myosin and relaxation have not been clearly determined [reproduced with permission from Brophy, C. M., Whitney, E. G., Lamb, S., and Beall, A. (1997). Cellular mechanisms of cyclic nucleotide-induced vasorelaxation. *J. Vasc. Surg.* **25,** 390–397].

is activated when cAMP binds to the regulatory subunit and releases the free catalytic subunit. The subunits have been classified as type I or II. A-kinase phosphorylates specific substrate proteins to initiate relaxation. MLCK is phosphorylated by A kinase. This phosphorylation decreases the affinity of MLCK for the $Ca^{2+}$–calmodulin complex and hence induces relaxation. Other phosphoproteins are phosphorylated by a substance which causes vasorelaxation. These might include small GTP-binding proteins, phospholamban aggregates, phospholamban, or other uncharacterized regulatory proteins (6).

The accumulation of cGMP in relaxation of bovine coronary arteries was inhibited by methylene blue. cGMP accumulation preceded the onset of relaxation elicited by NO and GTN, and it temporarily correlated with relaxation. In this study MetHb abolished cGMP accumulation and relaxation elicited by NO without altering responses to GTN, sodium nitrate, SNP, and sodium nitrite. cGMP formation in vascular smooth muscle relaxation is elicited by NO-containing vasodilators (120). NO, the reactive intermediate of the nitrovasodilators, binds to the heme moiety of guanylate cyclase and increases the activity of the enzyme. Hb in ferrous form competes for the binding of NO. Methylene blue inhibits relaxation by oxidizing guanylate cyclase. Activation of cGMP-dependent protein kinases may cause a cascade of protein phosphorylations resulting in the dephosphorylation of MLC and relaxation. cGMP increases $Ca^{2+}$ extrusion from the cell by activation of $Ca^{2+}$–ATPase. cGMP decreases the influx and the intracellular release of $Ca^{2+}$. In canine arteries basal levels of cGMP were reduced after SAH and by removal of endothelium (4,121).

NO and ANF increase cGMP in smooth muscle. These substances relax vascular smooth muscle by decreasing $[Ca^{2+}]i$, decreasing the $[Ca^{2+}]i$ sensitivity of phosphorylation, and uncoupling force generation from myosin phosphorylation (4). In 1979 it was shown that the dose-dependent relaxation of bovine mesenteric artery correlated with an increase in the cGMP content but not with the cAMP content (122). Schultz *et al.* suggested that cGMP acted as a mediator of relaxation in smooth muscle (123). cGMP mediates the relaxing action of a variety of endogenous substances and vasodilator drugs. cAMP mediates relaxation by β-adrenergic agonists and other activators of adenylate cyclase. Both second messengers reduce $[Ca^{2+}]i$. cGMP-dependent protein kinase is necessary for the reduction in $[Ca^{2+}]i$ by cGMP. Specific substrate proteins for cGMP-dependent protein kinase are not well characterized in vascular smooth muscle. $Ca^{2+}$–ATPase activation by phosphorylation of phospholamban by the cGMP – dependent protein kinase may be involved in cyclic nucleotide-dependent relaxation (124). cGMP decreases $Ca^{2+}$ influx by inactivating L channels independent of changes in $E_m$ and $[Ca^{2+}]i$. cGMP may act by inducing hyperpolarization or closing L type $Ca^{2+}$ channels (4).

cGMP-dependent protein kinase (G kinase) transduces cGMP signals into biological responses. G kinase occurs in high concentration in vascular smooth muscle cells, platelets, and a few brain areas. It does not have the ubiquitous distribution of cAMP-dependent protein kinase (A kinase) (6). The formation of cGMP from GTP catalyzed by guanylate cyclase occurs in both soluble and particulate fractions of cells. Two different cellular compartments for the particulate enzyme exist: the plasma membrane and cytoskeleton. The enzyme form found in the soluble fraction is a heterodimer regulated by both free radicals and nitrovasodilators. The membane form is a single-chain polypeptide which can be regulated by various peptides. The membrane form of guanylate cyclase which serves as cell surface receptor is the first protein recognized to directly catalyze formation of a low-molecular-weight second messenger in response to ligand binding (125). cGMP and PKG may inhibit agonist-evoked PLC formation. The mechanism by which cGMP inhibits $IP_3$ formation is unknown. PKG is

one of several types of receptor proteins for cGMP. cGMP regulates various phosphodiesterases. PKG is a family of enzymes found in eukaryotic cells. PKG can inhibit PLC, and $IP_3$ receptors and may stimulate BK channels and $Ca^{2+}$–ATPase activity through the phosphorylation of phospholamban (126).

### 2. *cGMP and Vasospasm*

Bloody cerebrospinel fluid (CSF) from patients caused reductions in cGMP levels of canine basilar arteries. OxyHb ($10^{-6}$ to $3 \times 10^{-6}$ M) caused dose-dependent inhibition of relaxation caused by A23187. Control canine basilar rings had 150 pmol cGMP/g tissue, whereas tissues containing Hb or methylene blue showed values of 13 and 48 pmol/g, respectively. The amount of Hb in human CSF was found to correlate with the inhibition of A23187-induced relaxation in the canine basilar artery (127). EDRF is impaired in arteries following SAH in animal models (128–130). Some spastic cerebral arteries appeared to release EDRF. Spastic cerebral arteries have reduced quantities of cGMP (131). The substrate for production of cGMP is guanosine triphosphate (GTP), a high-energy phosphate (128). Levels of GTP and creatine phosphate were markedly reduced in spastic canine arteries after SAH. The ratio of GTP to GDP was also significantly decreased. The content of high-energy phosphates was markedly reduced following incubation in anoxic circumstances without glucose (132). Porcine cerebral arteries were studied after exposure to SAH. cGMP levels were measured in intrathecal arteries after *in vitro* exposure, and Hb was applied either adventitially or intraluminally. The depression of cGMP levels by Hb was reversible and equivalent to the effect of endothelial denudation or incubation with L-NMMA, so the effect of Hb was attributed to a specific action on EDRF. cGMP levels in isolated arteries were unchanged after *in vivo* exposure to Hb for either 2 or 7 days or exposure to whole blood for 2 days. The levels of cGMP, however, were reduced by intraluminal infusion with 1 $\mu M$ Hb. In contrast, after 7 days of *in vivo* exposure to whole blood the cGMP levels were depressed and not further reduced by intraluminal perfusion of Hb. It was concluded that adventitially applied Hb can inhibit basal EDRF activity and that *in vivo* adventitial exposure to whole blood leads to a reduction in basal cGMP levels in association with vasoconstriction of the intrathecal arteries (133). EDRF was also inhibited during chronic VSP post-SAH in the canine basilar artery, although the luminal release of EDRF was apparently maintained. The loss of responsiveness of vascular smooth muscle to EDRF is considered to be due to an impaired production of cGMP. Resting levels of cGMP in rings with endothelium (reflecting spontaneous release of EDRF) and those evoked by BK in rings with endothelium and by NO and rings without endothelium were all diminished in SAH arteries. These results were interpreted as showing impaired activation of soluble guanylate cyclase, leading to reduced production of cGMP (132).

## J. cAMP

It used to be thought that cAMP relaxed smooth muscle after β-adrenergic stimulation, whereas cGMP would contract it after cholinergic stimulation. However, both cyclic nucleotide second messengers are now known to respond to endogenous hormones and regulatory substances by causing relaxation. Both reduce the concentration of $Ca^{2+}$ intracellularly and require the presence of cyclic nucleotide-dependent protein kinases to relax vascular smooth muscle. The kinases may phosphorylate to activate $Ca^{2+}$ –ATPase and increase SR $Ca^{2+}$ uptake (124).

Most tissues have at least two receptor classes, one depending on the generation of cAMP as a second messenger and another inducing rapid turnover of inositol phospholipids as well as the mobilization of $Ca^{2+}$. Stimulation of the latter class normally leads to release of AA and increases cGMP (73). Vasorelaxation is brought about by at least two pathways; (i) NO and nitrosovasodilators activate intracellular soluble guanylate cyclase and produce cGMP and (ii) the adenylate cyclase/cAMP pathway. Agents may contribute to relaxation by inhibiting phosophodiesterases, thereby raising cyclic nucleotide concentrations. The exact mechanism by which cyclic nucleotides induce relaxation is not understood. cAMP affects smooth muscle relaxation through receptor-mediated activation of a particulate guanylate cyclase. This is different from the soluble guanylate cyclase activated by NO (134).

In regulating cellular function, $Ca^{2+}$ and cAMP almost always function together. The flow of information from a cell's surface to its interior precedes by multiple distinct branches. $Ca^{2+}$ is a universal messenger in animal cells. If it accumulates in excessive amounts it can cause cellular dysfunction and death. Its rise in concentration in the cell cytosol is transient even in cells subsequently displaying a sustained response. The elevation of $Ca^{2+}$ is nearly always in concert with the elevation of cAMP. $Ca^{2+}$ activates many phospholipid-dependent protein kinases. Calcium receptor proteins include CaD and MLC. Interactions between cAMP and $Ca^{2+}$ messenger systems are multiple and complex. They can both activate phosphodiesterase and adenylate cyclase. They both increase plasma membrane influx and efflux. They can control the phosphorylation of sequential proteins in a cascade and may do so by phosphorylating separate protein kinases. A rise in

cAMP causes relaxation of many types of smooth muscle, including vascular smooth muscle. cAMP reduces the sensitivity of MLCK, stimulates the uptake of $Ca^{2+}$ by the endoplasmic reticulum, and stimulates the efflux of $Ca^{2+}$ by enhancing the activity of the $Ca^{2+}$ pump or indirectly enhancing $Na^+Ca^{2+}$ exchange via the activation of $Na^+$, $K^+$–ATPase (135). A reduction in $[Ca^{2+}]i$ can be induced by elevations of cAMP which are brought about by adenosine and activation of some cell membrane receptors. The primary mechanism for cAMP-mediated reduction in $[Ca^{2+}]i$ may be activation of cGMP-dependent protein kinase (4).

There is one type of serine/threonine kinase in which the site of phosphorylation is either a serine or theronine immediately followed by a proline. Members of this class include MAPKs, which are in the 40-to 45-kDa range and include extracellular signal-regulated kinases (136).

## IX. Membrane Potential

### A. General

In striated muscle an action potential propagates quickly over the entire sarcolemma, allowing the cell to contract synchronously. All cells producing action potentials have sizable resting membrane potentials ($V_m$) across the plasma membranes. A resting membrane potential of approximately −90 mV is usually necessary for a cell to fire an action potential. A resting potential of −50mV or less is associated with inability to produce an action potential (55).

Smooth muscles can generate potentials, but these vary considerable depending on the type. Smooth muscle action potentials have slower rates of depolarization and repolarization and less overshoot than skeletal muscle action potentials. There are no fast $Na^+$ channels in smooth muscle.The depolarizing phase of smooth muscle action potentials is caused primarily by channels that can conduct $Na^+$ and $Ca^{2+}$. The $Ca^{2+}$ entering the sarcoplasm via the slow channels is vital for excitation–contraction coupling in smooth muscle. Repolarization is caused by the closing of the slow $Na^+/Ca^{2+}$ channels and the simultaneous opening of $K^+$ channels (55). Smooth muscle membrane potential is mainly regulated by activation or inhibition of the different $K^+$ channels. Changes in the membrane potential lead to opening or closing of voltage-dependent $Ca^{2+}$ channels which couple excitation and contraction (8). Estimates of typical concentrations of the major physiological ions in extracellular fluid and VSMC cytoplasm give equilibrium potentials of −84 mV for $E_K$, −3 mV for $E_{Cl}$, 58 mV for $E_{Na}$, and 130 mV for $E_{Ca}$. At physiological pressures *in vivo* resting potential is about −40 to −50 mV, and it is about −60 to −70 mV *in vitro*. The membrane potential varies about the resting level in response to depolarizing and hyperpolarizing influences. The membrane potential is an important determinant of $[Ca^{2+}]i$ and contraction. Arterial smooth muscle cells have relatively low channel densities; estimated at a few hundred to a few thousand for $K^+$. Under physiological conditions, $K^+$ channels show low levels of activity. VSMCs have high input resistance so changes in the state of a few channels can have a major impact on the membrane potential (137).

$V_m$ is an important regulator of vascular tone. Membrane potential changes of a few millivolts can cause significant changes in vessel diameter. These changes can act synergistically with change in $Ca^{2+}$ sensitivity of the contractile process and with $Ca^{2+}$ released from cytoplasmic reticulum to affect changes in vessel diameter. A primary mechanism for altering membrane potential is through the alteration in $Ca^{2+}$ influx via L-type calcium channels. Membrane potential can often be influenced by the $Na^+$–$Ca^{2+}$ exchanger regulating $Ca^{2+}$ entry. A 3-mV depolarization or hyperpolarization can increase or decrease $Ca^{2+}$ influx as much as twofold. Membrane hyperpolarization through activation of $K^+$ channels causes vasodilation and lowering of blood pressure. Synthetic $K_{ATP}$ openers such as cromakalim hyperpolarize and dilate arteries. The contraction of vascular smooth muscle is potently regulated by the influx of extracellular $Ca^{2+}$ through voltage-dependent calcium channels. At −40 mV membrane potential in a vascular smooth muscle cell the current through a single channel is about − 0.2 pA, which is about 600,000 $Ca^{2+}$ ions per second. On average, only about 1–5 channels of the total of 3000 or more are open simultaneously in a single smooth muscle cell at physiological potential. These few open $Ca^{2+}$ channels can apparently maintain the tone under steady-state conditions (8). In the steady state, membrane potential is the principal determinant of $[Ca^{2+}]i$ but there is a feedback, with $[Ca^{2+}]i$ being one of the influences on membrane potential.

Hyperpolarization due to opening of ATP-gated $K^+$ channels closes voltage-gated $Ca^{2+}$ channels, reduces $Ca^{2+}$ influx, and lowers $[Ca^{2+}]i$. Changes in $K^+$ and $Cl^-$ permeability dominate the membrane potential (25). At physiological arterial blood pressure, cerebral arterial muscle is maintained in an active state because of membrane depolarization, compared to zero pressure load. Membrane potential changes with blood pressure. The membrane is relatively depolarized partly due to inhibition of $K^+$ channels. As blood pressure rises, so does cytochrome $P_{450}$ activity, which catalyzes the production of 20-hydroxyeicosatetraenoic acid from arachidonic acid, a potent inhibitor of large conductive $Ca^{2+}$-activated $K^+$ channels ($K_{Ca}$) (138).

### B. Calcium Channels

L-type $Ca^{2+}$ channels can be blocked by a variety of di- and trivalent cations (8). The open state probability of the L-type $Ca^{2+}$ channel increases with membrane depolarization. $Ca^{2+}$ pumps in the sarcolemma maintain a low cellular $[Ca^{2+}]i$ concentration, compensating for the tendency of $Ca^{2+}$ to move along a steep electrochemical gradient. $Ca^{2+}$ pumps in the endoplasmic reticulum membranes work in concert with the sarcoplasmic membrane pumps to bring the $[Ca^{2+}]i$ back to resting levels at the end of the stimulus. The pumps maintain a high $Ca^{2+}$ concentration within the lumen of the SR. This high luminal $Ca^{2+}$ plays a role in excitation of the cell as well as the regulation of protein synthesis and folding and degradation of secretory proteins. The plasma membrane $Ca^{2+}$ pump has a molecular weight of about 130 kDa and is stimulated by the binding of CaM. The SR $Ca^{2+}$ pump is smaller and does not bind CaM. The $Ca^{2+}$ transport ATPases are members of the class of transmembrane proteins and transduce the energy from ATP hydrolyzed into ion transport against steep electrochemical gradients. The $Ca^{2+}$ pumps exist in phosphorylated and unphosphorylated states. The number of $Ca^{2+}$ pumps in smooth muscle is much lower than that in striated muscle. This probably reflects their lower rates of contraction and relaxation. The sarcolemmal $Ca^{2+}$ pump operates in parallel with $Na^+/Ca^{2+}$ exchanger. These $Ca^{2+}$ pumps are considered to be susceptible to attack by reactive oxygen species (139).

The classical model of electromechanical coupling considers that membrane potential determines $[Ca^{2+}]i$. It is established that $[Ca^{2+}]i$ in turn can determine membrane potential by modulating the open probabilities of ion channels. There is a network of positive and negative feedback loops regulating $[Ca^{2+}]i$ as well as membrane potential. $[Ca^{2+}]i$ can regulate $K^+$, $Cl^-$ channels, and nonselective cation channels (140).

Spontaneous $[Ca^{2+}]i$ transients resulting from the release of $Ca^{2+}$ from intracellular stores have been termed "sparks." They are detected in single smooth muscle cells by the opening of $Ca^{2+}$-activated $K^+$ channels under voltage clamp and by the use of $Ca^{2+}$-sensitive dyes which show flashes of fluorescent light. Each event is associated with the opening of 10–100 $K^+$ selective channels (141). The $Ca^{2+}$ sparks result from the opening of a single or closely clustered ryanodine receptor(s) on the SR in arterial smooth muscle. They oppose the tonic contraction of blood vessels due to the intravascular pressure, causing a graded membrane potential depolarization to approximately −40 mV. The activation of $K_{Ca}$ channels in the plasmalemma hyperpolarizes it, opposing pressure-induced depolarization and stimulating relaxation. On the other hand, ryanodine receptor activation or $K_{Ca}$ blockade induce membrane depolarization, activation of L-type voltage-gated $Ca^{2+}$ channels, and vasoconstriction (142).

### C. Potassium Channels

#### *1. General*

The ions present in greatest concentrations within and outside of the smooth muscle cell, $Na^+$ and $K^+$, and their differential concentrations create a transmembrane electrical potential. Normally, these ions are transported across the sarcolemma by electrogenic forces which expel three $Na^+$ in exchange for two $K^+$. As each cycle of the pump removes one positive charge the membrane potential becomes progressively negative (2). $K^+$ conductance in the sarcolemma is also responsive to receptor-mediated mechanisms. When $K^+$ permeability is decreased the membrane potential becomes less negative. A decrease in negative membrane potential is associated with an increase in force because the change in potential influences the potential-dependent $Ca^{2+}$ channels, whose sum conductance is increased with depolarization. Increase in $Ca^{2+}$ influx and force generation can result from action potentials, reduced $K^+$ channels permeability, slowing of the $Na^+$ exchanger, and depolarization propagated via gap junctions from adjacent cells (2). The vascular smooth muscle membrane potential is determined by a balance between hyperpolarizing $K^+$ conductances and depolarizing conductances. When $K^+$ channels open and $K^+$ leaves the sarcoplasm the cell membrane is hyperpolarized, which reduces the open probability of $Ca^{2+}$ channels and thereby leads to relaxation. On the other hand, when $K^+$ channels close there is resultant depolarization and contraction. There are at least four types of $K^+$ channels. First, voltage-dependent ($K_v$) channels have an increased open probability with membrane depolarization and regulate sarcolemmal potential under physiological conditions. They are ubiquitous in smooth muscle. Second, $Ca^{2+}$-activated $K^+$ ($K_{ca}$) channels respond to changes in $[Ca^{2+}]i$ and are important regulators of tone in small arteries. Third, ATP-sensitive $K^+$ ($K_{ATP}$) channels respond to a variety of vasodilators. Finally, inward rectifier $K^+$ ($K_{IR}$) channels regulate membrane potential in some resistance arteries. Potassium channels regulate membrane potential and are an important determinant of arterial tone and consequently diameter. Vasodilators such as CGRP and adenosine act partly by activating $K^+$ channels. Inhibition of $K^+$ channels causes membrane depolarization and vasoconstriction. Vasoconstrictors have in common the fact that they cause membrane potential depolarization by inhibiting most $K^+$ channels.

$K^+$ channel dysfunction can lead to VSP as well as impair the ability of the artery to dilate (8).

Vascular arterial smooth muscle cells have stable membrane potentials between −40 and −60 mV at physiological blood pressures. Although very important, $K^+$ permeability is not the sole determinant of membrane conductance since membrane potential is considerably more positive than the $K^+$ equilibrium potential ($E_K$ is about −85 mV). The $Cl^-$ conductance is relatively high, and this may contribute to the actual membrane potential being more positive than $E_K$. At a physiological extracellular $K^+$ of 5 m*M*, the $E_K$ is approximately −85 mV and passive $K^+$ movement through any open $K^+$ channel will be out of the cell. Channels are portals between the extra- and intracellular space through which ions pass. They are selectively permeable to different ions and open or close in response to a wide variety of chemical, electrical, and physical influences. There are approximately 100–500 $K_{ATP}$, 100–500 $K_{IR}$, 1000–10,000 $K_V$, and 1000–10,000 $K_{Ca}$ channels per cell. Because the resting input resistance of arterial smooth muscle cells is so high, very few $K^+$ channels are open at any one time at physiological membrane potentials (8). Extracellular $K^+$ in CSF in normal circumstances is approximately 3–5 m*M* but can increase to >10 m*M* under conditions of ischemia and hypoxia. Increasing extracellular $K^+$ in the range 0–10 m*M* causes membrane hyperpolarization. This is associated with vasodilation (60). $K^+$ channels regulate membrane potential of smooth muscle, which in turn controls $Ca^{2+}$ passage through voltage-dependent $Ca^{2+}$ channels, thereby controlling contractility by changes in $[Ca^{2+}]i$ (60). Activation of $K^+$ channels mediates relaxation of cerebral vessels to a diverse group of stimuli, including receptor-mediated agonists, intracellular second messengers, and hypoxia. Several endothelium-derived factors produce relaxation by activating $K^+$ channels (143).

Activation of $K^+$ channels will lead to smooth muscle cell hyperpolarization and subsequent closure of voltage-dependent $Ca^{2+}$ channels. This reduces the steady-state $Ca^{2+}$ entry through L-type $Ca^{2+}$ channels, and membrane hyperpolarization therefore causes vessel relaxation. Membrane hyperpolarization also reduces $[Ca^{2+}]i$ by affecting the $Na^+/Ca^{2+}$ exchanger. This decreases agonist-induced $IP_3$ generation and $[Ca^{2+}]i$ release. Activation of $K_{ATP}$ channels causes vasorelaxation (60).

Each $K^+$ channel type has its own selective blocker. $K_V$ channels are selectively inhibited by 4-aminopyridine. Large-conductance $K_{Ca}$ channels are blocked by tetraethylammonium ions, charybdotoxin, and iberiotoxin. $K_{ATP}$ channels are inhibited by the sulphonylurea drugs glibenclamide and tolbutamide. Many antihypertensive drugs act through $K^+$ channel activation, including diazoxide, nicorandil, and cromakalim. These drugs potentially have antivasospastic actions. The vasodilation induced by them is blocked by glibenclamide. $K_{IR}$ channels are inhibited by external barium ions (8). It has been suggested that exposure to Hb can cause a dramatic increase in outward $K^+$ current (Fig. 7.8) (144). The depolarization of cerebral vascular muscle after SAH is thought to be due to inhibition of $K^+$ channels (143).

### 2. *Voltage-Dependent Potassium Channels*

Many smooth muscle cells in arteries do not generate action potentials but respond to stimulation with graded membrane potential changes. In the physiological range the $K_V$ channels provide important $K^+$ conductance. The inhibition of these $K_V$ channels depolarizes and constricts arteries (8). Voltage-dependent $K^+$ channels ($K_V$, also known as delayed rectifier $K^+$ channels) have been described in the cerebral vasculature. Both $K_{Ca}$ and $K_V$ have an increasing probability of opening as the membrane increasingly depolarizes. This results in an outward current that returns the membrane potential toward the resting level. The $K_V$ channels function as part of a negative-feedback system which includes $K_{ca}$ in modulating vascular tone. $K_V$ may contribute to regulation of membrane potential and the responses of cerebral arteries to changing arterial pressure (143).

Current passage through $K_V$ channels in response to a depolarizing voltage step initially increases to a peak over time due to voltage-dependent activation and then decays due to voltage-dependent inactivation. At a physiological tone the current through $K_V$ channels depends on a balance between channel activation and inactivation (8).

### 3. *Calcium-Dependent Potassium Channels*

$K_{Ca}$ exist in cerebral vessels. They are defined by their activation through increases in $[Ca^{2+}]i$. The activity of these channels increases with membrane depolarization and can be affected by other vasoactive stimuli. Application of inhibitors of $K_{Ca}$ channels produces contraction of cerebral arteries. The influence of $K_{Ca}$ channels on vasomotor tone may be more important in larger cerebral arteries. Activation of $K_{Ca}$ channels results in vasodilation in response to several stimuli, including forskolin (which directly activates adenlyate cyclase), cAMP, isoproterenol (a β-adrenergic agonist that also activates adenylate cyclase), CGRP, and adrenomedullin. These dilations are attenuated by inhibitors of $K_{Ca}$ channels. A variety of endogenous vasodilators increase cAMP in vascular muscle. NO may activate $K_{Ca}$ channels in some but not all cerebral vessels. NO and SIN-1 do not hyperpolarize vascular muscle in larger cerebral arteries. Reactive oxygen species may relax blood vessels by activating $K_{Ca}$ channels (143). Vasoconstrictors such as NE and Epi,

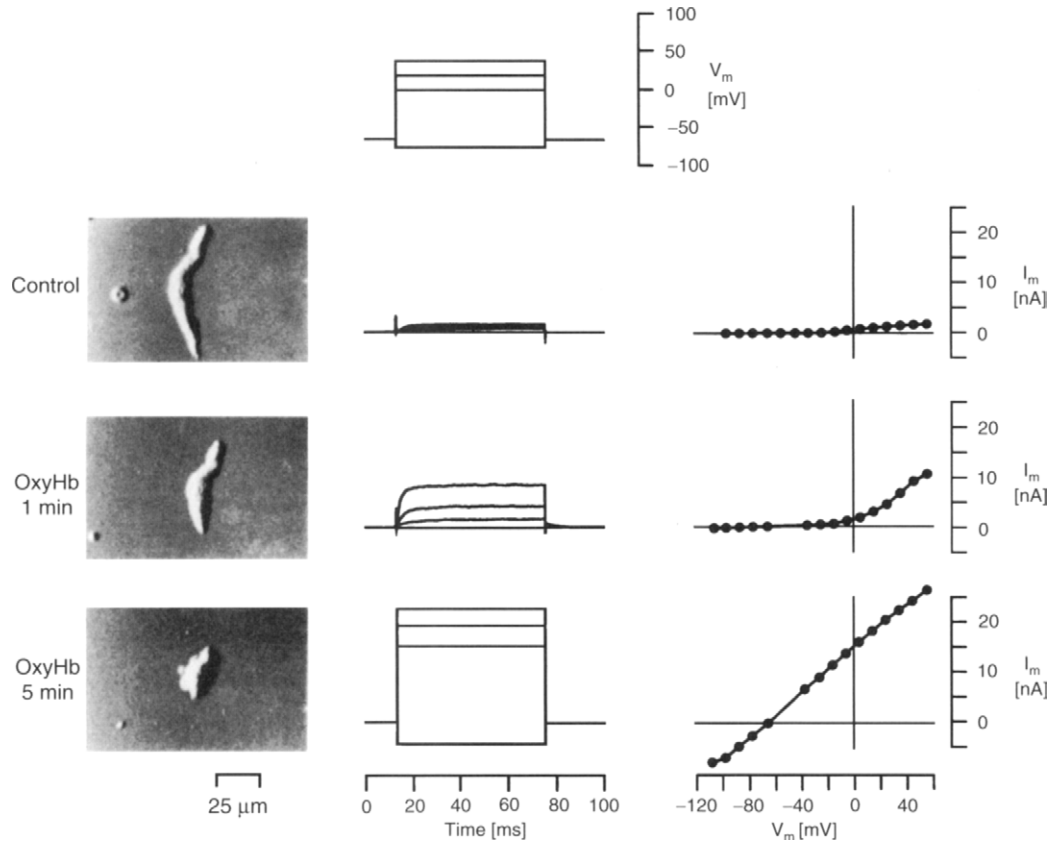



**FIGURE 7.8** Actions of oxyHb on an isolated cerebrovascular smooth muscle cell. (Left) Photographs of a single cell using an inverted microscope with Nomarski optics. The control cell was ~ 100μm. On exposure to 5 μm oxyHb, the cell contracted within approximately 1 min and showed further contraction and membrane blebs within 5 min. (Middle) Membrane currents recorded from a different single cell using the patch-electrode, voltage-clamp technique. Voltage-clamp command waveforms are shown in the top set of superimposed tracings. Shown below are three sets of membrane currents that were not corrected for leakage currents. Four current tracings are superimposed in each set. One minute after application of oxyHb to the solution bathing the cell, the magnitude of the outward $K^+$ currents (upward deflections) increased dramatically. The most likely explanation is that the intracellular $Ca^{2+}$ concentration increased and thus increased the number of $K_{ca}$ channels that were open. After several more minutes, the electrical resistance of the membrane decreased dramatically. (Right) Current-voltage plot of the data in the middle column. $V_m$, membrane voltage; $I_m$, membrane current. Brief exposure to oxyHb caused an increase in the magnitude of the $K^+$ currents over control values. Prolonged exposure caused an extremely large increase in the leakage conductance of the membrane, as evidenced by the increase in the slope of the line at 5 min. For this particular cell, the input resistance (an indicator of membrane permeability) in the control was 3.0 GΩ, after 1 min of oxyHb, it was 0.1 GΩ, and after 5 min it was 0.006 GΩ. This indicates that the permeability of the membrane increased dramatically after application of oxyHb [reproduced with permission from Steele, J. A., Stockbridge, N., Maljkovic, G., and Weir, B. (1991). Free radicals mediate actions of oxyhemoglobin on cerebrovascular smooth muscle cells. *Circ. Res.* **68,** 416–423].

angiotensin II, ET, and 5-HT depolarize vascular smooth muscle. They may operate by inhibiting $K_{Ca}$ channels, thereby contributing to membrane depolarization (8).

$K_{Ca}$ channels or large-conductance $K^+$ channels are present in all smooth muscles. The open state probability of these channels increases with membrane depolarization (2.7-fold per 12–14 mV depolarization) and elevations in $Ca^{2+}$]i in the physiological range (8).

#### *4. ATP-Sensitive Potassium Channels*

$K_{ATP}$ channels have been described in cerebral vascular smooth muscles. These channels are defined based on a sensitivity to increase intracellular ATP, which inhibits the channel. Dissociation of ATP from the channel results in its opening, hyperpolarization, and relaxation. Although elevation in intracellular ATP closes the channel, hypoxia and acidosis have the opposite effect and

produce relaxation. $K_{ATP}$ channels are activated by cromokalin. This activator produces hyperpolarization and relaxation of cerebral arteries and arterioles. The distribution of ATP-sensitive $K^+$ channels is heterogeneous and varies with the vessel size and location. Endogenous substances producing hyperpolarization and vasodilation through $K_{ATP}$ channel-opening mechanisms include EDHF, VIP, CGRP, adrenomedullin, prostacyclin, opioids, NE acting via β-adrenergic receptors, cAMP, and adenosine (143). Nonphysiological $K^+$ channel openers which can activate $K_{ATP}$ currents in vascular smooth muscle cells include cromakalin, pinacidil, nicoradil, and diazoxide (60). Activity of the $K_{ATP}$ channel is inhibited by glibenclamide. This substance does not inhibit vasodilation resulting from NO or nitrovasodilators, so these presumably do not act by activating $K_{ATP}$. $K_{ATP}$ channels are an important mechanism contributing to cerebral vasodilation during hypoxia. They probably do not influence resting tone in cerebral arteries since application of glibenclamide has no effect on it (143). $K_{ATP}$ channels are not apparently voltage dependent. They can respond to many metabolic changes within the cell as well as endogenous and synthetic vasodilators. In the absence of specific activators the open state probability of these channels is low (8). $K_{ATP}$ opening and closing states are subject to a wide variety of vasodilator and constrictor agents operating through cAMP or PKA and PKC, respectively (60). ATP levels are efficiently buffered in cytoplasm and are at millimolar levels, but they can decrease substantially under conditions of metabolic derangements. Vasoconstrictors such as NPY, NE, 5-HT, histamine, ET, and vasopressin can dock at receptors on the sarcolemma which are coupled to release PLC and DAG and ultimately elevate PKC, which closes $K_{ATP}$ channels, depolarizes the membrane potential, and causes vasoconstriction (60). Others have claimed that NO may act in part through activation of these channels (8). In canine basilar arteries obtained from dogs with or without prior SAH, levcromokalin decreased the resting $[Ca^{2+}]i$ and force more profoundly than did nicardipine, the $Ca^{2+}$ channel blocker. The effects of levchromokalin were completely antagonized by glibenclamide (145). Intravenous injections of chromokalin beginning 1 hr post-SAH in rabbits attenuated in a dose-dependent fashion the VSP present at 48 hr post-SAH; the effect was statistically significant at 0.1 and 0.3 mg/kg (146).

#### *5. Inward-Rectifier Potassium Channels*

$K_{IR}$ channels occur in arterial smooth muscle. These channels are activated by membrane hyperpolarization, in contrast to the other K channels which are activated by depolarization and which normally conduct an outward, hyperpolarizing membrane current. The $K_{IR}$ channels conduct inward current at membrane potentials negative to the $K^+$ equilibrium potential, and their activity depends on membrane activity as well as extracellular $K^+$ concentration (8). It is possible that they mediate vasodilation in response to elevations of extracellular $K^+$ (143). $K_{IR}$ are named from the steep inward rectification of their current–voltage relationship; they conduct inward $K^+$ much more readily than outward current (60). Since the $K_{IR}$ channel is increasingly active at negative membrane potentials, it may regulate membrane potentials in the absence of increasing pressure changes or constrictor influences which tend to depolarize the membrane potential. In nonarterial smooth muscle, the $K_{IR}$ channel prevents membrane hyperpolarization to values more negative than the $K^+$ equilibrium potential and reduces cellular $K^+$ loss and energy expenditure during sustained membrane depolarization. The membrane potential of arterial smooth muscle cells at <20 mmHg *in vitro* is in the −75 to −60 mV range. The function of the $K_{IR}$ channel in arterial smooth muscle is still not well understood (8).

#### *6. Potassium Channels and Vasospasm*

Cerebral vascular smooth muscle is reportedly depolarized after SAH, possibly indicating inactivation of channels. $K^+$ channel openers may act to release VSP (147–152).

Cortical $K^+$ was measured by an inserted microelectrode in a monkey SAH model. As blood flow decreased, the extracellular $K^+$ increased from 2.9 to 18.3 m*M* acutely and was still >10 m*M* 2 hr later. Such a change in $K^+$ across the arterial wall would impede $K^+$ efflux (153).

## X. Acidosis and Hypoxia

### A. Acidosis

A decrease in pH (extracellular acidification) due to hypercapnia can cause relaxation in many vascular beds. Cerebral arteries are particularly sensitive to hypercapnia. Hyperpolarization of the smooth muscle cell membrane increases $K^+$ permeability (60). $K^+$ efflux results in relaxation. There are differences in the response of large arteries of different vascular beds in response to altered pH due to differences in intracellular buffering power and sacrolemmal pH-regulating mechanisms (154). The roles of $[Ca^{2+}]i$ and membrane potential in the relaxation due to hypercapnia or normocapnic acidosis remain to be clarified. Both result in reduce myogenic tone. A decrease in $[Ca^{2+}]i$ in isobaric (constant transmural pressure) relaxation seems more likely that a consistent membrane potential shift. A direct effect of $CO_2$ is possible (155).

### B. Hypoxia

Hypoxia may cause vasodilation by a direct effect of $O_2$ on smooth muscle cells, hypoxia-induced release of vasodilators, or accumulation of metabolites such as adenosine and protons. An important component of hypoxic vasodilation is the activation of $K_{ATP}$ channels through the release of vasodilators from endothelial cells and surrounding tissues as well as the direct effect of hypoxia on the smooth muscle cell (60). On the other hand, endothelium-dependent relaxation to thrombin, substance P, and ACh is severely attenuated in porcine coronary arteries under hypoxic conditions. Relaxation to SNP, which is endothelium independent, is unimpaired by hypoxia. The internal pH of these endothelial cells increased in hypoxia, whereas the internal pH of smooth muscle cells did not (156). In contrast to pulmonary arteries, hypoxia causes systemic arteries to vasodilate. The responses of arteries to $O_2$ tension also depend on the specific site and stage of development (157).

Contraction of bovine cerebral arteries to whole blood or 5-HT was significantly depressed under hypoxic conditions (158). In an acute *in vitro* experiment on canine basilar arteries the relative tension developed to $10^{-5}$ M Hb was increased almost five-fold when the gas mixture was changed from 95% $O_2$–5% $CO_2$ to 95% $N_2$–5% $CO_2$ for about 10 min. There was less of an increase for $PGF_{2\alpha}$ and KCl. Endothelial denudation did not affect hypoxia effects (159). The difference in these results may reflect the duration and method of inducing hypoxia. Hypoxia induced contractions in dog basilar arteries were dependent on intact endothelium in both control and SAH animals (129) (Fig. 7.9).

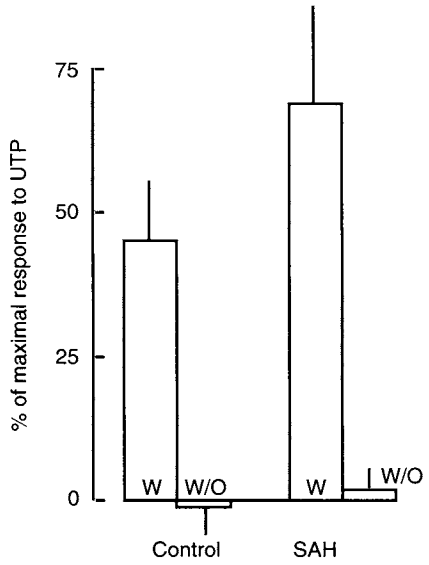



**FIGURE 7.9** Responses of the basilar arteries to hypoxia. In rings with endothelium (W), changing the gas mixture from 95% $O_2$/5% $CO_2$ to 95% $N_2$/5% $CO_2$ caused contractions which were not observed in rings without endothelium (W/O). The contractions, were maintained in the subarachnoid hemorrhage (SAH) group. Data shown are mean ± standard error of the mean for eight animals. UTP, uridine triphosphate [reproduced with permission from Kim, P., Sundt, T. M., Jr., and Vanhoutte, P. M. (1988). Alterations in endothelium-dependent responsiveness of the canine basilar artery after subarachnoid hemorrhage. *J. Neurosurg.* **69,** 239–246].

## XI. Growth and Contraction

Smooth muscle differs from cardiac and skeletal muscle because it exists in two phenotypes: contractile and synthetic or proliferative. The two phenotypes, in addition to differing markedly in their contractile activity, express different isoforms of contractile proteins and soluble enzymes. MAPK may induce CaD phosphorylation and lead directly to altered actomyosin activity. CaD may produce this effect by itself or in concert with CaP. Phosphorylation of CaD may alter the dynamics of actin filament organization. The cellular cytoskeleton may be altered during prolonged contraction by CaD phosphorylation. MAPK may be involved in both contractile and proliferative phenotypes of vascular smooth muscle (136). VSMCs dispersed in culture rapidly change from a contractile to a synthetic phenotype. This limits long-term *in vitro* studies. When smooth muscle is cultured with fetal calf serum, contractility decreases, ultrastructural changes occur, and there is diminished expression of plasmalemmal L-type $Ca^{2+}$ channels and increased release of $Ca^{2+}$ from SR via ryanodine receptors. Vascular segments or smooth muscle strips in organ culture can maintain contractility for up to a week, permitting long-term studies of protein expression or metabolism. Smooth muscle adapts to increases in functional load *in vivo* by increasing its synthesis of contractile proteins (hypertrophy) and by increased cell number (hyperplasia) (160).

## XII. Metabolism

### A. General

The metabolic activity of a muscle can change by several orders of magnitude in a fraction of a second. The signal for this is a change in $Ca^{2+}$ concentration. The importance of $Ca^{2+}$ as a chemical messenger was first identified in muscle and led to the discovery of a whole family of $Ca^{2+}$-binding proteins whose distribution is ubiquitous. All muscle contractions involve the interaction between actin- and myosin-containing filaments fueled by the hydrolysis of ATP. The filaments differ in

their arrangements and protein isoform. Muscles also generate ATP by different metabolic reactions depending on their type (3). Myosin is an ATPase. ATP and $H_2O$ reversibly change to ADP and $P_i$ and $H^+$. This reaction is the immediate source of free energy that drives muscle contraction.

Cross-bridge cycling in smooth muscle increases ATP consumption. The energetics of smooth muscle are quantitatively different from those of skeletal muscle because of very slow detachment rates of phosphorylated smooth muscle myosin as well as further slowing of cycling rates due to $[Ca^{2+}]$i-dependent phosphorylation rates. Detachment is the rate-limiting step in cross-bridge cycling. In smooth muscle almost all active cross-bridges generate force. Covalent regulation allows eight cross-bridge states in smooth muscle. Phosphorylation is obligatory for attachment. Phosphorylated cross-bridges cycle comparatively rapidly. MLCP is constitutively active and slows cycling rates. Phosphorylation rates are determined by $[Ca^{2+}]$i. ATP is required for both regulation and cycling. During contraction the metabolic needs are met by oxidative phosphorylation. Aerobic glycolysis with lactate production normally supports membrane ion pumps (2).

The energy for smooth muscle contraction comes from the hydrolysis of ATP. The phosphagen ATP and phosphocreatine content of smooth muscle is small compared to the energy demands of the tissue. There is probably a tight linkage between the rate of high-energy phosphate utilization and the resynthesis of high-energy phosphate by oxidative phosphorylation and glycolysis (161).The coupling of the energy use and supply is a necessity. Vascular smooth muscle has a relatively low level of phosphocreatine—about the same amount as that of ATP (1–3 μmol/g). Contractile activity in tonic vascular smooth muscle is associated with a two- or three-fold increase in ATP utilization and no change in ATP + phosphocreatine content because the rate of ATP breakdown is matched by the rate of aerobic synthesis. Tonic vascular smooth muscle could not attain peak isometric contraction if solely dependent on preformed high-energy phosphagen. Unstimulated vascular smooth muscle consumes ATP at 0.5–1 μmol/min/g. If it is stimulated to contract, consumption rates triple initially and then decrease to a steady rate of twice the unstimulated rate. An unusual aspect of smooth muscle metabolism is the production of a significant amount of lactate even under aerobic conditions. The rate of oxygen consumption is nearly universally correlated with the level of isometric force, whereas aerobic lactate production correlates more with the $Na^+$ pump. In smooth muscle, normal ionic gradients can be maintained by oxidative metabolism in the absence of glucose or by glycolysis in the absence of $O_2$. With adequate $O_2$ and glucose, aerobic glycolysis with lactate production is the source of ATP for membrane pump function (162).

### B. Arterial Metabolism after Experimental Subarachnoid Hemorrhage

In the initial development of VSP it seems probable that increased metabolic activity within the vessel wall would take place to support the active contraction. This could occur over minutes or hours. The long, maintained, chronic VSP lasting days is probably associated with decreased metabolic activity and a latch-like state.

Rabbit basilar arteries 2 days post-SAH showed a significant decrease in ATP which was not increased by hypoxia. More *l*-lactate was released from SAH-exposed arteries than control ones and the amount was increased by hypoxia (163). Canine basilar arteries were studied periodically for up to 14 days post-SAH. VSP was most severe at days 5–7 and partially resolved at day 14. The content of high-energy phosphates declined rapidly over the course of the study and a significant reduction in phosphocreatine was observed in the day 5 and 7 groups. The decrement of GTP was completed in the early phase and there was no recovery thereafter and no recovery through day 14. The total adenylate (ATP + ADP + AMP) and total creatine (creatine + phosphocreatine) diminished markedly over the course of the study (164).

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CHAPTER 8

# MEDICAL ASPECTS OF VASOSPASM



## I. Introduction

A clearer picture of the nature and importance of "vasospasm" has evolved over the past several decades. It is now well recognized that angiographic VSP may have no or profound effects on cerebral circulation depending on other anatomical or physiological factors. It is also clear that infarction, which is a pathologic basis for the clinically observed delayed ischemic deficits (DIDs), does not always follow angiographic VSP even when the latter is of a severe degree. There are a myriad of etiologies for infarction following aneurysmal rupture. It is not arguable that operative factors such as excessive retraction pressure, sacrifice of major veins, and the spilling of additional subarachnoid blood, as well as damage to small perforating vessels, can all have an additive effect to the ischemia resulting from long-lasting, large arterial vasoconstriction.

Vasospasm is only one of the factors contributing to adverse outcome. At the time of aneurysmal rupture, a veritable Pandora's box of adverse pathophysiological events is opened. The possible initial massive intracranial hypertension, anoxia from apneic interval, and hypotension from cardiac arrhythmia must all be factored into the additional damage done some days later when secondary

VSP becomes established. The extent of bleeding within the brain parenchyma and ventricles is at least as important in deciding whether infarction will occur with attendant permanent disability or death. Infarction develops when a region of the brain is subjected to a critically reduced blood flow for a sufficient time. To avoid infarction, the blood flowing to the brain must be adequately oxygenated, the hematocrit must be sufficient, and the cardiac function must be able to provide a safe pressure and flow. Intracranial pressure must not be sufficiently elevated to interfere with blood flow. Obviously, flow is reduced in older patients as a consequence of the abnormal physiology surrounding the ictus and as a result of prexisting medical conditions such as hypertension (1).

Perhaps the most significant advance in the medical management of patients following aneurysmal rupture was the recognition that previous practices were in fact contributing iatrogenically to the apparent significance of VSP as a poor prognostic factor. These practices included delaying clipping of aneurysms in a period of approximately 7–10 days post-SAH, using antifibrinolytics, employing deliberate profound hypotension, and keeping the patients "dry." It is also possible that we have gone through a phase of excessive enthusiasm for hypertension, hemodilution, and hypervolemia and pulmonary artery catheterization.

We think it likely that prophylactic measures aimed at removing the blood clot intraoperatively by suctioning and irrigation and postoperatively by instillation of fibrinolytic agents and cerebrospinal fluid (CSF) drainage have substantially reduced the risk of angiographically demonstrated VSP. Pharmacological clot dissolution may also prove compatible with endovascular techniques. Whether or not any drug is truly effective in preventing vascular smooth muscle constriction in those vessels surrounded by subarachnoid clot or in protecting the brain fed distally by such arteries is currently moot. With the rate of expansion of basic knowledge, it seems likely that such pharmacological approaches will ultimately prove useful. With all these caveats it remains true that VSP is a sufficient if not the sole cause of infarction post-SAH and it may result in permanent disability or death. The aim of medical management of patients with aneurysmal SAH is to preserve residual brain function and prevent neurological and systemic complications (2).

Patients with SAH frequently have serious preexisting medical illnesses (Table 8.1). In 2265 patients with ruptured aneurysms, the most common preoperative medical complications developed in the respiratory tract; pneumonia, 3%; atelectasis, 1%; asthma, 1%; pulmonary edema, 1%; and embolism, 2% (3). These complications were more common during the postoperative period. Hyponatremia occurred in up to 2% of patients preoperatively and slightly more often after clipping of the aneurysm. Diabetes insipidus occurred in about 2%. Gastrointestinal hemorrhage occurred in 1% of patients, hepatic failure in 0.4%, and hepatitis in 0.1%. All these complications were more than twice as common in the postoperative period. Renal failure occurred in about 1% of patients throughout the period of nonoperative care. Hematological complications preoperatively include anemia in 2% and bleeding disorders in 0.4%.

**TABLE 8.1 Medical Conditions Present before SAH ($N = 3521$)[a]**

| Medical problem | Percentage of patients |
|---|---|
| Hypertension | 21.2 |
| Diabetes mellitus | 2.0 |
| Asthma | 1.3 |
| Chronic lung disease | 1.3 |
| Arrhythmia | 1.2 |
| Gastrointestinal bleeding | 1.0 |
| Angina | 1.0 |
| Cardiac failure | 1.0 |
| Myocardial infarction | 0.8 |
| Anemia | 0.6 |
| Renal failure | 0.6 |
| Cerebral ischemia | 0.6 |
| Chronic liver disease | 0.4 |
| Hepatitis | 0.4 |
| Cerebral hemorrhage | 0.3 |
| Bleeding disorder | 0.1 |

[a]Reproduced with permission from Kassell, N. F., Torner, J. C., Haley, E. C. (1990). The International Cooperative Study on the timing of aneurysm surgery: Part 1. Overall management results. *J. Neurosurg.* **73,** 18–36.

A list of the medical illnesses developing during the hospitalization is given in (Table 8.2). The management of a particular phase of the patient's illness post-SAH should be appropriate to the time that has transpired since SAH and the relative likelihood of the different complications. The process is dynamic. The patient with DID from VSP requires even greater attention to medical detail in the acute period of the illness (4) (Table 8.3). The most important aspect of the management of patients with aneurysmal SAH is the avoidance of conditions that can increase the risk of VSP and/or cause it to become symptomatic. These iatrogenic factors include prolongation of the clot's existence by antifibrinolytic drugs, hypotension, inappropriate treatment of hyponatremia, hypovolemia, hyperthermia and increased intracranial pressure (5).

**TABLE 8.2 Medical Problems among 3521 SAH Patients during Hospitalization[a]**

| Medical problem | Percentage of patients |
|---|---|
| Hypertension | 18.3 |
| Pneumonia | 7.0 |
| Anemia | 4.9 |
| Gastrointestinal bleeding | 3.7 |
| Arrhythmia | 3.6 |
| Hyponatremia | 3.6 |
| Hypotension | 3.0 |
| Atelectasis | 2.3 |
| Diabetes mellitus | 2.2 |
| Adult respiratory distress syndrome | 2.0 |
| Cardiac failure | 2.0 |
| Hepatic failure | 1.9 |
| Pulmonary edema | 1.7 |
| Thrombophlebitis | 1.4 |
| Renal failure | 1.4 |
| Asthma | 1.2 |
| Hepatitis | 1.1 |
| Bleeding disorder | 1.0 |
| Pulmonary embolism | 0.8 |
| Myocardial infarct | 0.7 |
| Angina | 0.6 |

[a]Modified with permission from Kassell, N. F., Torner, J. C., and Haley, E. C. (1990). The International Cooperative Study on the timing of aneurysm surgery: Part 1. Overall management results. *J. Neurosurg.* **73,** 18–36.

**TABLE 8.3 Most Likely Complications by Time Interval after Aneurysmal Subarachnoid Hemorrhage[a]**

| Interval after hemorrhage (days) | Major complications |
|---|---|
| 0–3 | Brain edema and shift |
| | Rebleed |
| | Acute hydrocephalus |
| | Cardiac dysrhythmias |
| | Respiratory pattern abnormalities and/or arrest, pulmonary edema |
| 4–14 | Cerebral vasospasm |
| | Rebleed |
| | Hypovolemia |
| | Hyponatremia |
| | Subacute hydrocephalus |
| | Pneumonia |
| >15 | Chronic hydrocephalus |
| | Pneumonia, pulmonary emboli |
| | Rebleed |
| | Water and electrolyte disturbances |

[a]Reproduced with permission from Espinosa, F., Weir, B., and Noseworthy, T. (1990). Nonoperative treatment of subarachnoid hemorrhage. In *Neurological Surgery. A Comprehensive Reference Guide to Diagnosis and Management of Neurosurgical Problems* (J. R. Youngman, Ed.). Saunders, Philadelphia.

Four hundred and fifty-seven patients in the placebo group of a nicardipine study were admitted to the hospital within 7 days of SAH, most in the first 3 days. Symptomatic VSP was diagnosed in 46%. Seven percent rebled and the total mortality rate at 3 months was 19%. The frequency of at least one severe (life-threatening) medical complication was 40%. The proportion of deaths from medical complications was 23%. Medical complications now rank with direct effects of initial hemorrhage (19%), rebleeding (22%), and VSP (23%) as causes of death. Severe pulmonary edema occurred in 6% of patients. This complication occurs with greatest frequency on days 3–7. Pulmonary edema was nonsignificantly associated with the use of hypertensive hypervolemic therapy ($p = 0.10$) and had a significant association with the timing of surgery ($p < 0.05$). Thromobocytopenia occurred in 4% of patients, most of whom were septic. Pulmonary complications were the most common nonneurologic cause of death. The frequency of cardiac arrhythmia and pulmonary edema increases on the day of, or day after aneurysm surgery (6).

The Stroke Council of the American Heart Association delegated a writing committee to develop guidelines for the management of aneurysmal SAH. They recommended oral nimodipine (level of evidence II or III, grade A). Other calcium antagonists were believed to be of uncertain value. Hypertension/ hypervolemia/ hemodilution was recommended at a level of evidence III or IV, grade C. Clinical trials were recommended to further document the efficacy of this therapy. Transluminal angioplasty was recommended for the treatment of VSP in patients in whom conventional therapy failed (level of evidence IV or V, grade C). Other studies were recommended for intracisternal fibrinolysis, antioxidants, and anti-inflammatory agents. This report was made in 1994 (Table 8.4).

## II. Diagnosis

### A. Symptoms

The diagnosis of DID is based on sequential neurological examination by neurosurgical nurses who record

**TABLE 8.4 Levels of Evidence and Grading of Recommendations for Treatment of Patients with Subarachnoid Hemorrhage[a]**

**Levels of evidence**

*Level I:* Data from randomized trials with low false-positive (alpha) and low false-negative (beta) errors

*Level II:* Data from randomized trials with high false-positive (alpha) and high false-negative (beta) errors

*Level III:* Data from nonrandomized concurrent cohort studies

*Level IV:* Data from nonrandomized cohort studies using historical controls

**Strength of cumulative data**

*Grade A* Supported by level I evidence

*Grade B* Supported by level II evidence

*Grade C* Supported by level III or IV evidence

[a]From Mayberg, M. R., Batjer, H., Dacey, R., Diringer, M., Haley, C., Heros, R. C., Sternau, L. L., Torner, J., Adams, H. P., Feinberg, W., and Thies, W. (1994). Guidelines for the management of aneurysmal subarachnoid hemorrhage. A statement for health care professionals from a special writing group of the Stroke Council, American Heart Association. *Stroke* **25,** 2315–2328.

these observations on intensive care unit flow sheets. The data recorded, at a minimum, include systolic and diastolic blood pressure, heart rate, Glasgow Coma Scale (GCS), pupillary responses, strength in all limbs, and temperature. For patients at high risk, daily electrolyte and hematological indices should be recorded, and in patients who are deteriorating consideration should be given to the insertion of a ventricular catheter, a Swan Ganz catheter, and a bladder catheter to monitor appropriate physiological functions. Some causes of secondary deterioration are difficult to diagnose and may escape even the most astute clinician, including status epilepticus in a paralyzed ventilated patient, unusual drug reactions in patients frequently receiving many medications, and rare endocrinopathies or vitamin deficiencies (8). It is vital that patients be carefully observed for new onset neurological deficits, particularly in the period between the third and 14th days after SAH. The symptoms and signs of DID are myriad. The most common and important of these are diminishing level of consciousness or agitated delirium. New onset weakness and speech difficulties are readily observable if present. There is a tendency for patients to complain of increasing headache. They may evident hypertension and fever. Hyponatremia is frequently associated (1). Clinical features of VSP are unpredictable and not entirely dependent on the severity and extent of arterial narrowing. The volume and rate of the initial SAH with consequent cerebral ischemia and anoxia, the age of the brain, the presence of associated illnesses particularly chronic hypertension, the extent of collateral circulation, and the presence of small vessel disease within the brain are all modifying factors which relate to DID. Many patients with VSP remain asymptomatic. It has been estimated that progression to permanent cerebral infarction occurs in only half of untreated DID cases (8).

The initial and main symptom of VSP is headache. It is usually manifest as an increase in headache which had been previously improving since its onset as an ictus (9). Usually, the headache is described as diffuse rather than lateralized. There may also be a slight increase in discomfort from neck stiffness (10). Symptomatic VSP develops gradually over hours or days. Hyponatremia and hypovolemia may precede or accompany the ischemic symptoms (9). VSP is commonly manifest by a decrease in consciousness and by fever followed by focal symptoms and signs in the distribution of the constricted vessels (11–13). The deficit may remain unchanged or resolved within days or progress to permanent disability or death (11,14,15). Rarely, a patient will present with the symptoms of DID and the initial symptoms surrounding the SAH will be forgotten or unstated. If the subarachnoid clot has been absorbed, such patients are at risk of being misdiagnosed as having primary ischemic strokes and treated with thrombolytics or anticoagulant therapy (16). The differential diagnosis of focal or global neurological decline in patients with aneurysmal SAH is broad and it is often due to multiple causes (Table 8.5) (5).

### B. Signs

When the entire middle cerebral artery (MCA) territory becomes ischemic there is usually a hemiplegia with face and upper and lower limbs being equally affected. Head and eyes may deviate toward the side of the lesion. Hemianopia may develop. In the dominant hemisphere there is global aphasia. Neglect of the contralateral space is more common in the nondominant hemisphere. If the upper trunk is selectively involved, hemiparesis may be present, with face and arm being more involved than the leg. In the dominant hemisphere there is expressive aphasia and in the nondominant hemisphere neglect. For inferior trunk the weakness and sensory changes are less marked and the chance of the hemianopia or upper quadrantanopia is greater. In the nondominant hemisphere there can be constructional apraxia and delerium (17). The symptoms of anterior cerebral artery occlusion include sensory motor disturbances of the leg and foot and loss of control of urinary bladder manifest as urgency and incontinence. Abulia (reduced responsiveness and lack of spontaneity in an otherwise alert patient) is characteristic of bifrontal dysfunction. Lack of responsiveness to verbal and other

**TABLE 8.5 The Differential Diagnosis of Complications Following SAH[a]**

| Etiology | Diagnostic tests |
|---|---|
| Neurological complications | Physical examination in all |
| Vasospasm | Transcranial Doppler, ultrasound, angiography |
| Aneurysm bleeding | CT scan |
| Hydrocephalus | CT scan |
| Seizures | Electroencephalogram |
| Brain swelling | CT scan |
| Arterial thromboembolism | CT scan, angiogram |
| Complications of aneurysm surgery | Physical examination in all |
| Intracerebral, subdural, epidural hemorrhage | CT scan |
| Perforator injury | CT scan |
| Major arterial or venous occlusion | Angiogram |
| Intracranial infection | Culture of cerebrospinal or wound fluid |
| Systemic complications | Physical examination in all |
| Hyponatremia, hypoglycemia | Serum electrolytes and glucose |
| Hypoxemia, hypercarbia | Arterial blood gases |
| Infection | White blood count and different cultures |
| Endogenous toxins | Blood tests for renal and hepatic failure |
| Exogenous toxins | Assess medication records |

[a] Modified with permission from Macdonald, R. (1997). Cerebral vasospasm. In *Primer on Cerebrovascular Diseases* (K. M. A. Welch, M. R. Caplan, D. J. Reis, B. K. Siesje, and B. Weir, Eds.), pp. 490–497. Academic Press, San Diego.

stimuli is common. Speech may be soft and whispering. If Heubner's artery is in spasm there may be dysarthria as well as behavioral and cognitive disturbances (17). Anterior choroidal artery symptomotology includes hemiparesis, hemisensory loss, and homonymous hemianopia (17). Only rarely is the deficit apoplectic at onset or 13 days or more after SAH (5). Coma is often associated with bilateral spasm (9). The deficits seen from VSP mimic those from ischemic stroke in the same arteries. Unilateral anterior cerebral artery spasm can cause contralateral lower extremity weakness. Bilateral anterior cerebral artery spasm can be associated with akinesia, mutism, and incontinence, and frontal release signs may be added to the lower limb diplegia. Posterior cerebral artery spasm can be associated with obtundation. Visual field defects may occur, but cranial nerve palsies are distinctly uncommon (10).

Posterior circulation ischemia is suggested by coma, dizziness, vertigo, ocular and pupillary abnormalities, hemifacial paresis, bilateral motor weakness, ataxia, dysarthria, dysphagia, and crossed sensory loss (face and contralateral limbs). A basilar artery stroke may be associated with altered consciousness, altered sleep–wake cycle, impairment of gaze, diplopia, pupillary abnormalities, confusion, memory loss, delirium, and visual field defects (18).

Neurological physical examination may reveal fever and tachycardia. Low-grade fever is generally in the 38 to 39°C range. It may precede the development of neurological signs by several days (9). Clinical VSP was almost twice as likely to occur in patients with moderate fever compared to those without (19,20). In 262 patients post-SAH temperatures were initially normal and then rose to a plateau of 38 to 39°C. Of febrile patients, 85% developed clinical or radiologic signs of VSP or both. The onset of fever was simultaneous with the advent of headache and meningismus followed after a few days with clinical or angiographic signs of VSP. The resolution of fever preceded the resolution of angiographic VSP (13).

### C. Laboratory Findings

Patients who subsequently developed VSP presented with initially higher neutrophil counts (8500 vs 7000) and the development of VSP was accompanied by a rise in the neutrophil count to an average of 13,000 (21). Other studies have also noted a trend toward higher white blood cell (WBC) counts in patients who subsequently developed VSP (20,22). Admission blood glucose levels >120 mg/dl were associated with good recovery in only half the patients. If normal glucose levels were present on admission, 70% of patients had good recovery rates. Death rates of hyperglycemic patients were three times higher than those of normoglycemic patients (23).

The definitive diagnosis of symptomatic VSP (DID) is made by the concurrent angiographic demonstration of narrowing of intracranial arteries which were previously of larger caliber in association with neurological deficit attributable to a decrease in blood flow through the demonstrable narrowed arteries. The diagnosis of DID is frequently inferred by the exclusion of other causes of delayed deterioration. The angiographic demonstration of VSP is essential prior to the institution of balloon angioplasty and/or intraarterial papaverine (5).

Intracerebral microdialysis (MD) catheters are available for human use. They are usually placed at the time of operation in the region considered at highest risk of vasospastic ischemia. Limitations include the restricted sampling site, time delay in performing some of the biochemical tests, extra trauma to the brain, and potential

for infection. The benefits may be a definitive biochemical test for VSP, possible early warning of impending ischemic infarction, an objective basis to evaluate therapeutic maneuvers, and deeper insight into the pathophysiology.

In 7 patients MD monitoring commenced before symptoms of VSP, extracellular glucose decreased to 0.56 m$M$, lactate/glucose ratio increased to 8.46, and glycerol increased to 210 $\mu M$. A decrease in glucose to $< 1\ \mu M$ or an increase in lactate/glucose $> 3$ were very highly specific for symptomatic VSP; glycerol $> 100\ \mu M$ was less so (24). In patients with DID the MD values became secondarily pathological at varying times. Lactate increased from 3.5 to 5 $\mu M$. Glutamate was generally high (13.6 $\mu M$) during days 6–8 post-SAH (25). Increased lactate production and a decreased glucose/lactate ratio <1.0 correlated with Xe–CT data. Glutamate did not consistently reflect ischemia unless accompanied by increased lactate (26). NO in dialysate can differ from ventricular CSF levels and correlate better with brain tissue $O_2$. Levels of NO and $O_2$ presumably reflect $O_2$ delivery to the brain (27).

Lactate and glutamate seemed to be sensitive markers of impending ischemia, and increased glycerol levels were associated with severe ischemic deficits in 10 patients having MD catheters for 4–11 days (28).

## III. Differential Diagnosis

Estimates from the literature of the frequency of all types of complications are given in Table 8.6.

### A. Respiratory Complications

Approximately 8% of all patients with SAH developed pneumonia during their hospital stay. Decreased level of consciousness, immobility, invasive procedures and devices, diminished immunity, and colonization with hospital-acquired bacteria account for this common complication. For poor-grade patients particularly aggressive chest physiotherapy should be instituted early. Narrow-spectrum antibiotics should be used judiciously and in response to positive cultures. Early and adequate nutrition should be guaranteed and environmental sterility should be a goal (15). Mechanical ventilation may be associated with an increase in nosocomial pneumonia, barotrauma to the lung, or aggravation of intracranial hypertension. Impending cerebral infarction will obviously be aggravated by hypoxia resulting from pneumonia, aspiration, cardiogenic or noncardiogenic pulmonary edema, retained secretions, and lobar collapse from hypoventilation. Hypoxemia may be severe if a shunt is present in segments of the lung which are perfused but not ventilated. This complication requires the employment of positive-end expiratory pressure by mechanical ventilation (15). The reverse problem can result from pulmonary emboli causing ventilated but not perfused portions of the lung. All these respiratory complications must be watched for and treated aggressively in patients at risk from DID (4). In 254 patients studied post-SAH, 17% had loss of consciousness and cardiore-

**TABLE 8.6 Occurrence of Various Medical And Neurological Complications of Subarachnoid Hemorrhage[a]**

| Complication | Occurrence (%) |
|---|---|
| Fluid/electrolyte | 28–50 |
| Hyponatremia | 10–34 |
| Hypovolemia | 50 |
| Cardiovascular | 50–100 |
| Electrocardiographic alterations/arrhythmias | 50–100 |
| Wall motion abnormalities | 10–50 |
| Pulmonary | 15–25 |
| Pneumonia | 22 |
| Pulmonary edema | 23 |
| Thromboembolic | 2–15 |
| Deep venous thrombosis | 2–14 |
| Pulmonary embolism | 1–2 |
| Other medical | |
| Hepatic dysfunction | 2–24 |
| Renal dysfunction | 3–7 |
| Gastrointestinal bleediing | 0–4 |
| Secondary cerebral insults | >50 |
| Hypotension | 18–21 |
| Hypoxemia | 22–43 |
| Hyperglycemia | 21–46 |
| Increased intracranial pressure (beyond subarachnoid hemorrhage ictus) | 24–33 |
| Hydrocephalus | 6–67 |
| Seizure | |
| Initial | 4–25 |
| Early (<2 weeks) | 1.5–5.0 |
| Late | 1–3 |
| Epilepsy | 1–3 |
| Rebleeding (<30 days) | 7–19 |
| Vasospasm (symptomatic) | 28–46 |

[a]From McKhann, G. M., and LeRoux, P. D. (1998). Perioperative and intensive care unit care of patients with aneurysmal subarachnoid hemorrhage. *Neurosurg. Clin. North Am.* **9**, 595–613.

spiratory disorders. Thirty-seven percent of the patients who had respiratory arrests or ventricular fibrillation recovered with resuscitation (29).

### B. Electrolyte Disorders

Marked hyponatremia can cause disturbances of consciousness, seizures, and aggravate any concurrent neurological deficits from VSP. Cerebral salt wasting was first described in 1950 by Peters. Normally, serum $Na^+$ accounts for virtually all of the osmotically active solute in the extracellular fluid. Control of osmolality is synonymous with control of $Na^+$ concentration (30). Hemodynamic signs of hypovolemic hyponatremia are postural hypotension and postural accentuation of supine tachycardia (30,31). Hyponatremia can result from gastrointestinal losses, cerebral salt wasting, and from iatrogenic reasons including the use of diuretics, hypotonic intravenous solutions, fluid restrictions, inadequate fluid replacement, and blood loss due to laboratory investigations or operatively (4).

There were 88 instances of fluid and electrolyte disturbances in 1000 patients post-SAH who were operated on and reported on in 1979. Hyponatremia was the most common abnormality, occurring in 53%, and was associated with a mortality rate of 15%. The mortality rate of hypernatremia was 42% (32). In the same year, a series of 420 patients with delayed deterioration following aneurysmal SAH was reported. The causes were VSP (30%), Hyc (14%), hyponatremia and volume depletion (18%), and recurrent SAH or other intracranial bleeding (6%). Less common causes included aseptic meningitis, vascular occlusion, brain swelling, hypoxia, seizures, myocardial infarction, and pulmonary embolus (33).

In 134 SAH patients, 44 had a $Na^+$ <135 mmol/liter for at least 2 consecutive days between days 2 and 10 post-SAH. In 25 of the patients, criteria for the syndrome of inappropriate secretion of antidiuretic hormone (ADH) were fulfilled. Cerebral infarction developed in 61% of the patients with hyponatremia but in only 21% of those with normal serum $Na^+$ levels. Twenty-six of the 44 patients were treated with fluid restriction to correct low $Na^+$ levels for what appeared to be inappropriate secretion of ADH. It is likely that these patients were actually suffering from cerebral salt wasting, in which case fluid restriction is potentially dangerous and the correct therapy would be $Na^+$ and water replacement (34). Patients with the syndrome of inappropriate secretion of ADH and those with cerebral salt wasting syndrome both show low serum and elevated urinary $Na^+$ of >25 mmol/liter. However, because the patients with inappropriate secretion of ADH have retained $H_2O$, which explains the dilutionally lowered serum $Na^+$, they have normal blood volumes and appear well hydrated with normal blood pressures and normal or slow heart rates. Patients with cerebral salt wasting tend to have a decreased blood volume and appear dehydrated. Their body weight decreases. They may show a postural hypotension and postural tachycardia. They may also show raised blood urea nitrogen, whereas patients with inappropriate ADH would show a normal or decreased value (35).

Hyponatremia may be associated with anorexia, nausea, vomiting, irritability, personality changes, areflexia, weakness, and a decreased level of consciousness which can proceed to coma and convulsions. We attempt to maintain salt intake and to treat significant hyponatremia with saline solutions (250–500 ml of 3–5% saline infused over 48 hr) and sometimes furosemide (Lasix) in doses of up to 1 mg/kg. We believe that hyponatremia was mistakenly attributed to inappropriate ADH secretion in many instances in the past. This led to harmful institution of fluid restriction. Since the time course of hyponatremia can overlap that for the incidence of DID, this dehydration probably aggravated the ischemia due to VSP (4).

Nineteen patients were studied within 3 days after aneurysmal SAH. Thirty-two percent developed hyponatremia, but only 2 had a negative sodium balance. In most patients, the level of atrial natriuretic factor (ANF) was elevated while plasma renin activity and aldosterone concentrations were generally suppressed. Plasma arginine vasopressin levels were not suppressed during hypoosmolality and did not correlate with serum osmolality in hyponatremic patients. Only 1 patient had a decrease in blood volume associated with a marked rise in aldosterone and plasma renin activity but not serum $Na^+$ and plasma ANF. It was concluded that following SAH hypervolemic therapy prevents volume contraction but not hyponatremia, humoral factors favor both $Na^+$ loss and $H_2O$ retention, and arginine vasopressin regulation is disturbed and may contribute to hyponatremia (36).

Hyponatremia occurred in one-10th and one-third of patients following SAH (34,37). The majority of hyponatremia results from cerebral salt wasting, which is a consequence of excess secretion of atrial natriuretic factor (38–42) or a digoxin-like substance (43). The elevation of ANF may be associated with depressed plasma renin activity and aldosterone concentrations (36,38).

Natriuretic peptides are classified as (i) atrial, produced in the right atrium in response to hypervolemia and cardiac overload; (ii) brain, which is produced in cardiac ventricles, and (iii) C type, produced by vascular endothelium in response to inflammation or injury. Brain natriuretic peptide plasma concentrations were measured serially post-SAH. In most patients concentrations decreased progressively throughout four time periods to 10–12 days. However, in six patients in whom transcranial

Doppler (TCD) velocities were high and who showed DID and had delayed brain infarction on CT scan, the ratio of brain natriuretic peptide on days 7–9 over that on days 1–3 increased progressively and significantly. There was a sixfold increase in plasma concentration in patients with severe, symptomatic VSP (44).

### C. Infection and Fever

Fever can be evidence of hemogenic aseptic meningitis as well as true infection. It can be due to VSP and infection. Untreated, it can contribute to infarction. At least one-third of patients have fever following SAH. Acetylsalicylic acid, acetominophen, cooling blankets, and treatment of specific causes of fever are routine. Surveillance for pneumonia, wound infections, meningitis, ventriculitis, and line sepsis should be performed (45). Those caring for SAH patients must be constantly vigilant with respect to the possibility of infection. Organisms can be introduced at angiography, operation, and intubation of all types. Indwelling catheters add to the risk. Immobilization interferes with proper clearing of secretions from the respiratory, gastrointestinal, and genitourinary tracts. Additional patients are stressed by dehydration and malnutrition. Although VSP is frequently associated with systemic fever, one must never assume that the fever is entirely due to VSP and not some associated infection requiring treatment (4).

### D. Cardiac Complications

Cardiac failure can aggravate the symptoms and signs of DID. It can occur at any time in the acute period following SAH. Patients with a large volume of SAH are particularly stressed. SAH and therapeutic interventions with fluid loading, induced hypertension, and central line placements may all result in cardiac failure, arrhythmias, myocardial infarction, and hypertension or hypotension. Cardiac output is the product of heart rate and stroke volume; the latter is determined by preload, afterload, and contractility (46). Preload is the initial cardiac muscle fiber length, which is assessed by pulmonary capillary wedge pressure since this is equivalent to left ventricular end diastolic pressure (normally less than 12 mmHg). Afterload is the resistance to ventricular ejection, which is measured as total peripheral resistance (normally 900–1200 dynes/sec/cm$^{-5}$). Afterload may be reduced by vasodilators, and this is done in critically ill patients whose pulmonary capillary wedge pressure exceeds 18 mmHg and who have elevated systemic vascular resistance and reduced cardiac index. Contractility is the ability of the heart to change its contractile force and velocity independently of the fiber length. It is assessed by measuring the systolic ejection fraction and is modestly altered by drugs such as glucagon and digitalis. Of the 2265 patients with SAH studied by Torner and associates, cardiovascular problems included hypertension in 16%, arrhythmia in 2%, hypotension in 1%, cardiac failure in 1%, and myocardial infarction in 1%. Virtually any type of cardiac arrhythmia can be associated with SAH. Stroke volume and rate are important determinants of $O_2$ delivery to the brain, as is blood pressure (47). Acute stroke (thromboembolic) patients are three times as likely to have ischemic heart disease as age-matched controls with other pathology. In one study of aneurysm cases none who died post-SAH and who developed ECG changes showed autopsy evidence of coronary artery disease or acute myocardial ischemia (48). In another series of fatal cerebral infarctions, none of the patients who had ECG abnormalities showed evidence of coronary occlusion at autopsy (49). After SAH, ventricular arrhythmias are exceptionally common and correlate with prolongation of the QT interval. Post-SAH, more than half of patients have multifocal ventricular premature beats and 29% show unsustained ventricular tachycardia (50).

### E. Hypertension and Hypertensive Encephalopathy

Aside from age, hypertension is the greatest risk factor for all types of stroke. High intraluminal pressure increases the stress on the endothelium in small intracerebral vessels and can alter the blood–brain barrier (BBB) and introduce multifocal brain edema. Degenerative changes in the same vessels predispose to intracranial hemorrhage (ICH). BBB leakage in experimental animals can be produced by a variety of agents which are used to increase blood pressure therapeutically. In experimental animals there is no absolute level at which breakdown in the BBB will occur. The response varies with vascular tone, abruptness of the pressure increase, and the type of drugs employed (51). Hypertensive encephalopathy is an acute syndrome characterized by severely elevated blood pressure with rapidly progressive neurological signs and symptoms such as headache, seizures, altered mental status, and visual disturbances. It is likely that BBB disturbances and cerebral edema play a role in the development of hypertensive encephalopathy. Rapidly developing, fluctuating, or intermittent hypertension are particularly likely to cause hypertensive encephalopathy. CT scans of patients with hypertensive encephalopathy reveal hypodense regions, often symmetric and frequently in the occipital lobes. Regions of BBB breakdown may be found in gadolinium-induced focal enhancement on magnetic resonance imaging. It is a reasonable conjecture that in some patients who deteriorate on aggressive treatment an iatrogenic hypertensive encephalopathy is substituted for their initial vasospastic ischemia (52). We believe in urgently

treating elevated blood pressure prior to definite treatment of the recently ruptured aneurysm (Table 8.7) but only lower pressure at the extreme levels in the acute postoperative period when VSP is a major risk. Treatment for chronic hypertension is resumed after a couple of weeks if all has gone well. The agent(s) of choice depends on the previous medical history (Table 8.8).

### F. Seizures

In 3–5% of patients with SAH, seizures occur during hospitalization (53). The use of anticonvulsants prophylactically is common in North America and relatively rare in Europe. Ictal activity at the time of SAH is not prognostic for the subsequent development of epilepsy. Some series have reported epilepsy to develop in up to 15% of patients, particularly those with infarcts (53,54). An unobserved seizure or status epilepticus may account for a secondary deterioration not due to VSP (10). In 138 patients the interval between surgery and the onset of seizures ranged between 2 and 57 months, with a mean of 20 months (55). Seizures during the acute phase were uncommon. The value of routine anticonvulsants has been questioned (56–58). We use early anticonvulsants for patients with a history of preexisting seizures or those in a high-risk group.

### G. Rebleeding

One review listed 39 potential causes for delayed-onset neurological deficit. If the aneurysm is unclipped, clearly the principal alternative diagnosis of DID is rebleeding. Hydrocephalus may also become evident at any point in the course (33). The risk of rebleeding is highest in the hours after the first SAH and decreases progressively. The risk is about 4% in the first 24 hr and decreases quickly to about 1.5% per day in the first couple of weeks. Documented incidence of rebleeding in one series averaged 19% during the first 2 weeks, 64% by the end of the first month, and 78% by the end of the eighth week after initial SAH (47,53,59,60). Even in a patient in whom an aneurysm has been initially clipped during early operation or coiled, a secondary deterioration occurring in the time span when VSP is likely is sometimes due to rebleeding from inadequate initial treatment or the rupture of a lesion other than the treated one. When a patient has marked secondary deterioration likely due to VSP, it is essential to take immediate measures to get a CT scan to rule out rebleeding or a mass lesion such as Hyc or an intracranial clot (4).

### H. Gastrointestinal Complications

In a series of 29 fatal SAH cases, 83% showed gastroduodenal lesions at autopsy (61). The incidence of gastrointestinal hemorrhage in patients post-SAH is estimated to be 4% (15). In more than 500 patients undergoing neurosurgery, 6.8% had postoperative intestinal bleeding. Complications associated with gastrointestinal bleeding included hyponatremia, coma, higher age, other postoperative complications, and infections (62).

In patients with decreasing hematocrits, it is important to rule out sources of bleeding such as occult gastrointestinal hemorrhage (63).

**TABLE 8.7 Treatment for Hypertension after SAH before Definitive Aneurysm Ablation**

| Blood pressure | Drug of choice |
|---|---|
| When systolic blood pressure >160 mmHg and/or diastolic blood pressure >100 mmHg and patient is at risk of recurrent SAH | Sodium nitroprusside, 0.25–10μg/kg/min, or labetalol, 10–20 mg, IV every 10–20 min (maximum daily dose = 300 mg) |

**TABLE 8.8 Chronic Therapy of Hypertension: Preferred Agents According to Concurrent Disease States[a]**

| Disease state | Diuretics | β-Blockers | Calcium channel blockers | ACE inhibitors |
|---|---|---|---|---|
| Diabetes mellitus | | | Preferred agent (not nifedipine) | More preferred agent |
| Congestive heart failure | Preferred agent | | | More preferred agent |
| Previous myocardial infarct | | More preferred agent | | Preferred agent |
| Isolated systolic hypertension | More preferred agent | Preferred agent | | |

[a]Modified with permission from Fagan, S. (1997). Management of hypertension in stroke. In *Primer of Cerebrovascular Disease* (K. M. Welch, L. R. Caplan, D. J. Reis, B. K. Siesjo, and B. Weis, Eds.), pp. 687–689. Academic Press, San Diego.

### I. Endocrine

#### *1. Hypopituitarism*

Deficiencies in pituitary functions have been found with fairly high frequency when they have been searched for post-SAH. Such deficiencies are relatively more common with anterior communicating artery aneurysms and in the presence of VSP (64).

#### *2. Diabetes*

The management of the diabetic patient does not differ from that of the nondiabetic patient except for the need to avoid hyperglycemia. Blood glucose levels should be maintained between 100 and 250 mg/dl. Blood glucose tends to be elevated as a result of the metabolic stress from SAH (65). In 617 patients admitted within 3 days post-SAH, glucose levels were measured on admission and 3–7 days post-SAH. Normal glucose was defined as $\leq$120 mg/dl for those whose glucose was normal on admission, and subsequently 81% had good recovery. For those with normal values on admission but elevated subsequently, 58% had a good outcome. If the admission levels were elevated but the subsequent levels were not, 71% had a good outcome. The poorest good recovery rate was 48% for the patient in whom glucose was elevated on admission as well as subsequently. The association between hyperglycemia and poor outcome was still maintained after adjustment for age and clot thickness. In patients who had VSP, the outcome was worse in those who had elevated glucose levels between days 3 and 7 compared to those with normal glucose levels (23).

### J. Metabolic

SAH produces a nonspecific stress response. Nitrogen utilization after surgery for SAH is subnormal and there is a strongly negative nitrogen balance and a failure of exogenous amino acids to improve it (66). A catabolic response is induced by a SAH, aggravated by steroids. Parenteral nutrition is indicated in the course after SAH. Thiamine-deficient encephalopathy may develop after aneurysmal SAH if vitamin supplements are not given, particularly in alcoholic patients (67).

### K. Intracranial Hypertension and Hydrocephalus

To prevent intracranial hypertension in poor-grade patients, catheters are placed in the ventricles at the time of surgery and left in place for postoperative monitoring, instillation of tissue plasminogen activator (t-PA), and ventricular drainage when necessary. Intracranial pressure (ICP) is usually maintained below 15 mmHg. Frequently, a patient who appears to be grade IV or V will improve significantly after a few hours of ventricular drainage. Ventricular drainage can be hazardous in the presence of an unsecured, recently ruptured aneurysm but is sometimes essential. Chronic Hyc is unusual in the time frame of DID (<2 weeks).

## IV. Prophylaxis

Some aspects of the presentation of infarction after aneurysm rupture are given in Table 8.9. Some specific recommendations regarding VSP are presented in Table 8.10.

### A. Calcium Antagonists

In 1964, Fleckenstein found that the compound verapamil (subsequently called a $Ca^{2+}$ antagonist) was a coronary vasodilator (68). The intracellular free $Ca^{2+}$ concentration is maintained 10,000 times lower than the extracellular space. Intracellular $Ca^{2+}$ homeostasis is regulated by voltage-dependent $Ca^{2+}$ channels, receptor-operated $Ca^{2+}$ channels, and intrinsic mechanisms. The distinction between receptor and voltage channels is not absolute in that they influence one another. Voltage-dependent channels have been classified as transient, intermediate, and long-lasting or slow. Calcium antagonists investigated in the treatment of stroke include the dihydropyridines—nimodipine, nicardipine, nilvadipine, PY 108–068, and PN 200–110—and the diphenylalkylamines—flunarizine and cinnarizine (69).

When $O_2$ falls below 1 $\mu M$, $[Ca^{2+}]i$ increases within 30–45 sec to double its initial value and stays at this level for approximately 1 min. The plasma membrane then abruptly depolarizes, $K^+$ rapidly leaves neurons, and this is immediately followed by a massive fall in $[Ca^{2+}]e$ and a corresponding rise in $[Ca^{2+}]i$. These changes plateau within 5 min, with extracellular values of 0.2–0.5 m$M$ and highly variable regional intracellular levels. The levels of $[Ca^{2+}]i$ 8–10 min after induction of hypoxia/ischemia are unknown because of technical difficulties with measurements. Reperfusion of brain which has been ischemic for a few minutes is followed by restoration of $[Ca^{2+}]e$ levels within 30 min to near preischemic values. $[Ca^{2+}]i$ levels in neurons more than 6 hr postinsult have not been determined (70). Levels of ionized calcium in the whole blood of patients post-SAH tend to be lower in those with poor neurological grades or VSP. The average $Ca^{2+}$ value in controls were 1.23 $\pm$ 0.02 m$M$/liter (71).

#### *1. Nimodipine*

Intravenous infusions of nimodipine at rates of 2 mg/hr result in mean steady-state plasma concentrations of

**TABLE 8.9 Prevention of Cerebral Infarction after Aneurysmal Rupture[a]**

| | |
|---|---|
| Acute elevation of ICP[b] with SAH | Prevent rebleeding by early definitive surgery |
| | Evacuation of resultant discrete SDH or ICH |
| | Ventricular and/or subarachnoid drains |
| Cardiac arrhythmia | Monitoring, appropriate medication |
| Hypoxia (pulmonary edema, atelectasis, pneumonia, embolism) | Intubation, oxygenation, appropriate antibiotics, diuretics, antiembolic measures |
| Intraoperative complications (retractor injury, arterial occlusion, venous infarction) | Avoid hypotension |
| | Use temporary clips to avoid intraoperative rupture, consider cerebral protection by hypothermia (32–35°) and propofol |
| | Use ventricular drain in acute surgery to reduce brain volume at surgery and to monitor and control ICP postoperatively; preserve bridging veins if possible |
| Postoperative clots | CT scan; evacuation if necessary |
| Late elevations of ICP | Shunt chronic hydrocephalus |
| | Drain chronic subdural hematomas |
| Fever, infection | Acetylsalicylic acid or acetaminophen suppository, cooling blanket, appropriate antibiotic |
| Dehydration | Adequate fluid replacement |
| Hyponatremia | Saline, hypertonic saline |
| Anemia | Blood transfusion |
| Seizures | Anticonvulsants |
| Hypertension | Treat cautiously if at all days 4–14 post-SAH |

[a]From *Subarachnoid Hemorrhage: Causes and Cures*, p. 190, by Bryce Weir, copyright © 1998 by Oxford University Press, Inc. Used by permission of Oxford University Press, Inc.
[b]ICH, intracerebral hematoma; SDH, subdural hematoma; ICP, intracranial pressure.

**TABLE 8.10 Prevention of Vasospasm**

Prevent aneurysm formation and rupture by avoiding risk factors such as smoking, hypertension, and cocaine use

Clip appropriate symptomatic and unruptured asymptomatic aneurysms, screen for aneurysms in groups at significant risk for aneurysmal development and rupture

Diagnose and treat patients with warning leaks before they suffer catastrophic SAH

Clot removal
- Intraoperative irrigation
- Surgical clot removal
- Pharmacological removal of clot with plasminogen activator or other fibrinolytic agent(s)
- Ventricular drain to remove bloody CSF

Pharmacological prevention of vasospasm or its effects
- Calcium channel antagonists (nimodipine, nicardipine)

Avoid hypovolemia and hyponatremia, induce modest hypervolemia, increase cardiac output

Avoid antifibrinolytics

Avoid hypotension and antihypertensives during period of maximum risk of vasospasm

Avoid increased intracranial pressure, consider ventricular drainage

Treat fever

Normalize hematocrit for the individual patient

27–53 μg/liter in patients with SAH. Single oral 60-mg doses produce plasma concentrations of up to 31 μg/liter. Mean area-under-the-curve values following the administration of 60-mg oral doses range from 42 to 125 μg/liter/hr. Only a small proportion of nimodipine passes into the CSF. CSF concentrations of 0.3 μg/liter exist at plasma concentrations of 77 μg/liter in SAH patients. The incidence of hypotension in studies of patients with SAH has range from 4.7 to 8%. Usual oral treatment for adults is 60 mg every 4 hr for 21 days. Intravenous treatment is begun with infusions of 1 mg/hr and these increase to 2 mg/hr after 2 hr if blood pressure remains stable (72).

In various animal and human preparations, nimodipine produces dilatation of arterioles more than venules and larger arteries. In animal experiments and in some human studies, CBF tends to increase with intracarotid infusion. However, in several studies there was no effect on CBF. Total CBF in healthy volunteers increased after a single oral dose of 80 mg (72).

Allen suggested that cerebral arterial smooth muscle might have a greater dependency on $Ca^{2+}$ than do systemic arteries. It was suggested that the family of $Ca^{2+}$ antagonists may selectively dilate cerebral arteries or reverse VSP (73).The initial studies on nimodipine represented the first prospective, multicentered, placebo-controlled trials in neurosurgery. It is fascinating to conjecture whether or not the U.S. Food and Drug Administration would approve this medication were the same data presented to it today. There still exists some skepticism within the profession as to the efficacy of $Ca^{2+}$ antagonists (74). Currently it is almost universal practice not to continue the medication after discharge in patients who are doing well (75). In a prospective, multicentered, randomized, controlled trial of nimodipine in 125 patients with SAH who were in good grade and who had therapy started within 4 days of SAH, nimodipine was apparently effective. A neurological deficit from VSP which persisted and was severe or caused death developed in 8 of 60 patients given placebo and in only 1 of 56 patients given nimodipine ($p < 0.03$). Conclusions could not be drawn regarding the frequency and/or severity of VSP (76). In a subsequent trial of three different doses of nimodipine, only doses of 60 or 90 mg every 4 hr for 21 days were believed to be effective. About 10% of the patients had a drug-related decrease in blood pressure (77). Of the seven prospective, randomized, controlled studies of nimodipine post-SAH published between 1983 and 1988, five involved oral dosage and two intravenous dosage. There were no differences between nimodipine and the placebo groups in deficits from all causes in two of the studies, in poor outcome from all causes in three other studies, and in only a British study was poor outcome significantly reduced by nimodipine. In one early study poor outcome and death from ischemic stroke were significantly reduced by nimodipine (78). The British SAH nimodipine trial compared placebo with 60 mg oral nimodipine every 4 hr. Symptomatic VSP affected 33% of controls and 22% of treated patients. Death attributed to ischemia occurred in 25% of the controls and 17% of the treated group. Deaths were reduced by 32% and severe disability Glasgow Outcome Scale (GOS; 3–4) by 42%. Poor outcomes occurred in 33% of the placebo group and 20% of the nimodipine-treated patients (79). This trial, performed between 1985 and 1987, used as an end point infarction at 3 months, DID and outcome. Of the 1115 SAH patients admitted to the participating centers, only 554 were admitted within 4 days post-SAH. In the 130 patients in whom therapy or placebo were discontinued early in 1987, this was done because the angiogram was negative. These patients tended to be younger than those in most North American multicenter trials. Nimodipine-treated patients had a mean age of 46 compared to 48 years for the placebo group. The time from SAH to initial CT scan averaged 1.5 days for both groups. The time from SAH to angiography averaged 5.5 days for the nimodipine group and 5.1 days for the placebo group. The time from SAH to operation averaged 10.8 days for nimodipine and 11.3 days for placebo. Many of these patients were subjected to very late operation. The use of intravenous fluids was not documented in the report. Cerebral ischemia and infarction were lumped together and were defined by CT, operation, and necropsy. Deaths from this cause occurred in 6% of the nimodipine patients and 9% of the placebo-treated patients. Interestingly, death from initial bleeding or rebleeding was much more common in the placebo group (13 vs 8%). Disability attributed to cerebral ischemia and/or infarction occurred in 7% of the nimodipine patients and 12% of the placebo group. The use of nimodipine was associated with a reduction in cerebral infarction from 33 to 22%, reduction in poor outcome from 33 to 20%, and reduction of rebleeding 14 to 9%. Only 9% of the nimodipine-treated patients were grade IV or V compared to 12% in the placebo group. In a dramatic difference from the North American studies, 66% of the placebo patients were not operated, whereas in the nimodipine group 59% were not operated (79).

In the Finnish study of 213 grade I–III cases of patients having follow-up CT scans at a mean of 1.4 years, nimodipine was associated with a decrease in mortality from DID and a reduction in infarction as judged by CT scan in the whole patient population. This tended to be a young patient group with a relatively low population of females. Only 30% of the nimodipine patients and 29% of the placebo patients were operated on in the first 3 days. Mortality rate for nimodipine patients was 13% and for

the placebo patients 16%. The overall outcome did not differ significantly between the treatment groups. Although nimodipine was apparently associated with a reduction in deaths due to DID (from 59% in placebo to 8% in nimodipine), the opposite was true for death from rebleeding (24% for placebo and 69% for nimodipine) (80).

In the Canadian trial of poor-grade patients 188 were registered and 34 were excluded for various reasons. Therapy was carried out on 72 patients receiving nimodipine and 82 placebo patients. The average age of the nimodipine patients was 54 and for placebos 56 years, making this a relatively old group of patients. Females made up 63% of the nimodipine group and 71% of the placebo. Hypotension was reported as a side effect in 8% of the nimodipine versus 4% of the others. At 3 months, 47% of the nimodipine-treated patients were dead versus 39% of the placebo patients. The difference occurred mainly in grade III patients, in whom 28% of the nimodipine patients treated died versus 5% of the placebo group. Early operation was performed on 43% of the nimodipine patients as well as the placebo group. No operation was performed on 36% of the nimodipine patients and 43% of the placebo cases. Permanent deficit attributable to VSP alone occurred in 7% of the treated and 27% of the untreated patients. No DID occurred in 63% of nimodipine patients versus 44% in placebo ones. There was no significant difference in the occurrence of moderate to severe angiographic VSP after day 4 which occurred in 64% of the nimodipine-treated patients. Despite the higher death rate in the nimodipine-treated patients, good outcomes occurred in 29 versus 10% for placebo. This study suggested that nimodipine could reduce the infarction rate on angiographic VSP but not directly affect angiographic caliber. It was of concern that there was no overall reduction in mortality with treatment and that the higher death rate in the nimodipine patients might have been a factor in the improved outcome in the nimodipine patients (81).

Despite the fact that none of the prospective studies of nimodipine taken individually established the efficacy of nimodipine to a degree that would meet contemporary standards for such randomized clinical trials, a veritable cottage industry of meta-analyses was nevertheless spawned (82–84). The meta-analysis by Tettenborn and Dykca of 7 placebo-controlled trials suggested an odds ratio for poor outcome of nimodipine of 0.58 (confidence interval, 0.45–0.74) (79,81,86–89). The analysis by Barker and Ogilvy suggested that nimodipine improved all eight outcome measures that they selected. The odds of good and good plus fair outcomes were improved by ratios of 1.86:1 and 1.67:1, respectively, for nimodipine versus control ($p < 0.005$ for both measures). The odds of deficit and/or mortality attributed to VSP and CT-assessed infarction rates were reduced by ratios of 0.46:1 to 0.58:1 in the nimodipine group ($p < 0.008$ for all measures). However, there was no significant reduction in overall mortality associated with the use of nimodipine. Although the individual trials examined did not have statistically significant results at the $p < 0.01$ level according to most outcome measures, the meta-analysis confirmed the significant efficacy of prophylactic nimodipine in improving outcomes after SAH under the conditions used in these trials (83). The most recent systematic review of calcium antagonist by Feigin and colleagues analyzed 10 trials in which calcium antagonists were compared to controls and treatment was begun within 10 days post-SAH. All calcium antagonists (nimodipine, nicardipine, and AT877) were included. This review analyzed 10 trials totaling 2756 patients. The relative risk reduction for death or dependency was 16% (95% confidence interval, 6–27%) and that of case fatality was 10% (95% confidence interval, 6–25%). Calcium antagonists gave a 33% relative risk reduction in the frequency of ischemic neurologic deficits and a 20% relative of risk reduction in the frequency of CT – documented cerebral infarction. In the analysis for nimodipine alone, treatment was associated with a 24% relative risk reduction of poor outcome. The relative risk reduction for angiographically detected cerebral VSP was statistically significant for AT877 (38%; 95% confidence interval, 17–54%) and nicardipine (21%; 95% confidence interval, 6–34%) but not for nimodipine (84). In 10 trials (mostly the same) reviewed by Dorsch, the death rates for controls were 17% of 1159 compared to a 12% rate for 978 patients treated with nimodipine. The combined permanent deficit and death rate for the 1159 control patients was 28 versus 20% of 923 nimodipine-treated cases (90). DID occurred in 14% of 558 patients treated prophylactically with nimodipine. Where the drug was administered intravenously, the rate was 12 versus 20% when it was used orally (90). Nimodipine has also been used as treatment for established DID. Six studies describe 343 cases in whom the outcomes were death (13%), permanent deficits (20%), and good recoveries (67%) (90).

Mechanisms of action for nimodipine did not include significant prevention or reversal of VSP. It has therefore been hypothesized that small vessel collaterals may be opened up, neurons may be protected against ischemia, and the use of nimodipine with resultant hypotension may have necessitated a greater fluid administration.

In nine randomized and controlled studies, in which nimodipine was used as a treatment for acute ischemic stroke, only the two smallest and earliest studies suggested a favorable outcome (78). Many other potential cerebral protectant medications are under active study, so far with little success (Table 8.11). Since the publication of the

**TABLE 8.11 Cytoprotective Agents in Clinical Testing**[a]

| Agent | Clinical testing in stroke completed or in progress |
|---|---|
| Hypothermia | No human studies in stroke |
| Hyperbaric oxygen | Phase II |
| Calcium antagonists | |
| Nimodipine | Phase III |
| Nicardipine | Phase II |
| NMDA antagonists | |
| Selfotel | Phase III |
| Dextrorphan | Phase II |
| Cerestat | Phase III |
| Eliprodil | Phase III |
| Magnesium | Phase II |
| Lamotrigine | No human studies in stroke |
| Clycine site antagonist | Phase II |
| Fosphenytoin | Phase II/III |
| Glutamine release inhibitors | No human studies in stroke |
| AMPA antagonists | No human studies in stroke |
| Adenosine agonists | No human studies in stroke |
| GABA agonists | Phase II |
| Kappa-selective opioid antagonists | Phase III |
| Lubeluzole | Phase III |
| Nitric oxide synthase inhibitors | No human studies in stroke |
| Free radical scavengers | Phase III |
| Antiadhesion molecules | Phase III |
| GM-1 ganglioside | Phase III |
| Calpain inhibitors | Human testing planned |
| Basic fibroblast growth factor | Human testing planned |
| CDP – choline | Phase III |
| Combined cytoprotective strategies | No human studies in stroke |
| Cytoprotection plus thrombolysis | No human studies in stroke |

[a]Modified with permission from Chui, D., and Grotta, J. (1997). Current clinical status of cytoprotection. In *Primer on Cerebrovascular Disease* (K. M. A. Welch, L. R. Caplan, D. J. Reis, B. K. Siesjo, and B. Weir, Eds.), pp. 731–737. Academic Press, San Diego.

major studies, there have been isolated case reports that nimodipine might be associated with pulmonary vasoconstriction (91) and pseudo-obstruction of the colon (92,93). The most frequent side effect of significance is dose related hypotension. Some disturbances of liver function and hyperglycemia have also been reported. These do not increase the rebleeding rate.

### 2. *Nicardipine*

Between 1987 and 1989, 449 patients were randomly assigned to receive nicardipine intravenously (0.15 mg/kg/hr) and 457 were assigned a placebo. Antihypertensive agents were used in 26% of the nicardipine-treated group versus 43% of the placebo group. Fewer patients in the nicardipine group required hypervolemia, hypertension, and hemodilution for symptomatic VSP (25 vs 38%). Symptomatic VSP occurred in 46% of the placebo cases and 32% of nicardipine-treated patients. Despite the reduction in symptomatic VSP in treated patients, overall outcome at 3 months was similar between the two groups. In nicardipine patients 55% had a good recovery and 17% were dead. This compared to 56 and 18%, respectively, in the placebo-treated group, and these differences were not statistically significant (94). Angiograms obtained between days 7 and 11 were done in 23% of the nicardipine and 26% of the placebo patients. Fifty-one percent of the placebo-treated patients had moderate or severe VSP on days 7–11 angiograms compared to 33% of the nicardipine-treated patients. This difference was not statistically significant ($p < 0.01$). TCD mean flow velocities exceeding 120 cm/sec occurred in 23% of the nicardipine-treated patients and 49% of the placebo-treated patients. Patients were examined by TCD between days 7 and 11 (95). In the high-dose nicardipine trial, pulmonary edema in combination with azotemia was observed in 6% of nicardipine-treated patients versus 2.4% of placebo patients ($p < 0.01$). Rarely were these pulmonary/renal dysfunctions life-threatening (94). The high-dose study was followed by a low-dose comparison study. One hundred and eighty-four patients were randomly assigned to receive 0.15 mg/kg/hr nicardipine and 181 patients received a 50% lower dose (0.075 mg/kg/hr). The incidence of symptomatic VSP was 31% in both groups and overall 3 month outcomes were nearly identical. The administration of low-dose nicardipine was accompanied by fewer side effects. In the high-dose group, hypotension, renal dysfunction, and pulmonary edema occurred in 3.3, 2.2, and 5.4% of patients; for the low-dose group the comparable percentages were 3.3, 1.7, and 3.3%. Unlike nimodipine, nicardipine appeared to have a relatively robust effect in preventing or treating angiographic VSP. The fact that such effective treatment did not make any difference to the outcome has been attributed to the possibility that hypertensive/hypervolemic therapy was efficacious for the patients who had angiographic VSP. It is also conceivable that although the amelioration of vasospastic ischemia would have improved outcome, there may have been additive adverse factors which counterbalanced this effect. Scientific rigor also demands consideration of the possibility that VSP was not a significant prognostic factor for poor outcome during the time that this study was performed (96). In the second trial of nicardipine given at two doses, the incidence of DID was 31% in the 365 patients. In 14 other reports of nicardipine the

incidence of DID was 0–28%. These other series were small and mostly uncontrolled. DID was often poorly defined. Combined reports of prophylactic nicardipine give an incidence of DID of 25% (404/1643) (7). Twelve references provided 1045 patients given nicardipine as prophylaxis against DID. The incidence of DID was 24%. The outcomes in such patients were dead (12%), permanent deficit (17%), and good outcome (71%) (90).

#### *3. Other Calcium Antagonists*

The incidence of DID in four studies with diltiazem was 32% of 166 cases. For flunarizine (three studies) the incidence was 6% of 258 patients. For verapamil it was 15% of 34 patients (7). Flunarizine was used to treat 37 Fisher grade III patients and results were compared with those of 37 historical controls. Severe angiographic VSP occurred in 18% of the treated group versus 57% of the controls. Delayed ischemic deficits were present in only 1 flunarizine-treated patient but in 8 control patients (97).

### B. Models Using Calcium Antagonists

#### *1. Dihydropyridines*

Allen and Bahr (98) treated dogs with 1 mg/kg nifedipine. In 4 dogs 2 days after SAH, the cross-sectional area of the basilar artery was 57% of its control value and increased to 111% 30 min after an oral dose of nifedipine. In 3 of 12 dogs, mean systolic blood pressure fell from 170 to 140 mmHg within 10 min of administration of the drug; blood pressure remained stable or rose slightly during the next 20 min. Nagai and investigators (99) induced SAH in dogs and then injected one dose of a $Ca^{2+}$ antagonist into the vertebral artery and performed angiography for up to 30 min. Cinnarizine, nifedipine, and verapamil, in a dose of about $10^{-5}$ M, caused relaxation; nifedipine and verapamil depressed the blood pressure; and verapamil increased ICP. Kamiya (100) produced VSP in adult dogs by injecting fresh arterial blood into the cisterna magna. Various $Ca^{2+}$ blocking drugs were then given as single bolus injections into the vertebral artery. Angiographic monitoring was then carried out up to 30 min after the administration of drug. The $Ca^{2+}$ antagonists, cinnarizine, verapamil, sodium nitroprusside (SNP), and nifedipine all released the spasm *in vivo* for 15–30 min. SNP markedly decreased blood pressure and increased ICP. Cinnarizine caused relaxation without affecting blood pressure, ICP, or pulse rate. Brandt and associates (101), using a television image-splitting technique to measure the caliber of resting pial arterioles after acute SAH in cats, recorded an increase of 33–55% from baseline values after perivascular application of nifedipine (0.1–10$\mu M$). In studies of the effect of nimodipine on VSP in cats, Tanaka and coworkers (102) induced VSP by injection of 0.2 to 0.3 ml of fresh autologous blood into the cisterna magna and monitored the diameter of pial vessels radiographically with a television camera. Nimodopine, 0.1 mg/kg given intravenously 20–30 min after SAH, abolished the spasm. The vasodilation was greater in arteries under 100$\mu$m in diameter than those that were larger. Auer and colleagues (103) applied a solution of nimodipine, ($2.4 \times 10^{-5}$ M), to exposed cerebral vessels in 17 patients and inserted a plastic cannula for topical administration postoperatively in 13 of them. This was continued while ruptured aneurysms were being clipped 42–72 hr after the SAH. In all cases, CT scans showed blood in the basal cisterns. Vasodilation, which occurred in all instances, was greater in the smaller vessels. Brandt and colleagues measured cortical arterioles and venules under normal conditions and then in focal ischemia in cats. Nifedipine applied topically induced marked concentration-dependent dilation of the arterioles, and its microapplication around venules dilated them as well but to a lesser degree. In some arterioles that had constricted after occlusion of the MCA, the dilation in response to nifedipine overcame stasis and restored blood flow (104).

Varsos and colleagues, using a two-hemorrhage model of chronic VSP, failed to reverse vasoconstriction induced by SAH 5 days after the second injection, with either intravenously administered aminophylline (10 mg/kg/hr) or nifedipine (1 mg/kg) or an intraarterial bolus injection of 2 mg/kg of papaverine (105). Comparing the effects of nifedipine and nimodipine (0.28 mg/kg), in dogs, Cohen and Allen (106) found that at this reduced dose, nimodipine but not nifedipine significantly relieved VSP . Svendgaard's group (107) produced late cerebral arterial spasm by injections of autologous blood, to a total of 13–33 ml, into the basal cisterns in baboons. In the experimental group, 1 week after the first injection vessel caliber was reduced by 10–20%. Varying in individual areas, CBF had decreased 18%, and brain metabolism had also decreased. During hypercapnia before and after SAH, CBF increased (mean 3.7 and 1.8 ml/100 g/min, respectively, for each 1-mm Hg elevation of $p_aCO_2$). After SAH, CBF autoregulation was impaired in five of six animals.

Espinosa and coworkers (108) studied in a randomized, placebo-controlled, double-blind trial the effect of oral nimodipine after SAH in monkeys. The 30 monkeys studied for 7–14 days after the SAH were allocated into two groups of 15. One group of animals was given nimodipine, 1 mg/kg every 8 hr, and the other was given placebo at the same time interval. Significant VSP developed in 87% of the animals, and overall this was more common in the placebo group. In this group, the incidence of VSP was significantly higher by days 7 and 14 post-SAH compared with the nimodipine group. However, the effect of

nimodipine on vessel caliber at this dosage was equivocal; the average percentage reduction in vessel caliber in each monkey was not significantly different, and when VSP on the clot side in both groups (treated vs placebo) was compared, there was no significant difference between the two groups. DID developed in one monkey (placebo group) 4 days after clot placement, which lasted until sacrifice on day 14. No such deficits occurred in the nimodipine group. No serious side effects were noted. In a study of a higher dosage, 3 mg/kg orally every 8 hr, starting 14 to 20-hr post-SAH no significant effect of treatment was evident (109). When nimodipine was administered intrathecally at a dosage of 0.2 mg every 8 hr for 6 days, there was no significant difference in the severity of VSP between control and treated animals. Nimodipine produced slight dilatation of mildly spastic basilar arteries in 3/8 animals (110). The MCA vessels under the clot, which was placed on one side only, and contralateral vessels were studied *in vitro* following sacrifice (111). There was a highly significant reduction in contractile response of this MCA segment on the clot side (the vessels in severe VSP) compared with the contralateral nonclot side in response to 5-HT, NE, and KCl. Clot side contractility was not influenced by previous exposure to nimodipine. Oral nimodipine did appear to enhance the contractility of the nonclot vessels.

The intrathecal injection of 4 ml of $10^{-3}$ M nimodipine promptly and completely reversed VSP on days in which it was present 1–4 days after a single blood injection or 3–6 days after multiple injections given 2 days apart (112). The vasodilation lasted at least 4 but less than 24 hr. The intrathecal administration produced a transient decrease in blood pressure. Sublingual (0.28–0.58 mg/kg) or intravenous (0.1 mg/kg) drug produced persistent hypotensive effects without affecting VSP. In various animal models of cerebral ischemia, nimodipine usually failed to affect neuronal damage, neurological outcome, or metabolic responses when given after the onset of ischemia (72).

The authors consider that the bulk of evidence from the animal experimentation does not suggest that nimodipine would be a useful agent in the prophylaxis of vasospasm. Whether it has a neuroprotective effect in humans remains moot and is discussed elsewhere.

#### 2. *Other Calcium Antagonists*

The calcium blocker HA 1077 [1-(5-isoquinolinesulfonyl) homopiperazine] antagonized contraction of canine basilar artery to calcium ionophores. It produced marked dilatation of basilar artery in dogs subjected to hemorrhages. The mean arterial blood pressure (MABP) was lowered at higher doses. HA compounds are now known to inhibit multiple intracellular kinases. They are prepared by modifying the structure of a CaM antagonist (113). Another $Ca^{2+}$ antagonist, diltiazem, was used in a monkey model of SAH. Six animals received oral doses of diltiazem (20 mg/kg t.i.d.) starting 24 hr after SAH induced by a needle-removal technique. Treated animals showed less hypotonia and hyperreflexia and greater vessel diameters than untreated animals (114). HA 1077 was found to dilate parenchymal aterioles of 50 μm average diameter isolated *in vitro*. Vasoconstriction induced by synthetic thromboxane A2 was completely inhibited by HA 1077 (115). In a canine SAH model, 7 days after induction intravenous administered bolus of HA 1077 dose dependently increased rCBF without significantly changing the blood pressure after SAH (116). Intravenous infusion of HA 1077 twice daily after the first intracisternal injection of blood in a canine model prevented the occurrence of chronic VSP. Bolus intravenous administration dose dependently increased local CBF (117).

### C. Avoidance of Antifibrinolytics

The most widely used antifibrinolytic agent is ε-aminocaproic acid, which is given intravenously and achieves a peak level within 20 min. About 75% is excreted unchanged within the urine within 12 hr with a single injection. It crosses the BBB and achieves a maximal state of antifibrinolytic activity in CSF within 2 days of the start of therapy (118,119). Administering a loading dose results in more rapid attainment of therapeutic CSF levels. A review of 25 reports of treatment with antifibrinolytic agents after SAH reported a reduced incidence of rebleeding in 92% of the series. A decreased mortality rate was noted in 31% of the trials. Discrepancies arose from the multiple clinical variables and the methodological errors which precluded assessment of whether the natural history of rebleeding was really affected (120). Fodstad observed rebleeding in 13% of cases treated with antifibrinolytic drugs and in 31% of those not treated. However, 16% of the treated patients and only 7% of the untreated patients died of cerebral ischemia due to VSP. Overall mortality from rebleeding and ischemia was 25% among patients receiving the antifibrinolytic agent tranexamic acid and only 19% in the untreated control group. One hundred and five patients were in this study (121). In the International Study on the Timing of Aneurysm Surgery conducted in the early 1980s, 672 patients were observed with reference to antifibrinolysis. Twelve percent of those treated rebled within 14 days, whereas 19% of those not given antifibrinolytic agents rebled. The rate of focal ischemic deficits was significantly higher in the treated patients (32 vs 23%). Treated patients also had a higher incidence of Hyc (14 vs 7%) (122). Similar conclusions were reached in a study of 479 patients with SAH in a multicenter, randomized, double-blind, placebo-controlled trial of tranexamic acid. Rebleeding was reduced

from 24 to 9% but ischemic complications increased from 15 to 24% in the treated patients. In this trial the incidence of Hyc was not significantly different between groups (123).

The disadvantage of antifibrinolytic agents appears to be the prolonged life of the clot around the arteries which is the etiological factor for VSP. By preserving the fibrin plug in the ruptured aneurysm these agents reduce the rebleeding tendency, but with early effort at intervention now being the norm there is no longer any reason to use antifibrinolytic agents. In the event that early definitive treatment is impossible and if the risk of VSP is judged to be small, there is still a place for consideration of the use of antifibrinolytic agents. The minor adverse effects can include increased bleeding time, diuresis with resulting hypovolemia, and undesirable side effects such as diarrhea, nasal stuffiness, abdominal discomfort, nausea, vomiting, dizziness, and, uncommonly, arrhythmia, hypotension, myalgia, weakness, and myoglobinuria. We see no rationale for the use of antifibrinolytics if early surgery is planned.

### D. Avoidance of Dehydration

#### *1. Fluid Replacement*

The recognition that strokes could be aggravated by hypotension preceded the suggestion that they could be improved by induced hypertension and manipulation of fluid intake. Our ability to measure all the critical physiological parameters for a period of 2 weeks or more following SAH is very limited. Extrapolation from animal experiments to patient management has sometimes been simplistic. It seems unequivocal, however, that a patient on the brink of ischemic infarction from VSP can be harmed by excessive fluid restriction or the induction of a lowered cerebral perfusion pressure. Two hundred and forty-four consecutive patients admitted within 3 days post-SAH between 1977 and 1987 were not treated with antifibrinolytics. In the first 5 years daily fluid intake was maintained at 1.5–2 liters and fluid restriction was applied when hyponatremia developed. Antihypertensive medication was administered to all patients who were hypertensive. In the second study period, daily fluid intake was increased to at least 3 liters and patients were not fluid restricted. Antihypertensives were administered only when patients were on such medication before admission. Calcium antagonists were not administered. Although prognostic variables were believed to favor the early group of patients, cerebral ischemia occurred less frequently among patients during the second study period. Cerebral ischemia occurred among 10% of 155 patients treated with more liberal fluid intake compared to 21% of the 89 treated by relative fluid restriction. Overall mortality decreased from 46 to 36%, whereas mortality among patients with cerebral ischemia decreased from 60 to 31%. Rebleeding and acute Hyc did not change in frequency between the two study periods. It was concluded that the combination of increased fluid intake and the avoidance of antihypertensives helped maintain cerebral perfusion and prevented ischemia post-SAH (124). Buckell found that in 134 patients with recent SAH the hematocrit tended to be increased in comatose compared to alert patients. Hb was above the upper limit of normal in 22% of comatose patients but in none of the alert patients. Differences between comatose and alert patients were observable in tests indicating dehydration (125). Fluid restriction can aggravate the ischemia due to VSP and produce cerebral infarction. The evidence that volume expansion and blood pressure elevation prevent DID and can resolve some deficits without infarction is found in case series (126–128). The risks of deliberate hypervolemia include aggravation of cerebral edema, pulmonary edema, and congestive heart failure (129,130).

Forty-two blood volume determinations using $^{51}$Cr-labeled autologous red blood cells (RBCs) were performed on 11 control and 25 SAH patients. The females, but not the males, showed significant reductions in mean RBC volume and total blood volume after SAH. One patient had asymptomatic VSP with a below normal RBC volume and total blood volume, whereas 6 of 7 patients with symptomatic VSP had subnormal values. It was concluded that patients with normal blood volumes are far less likely to develop signs of cerebral ischemia than those with lowered volumes (131). The plasma volume of 25 patients admitted within 4 days post-SAH was measured using radioiodinated serum albumin. Normal plasma volumes were measured in an out-patient setting 6 months later or predicted from total body water. Thirty-six percent of the patients were found to be hypovolemic on admission, which was defined as a decrease in plasma volume exceeding 10% of normal levels. The basal cisterns were compressed or obliterated on the initial CT scans of the hypovolemic patients compared to only 13% of the normovolemic ones. All the patients with compression of basal cisterns associated with ICH or midline shift were hypovolemic. More than 80% of the patients with Hyc or compression of the basal cisterns were hypovolemic, 20% of the hypovolemic patients had Hyc (132). Patients who are in good neurological condition and who have small-volume SAH are generally treated simply by avoiding hypovolemia. Normal volemia is maintained by administration of 5% albumin and normal saline. Potassium supplements are added. Ringer's lactate is not used since it is hypotonic. Dextrose is avoided because of evidence that glucose >200 mg/dl can aggravate cerebral ischemia and worsen the outcome (23).

At the Barrow Neurological Institute patients judged to be at low risk for VSP (Fisher CT grade 1 or 2) are treated with normal saline (150 ml/hr) and their serum $Na^+$ levels are checked daily. If the $Na^+$ level decreases to <135 mEq/liter, patients are switched to a high-risk protocol. This is used as well for patients who are a Fisher grade III on admission. A Swan–Ganz catheter is inserted with continuous monitoring of pulmonary artery diastolic pressure and cardiac output, and parameters are measured daily. Normal saline is administered at 150 ml/hr and plasmanate (5% plasma protein fraction-human) is administered at 100 ml/hr as required to keep the pulmonary artery diastolic pressure or pulmonary capillary wedge pressure >10 mmHg. Serum $Na^+$ is checked twice daily and if it declinese below 135 mEq/liter 3% saline solution is administered at 30–50 ml/hr as long as the pulmonary artery diastolic pressure is not above 16 mmHg. DDAVP (desmopressin acetate) injection is administered as 1 ml intravenously at 12-hr intervals for urinary outputs >200 ml/hr for 2 consecutive hours. It is suspended if the pulmonary artery diastolic pressure is >16 mmHg or serum sodium is <135 mEq/liter. To maintain blood pressure between 180 and 220 mmHg and systolic and reverse ischemic deficits phenylephrine (50 mg in 250 ml normal saline) is used to maintain systemic vascular resistance. Dopamine is infused to maintain cardiac output ≥5 liters/min and is held for heart rates >120 beats/min (133).

Intravenous fluids are usually given as 0.9% sodium chloride or Ringer's lactate and for adults the University of Chicago infuses fluids intravenously at a rate 100–150 ml per hour to prevent hypernatremia and hypovolemia. Accurate fluid balance should be maintained. Patients should be kept normally hydrated. Foley catheters and nasogastric tubes are used as required. Daily weighing of the patient is indicated (4). The 31 reports specifying prophylactic fluid loading gave an incidence of DID of 18% of 2516 cases, which is considerably lower than the overall incidence without the treatment being specified (33% of 12,449) (7).

### *2. Inhibition of Natriuresis*

Plasma volume may be decreased in association with excessive natriuresis. Fluid restriction in this situation is associated with an increased risk of cerebral infarction (34). The decreased plasma volume increases hematocrit and blood viscosity and potentially reduces CBF. Attempts to maintain plasma volume purely by fluid replacement may be thwarted by a reactive rapid and substantial diuresis. Half the patients with SAH become hyponatremic and hypovolemic (34). Fludrocortisone acetate has been used to ameliorate volume depletion (127). In half of SAH patients volume decreases more than 10% in the first 6 days (34). Volume contraction is a risk factor for VSP and increases RBC aggregability, which results in microcirculatory changes and hemodynamic depression (34,131,134). Fludrocortisone acetate is a mineralocorticoid that enhances $Na^+$ reabsorption in the distal renal tubules. Forty-six of 91 patients post-SAH were given 0.4 mg/day in two doses for up to 12 days. Negative $Na^+$ balance in the first 6 days post-SAH was reduced from 63% in controls to 38% in treated patients. The negative $Na^+$ balance correlated with decreased plasma volume for up to 12 days. Control patients had more cerebral ischemia (31 vs 22%) and more requirement for therapeutic plasma volume expanders (24 vs 15%) (135). In a different series, 15 of 30 patients were given 0.3 mg/day of fludrocortisone; these patients had reduced $Na^+$ and $H_2O$ intake, and reduced $Na^+$ excretion and urinary volume as a result. The average $H_2O$ intake for 14 days in the treated group was 5159 compared to 6612 ml/day for untreated cases. The mean $Na^+$ intake in treated patients was 487 vs 634 mEq/day in the others. The serum $Na^+$ level fell to 135 mEq/liter on average in the untreated groups but remained normal in the fludrocortisone group. Hyponatremia was always seen in untreated patients when the CVP was in the target range of 8–12 cm $H_2O$ (136). The evidence appears to support the use of this agent.

## E. Optimal Hematocrit

Hematocrit (Hct) tends to fall progressively during hospitalization for SAH; It is very important to optimize it in patients at high risk of DID. A decrease of 10% is common. Twenty-seven patients with a variety of ischemic stroke syndromes were subjected to Xe-133 studies of CBF. Hct values in the patients ranged between 31 and 53%. There was an inverse correlation between CBF and Hct in this range. $O_2$ delivery (the product of arterial oxygen content and CBF) increased with Hct elevation and reached the maximum level in the Hct range of 40–45%, beyond which it declined (137). In 6 patients with high Hct (mean, 51.1%) CBF was measured by Xe-133 intracarotid injection. Rapid two-stage hemodilution which lowered Hct by 9 and 13% increased CBF by 19 and 23%, respectively. The cerebral metabolic rate for oxygen ($CMRO_2$) increased slightly following the initial stage of hemodilution but returned to baseline value following the second one (138). CBF does not equate with $O_2$ delivery to the ischemic brain, however. Kudo and associates used the isotope dilution technique to monitor circulating blood volume in patients post-SAH. In 2, the predeterioration circulating blood volumes were determined. Their data suggested that depleted RBC volumes were more responsible for neurological deterioration than

were lower plasma volumes, but both were diminished. They suggested that there should be serial monitoring of this parameter to detect and prevent hypovolemia and anemia in aneurysm patients (139).

Increase in blood viscosity reduces CBF (140). This may be clinically significant in polycythemia vera. Conversely, in anemia, cerebrovascular resistance is reduced and CBF increases. There may also be a compensatory increase in CBF to match the reduced $O_2$-carrying capacity of blood. Normally, the ratio $CBF/CMRO_2$ is 14 or 15 ml blood/1 ml $O_2$. The optimum Hct for $O_2$ delivery is unknown, with some authors favoring the 30–32% (141,142) range and others suggesting 40–45% (143). Gaehtgens and Marx (144) concluded that the optimal Hct for $O_2$ delivery is approximately 42% and that hemodilution below the physiological level tends to reduce tissue $O_2$ supply. It is likely that there is a range of Hct (35–50%) over which cerebral $O_2$ delivery is normal. At Hct values above this, the increase in $CaO_2$ may be more than offset by the increased viscosity. At Hct values below this range the decreased $O_2$ carrying capacity is not offset by the increase in flow resulting from the decreased viscosity. In thromboembolic stroke the Hct tends to be elevated in the 44–48% range. Hct values in three clinical studies were 37, 38, and 39% (60,126,145). There is a view that DID can be provoked by decreases in blood volume even without obvious changes in blood pressure.

Maroon and Nelson found that RBC mass and total blood volumes were significantly decreased in 15 patients with SAH. The causes of contracted blood volume were considered to be bed rest, a supine diuresis, peripheral pooling, negative nitrogen balance, decreased RBC production, and iatrogenic blood loss (146). Total circulating blood volume and total body: venous Hct ratio were determined by simultaneous measurements of RBC volume and plasma volume in 10 SAH patients, 10 supine bed-resting control patients, and 20 ambulant outpatients. The mean total body: venous Hct ratio of the SAH patients was 0.866, which was significantly lower than that of the supine controls (0.908) and the ambulant patients (0.909) (147).

The relationship between blood viscosity and clinical condition was examined in 17 patients after surgical treatment with ruptured intracranial aneurysms. Two hundred and thirteen blood samples were analyzed. Hct was related to clinical condition and the correlation was not strong. Postoperative plasma viscosity was higher in patients with focal neurological deficits. Blood viscosity low, middle, and high shear rates were significantly higher when impaired level of consciousness was observed. Plasma viscosity increased rapidly to 1.58 mPa/sec on the second postoperative day, peaked at 1.6 on the seventh day, and then declined gradually to 1.56 on day 12. Postoperative angiography was performed on 65% of the patients and the incidence of VSP was 64%. Slight to moderate VSP was found in 6 patients and severe VSP was found in only 1. Patients with angiographic VSP had mean blood viscosity values of 32.6 mPa/sec at low shear rates, 17.6 mPa/sec at moderate shear rates, and 4.15 mPa/sec at high shear rates. The Hct values had a mean of 34%. Blood viscosity was determined by Hct, plasma viscosity, RBC aggregation, and RBC deformability. Mean postoperative plasma viscosity was higher in patients with focal neurologic deficit (148). One hundred and sixty-five cases, all in neurological grades I–IV were operated on within 7 days post-SAH. Forty-five percent developed symptomatic VSP. Twenty percent showed ischemic changes on CT scan. The ischemic CT findings were as follows: watershed zone infarction (13%), major artery area infarction (2%), perforator area infarction (2%), hemorrhagic infarction (2%), and brain swelling (7%). The mortality rate for the patients with brain swelling was 67%. The Hcts by patient groups were as follows: reversible symptomatic VSP (36%), complete infarction by CT scan (31%), and brain swelling (27%). The Hcts in the group with reversible symptomatic VSP were significantly higher than the Hcts in the other groups (149).

### F. Sickle Cell Disease

SAH is an uncommon manifestation of sickle cell disease, but such patients are thought to have twice the risk of developing aneurysms compared to the general population (150). Sickle cell patients are also two or three times more likely to have multiple aneurysms than are other people. Careful hydration, transfusions, and standard surgical approaches are recommended for patients with SAH who have associated sickle cell disease. The percentage of sickle hemoglobin should be maintained less than 30% (151).

### G. Avoidance of Hypotension and Hypertension

We believe the critical aspects of post-SAH care are to avoid hypovolemia, hypotension, and hemodilution, and not to induce hypervolemia, hypertension, and hemodilution. Hypertension should not be blindly continued in the face of continued clinical deterioration and should be maintained at the lowest level of increased blood pressure consistent with maintained recovery of function (152).

When blood pressures are in the range considered to be a hypertensive emergency and are accompanied by a high risk of hemorrhage, we prefer to use intravenous SNP starting with low doses. Agents known to decrease CBF, such as propranolol and metoprolol, should be avoided, as should the use of diuretics except in the presence of

heart failure. A slow, modest reduction in blood pressure should be the goal (153). Because of their negative inotropic effect, pure β-blockers should be avoided; if used, it is recommended that the cardiac output should be monitored (45).

Denny Brown was the first to notice that hypotension could have disastrous effects when superimposed on structural narrowing of major cerebral blood vessels. Clinical worsening was observed to accompany syncope, gastrointestinal hemorrhage, or sleep. He related symptoms from stenotic lesions to anatomical defects in the collateral circulation rather than VSP. In such circumstances, neurological dysfunction directly reflected changes in systemic blood pressure.

In a Swedish consecutive series of 219 patients, 17 developed DID between 4 and 13 days post-SAH and 8 of these patients died. Their poor outcome correlated with a history of elevated blood pressure before SAH. Of 18 patients having early surgery who had diastolic blood pressure >110 mmHg on at least two separate measurements before SAH or who had a history of previous treatment for arterial hypertension, DID developed in 50% of cases as opposed to only 13% of 63 normotensive patients. Of the 8 hypertensive patients who received antihypertensive medication at the time of SAH, 75% developed DID. Fifty percent of the hypertensive patients died (154). One hundred and thirty-four patients were studied; Some were treated by antihypertensive medication aimed at reducing the diastolic blood pressure to levels below 100 mmHg. The use of antihypertensive drugs did not produce a difference in outcome. Fifteen percent of the 80 patients treated with antihypertensive medication rebled compared to 33% of the 54 patients not treated with antihypertensives. Patients on antihypertensive treatment had on average higher blood pressure than the untreated patients. Infarction occurred with antihypertensive treatment in 43% of 80 patients versus 22% of 54 patients without antihypertensives. This relationship was considered partly due to coexisting hyponatremia. These patients were not treated by early surgery. In this series the use of antihypertensives during the period of the development of infarction appeared to be a risk factor (155).

Aggressive blood pressure reduction may compromise cerebral perfusion pressure. Although blood pressure should be vigorously controlled in the pretreatment phase of SAH, once the aneurysm is secured and the patient enters the time of maximum risk for VSP aggressive treatment of hypertension should be avoided. The selection of the level of blood pressure at which antihypertensive medication should be employed will vary with the patient's medical history. Since arterial hypertension post-SAH may be a homeostatic response to reduction in cerebral perfusion pressure, it is important not to be too aggressive in lowering arterial blood pressure. This is particularly true in the period of 4–14 days post-SAH if angiographic VSP is present (4).

### H. Salicylates

Of 242 aneurysm patients surviving more than 4 days post-SAH, 37% had DID, which caused permanent deficit or death in 22%. Forty-four percent of the patients having follow-up CT scans showed cerebral infarction which had not been seen on the postadmission CT scan. Interestingly, those who had salicylates in the urine on admission had a relative risk of DID with fixed deficit of 0.40 (95% confidence interval, 0.15–1.10) compared to those who did not have salicylates in their urine. The risk of cerebral infarction was similarly reduced. The reduced risk of ischemic complications was apparently restricted to those patients who used aspirin before the hemorrhage. Patients who had taken aspirin before the SAH had an ischemic risk of 0.21 (95% confidence interval, 0.03–1.63) and a risk of infarction of 0.18 (95% confidence interval, 0.04–0.84) compared to those who did not use aspirin. The reduced risk of cerebral infarction was significant even after adjustment for several potential confounding factors. Juvela suggested that platelet function at the time of SAH may be associated with delayed cerebral ischemia after aneurysmal rupture. Platelet aggregation and thromboxane production are reduced for at least 4–6 days after cessation of aspirin use (156).

### I. Cisternal Drainage

Theoretical advantages of prophylactic cisternal drainage include removal of spasmogen and maintenance of adequate perfusion pressure in the face of a potential reduction in regional blood pressure due to VSP. In 16 reports of 1169 patients having cisternal drainage, the incidence of DID was 28% (range, 9–47%). When drainage was combined with irrigation in 6 reports of 359 patients, the incidence of DID was 17% (7). A theoretical disadvantage might be an increased tendency to develop Hyc.

### J. Fibrinolytics

#### *1. Clinical Studies of t-PA*

We continue to employ t-PA in selected patients. We believe that such patients should be at significant risk of VSP and infarction as evidenced by thick SAH. In addition, it must be possible to secure the ruptured aneurysm within a couple of days of ictus. The operation must be performed without a significant production of raw, ooz-

ing brain surfaces. We no longer give t-PA as an intraoperative bolus but confirm complete exclusion of the aneurysm with intraoperative angiography and obtain an immediate postoperative CT scan to exclude any new intraoperative bleeding. In such patients we routinely place ventricular drains at the start of the clipping procedure. In the vast majority of patients the ventricular catheter can be placed atraumatically in a single pass. We would be more reluctant to employ t-PA if multiple passes are required. In patients fulfilling the aforementioned criteria and in whom the postoperative CT scan demonstrates significant residual SAH, we instill 0.5 mg t-PA in 0.5 ml of sterile water every 8 hr through the ventricular catheter if there continues to be a significant residual clot. CT scans are done on a daily basis to ensure that there are no adverse bleeding complications and that there is a continuing requirement for t-PA. Although this approach delays the administration of t-PA, we believe that safety is enhanced and that the t-PA in ventricular CSF will diffuse widely throughout the basal subarachnoid cisterns.

It appears unlikely that large, randomized clinical trials could be organized in view of the overwhelming experimental evidence and the very favorable results achieved in the larger studies from experienced aneurysm centers with extremely low rates of severe angiographic VSP, permanent DID, and death attributable to VSP. In cases in which aneurysms are treated by open methods with clipping, the onus is on the surgeon to use meticulous hemostasis, very careful dissection, and water-tight dural closure if t-PA is to be employed.

In the first published clinical report of t-PA, eight poor-grade patients were operated on early post-SAH. Clot clearance was observed at 24 and 48 hr in two cases. VSP developed in three patients and was symptomatic in one. Outcomes were as follows: good, three patients; poor, two patients, dead, one patient; and unstated, two patients (157).

Tanabe *et al.* gave 12 patients varying doses of intracisternal t-PA, amikacin, and "CBPC." Patients had Fisher grade III SAH. Clinically, they were neurological grade II or III. From 10 to 75 mg of t-PA was injected over several days depending on the color of CSF drainage from the cisternal catheter. One patient developed symptomatic VSP. The authors believed that simultaneous administration of intravenous tranexamic acid prevented subgaleal hematoma formation in 2 cases. Overall outcome was excellent in 11 and fair in 1 (158). We do not agree with the simultaneous use of antifibrinolytics.

Findlay and collaborators reported on 15 patients who underwent surgery in the first 2 days post-SAH and received t-PA intraoperatively. Preoperatively, 13 patients had diffuse or localized thick SAH by CT scan and 2 had diffuse thin clots. The t-PA was given as a single intraoperative injection of 7.5 mg in 1, 10 mg in 9, and 15 mg in 5 other patients. Postoperative cisternal drainage was employed in 3 patients. All patients except 1 demonstrated partial or complete cisternal clot clearance on CT scans within 24 hr after surgery (Figs. 8.1 and 8.2). The patient who showed no clot reduction developed symptomatic VSP and died 8 days after rupture. No VSP was seen on postoperative cerebral angiography in 6 of the 14 patients. Mild to moderate VSP was seen in at least one major cerebral artery in the remaining 8 patients. Severe angiographic VSP was not seen, although the patient who died did not undergo repeat angiography. One patient developed a large extradural hematoma within several hours of the craniotomy probably due to the t-PA. Thirty patients with one angiographically verified aneurysmal SAH who received intrathecal t-PA at the time of surgery were reported by Ohman and colleagues. Patients were operated on within 3 days post-SAH. Patients were divided into groups of 10, with patients receiving 3, 10, or 13 mg of t-PA in a single intracisternal injection after the clipping. There were no differences between the treatment groups and overall outcome. One patient in the 3-mg t-PA group developed postoperative ICH, while 1 patient in the 10-mg t-PA group had a postoperative epidural hematoma. The only death in the 13-mg t-PA group was attributed to inclusion of a segment of the pericallosal artery in the aneurysm clip. In all groups a reduction was observed in the amount of blood seen on the postoperative CT scans compared to the preoperative CT scans. The higher t-PA dosage group showed less angiographic VSP (159).

Ten patients treated with intracisternal t-PA at the Barrow Neurological Institute were clinically neurological grades III or IV and had thick clots or layers of blood in the basal cisterns and major cerebral fissures. One patient had 10 mg t-PA instilled into the subarachnoid cisterns prior to closing the dura, whereas in the other 9 patients a small catheter was left in the subarachnoid space and t-PA was instilled 12–24 hr postoperatively. Four patients had 5 mg and 5 had 0.5 mg every 8 hr for three infusions. Minor local bleeding complications were noted in all patients receiving 5 or 10 mg t-PA. Oozing was noted at the operative incision in 4 of 5 patients and at the drain site in 2 patients. A small epidural hematoma was treated by delayed drainage in 1 patient. No bleeding complications were noted in the patients receiving the three infusions of 0.5 mg each. Cisternal CSF samples demonstrated high t-PA levels for 24–48 hr. Angiography 7–8 days after rupture showed mild to moderate VSP in 9 patients whereas 1 patient with hemorrhage from a posterior inferior cerebellar artery aneurysm had severe focal spasm of the vertebral arteries that was asymptomatic. The results

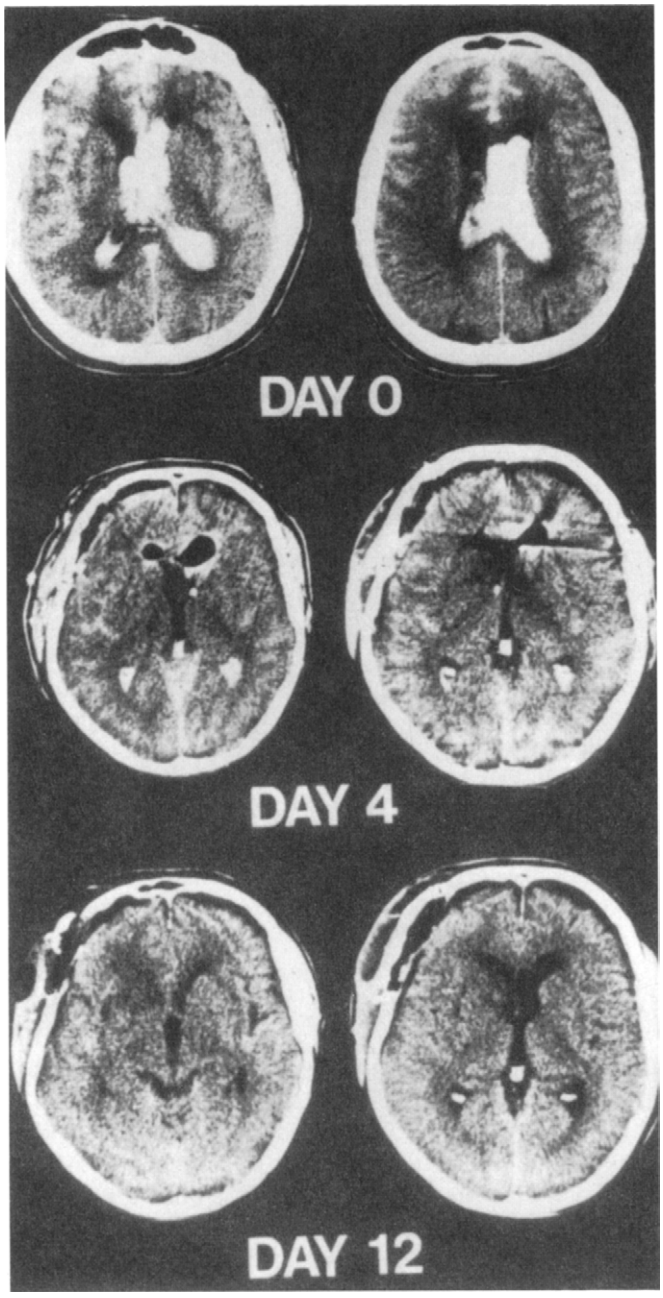


**FIGURE 8.1** Computerized tomography scans in Case 11. (Top) Scans obtained at admission showing large intraventricular blood clots from rupture of an anterior communicating artery aneurysm. These were treated with two separate intraventricular injections of 5 mg rt-PA 24 and 48 hr after aneurysm clipping through a ventriculostomy catheter. Combined with ventricular drainage, this resulted in rapid and complete clot clearance. (Middle and bottom) Scans obtained on days 4 and 12 after rupture showing resolution of the clots [reproduced with permission from Findlay, J. M., Weir, B. K. A., Kassell, N. F., Disney, L. B., and Grace, M. G. (1991). Intracisternal recombinant tissue plasminogen activator after aneurysmal subarachnoid hemorrhage. *J. Neurosurg.* **75**, 181–188].

of serial t-PA levels suggested that the lower dosage regiment with divided dosages at 8-hr intervals is well tolerated and that even lower dosages may be effective (160).

Mizoi *et al.* studied 10 patients with SAH and Fisher grade III. Some patients had associated ICH. Bilateral craniotomy was performed and multiple subarachnoid catheters were placed for subsequent daily infusions of t-PA in total doses between 4 and 26 mg for up to 9 days. Based on detailed angiographic, CT, and clinical follow-up no patient developed VSP or DID and there were no hemorrhagic complications attributed to t-PA. There were 4 good and 6 excellent outcomes (161). Twenty patients were reported by Stolke and Seifert. Patients were Fisher grade III, surgery was performed within 72 hr, and 19 mg of t-PA was instilled into the cisterns after aneurysmal clipping. VSP was diagnosed on the basis of TCD velocities. One patient died. DID was attributed to VSP, and there were no complications attributed to the t-PA. Increased TCD velocities were frequently noted. They suggested that to achieve the most rapid and complete blood removal early use of high drug doses might be required or multiple postoperative cisternal t-PA injections may be necessary (162). A multicentered dose escalation study of t-PA involved 17 patients admitted within 48 hr of SAH. Neurological grades ranged from II to IV. CT grades were Fisher III or IV. At surgery a small silicon catheter was left in the subarachnoid space and 24 hr later bolus infusions of t-PA were started at 6 hr intervals for 3 days. Between 25,000 and 600,000 IU of t-PA was given to four groups composed of 3 to 6 cases. There was no significant difference in the clearance of subarachnoid clots between the groups. The occurrence of both symptomatic and angiographic VSP was less in the 75,000 IU group than in the other groups. Intracranial bleeding was noted in 1/6 patients of the 75,000 IU group, 2/4 patients in the 200,000 IU group, and 1/3 patients in the 600,000 IU group. Serial coagulation studies demonstrated no evidence of systemic fibrinolysis (163).

In a report from Korea, 6 patients had preoperative neurological grades II and III and Fisher grades II–IV. Only 2 were Fisher grade III. Aneurysms were clipped within 4 days of rupture, and after clipping 10 mg of t-PA was administered directly into the subarachnoid space. Three patients had clinical VSP, of whom 1 had a diffuse infarct. They noted hemorrhagic infarction in 1 case, cerebellar hemorrhage in 2 cases, and a small epidural hematoma in 2 cases (164). One would question the administration of t-PA in the absence of thick diffuse SAH preoperatively and in operations being carried out as late as 4 days after SAH. The large number of

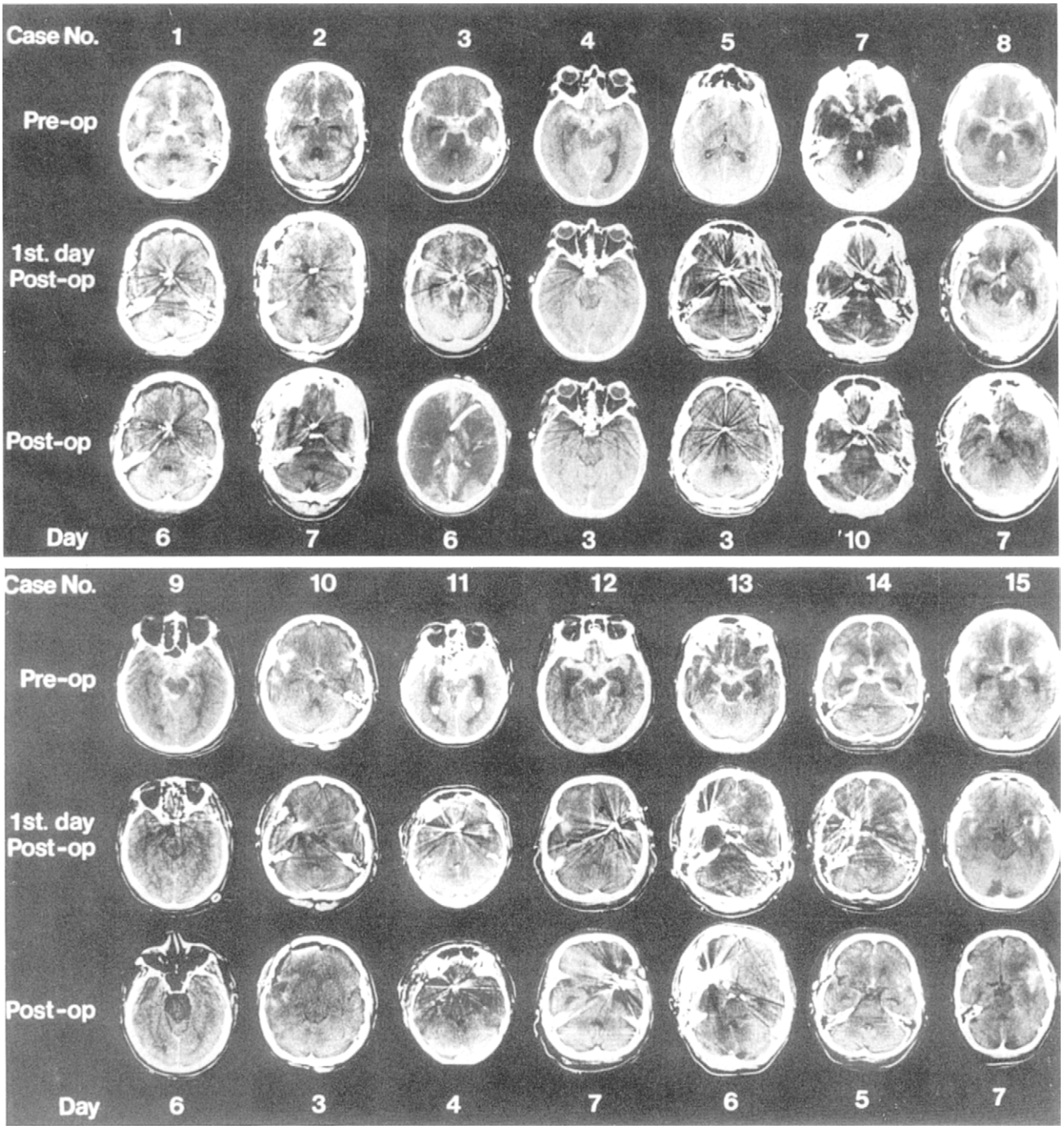


**FIGURE 8.2** Serial computerized tomography (CT) scans. Preoperative CT scans were obtained within 24 hr after subarachnoid hemorrhage (SAH); the day 1 postoperative CT scans were obtained within 24 hr after surgery and rt-PA administration and the follow-up CT scans were obtained on the days shown. All patients show partial to complete subarachnoid clot lysis within 24 hr after surgery except Case 3, in which there was no clot reduction on the first postoperative day. This was the only patient in the series to develop severe vasospasm, and she died 8 days after SAH and 6 days after surgery [reproduced with permission from Findlay, J. M., Weir, B. K. A., Kassell, N. F., Disney, L. B., and Grace, M. G. (1991). Intracisternal recombinant tissue plasminogen activator after aneurysmal subarachnoid hemorrhage. *J. Neurosurg.* **75,** 181–188].

postoperative hemorrhages makes this account very atypical. Mizoi *et al.* examined 105 patients undergoing surgery within 48 hr post-SAH who had Fisher grade III CT findings. The patients were classified by the degree of density of their clots (> 75 and < 75 Hounsfield units). The latter constituted the control group. In both groups, cisternal drainage was instituted. On the day following operation the t-PA was given by intrathecal injection as a 2-mg dose and this was continued for several days until all cisterns exhibited low density on CT scan. Follow-up angiography demonstrated that 87% of the t-PA group had no VSP, 10% had moderate VSP, and only 1 case (3%) had severe VSP. All 4 patients who had received t-PA and showed angiographic VSP were asymptomatic, and there were no cases of DID. In contrast, 15% of the patients in the control group (less dense clots) developed DID. In 1 patient in the t-PA there was a single SAH caused by a drainage catheter removal, 1 patient had a small epidural hematoma, and 1 had a subgaleal fluid accumulation. All of these events were treated conservatively with favorable results. There were no infectious complications. On the basis of this excellent experience the authors recommended the injection of 2 mg of t-PA daily for 5 days for a total of 10 mg beginning on the first postoperative day. The 30 patients in the t-PA group had a mean age of 59 years, 37% were grade IV, and the mean CT density of clot was 80 Hounsfield units. In comparison, the 75 patients in the control group had a mean age of 56 years, only 7% were neurological grade IV, and the mean clot density was 67 Hounsfield units. Despite these imbalances all favoring the "control" group, 15% of the control group developed DID, whereas none of the t-PA group did; good recoveries occurred in 75% of the controls and in 77% of the t-PA group and 5% of the control group died compared to none of the t-PA-treated group (165). This study from a very busy aneurysm service (Tohoku University, Sendai) suggested that multiple injections postoperatively until clot clearance was observed were superior to a single intraoperative bolus administration—a view with which we concur.

In another study, t-PA was given intracisternally in 15 patients operated within the first 4 days post-SAH. Good results were seen in 80% of the t-PA patients compared to 70% of a similar group of patients not given t-PA (166).

Ten neurosurgical units studied 53 patients between 1989 and 1991. Surgery was performed within 3 days post-SAH. The study was designed to examine differences in efficacy and complications between three different t-PA dose regiments. 0.1, 0.2, or 0.4 mg given three times daily for 5 days. The drug was administered intrathecally via drains left *in situ* at the time of clipping. Administration via ventricular catheters was also permitted if there were ventricular clots. Spontaneous drainage was instituted after the drug had been injected and the tube clamped for 4 hr. Active perfusion drainage was used. The majority of patients were admitted during the first 2 days after SAH, were in poor neurological grades, and had thick subarachnoid clots on CT scans. On follow-up angiography performed between days 7 and 14, none of the patients showed severe angiographic VSP and 51% had no or only slight VSP. Only 2% of the cases showed severe symptomatic VSP and 4 showed moderate symptomatic VSP. CT scans performed more than 1 month later showed no multiple or large infarcts, although 8% showed intermediate-sized infarcts. t-PA was believed to be completely ineffective in clearing clot from the basal cistern in 6% of cases and effective in 51% of cases. Three patients showed an increase in SAH after the use of t-PA and there was 1 case of epidural hematoma possibly related to t-PA. There was no significant difference in safety or efficacy between the groups, which suggested that 0.1 mg every 8 hr for 5 days was a reasonable therapeutic selection (167). There was no placebo group in this study since the participating neurosurgeons believed that efficacy had already been established. Since 15 patients in the study were Fisher grade IV, they could have had only diffuse thin or no SAH but ICH and/or IVH. The number of patients at significant risk, therefore, was only 9,10, and 12 in the three dosage groups.

From a series of 224 patients with aneurysmal SAH, 52 were selected for intrathecal t-PA. These patients were compared to a control group of 68 patients. All were treated within 72 hr of SAH. All patients were Fisher grade III, and in the t-PA patients 40% had some blood noted in the ventricles. In all patients 10 mg of t-PA was injected into the basal cisterns at the termination of surgery, and in cases with severe IVH 5–10 mg of t-PA was injected via the ventricular catheter at the end of the operation. Outcomes were assessed at 3 months postsurgery. Overall results for the t-PA and control (parentheses) groups were as follows: neurological grade I, 75% (60%); II, 13.5% (9%); III, 11.5% (21%); IV, 0 (1.5%); and V (dead), 0 (4%). DID attributed to VSP occurred in 8% of t-PA patients and 13% of controls. Permanent neurological deficits with CT evidence of infarction attributed to VSP occurred in 2% of treated patients and 6% of control patients. In addition, 4% of the controls died of severe VSP. All of these deficits occurred despite vigorous hypertensive/hypervolemic treatment. In the 15 patients who had severe IVH, after injection of the t-PA the intraventricular catheter was closed for 60 min. In 2 patients with complete clotting of both ventricles, 13 additional intraventricular injections of t-PA were made on days 1–3 postoperatively. In the patients with severe IVH but not complete clotting of the ventricles, the t-PA injection resulted in complete clot removal in 5 patients and incom-

plete although radical clot removal in an additional 7. In the 2 patients with complete ventricular casts, multiple injections of t-PA over 4 days resulted in radical but still not complete clot removal. In all 15 patients it was possible to keep the ICP only moderately elevated and under 20 cm $H_2O$. Using the dosage of 10 mg of t-PA the only complication was minor oozing from the incision sites of the craniotomy. The authors considered that the conversion of a SAH from a Fisher grade III to a Fisher grade II was sufficient for a significant reduction in the incidence of post-SAH DID (168).

Four patients out of eight treated at one center with t-PA developed angiographic and clinical VSP. One patient had a massive SAH and was operated on with a t-PA installation on day 2. An additional 8 mg of t-PA was instilled through a ventriculostomy on day 3. On post-SAH day 6, she developed a right hemiparesis requiring volume expansion and hypertension. Eighteen months later she had made an excellent recovery with only mild memory problems. The second patient, considered to be a failure of t-PA, had an occluded right internal carotid artery and an 80% stenotic lesion of his left internal carotid artery. He was operated on day 2 post-SAH. Clearing of the SAH was demonstrated by post-SAH day 3. On post-SAH day 7 progressive lethargy and left hemiplegia developed. Angiography on day 8 demonstrated severe VSP of the posterior communicating artery. Hypertension and hypervolemia were used and the patient recovered with a residual moderate right hemiparesis. A third patient was admitted 12 days after a possible SAH with a major bleed. He was operated on day 2 post-SAH (day 15 post-initial hemorrhage). On day 6 an angiogram demonstrated severe VSP and he was treated with volume expansion and hypertension. He was neurologically normal 1 year later. The fourth patient was admitted 7 days following an initial hemorrhage. On day 1 post-second SAH she had craniotomy and t-PA installation. Her SAH had disappeared by postoperative day 1 except for a small amount of residual blood in the interhemispheric fissure. On postoperative day 6 she developed progressive confusion and right hemiplegia. Angiography 2 days following this demonstrated severe bilateral cerebral artery spasm. She was treated with hypertension/hypervolemia and made a complete recovery (169). Although these were considered to be failures of t-PA, it is entirely possible that the degree of VSP was lessened by earlier clot resolution and that the outcome might not have been as favorable as it was without the use of t-PA.

In the first account of t-PA in association with endovascular aneurysm occlusion, thrombosis of aneurysms using cellulose acetate polymer within 23 hr of aneurysmal rupture was reported. Nine patients having diffuse localized thick subarachnoid clots, 2 patients with diffuse thin clots, and 1 with intraventricular hemorrhage (IVH) were treated immediately after therapuetic aneurysmal thrombosis with the administration of t-PA through spinal or ventricular catheters. Lumbar CSF pressure was maintained at 100–150 mm $H_2O$. t-PA was given as multiple injections of 2 mg on day 0 and 1–2 mg on the following 1 or 2 days. In 2 patients the second injection of t-PA was not given because of severe brain damage resulting from the initial SAH. Ten patients showed complete clearance of cisternal clot on CT within 72 hr after aneurysmal thrombosis and t-PA injection. Seven partially thrombosed aneurysms and five multiple aneurysms were clipped during delayed surgery. There was only one instance of mild VSP on the follow-up angiography. Outcomes were as follows: good recovery, 67%; severe disability, 17%; and death, 17%. Urgent endovascular thrombosis of a ruptured aneurysm followed by immediate postthrombotic administration of t-PA was considered to be a safe and reasonable means of preventing VSP (170).

Fifteen patients treated within 3 days of SAH with cisternal installation of 10 mg t-PA were reported. All were Fisher grade III. In comparison with a control group, the patients who received t-PA rarely experienced radiological signs of VSP or DID (171). In a Japanese series, 104 patients undergoing surgery within 48 hr post-SAH were randomized into four groups: cisternal drainage, ventricular or cisternal irrigation combined with cisternal drainage, cisternal urokinase (UK) irrigation with cisternal drainage, and single intracisternal t-PA injection with cisternal drainage. Cisternal drainage was performed until 20 days post-SAH. In the t-PA group 29 patients received a single intraoperative injection of 8 mg, and postoperative cisternal drainage was employed for 7 days. DID occurred in 41% of patients having only cisternal drainage, 23% of patients having irrigation and drainage, 32% of the UK group, and 17% of the t-PA group. Good outcome was more common in the t-PA than in the other groups. Of the 26 t-PA-treated patients, 7 died from premature and intraoperative rupture (4%), large ICH preoperatively from initial rupture (15%), and severe VSP (8%). Of these, 7 deaths were Fisher group IV prior to operation (172).

Nine North American centers contributed patients to a double-blind, randomized, placebo-controlled trial of cisternal administration of 10 mg t-PA using block randomization. End points included angiographic VSP and postoperative and intraoperative intracranial hemorrhage. One hundred patient were randomized, 49 to placebo and 51 to t-PA. Both placebo and t-PA patients had an 87% incidence of thick subarachoid clot. The placebo group had 16% ICH and 23% IVH compared to 24 and 22% for the t-PA group. Nine randomized patients did

not receive the anticipated t-PA in the operating room. In 8 this was due to conditions believed to be unsafe for the administration of a fibrinolytic agent. Angiography performed between days 7 and 11 post-SAH showed an incidence of angiographic VSP of 74% in placebo and 65% in t-PA-treated patients. The rates for no or mild, moderate, and severe VSP were 69, 16, and 15%, respectively, in the t-PA group versus 42, 35, and 23% in the placebo group. There is a trend to a lesser degree of VSP in the t-PA group but this did not achieve significance. When only those patients with thick subarachnoid clot were considered there was a 56% relative risk reduction of severe VSP in the rt-PA-treated group which was significant. Considering just the dosed patients with thick clot, 56% made a good recovery and 12% died in the t-PA group versus 38 and 22% in the placebo group. Most important, overall bleeding complications were the same between groups. Given the relatively small size of the prospective trial, it is not surprising that efficacy was not demonstrated in a statistically significant fashion. Mortality at 14 days for those patients was 12% in the placebo group and 7% in the t-PA group. At 3 months, 57% of the t-PA group had made a good recovery versus 43% of the placebo group. Bleeding complications occurred in 16.3% of the placebo patients and 18.8% of the t-PA patients. No intracranial epidural bleeding occurred in either group. Intraoperative blood transfusions were used in 5% of the placebo patients and 4% of the t-PA patients. The mean Hct declined from 41% preoperatively to 32% postoperatively in the t-PA patients and from 39 to 32% in the vehicle patients (173). Angiographic VSP occurred in 73% of the thick clot vehicle patients and 63% of the thick clot dosed patients, and severe angiographic VSP occurred in 27 and 12%, respectively. This study was underpowered to demonstrate efficacy, but the trend in favor of t-PA was encouraging and consistent with prior studies. More important, there were no striking differences with respect to postoperative hemorrhagic complications (174).

### 2. *Urokinase*

Between 1986 and 1991, a retrospective review of 60 patients treated with UK (60,000 IU/day for 7 days), 22 patients treated with t-PA (0.42–1 mg t-PA every 6–8 hr for 5 days), and 29 patients not receiving fibrinolytic treatment was performed. The no treatment group comprised historical controls from prior to 1986 and patients in whom fibrinolytic therapy was not used because of small-volume SAH. UK was employed prior to 1991 and t-PA thereafter. The incidence of infarction was less in both treated groups compared to the no treatment group despite the larger amount of initial SAH in both UK and t-PA groups. This was attributed to the more rapid clearance of the cisternal clot by fibrinolysis. Only t-PA therapy reduced the incidence of symptomatic VSP in a statistically significant fashion. No serious complications were observed, although in the t-PA group asymptomatic IVH occurred in 1 patient. Meningitis was suspected in 27% of the UK group. There were no differences in overall outcome among the three groups at 3 months. It was concluded that postoperative intrathecal thrombolytic therapies, especially with <4 mg/day of t-PA, were effective in lysing subarachnoid clot and safely prevented VSP and infarction (175).

Ikeda and Shida used a ventriculocisternal intermittent drainage system with UK and sodium nitrite infusion once or twice daily. In a third group, a thromboxane $A_2$ synthetase inhibitor was used in addition to the fibrinolytic and vasodilator regimen. All 32 patients were Fisher grade III. Symptomatic VSP occurred in 30% of the once-daily infusion and 7% of the twice daily. In the once-a-day infusion group there was one death from meningitis and one from hemorrhagic infarction. In the twice-a-day infusion group there was one death from VSP, and in the third group there were no VSP or deaths. These are relatively small groups, so it is difficult to attribute significance to the difference in outcomes (176). In 1983, Yoshida and colleagues reported on the use of postoperative intrathecal irrigation with UK after early aneurysm surgery (177). Five years later, Kodama *et al.* reported that higher concentrations of UK were required. Fifty patients with Fisher grade III CT scans and Hounsfield units >60 were selected for UK therapy, only 6% developed symptomatic VSP and only 1 showed permanent sequelae. The UK was delivered through a cisternal irrigation system. The average duration of therapy was 10 days and complete bed rest was required during the treatment period. The safety of irrigation was dependent on meticulous nursing and medical care. Two-cm-diameter columnar clots *in vitro* were dissolved by 60–120 IU/ml UK. At the higher dose, clots were reduced by 83% in weight in the first 24 hr (178).

Sixty patients in neurological grade III underwent surgery in the first 3 days post-SAH. Permanent DID caused by VSP occurred in 31% of patients without cisternal drainage, 15% with such drainage, and only 10% of patients with combined drainage and UK injection. The amount of blood on the initial CT scan correlated closely with the occurrence of symptomatic VSP. The volume of bloody CSF drained during the first 10 days after surgery and the duration of drainage placement were related to the prevention of VSP. The greater the volume of CSF drained, the greater the reduction in the incidence of permanent neurological deficits from VSP. On the other hand, there appeared to be an increased require-

ment for shunts in patients with the largest volumes drained (179).

Kodama and colleagues recently reported on the results of cisternal irrigation therapy with UK and ascorbic acid. The latter was added to convert oxyHb into verdoheme-like products, considered to be less spasmogenic. Two hundred and seventeen Fisher grade III patients were studied within 3 days post-SAH. Through and through irrigation inlet tubes were placed in the Sylvian fissure unilaterally or bilaterally and the prepontine or chiasmal cistern for outlet drainage. The UK and ascorbic acid were infused in a lactated Ringer's solution at a rate of 30 ml/hr/side for approximately 10 days. Symptomatic VSP occurred in 3% of patients and only 2 of them (1%) demonstrated a sequella. The average total volume of blood removed from the drainage fluid was calculated to be approximately 114 ml. Two patients experienced seizures, 2 developed meningitis, and 4 had an intracranial hemorrhage. All these patients recovered without neurological deficits.

## V. Management of Delayed Ischemic Deficit

Table 8.12 summarizes our current approach.

### A. Monitoring for Delayed Ischemic Deficit

Following SAH, patients are monitored in an intensive care or a step-down unit during the time of risk of VSP. Patients whose initial CT scan showed no or minimal SAH in the basal cisterns are sometimes discharged in the 7- to 10-day period if they have done uneventfully well following clipping or coiling. Patients with thick clots on the initial CT scan are kept hospitalized under close observation for at least 2 weeks. It is essential to avoid hypovolemia, hypotension, increased intracranial pressure, hyponatremia, and the use of antifibrinolytics if possible. Calcium channel antagonists are administered routinely while the patient is in hospital (63). For all patients at risk frequent vital signs assessment should be performed and recorded by the nursing staff. The Glasgow Coma Scale should be part of the nursing assessment (1). Particular vigilance is warranted for days 4–14 (Fig. 8.3). Nurses should be included in the management discussions, and they should be instructed on the specific clinical signs to be monitored if a specific vascular territory is suspected to be at higher risk because of the pattern of SAH.

### B. Immediate Actions on Detection of Delayed Ischemic Deficit

In the event of a new-onset neurological deficit possibly due to VSP, the patient's blood pressure should be raised even as he or she is being taken for an emergency CT scan. In the absence of a surgical lesion such as ICH or Hyc, infection, metabolic aberrations, and seizures, it is important to optimize the patient's blood volumeblood pressure, and oxygenation. Any cardiac arrhythmia should be treated (1). A peripheral blood specimen should be examined for evidence of infection, anemia, and electrolyte or metabolic disorder.

The prophylactic and therapeutic response to SAH from the perspective of VSP depends on the amount of

**TABLE 8.12 Preventing and Managing Delayed Ischemia**[a]

| |
|---|
| Surgery or coiling for aneurysm obliteration as soon as possible after diagnois except in some grade 5 patients or those with severe VSP and recent infarction |
| Consider postoperative administration of t-PA or urokinase after aneurysm clipping or coiling in patients with diffuse, thick SAH |
| Nimodipine, 60 mg enterally every 4 hr |
| Administer at least 3 liters of fluid per day; do not administer antihypertensive agents after aneurysm is clipped; allow blood pressure to increase spontaneously |
| Maintain normal ICP with ventricular drainage, mannitol, evacuation of clots, and short periods of hyperventilation as needed |
| Maintain Hct over 33% |
| Consider daily transcranial Doppler (TCD) ultrasound studies to monitor middle cerebral artery flow velocities |
| If deterioration due to VSP occurs, induce hypertension to levels at which DID clears and administer fluids to maximize cardiac output; maintain for at least 3 days or until TCD flow velocities begin to decrease |
| Consider angiography to diagnose VSP prior to aggressive therapy |
| Obtain angiogram if deficit does not improve with hemodynamic therapy, or if it recurs consider balloon angioplasty and/or intraarterial papaverine in these cases |

[a]Modified with permission from Macdonald, R. (1997). Cerebral vasospasm. In *Primer on Cerebrovascular Diseases* (K. M. A. Welch, M. R. Caplan, D. J. Reis, B. K. Siesjo, and B. Weir, Eds.), pp. 490–497. Academic Press, San Diego.

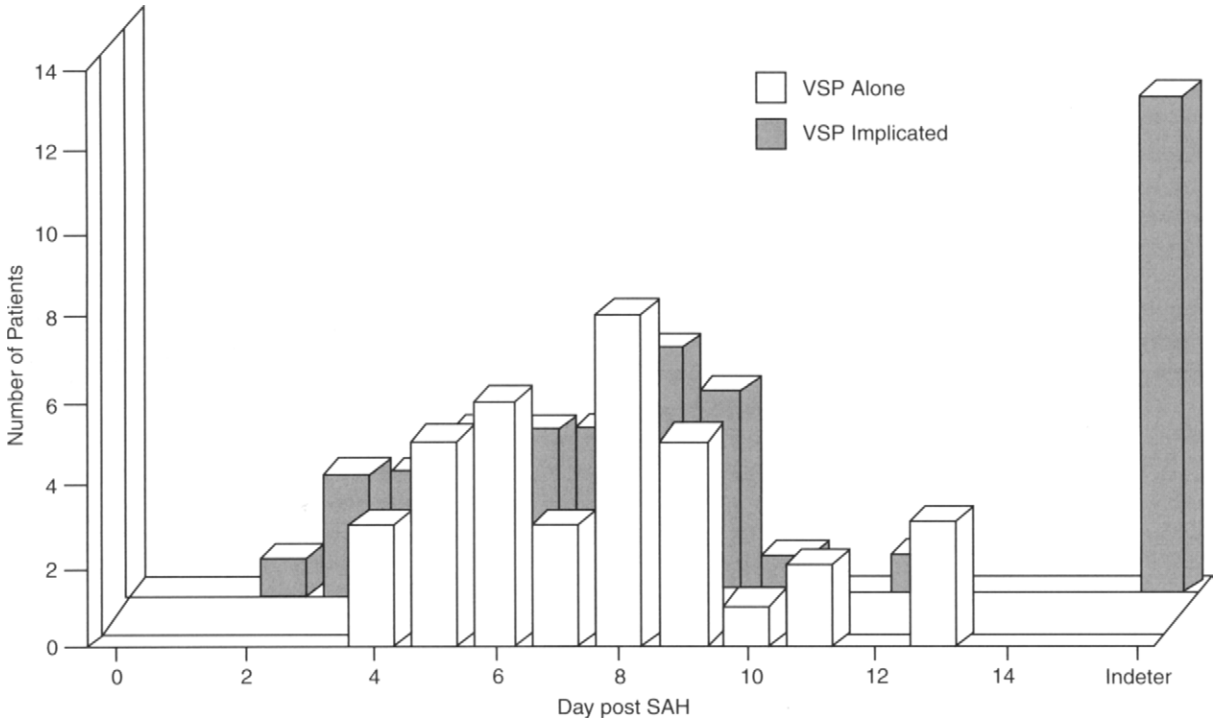


**FIGURE 8.3** An illustration of the day of onset of neurological deficits from VSP in 154 patients admitted in poor initial condition (grades III and IV). When VSP was considered to be the cause of delayed ischemia, the earliest day of onset was day 4 post-SAH. The last day of onset for ischemic deficit from VSP was day 13 (3 patients). Indetet, indeterminate day of onset [from *Subarachnoid Hemorrhage: Causes and Cures*, by Bryce Weir, copyright © 1998 by Oxford University Press, Inc. Used by permission of Oxford University Press, Inc.].

blood clot evident on the initial CT scan. If no blood is shown on the initial CT scan, it is adequate simply to avoid adverse factors such as hypovolemia and hypotension. When thin clot is present on the initial CT scan we, would add nimodipine and monitor by TCD. Modest hypervolemia would be induced during the time of risk for VSP. Treatment of early DID would be with hypertension and more vigorous hypervolemia. This approach would be used for patients with thick clot on CT, although angioplasty would be employed at an earlier stage if a significant neurological deficit developed (180).

At the first sign of DID, blood pressure is usually increased to over 150 mmHg systolic and sometimes up to a level of 240 mmHg systolic. The optimal level is the lowest blood pressure tolerated by the patient, at which the ischemic symptomatology is reduced. Occasionally, flat or Trendelburg head positioning can be helpful in improving DID symptoms acutely, but this should be done with caution in patients with airway problems, an open ventriculostomy to drainage, or severe brain swelling. Normally, crystalloid, albumin, plasma, and/or packed RBCs are administered in addition to the than 3 liters/day which maintains normovolemia or a mild degree of hypervolemia. Target parameters frequently advocated include a central venous pressure of up to 10–12 mmHg and a pulmonary capillary wedge pressure up to 15–18 mmHg. Patients receiving ionotropes require central administration and monitoring of cardiac function with a Swan–Ganz catheter. Active hemodilution should not be sought. The Hct usually decreases spontaneously. Packed RBCs should be administered to keep the Hct >33% (5).

ICP should be normalized to obtain an optimal cerebral perfusion. Blood pressure is elevated until the deficit improves, although we do not recommend protracted use of inotropes if the deficit is fixed or worsening. Supplemental $O_2$ is administered. If TCD does not demonstrate changes consistent with VSP in appropriate vascular territory from the patient's symptoms angiography should be considered.

Transluminal angioplasty is a reasonable therapeutic option if a clinically significant new deficit does not improve with hypertension and hypervolemia and if there is no established low-density area on the CT scan. Fever should be vigorously treated with ibuprofen, acetylsalicylic acid and/or acetaminophen and a cooling

blanket. Shivering should be treated pharmacologically (5). Even if VSP is present and severe, it must always be considered that it can be aggravated and rendered symptomatic by coexisting cardiorespiratory problems, intracerebral postoperative complications, and other late consequences of SAH such as Hyc.

Wilkins (181) summarized the hundreds of investigations performed over several decades involving potential therapies such as vasodilators, vasoactive antagonists, and other pharmacological efforts to inhibit vascular smooth muscle contraction. Many of these approaches were apparently successful in experimental models, but virtually none have stood the test of clinical application or indeed have appeared to be promising enough to justify clinical trials. He subsequently discussed more than 80 different treatments which have been used in an attempt to treat VSP (181,182). A wide variety of pharmacological agents have been utilized as therapy for VSP and DID with only moderate success.

### C. Likelihood of Delayed Ischemic Deficit Developing

An extensive literature review suggested that angiographic VSP occurs in 67% of cases when angiography is timed for the highest likelihood. DID or symptomatic VSP occurred in about one-third of cases. For the patients developing DID, approximately one-third had a good outcome, one third had permanent deficits, and one-third died. The natural history of aneurysmal SAH is therefore as follows: About 10% of patients have a permanent deficit and 10% die from VSP. These data were drawn from the literature as a whole and no specific therapies were analyzed (183). Recent studies suggest a better outlook if patients develop DID (Table 8.13).

**TABLE 8.13 Correlation of Delayed Ischemia with Cerebral Infarction on Follow-up CT Scan[a]**

| Infarct on follow-up CT scan | Ischemic symptoms | | | |
|---|---|---|---|---|
| | None | RIND | FND | Total |
| | No. (%) | No. (%) | No. (%) | No. (%) |
| No | 91 (82) | 13 (41) | 6 (12) | 110 (56) |
| Yes | 20 (18) | 19 (59) | 46 (88) | 85 (44) |
| Total | 111 (100) | 32 (100) | 52 (100) | 195 (100) |

[a]Reproduced with permission from Juvela, S. (1995). Aspirin and delayed ischemia after aneurysmal subarachnoid hemorrhage. *J. Neurosurg.* **82,** 945–952. $p < 0.0001$ for the correlation of ischemic symptoms with the presence of cerebral infarction. CT, computerized tomography; RIND, reversible ischemic neurological deficit; FND, fixed ischemic neurological deficit.

### D. Delayed Ischemic Deficit after Coiling

Sixty-nine patients treated by aneurysmal coiling within 72 hr of rupture showed a symptomatic VSP rate of 23%. Admission neurological grade and the amount of blood on the initial CT scan were both associated with the incidence of subsequent VSP. At 6 months clinical follow up of patients with VSP, 75% had a good recovery and 13% had died of VSP. The mortality rate due to VSP for the entire group of patients was 3% (184).

### E. Neuroprotective Strategies

Ischemia is an evolving process and not an instantaneous event so that the possibility exists to modify the process favorably after the clinical ictus. On the basis mainly of animal models, cellular injury may be limited by inhibiting glutamate release, antagonizing postsynaptic glutamate effect, altering NO synthesis, preventing inflammatory cell recruitment, neutralizing free radicals, potentiating adenosine activity, blocking apoptosis, or stimulating neuronal repair (185). However, these therapies, although promising in the laboratory, are not at the point of therapeutic application.

## VI. Therapy

### A. Hypertension

Shanbrom and Levy studied two patients with advanced antherosclerosis in whom maintenance of systemic arterial pressures above a critical level was necessary to overcome cerebral ischemic symptomatology (186). The use of vasopressors is generally reserved for patients with evolving new deficits. In patients with developing ischemic deficits the systolic blood pressure should be raised to the point at which the deficits recede but not higher than 240 mmHg systolic. In the presence of unclipped ruptured aneurysms a maximum of 180 mmHg is used. It is critically important to observe patients clinically minute by minute when hypertension is being induced since in some patients there is a paradoxical deterioration (4). If the patient is treated within minutes of onset with hypertension and hypervolemia, the symptoms and signs may resolve within minutes to hours (10). Patients on vasopressors should have daily electrocardiograms to detect cardiac ischemia. Patients with a history of cardiac contractile dysfunction require careful analysis of risks and benefits of vasopressors, and Swan–Ganz catheter-facilitated monitoring may be advisable in such patients.

Hypotension has disastrous effects on patients suffering from recent strokes of all types. It was therefore

hypothesized that raising systemic blood pressure would be helpful. Efficacy of systolic blood elevations in the vicinity of 50–60 mmHg was analyzed in patients with strokes other than SAH. By the mid-1970s, deliberate attempts were made to increase blood pressure and volume in patients post-SAH to reverse neurological deficits (130,187). Dopamine became the most commonly used hypertensive agent. In the dosage of 2 μg/kg/min or higher, dopamine has an ionotropic effect with renal vasodilation. In the dose range of >10 μg/kg/min its effects resemble those of norepinephrine, of which it is the normal precursor. It commonly causes tachycardia. The actual dosage must be titrated against the desired pressure response (152). Although dopamine is most commonly used, phenylephrine is an alternative. If a cardiac stimulant is required, dobutamine is employed (152), but its effect of peripheral vasodilation can lead to consequential BP lowering in VSP patients.

In 1993 and 1994, 24 patients with DID were treated using phenylephrine as part of a hypertensive – hypervolemic protocol. Patients with signs of ongoing myocardial ischemia were excluded. Also, patients with a baseline mean arterial pressure consistently >135 mmHg without the use of vasopressor drugs were excluded. Colloids or crystalloids were infused at >35 ml/kg/day. Fluid input was kept greater than output by approximately 0.5 liters/day. Patients were transfused for Hct <30%. Hypertensive therapy was instituted if a rapid bolus of crystalloid or colloid did not result in prompt neurological improvement. Phenylephrine was administered by continuous iv infusion into a central vein with a starting dose 20 μg/min. The infusion was rapidly titrated to initially increase MABP to 20–25% above baseline. After 2–4 hr of maintained pressure elevation, if the patient did not improve neurologically the MABP was further increased for a similar period. There was no absolute ceiling for MABP. However, therapy such as angioplasty was considered if the patients did not have clinical improvement at a blood pressure elevation of >35%. Once maximal improvement had been obtained the associated blood pressure was maintained for at least 2 days. Sixty-three percent of patients were Fisher grade III on admission. Fifty-eight percent were neurological grades III and IV. The mean age was 55 years, and the mean fluid intake on active therapy was 9.2 liters/day. The mean maximum dose of phenylephrine was 7.56 μg/kg/min. This resulted in a change in MABP from 99 to 123 mmHg, and systemic vascular resistance increased from 1234 to 1739 dyne/sec/cm$^{-5}$. Pulmonary artery wedge pressure increased from 13 to 16 mmHg and cardiac index increased from 4 to 4.1 liters/min/m$^2$. The duration of induced hypertension was a mean of 5 days. In 1 patient with no clinical improvement, the phenylephrine was stopped after 2 days. Complications were remarkable few. One patient had a transient one-time elevation of CPK-MB fraction to 3%. A second patient had T-wave inversions in several ECG leads. A third patient had intermittent bradycardia. None of these complications were serious or permanent. Mild to moderate interstitial infiltrates occurred on chest X-ray in 38% of patients at some point. Only 4 patients had transient increased $O_2$ requirements. In all cases fractional inspired $O_2$ was $\leq$50%. No patient required intubation. Eighty-eight percent of the patients in this series improved. The complication rate may have been relatively low in this series because the hypertension and hemodilution were introduced therapeutically and not in the first few days following SAH when patients may be relatively susceptible to cardiorespiratory failure (188).

A meta-analysis of 14 peer-reviewed studies was performed by Pritz *et al.* The overall success of hyperdynamic (induced hypertension and hypervolemia) therapy was 77%, with a 95% confidence interval of 69–86% (189). We observe, however, that the success rate of no treatment of symptomatic VSP may be as high as one-half to two-thirds. Pritz *et al.* used the Swan–Ganz catheter to perform cardiac output measurements which were averaged on days 2 or 3 for different pulmonary wedge pressures, including repetition of cardiac output at the same pulmonary artery wedge pressure. After an optimum pulmonary wedge pressure was ascertained, the pulmonary artery diastolic pressure that correlated with the optimal pulmonary artery wedge pressure was determined. The authors did this because repeated wedging of the pulmonary artery catheter can occasionally cause balloon malfunction, which would prevent obtaining a wedge tracing, and also pulmonary artery diastolic pressure can be recorded continuously. Volume status is then geared to that pulmonary artery diastolic pressure. Cardiac output measurements were not routinely used providing the patient's status was stable and no new factors influencing these measurements were introduced.

Blood flow through large-diameter blood vessels, the conductance vessels, may be characterized by the Hagen–Poiseuille equation in which $Q$ is blood flow, $P$ is pressure, $r$ is the vessel radius, $L$ is the vessel length, and $\eta$ is viscosity:

$$Q = \frac{\Delta P \pi r^4}{8 L \eta}$$

This equation has been used by many workers to suggest that blood flow can be manipulated. The limitation to the application of this formula is that it is based on non pulsatile flow of Newtonian fluids through rigid vessels. Actually, blood behaves as a non-Newtonian fluid and

CBF is principally regulated by multiple small resistance vessels and not the larger conducting ones (190). In addition, the hematocrit in the microvasculature or in ischemic regions may be different from that in peripheral venous samples. The optimal hematocrit for $O_2$ delivery to the ischemic portions of brain is unknown and may vary from site to site, moment to moment, and patient to patient. Pritz (191) employed hetastarch infusions of $\leq$500 ml infused over 4 hr in a single 24-hr period. The Hct is maintained at a minimum of 30%, and antihypertensive medication is discontinued. If symptoms of ischemia are not reversed, dobutamine (2.5 μg/kg/min) is instituted. The dose is titrated against blood pressure and response to induced hypertension. Blood pressure is raised to as high as 228 mmHg systolic in patients with obliterated aneurysms. In certain circumstances, if Hct is <30% transfusion is given (191). We do not recommend hetastarch.

### B. Hypervolemia

The most widely used therapy for reversing neurological deficits due to VSP is the augmentation of cerebral perfusion pressure with volume expansion and pharmalogically induced hypertension (60,130,187,192). Pathologically elevated ICP is also reduced to increase cerebral perfusion pressure. Patients have depressed circulating blood volumes and decreased RBC mass after SAH (193). Blood volume status is a key factor in predicting patients who will develop neurologic deficits from VSP (34,131,194,195). In 1979, Maroon and Nelson demonstrated blood volume in 15 nonselected patients post-SAH (146). Hypovolemia was measured and the contracted blood volumes were attributed to bed rest, supine diuresis, peripheral pooling of blood, negative nitrogen balance, decreased RBC production, and iatrogenic blood loss. Other workers have presented similar findings (131, 139). Increased sympathetic activity has been suggested as a factor in the reduction of total blood volume post-SAH (196,197).

Dorsch reviewed 31 reports specifying fluid loading and/or hypertensive treatment. Eighteen percent of the patients developed DID. It was suggested that this was a moderately lower risk than expected from the natural history. More commonly, these entities are used as prophylaxis. Dorsch found 73 references with 2111 patients. Outcomes were as follows: good, 54%, permanent deficit, 29%; and dead, 18% (7).

The effects of hypervolemia are difficult to dissect from the effect of altered viscosity. Increasing the blood volume should increase CBF if the viscosity is reduced and also if cardiac output is augmented. There is always a risk that hypervolemic can aggravate cerebral edema and elevate ICP (63). It has been suggested that fluid administration in patients with SAH should be 2.5–3.5 liters/day of normal saline and then increased to 6–8 liters/day after aneurysm clipping. Such a regimen maintained plasma volume and decreased serum concentrations of ADH, aldosterone, and plasma renin. ANF factor concentrations were increased (198). Hct is the most important determinant of whole blood viscosity (199). CBF increases following hemodilution but it is uncertain whether $O_2$ delivery or the blood viscosity is the primary determinant. Viscosity and $CaO_2$ may be independent variables. Active vasodilation of the cerebrovascular bed may occur in response to reduced bulk $O_2$ transport or to a decrease in $pO_2$ (200).

Induced hypervolemic hypertension is a physiological stress and the body tends to counter it by diuresis and reflex bradycardia. In a therapeutic volume expansion the most reasonable end point is neurological function. There are no clear guidelines for volume loading with crystalloid or colloid in patients with symptomatic VSP. It is considered likely that changes in colloid osmotic pressure are associated with volume expansion, which could aggravate ischemic cerebral edema (201). Prospective trials to evaluate the effectiveness of the independent contributions of hemodynamic and rheologically based therapy for delayed cerebral ischemia from VSP have been called for but not carried out (201).

Thirty hypertensive patients were classified into two groups at one center. In one group volume expansion was induced and hypertension was controlled with vasodilators and centrally acting drugs, whereas patients in the control group were treated with the same antihypertensive agents but without volume expansion. Blood pressure averaged above 150/95 mmHg. Pulmonary artery catheters were inserted. Various hypertensive agents were used to maintain systolic blood pressure above 120 mmHg in previously hypertensive patients. Volume expansion was obtained with packed RBCs and 5% albumin in crystalloid solutions. Pulmonary capillary wedge pressures were increased from an average of 5 to a range of 12–15 mmHg. If initial wedge pressures were above 18 mmHg, furosemide was given to lower it. The incidence of VSP preoperatively was 20% in the treated group versus 60% in the control group. Fully 87% of those in the group treated with volume expansion survived eventual operation, only 53% of the control group did so (4).

In a unique, prospective, randomized, controlled study CBF was measured in patients in whom an attempt was made to render them either hypervolemic or normovolemic. On the first postoperative day, 82 patients were randomized to receive 80 ml/hr of isotonic crystalloid (5% dextrose and 0.9% saline) until post-SAH day 14 plus 250 ml of 5% albumin every 2 hr if pulmonary artery diastolic pressure fell below 14 mmHg on postoperative

days 0–3 or central venous pressure fell below 8 mmHg on days 4–14. These patients comprised the "hypervolemic" group. Patients in the "normovolemic" group were only given albumin at pressures lower than 7 mmHg. Although the hypervolemic group received more fluid and had higher pressures on postoperative day 3 there was no difference in net fluid balance or blood volume. Mean global CBF did not differ during the treatment period. Symptomatic VSP occurred in 20% of each group. Ten percent of the normovolemic patients developed infarction from VSP versus 17% of the hypervolemic patients. It was concluded that prophylactic hypervolemic therapy was unlikely to confer an additional benefit over simple avoidance of hypovolemia (202).

### C. Hemodilution

Wood stressed the potential importance of lowering blood viscosity to improve blood flow in areas of the brain that have been damaged. Viscosity is the thickness of blood, and it is determined by factors such a hematocrit, RBC aggregation, RBC flexibility, platelet aggregation, plasma viscosity, and shear rate (velocity gradient). In general, CBF falls as blood viscosity increases. Blood flow distal to a vasospastic arterial segment will be adversely affected by depression of shear rate gradients. This can result in increased viscosity within ischemic brain. Both Hct and the extent of collateral channels available will be important factors in determining the outcome from focal ishemia (203). Wood recommended 250 ml of 5% albumin intravenously over 30 min followed by 250 ml every 4 hr. The infused volumes were used to try to maintain a Hct of approximately 33%. Salt-free albumin was employed for hypernatremic patients. He did not employ whole blood transfusions for symptomatic patients unless the Hct was under 30% (203). Hemodilution can have deleterious effects on patients with established VSP. If the Hct falls sufficiently, the $O_2$ carrying capacity of the blood will result in decreased $O_2$ delivery to the brain. Although flow may increase as a result of the reduced Hct, the reduction in $O_2$ delivery may more than offset its potentially beneficial effect (63). Changes in blood viscosity determined by coaxial viscometry cannot be used to quantitatively predict changes in CBF. Characteristic rheological phenomena such as red cell aggregation and deformability, plasma viscosity, and protein composition are important for the rheological aspects of microcirculatory supply function, which is reflected by their contribution to macroscopic viscosity of the blood (144).

Hemodilution reduces Hct and thereby improves CBF; however, it reduces $O_2$ carrying capacity. Tissue $O_2$ supply follows an inverse U-shaped curve. $O_2$ delivery reaches its peak as Hct falls, but as Hct is reduced below a critical point $O_2$ delivery starts to fall. Although this relationship has been demonstrated in many peripheral tissues in various species, it has not been clearly established to exist in the human brain. The optimum Hct level for $O_2$ delivery to ischemic brain tissue is unknown. Experimental data suggest that the optimal Hct in providing maximal $O_2$ delivery to normal tissues is in the 30–33% range but it may be higher for $O_2$ delivery to brain tissue (137,204). Hemodilution can be obtained by infusion of a plasma volume expander and may include concomitant venesection. If the volume of the liquid infused equals the volume of blood removed the hemodilution is referred to as being isovolemic. In most instances following SAH deliberate venesection is not employed so that most hemodilution is hypervolemic. Plasma expanders in ischemic stroke studies have included dextran 40, hydroxyethyl starch (HES; pentastarch), and albumin. Hemodilution undoutedly can increase blood flow to both ischemic and normal brain, but the critical unanswered question is whether $O_2$ supply is thereby increased.

In ischemic stroke studies, 2756 patients were randomized in 15 trials. The reduction in Hct was similar in all but one trial and resulted in a decrease of 4–7% in absolute terms. There were more deaths in the treated group, and the odds ratio for death was 1.14. There was clearly no beneficial effect of hemodilution on survival. Anaphylactic reactions to dextran or HES occurred in 6 of 969 patients, although none of these reactions were fatal. In only one study was albumin part of the hemodilution regimen. There was an insignificant trend toward lower case fatality among treated patients at late follow-up. No beneficial effect of hemodilution was observed in a group analysis with the highest Hct levels at entry. Trials reporting cardiovascular events in a systematic manner failed to document any major increase in adverse circulatory events in hemodiluted patients. The majority of the patients in these trials were treated more than 6 hr after onset of stroke symptoms. However, from the available data for patients treated within 6 hr, it was believed by the reviewers that there was insufficient support for another large-scale clinical trial restricted to earlier initiation of therapy (205).

Fisher and colleagues demonstrated a significant elevation in the values of fibrinogen, plasma viscosity, and zeta sedimentation between the fourth and seventh days post-SAH in 12 patients with ruptured aneurysms (206). Positron emission tomography (PET) studies were performed on 5 patients with unilateral internal carotid artery occlusion and minor stroke. Hemodilution was performed by a 400-ml phlebotomy and infusion of 400 ml hydroxyethyl starch. After hemodilution the Hct decreased from 41 to

36% and the arterial $O_2$ content decreased from 18.6 to 16.5 ml/dl. CBF and $O_2$ transport were increased and OEF was decreased without any change in $O_2$ consumption. In this circumstance, hemodilution improved $O_2$ transport as well as CBF. Such improvement may be more prominent in patients with severely compromised hemodynamic states (207).

### D. HHH

Origitano and colleagues introduced the term "triple-H" therapy for prophylactic hypertensive hypervolemic hemodilution. They described 43 patients with SAH cared for between 1987 and 1990. Patients were not treated with nimodipine. Forty of their patients had proven aneurysms. In contrast to when the term triple-H is usually employed, the authors of this paper actually did phlebotomize their patients. During the 24 hr after admission Hb was lowered an average of 3 g/dl and Hct was lowered 9%. These values were maintained at 10 g/dl and 30% throughout the treatment. Weaning of triple-H therapy was attempted 10–14 days after surgery. Venesection of 150–250 ml of blood was performed concurrently with the administration of 250–500 ml of 5% albumin. Five-percent albumin was infused every 6 hr to maintain the Hct at 30% and a central venous pressure (CVP) between 8 and 12 mmHg. Intravenous fluids consisted of 5% dextrose and 0.45% saline with added KCl were administered at 100–125 ml/hr and adjusted by cardiovascular parameters. Systolic BP was maintained at 130–150 mmHg for unclipped aneurysms. CBF for normals in their laboratory was a mean of 45 ml/100 g/min. For the study patients the mean on admission was 39 ml/100 g/min. With the triple-H protocol employed, they were able to maintain mean CBF between 39 and 49 ml/100 g/min from days 2 to 11 post-SAH. Thirty-five percent of the patients showed signs of DID which resolved with more aggressive application of the protocol. Patients averaged 14 days on the protocol. The patients in this series were not analyzed by Fisher grade, so the likelihood of development of DID cannot be accurately estimated. In addition, 9% were admitted more than 9 days after SAH, beyond the time for the greatest likelihood of developing DID. The patients tended to be in good neurological condition on admission, with only 12% grade III and 9% grade IV. Two percent of the patients had permanent neurological deficits at days 9 or 10 post-SAH. Thirty-seven percent had evidence of angiographic VSP. Complications attributed to the protocol included urinary tract infection (30%), hyponatremia (126–128 mEq, 16%), diabetes insipidus (7%), delayed cerebral ischemia resulting in infarction (5%), pneumothorax (2%), and fatal rebleeding (5%) (208). Twenty-four patients who received Triple-H therapy with a variety of pharmacological agents to induce hypertension had no clinically significant evidence of pulmonary edema or myocardial infarction despite the fact that two-thirds of these patients had underlying hypertension, cardiac failure, or vascular disease (188).

The stroke and death rates from DID approach 15% in some series, with the best outcomes following hypertension, hypervolemia, and hemodilution (60,126, 145,209). When triple-H was employed deliberately as soon as possible after hemorrhage ( 28 references accumulated by Dorsch) the incidence of DID in 2361 cases was 18%. This was considered to represent a decrease by nearly half of the anticipated occurrence rate for DID. From 67 reports in which triple-H therapy was employed for established DID, outcomes were as follows: death, 17% of 1920; permanent deficits, 39% of 549; and good outcomes, 55% of 1046. Dorsch considered these to be improvements on the natural history (90).

### E. Cerebral Blood Flow

During VSP there is a loss of regulation of CBF to blood pressure (autoregulation) and possibly to blood volume, cardiac output, and viscosity. Altering these parameters can therefore augment CBF in some circumstances and thereby prevent cerebral infarction. Induction of hypertension will directly augment CBF in most circumstances. The blood–blood vessel barrier normally prevents adrenergic agents from inducing vasoconstriction of cerebral blood vessels, but it is possible that in the arterial injury following SAH vessel reactivity may become abnormal (63). If there is loss of autoregulation to blood pressure and possibly blood volume and cardiac output, manipulation of these parameters may augment CBF and prevent infarction from VSP. Cerebral perfusion pressure can be increased by elevating blood pressure, lowering intracranial pressure, increasing cardiac output by increasing blood volume, and administering cardiac stimulants such as dopamine and dobutamine. The numerous approaches to augmenting hemodynamic status post-SAH have in common a requirement for intensive care and a substantial risk of iatrogenic complications (5). Caution in using this therapy is required since raising the blood pressure occasionally results in reduction in blood flow in critical brain areas. This has been well documented by stable Xe-CT scans. If this technology is unavailable, the mainstay of assessment is continuous neurological observation (152).

rCBF measurements were performed before and after volume expansion on 35 patients post-SAH. Five hundred milliliters of 5% human serum albumin was infused over 30 min. The Hb decreased significantly, but mean arterial

BP did not change. During the first 2 weeks post-SAH, CBF decreased significantly with volume expansion. During the third week after SAH and up to the fourth, volume expansion produced no change in CBF. In patients with symptomatic VSP, CBF decreased significantly with volume expansion. In patients without symptomatic VSP, volume expansion produced no change in CBF. This study was unique in suggesting that increasing the intravascular volume above normal by volume expansion does not increase CBF or reverse symptomatic VSP. It was considered by the authors that CBF would have been increased if the only factors were hemorrheological ones, but they assumed that because this did not occur there may have been an increase in cerebral edema or cerebral blood volume with subsequent elevation of ICP that adversely affected CPP to prevent an increase in CBF (210). Rosenstein *et al.* used bed-side CBF determinations to document increases in CBF following hypervolemic therapy which reversed neurological deficits (211). $^{133}$Xe CBF measurements were performed in four cases with DID. Therapy consisted of fluid administration and induction of hypertension with phenylephrine. The MABPs before (after) treatment were 95, 90, 98, and 123 mmHg (112, 125, 126, and 173 mmHg). The Hcts before (after) treatment were 45, 33, 34, and 33% (31, 33, 32, and 33%). Blood flow was improved by these therapeutic maneuvers. CBF left/right in cc/100 g/min before (after) were as follows: 21/25, 20/18, 19/20, and 19/17 (33/38, 47/41, 26/32, and 26/33). Three of these cases had a good outcome, and one was moderately disabled. It should be noted that the diagnosis of VSP was made solely on the basis of CBF. This therapy was able to raise mean CBF in the most affected hemisphere by 64%. In another study, three of the four patients required hypertension for an average period of 8 days (145).

CBF was measured in eight cases post-SAH who were being treated with dopamine as therapy for DID following intracranial aneurysm surgery. Clinical deterioration occurred in seven of nine instances when CBF fell by more than 25% of its dopamine value but never occurred in six cases of withdrawal of dopamine when the CBF fell by less than 25% of its value on the hypertensive therapy (212).

Between 1983 and 1987, 60 patients had aneurysm clipping within 5 days post-SAH. The mean age was 53 years. Seven (12%) were moderately disabled, but none because of VSP. Three (5%) were severely disabled, one because of VSP. Of the 8 patients (13%) who died, 3 were well postoperatively and subsequently developed signs and symptoms of VSP (5% overall mortality from VSP). CBF measurements were performed on these patients. Seven patients diagnosed clinically with VSP had confirmatory reduction in CBF, whereas for 4 patients the diagnosis of VSP was considered but they were found to have normal CBF. In almost all cases the CBF was lower on the operated side and the increase in CBF with induced hypertension was 42% on the operated side versus 35% on the nonoperated side (213).

The product of CBF and total $O_2$ content of arterial blood ($CaO_2$) was computed as an index of $O_2$ delivery to cerebral tissue. The ratio CBF/CBV can be used as an index of regional cerebral perfusion pressure: $CaO_2 = (1.39 \times Hb \times SaO_2) + (0.0031 \times P_aO_2)$. In eight normal volunteers averaging 25 years of age, studies were performed before and after hemodilution. Hemodilution was accomplished by phlebotomy of 400 ml and intravenous infusion of 500 ml of low-molecular-weight dextran. Hematocrit was reduced from 42.5 to 37.2% and $CaO_2$ from 19.1 to 16.9 ml/dl. CBF increased from 45.2 to 47.7 ml/100 ml/min. Tissue $O_2$ delivery was decreased from 8.7 to 8.0 ml/100 ml/min and CBV from 4.9 to 4.6% in the overall cortical gray matter. This important sudy demonstrated that hemodilution in the tested range did not improve $O_2$ transport or tissue oxygenation in normal human brain despite the increase in CBF (214). In another PET study, hemodilution was shown to increase CBF but unfortunately actually reduced the $O_2$ delivery to the brain (215).

In 20 patients with aneurysmal SAH, serial CBF measurements were made using stable Xe-CT scans. All patient showed angiographic VSP. Sixty percent of patients did not have symptomatic VSP. They showed the lowest hemispheric CBF on the craniotomy side (32 ml/100 g/min) on days 4–9. The other 40% of patients with symptomatic VSP had the lowest hemispheric CBF also on the craniotomy side (25 ml/100 g/min) on days 10–14. The critical hemispheric CBF for DID was considered to be 20 ml/100 g/min in the group with symptomatic VSP. CBF was lower on the operated side at all time periods after surgery regardless of whether or not the patient had symptomatic VSP. The patients with symptomatic VSP had the lowest CBF recorded and the nadir was later (days 10–14) than that of patients who did not develop symptomatic VSP. In the period of lowest blood flow for the patients with symptomatic VSP, the induction of hypertension increased CBF on the craniotomy side from 25 to 34 ml/100 g/min and on the contralateral side from 32 to 36 ml/100 g/min. Therapy was induced with packed RBC transfusions, albumin, and induced hypertension. Therapy was continued for 3–5 days after onset (216).

Xe CT studies were performed on 51 patients post-SAH from aneurysms. Symptomatic VSP occurred in 27% of patients. In all of these patients with VSP, the

first postdeficit Xe CT study found an abruptly reduced CBF, either regionally or globally. Of patients with VSP, 64% had flow values below 50 ml/100 g/min in two or more adjacent cortical regions of interest. In all the patients with reduced flows, concurrent follow-up CT scans showed infarction in these regions. Eighty-nine percent of the patients with such reduced flow had paralysis and a severe sensory deficit. No patients with a CBF >18 ml/100 g/min developed infarction. The correlation of physiological and anatomical details in these studies is extremely advantageous. The drawbacks are the necessity of transporting patients, of maintaining the absence of motion, and of using radiation. There is a skin dose of 12–24 rads for each level of the brain being studied. This is comparable to the skin dose from conventional angiography. In CBF studies, however, the radiation is highly collimated to exclude cornea and thyroid exposure (217).

### F. Clinical Series

Farhat and Schneider reported prompt reversal of neurologic symptoms in patients suffering from acute cerebral vascular insufficiency when blood pressure was increased by 50–60 mmHg in systolic pressure (218). Wise and coworkers also reversed the neurologic deficit by employing vasopressors in patients suffering from cerebral ischemia. Vasopressors were given to 13 patients shortly after the development of focal brain ischemic signs, even in the absence of hypotension. In 38% of the patients, neurological function improved with hypertension to levels of 150–170 mmHg systolic and 85–100 mmHg diastolic. Symptoms from focal brain dysfunction occurred whenever the blood pressure was allowed to fall to the initial level right after the insult. Clinical improvement was usually observed within an hour of administration of therapy. They recommended that since vasoconstrictor drugs could produce volume depletion careful monitoring and adequate volume replacement should be done (219). Wernick and Sugar also treated a postangiographic hemiplegia with success using vasopressor medication (220).

Kosnik and Hunt (130) described 7 patients with SAH who developed neurological deficits. In 6 of the 7 there was a marked improvement in their state following the raising of blood pressure volume and central venous pressure. It was assumed that autoregulation was at least partly lost in patients with a "cerebral hemodynamic crisis." Blood volume was employed to augment the vasopressors in maintaining systemic hypertension. They used norepinephrine (16 mg), in 500 ml of D 5/0.45 normal saline given intravenously through a central venous line. It was suggested that if ischemia and infarction were already well developed when this treatment was employed, the hypertension might be useless and might in fact even accelerate brain swelling. Other hazards include the nonspecific ones associated with an indwelling central venous line and the possibility of fluid overload and congestive heart failure. Giannotta and colleagues (192) studied 17 patients who developed severe neurological deficit from postoperative cerebral VSP. All patients had initial angiograms. They were able to completely reverse neurological deficit in 12 patients and partially reverse it in 3. In their 17 cases, the initial CVP following the onset of ischemic symptoms was <5 cm water. They elevated the pressure to 8–10 cm water by infusion of 1 or 2 units of whole blood and maintained this level of CVP by the use of blood, plasma, or albumin. This elevated the systolic blood pressure in 10 of 17 patients to 140–170 mmHg. Low-molecular-weight dextran was infused in doses of 29 mg/kg/day for the first day and in decreasing doses over the following 2 days. If neurological deficit was not immediately improved and systolic blood pressure remained below 140 mmHg, the patients were treated intravenously with phenylephrine or dopamine to maintain a systolic blood pressure above 150 mmHg. In the presence of continued neurologic deterioration, the patients were intubated and mechanically hyperventilated. The $p_aCO_2$ was maintained in the 20 to 25 mmHg range, and $O_2$ was maintained at 100 mmHg or more. In 2 patients with increased ICP, subdural pressure monitors were installed and mannitol and urea were used.

Pritz and coworkers (187) studied four patients with SAH due to ruptured aneurysm in whom neurological deficits developed from VSP proven with cerebral angiography, and these were treated with intravascular volume expansion. After treatment, all four patients improved promptly, and none developed cardiac or pulmonary dysfunction despite aggressive treatment with increase in intravascular volume, cardiac symptoms, electrocardiographic abnormalities, or advanced age. Brown and associates (221) treated three patient with severe postoperative hemiplegia and one with hemiplegia following SAH with a combination of dopamine-induced hypertension, mannitol, and large quantities of intravascular fluids. All patients had large, surgically treated aneurysms. The three patients with postoperative hemiplegias did not have clots. They all woke from operation neurologically intact and subsequently deteriorated in the first 24 hr. Dopamine was given at a rate of 9–46 μg/kg/min to maintain a systolic blood pressure of 160–200 mmHg. The level of blood pressure capable of preventing further deterioration varied. The critical pressure for each patient had to be discovered by trial and error. Mannitol was used to keep ICP below 26 mmHg. Central venous pressure was

maintained at 8–15 cm water by intravenously adding fluids consisting of 0.2% normal saline with 5% dextrose in water.

Kassell and associates (60) reviewed 58 patients with progressive neurological deterioration from angiographically confirmed VSP. Treatment with intravascular volume expansion and an induced arterial hypertension was carried out. Therapy included blockade of vagal depressor response and the administration of antidiuretics and vasopressor agents. Arterial blood pressure was sustained for prolonged intervals in these patients. Neurological deterioration was reversed in 81%, transiently in 7%, and permanently in 74%. Blood pressure was raised in the presence of an unsecured aneurysm in 38% of patients. Overall, 16% of patients were unchanged and 10% deteriorated with this therapy. The causes of failures were listed as preexisting infarction (17%), progression of VSP (5%), rebleeding (2%), and inability to produce hypertension (2%). The complications of therapy included aneurysmal bleeding (19%), pulmonary edema (17%), dilutional hyponatremia (3%), coagulopathy (3%), pneumothorax (2%), and myocardial infarction (2%). Initially, low-molecular-weight dextran was used, but it was later abandoned because of adverse alterations in blood coagulation properties. Central venous pressure was maintained at approximately 10 mmHg or a pulmonary artery wedge pressure of 18–20 mmHg. Aqueous pitressin, 5 units administered intramuscularly, was used to keep urinary output below 200 ml/hr. Only 28% of patients needed this drug. Their preferred agent to induce hypertension was dopamine, although dobutamine was also used. Pressure increases to 240 mmHg systolic (150 mean) were employed. Some patients developed headaches at this level. A hysteresis effect was frequently observed with deficits resolving at a particular blood pressure level, although when blood pressure was subsequently lowered below that level the deficit did not reappear for several hours. Increase in infarct size or conversion of a bland to a hemorrhagic infarct with hypertension were not observed. In previous work, however, some authors noted a fatal intracerebral hematoma consequent to induced arterial hypertension. Rupture of a previously unruptured multiple aneurysm following induced hypertension had also been noted. All 3 patients with unsecured aneurysms who rebled on this therapy had arterial pressures in excess of 160 mmHg systolic at the time of repeat SAH. Frank cases of congestive heart failure were not seen. In some of their patients, improvement occurred following intravascular volume expansion but prior to the induction of hypertension.

Thirty hypertensive patients with SAH from ruptured aneurysm entered one study and were classified into two groups of 15 each. Blood pressure range was 300–160/150–95 mmHg. One group was treated for control of hypertension with vasodilators such as hydralazine (5–10 mg every 4–6 hr), methyldopa (250–500 mg every 4–12 hr), and, in 4 cases, sodium nitroprusside (3 μg/kg/min intravenously). To counteract the tachycardia induced by hydralazine, propanolol (1 mg every 4–6 hr), was used. Once hypertension was controlled, volume expansion was obtained with the use of packed cells to increase the Hct above 45% and crystalloid and albumin (5%) were used to increase pulmonary capillary wedge pressure to about 12–15 mmHg. The nontreated or control group received diuretics for hypertension, and pulmonary capillary wedge pressure was measured and blood pressure was reported after the therapy was started. In the treated group, angiography revealed VSP in 13 of 15 patients (87%), and in 3 of these patients (20%) neurological signs from VSP developed. In 1 patient, clinical signs of VSP were reversed by moderate induced hypertension. In this group, 2 of 15 patients (13%) died after surgical clipping of the aneurysm. In the nontreated group, VSP developed in 12 of 15 patients (80%) and in 9 (60%) DID developed. In this group, 7 patients (53%) died after surgical intervention. The poor rate of survival in the patients with hypertension was attributable to irreversible VSP and subsequent cerebral ischemia and infarction (222). In a study of over 100 patients treated with hypertension and hypervolemia, one-third had sustained improvement and one-fourth were stable. One patient in 6 worsened. Death or major neurological deficit from DID occurred in 7% of patients treated in this fashion. The therapeutic aims were Hct of 33–38%, pulmonary artery wedge pressure of 15–18 mmHg, and systolic arterial pressure of 160–200 mmHg for the duration of clinical VSP (126).

In 45 patients with Fisher grade 3 SAH, clipping was performed within 4 days post-SAH. Average age was 49 years. They were compared to 47 patients with similar Fisher SAH grade but who were not operated and averaged 53 years. All patients were treated with hypervolemic therapy without deliberate hypertension. Prophylactically 25% albumin was administered intravenously in a dose of 1–1.5 g/kg/day. If DID developed the dosage was increased to 1.5–3 g/kg/day. Albumin was administered for a mean of 11 days (5–19 days). Antihypertensive medication was administered to maintain systolic BP $<160$ mmHg and antifibrinolytic medication was employed in the patients who were not operated. Hematocrit was maintained $>32\%$. Patients were digitalized if CVP exceeded 15 cm $H_2O$ or the PWCP exceeded 15 mmHg. Symptomatic VSP occurred in 11% of the operated patients, although increasing the dose of albumin improved neurological function in 3 of these 5 patients. Thirteen percent of patients developed pulmon-

ary edema which required digitalization for control. In the nonoperated patients symptomatic VSP developed in 43% which was controllable with the higher dose of albumin in 15 of the 20 patients. Five (11%) of the unoperated patients had symptomatic VSP resistant to this therapy. The authors believed that the incidence of symptomatic VSP was lower than that of historical controls (110). Solomon *et al.* reported on 56 patients presenting earlier than 7 days post-SAH who were operated. Only 4% were neurological grade IV. Eighteen percent had DIDs which were reversible in 75% of these cases. Thirty percent of the DID patients had infarcts and 1 died. All patients had been treated with prophylactic volume expansion therapy and induced hypertension with central monitoring until day 14 post-SAH. The CT grades of SAH were not given (128).

In 199 patients, early surgery for ruptured aneurysms was performed. Thirty-one unruptured and unclipped aneurysms were present postoperatively. These patients were treated with prophylactic volume expansion. Mean CVP during treatment was 10.3 cm $H_2O$ and MABP 141/76 mmHg. Volume expansion was continued from 7 to 10 days. Eight patients developed DID requiring additional volume expansion and induced hypertension. Fifty percent of these patients had reversal of symptoms, whereas the others developed cerebral infarcts. One patient died from massive cerebral infarction attributed to refractory VSP. None of the unruptured aneurysms ruptured during the hypervolemic/ hypertensive therapy (223). Seventy-eight patients had surgical clippings of aneurysms between 1990 and 1992. Twenty-three percent developed DID. All patients were on nimodipine for 3 weeks. Hemodilution, hypervolemia, and hypertensive therapy was instituted using phenylephrine and colloid (224).

### G. Complications

Although hypervolemic hypertension is unquestionably capable of reversing neurologic deficits in some patients, in others it appears to aggravate cerebral edema, induce cardiogenic or neurogenic pulmonary edema, cause adult respiratory distress syndrome, and increase intracranial pressure. Deliberate hemodilution can decrease the $O_2$ carrying capacity of the blood (4). The agents which are used to induce hypertension can pose serious risks to patients. These include dopamine, dobutamine, phenylephrine, and norepinephrine, which have all been associated with intracranial hemorrhage, rebleeding, cerebral edema, increased ICP, arrhythmias, and myocardial infarction (45). Complications of hemodynamic therapy can occur in up to 25% of patients and include, in order of decreasing frequency, pulmonary edema, aneurysm rebleeding in patients with an unsecured ruptured aneurysm, hemorrhagic infarction, dilutional hyponatremia, coagulopathy, and complications of central venous catheterization. Induced hypertension has also been shown to aggravate regional ischemia and elevate intracranial pressure in certain patients. Cerebral edema may result from aggressive fluid loading and administering of vasoconstricting adrenergic agonists in the presence of BBB disruption (5). Table 8.14 lists some of the concerns.

Two cases of ruptured aneurysms were reported who developed IVH and ICH as a complication of hemodynamic therapy. They both had a long history of arterial hypertension. The cause of the secondary ICH/IVH was considered to be an aggravation of hypertension plus volume expansion. Patients experienced a deterioration in neurological status after infarction from VSP. Hemodynamic therapy was considered to be particularly risky

**TABLE 8.14 Problems with Hypervolemic, Hypertensive, Hemodilution Treatment**[a]

| |
|---|
| Not based on randomized, controlled trials |
| High incidence of serious complications: pulmonary edema, fluid overload, myocardial infarction, dilutional hyponatremia, cardiac and vessel injury, sepsis, cardiac arrhythmias, renal medullary washout, hemorrhagic cerebral infarction, cerebral edema |
| High pulmonary artery wedge pressures (often advocated) frequently impossible to obtain even with normal heart function |
| Tachyphylaxis often developing to commonly used drugs |
| Possible exacerbation of cardiac ischemia by vasoactive agents such as dopamine, dobutamine, norepinephrine, and phenylephrine, causing tachyarrhythmias, angina, severe hypertension, and profound vasoconstriction |
| Extremely expensive and time-consuming |
| Central venous pressure and pulmonary capillary wedge pressures are poor indicators of intravascular volume and pressure and correlate poorly with preload (left ventricular end diastolic volume) |
| *Reductions* with induced hypertension of regional cerebral blood flow in ischemic areas sometimes shown by direct measurements (xenon-CT) |
| *Reduction* in oxygen delivery despite increased blood flow brought on by hemodilution, sometimes shown in PET studies |

[a]From *Subarachnoid Hemorrhage: Causes and Cures*, p. 192, by Bryce Weir, copyright © 1998 by Oxford University Press, Inc. Used by permission of Oxford University Press, Inc.

in patients with a long history of arterial hypertension and increased catecholamine release after SAH (224a). The complications of induced hypervolemic hypertension also include cardiovascular ones—pulmonary edema, myocardial ischemia, and infarction—as well as the complications arising from the invasive monitoring devices. In one study, cardiovascular complications developed in 11 of 58 patients, pulmonary edema may be symptomatic or only detected on chest X-rays (60). In another study, 3 of 39 patients developed pulmonary edema, and 1 case required intubation (126).

Twenty-four patients were operated within 24 hr of SAH. They all received 500 ml of low-molecular-weight dextran daily for 2 weeks postoperatively. In addition, 16 patients were given 200 ml of 25% albumin daily. The average plasma volume was 58 ml/kg for the albumin group and 48 ml/kg for the nonalbumin group. The average CVP was 11 and 5 cm, respectively, for the two groups. Water balance was positive for half of the albumin group and was negative for the nonalbumin group. Three patients in the albumin group experienced pulmonary edema, and 1 patient in the nonalbumin group had congestive heart failure (225). A previously clipped large distal basilar aneurysm recurred following prolonged hypervolemic and hypertensive therapy for VSP (226).

Forty-one patients with DID attributed to VSP were treated by normovolemic-induced hypertension. In 6 cases monitored with Swan–Ganz catheters during this induced hypertension, the systolic blood pressure rose on average 62%, the mean CVP was 3.7 mmHg, the mean pulmonary artery wedge pressure was 5.7 mmHg, and the maximum cardiac index was 5.46 liters/min/$m^2$. Volume expansion was accomplished by whole blood transfusion, plasma or albumin, low-molecular-weight dextran for 1 week and maintenance of osmolarity within the range 290–310. Hb was maintained below 15g/dl and Hct was kept under 45%. Dopamine was given at a maximum rate of 20 μg/kg/min to maintain the blood pressure and dobutamine was added as necessary to a rate of 20 μg/kg/min. Therapy was maintained for approximately 2 weeks. Of the 21 neurological grade I and II patients, 57% had a good response to this treatment. For the 20 grade III and IV patients, 50% responded favorably. When the systolic blood pressure was elevated to a range of between 25 and 50% greater than baseline, 71% of the patients had a favorable neurological remission. If the blood pressure was raised to <25% baseline only 25% of patients improved. When the blood pressure was raised to more than 50% only 33% improved. On the other hand, intracerebral hemorrhage increased as the blood pressure was raised. Thirteen percent of patients whose blood pressure was raised to <50% more than baseline had hemorrhagic transformation of infarcts, whereas 44% of the 9 patients whose blood pressure was raised more than 50% suffered this complication. Overall, 20% of patients had hemorrhages as a complication of induced hypertension and half of these patients had hemorrhagic infarction. One patient of the 15 complications had rerupture of an aneurysm. Other complications included coagulopathy (7%), arrhythmia (7%), and pulmonary edema (2%). The chance of a hemorrhagic infarct developing during hypertension seemed to relate to the presence of low-density areas on the CT scan prior to inducing hypertension. When such low-density areas were present, 44% had hemorrhagic transformation compared to only 12% when no such low densities existed prior to treatment (227).

Between 1986 and 1989, 146 patients with aneurysm and/or SAH were treated. Forty-seven patients had clipping of the aneurysm within 3 days post-SAH with neurological grades of I–III and prophylactic hypervolemia with a pulmonary artery catheter to optimize fluid management. Of these 47 patients, 85% had an excellent or good outcome and 6% died. All of those who died had DID from VSP. Nineteen percent of the patients developed DID. These patients had postoperative Swan–Ganz catheter placement, plasmanate 250 ml every 6–8 hr, maintenance of pulmonary wedge pressure between 14 and 16 mmHg, and a Hct between 30 and 33%. When DID developed, CT scan, blood gases, electrolytes, further fluid expansion as tolerated, and pharmacological hypertension were performed. Forty-four patients grade IV or V were not treated with volume expansion. Of the 9 patients who developed DID, the average age was 60 years compared to 50 years for the 38 patients without DID. Patients with DID had 11.7 mean days of hypervolemia compared to 8.6 days for the patients who did not develop DID. There were no significant differences in surgical grade, mean days to operation, or initiation of volume expansion between DID and non-DID groups. Of the 47 patients receiving aggressive volume expansion in this series, 26% developed pulmonary edema sufficient to require some form of change in management such as decreasing fluids or $O_2$ therapy. Of the 16 patients developing complications of prophylactic hypervolemia, the types of complications were pulmonary edema in 12, bacteremia in 3, pleural effusion in 2, and pneumonia, pneumothorax, Swan–Ganz replacement, and death in 1 patient each. When comparing their results to those of contemporary reports, the authors found that despite aggressive volume expansion to the point of cardiovascular compromise, there was no appreciable decrease in neurological morbidity or mortality (228).

DID developed in 35% of 323 SAH patients. Ninety-four underwent hypervolemic therapy. Ultimately, infarction developed in 46% of these treated patients.

Twenty-eight percent developed an intracranial complication during hypervolemic therapy: Cererbral edema was aggravated in 69% and a hemorrhagic infarction developed in 31%. Eighteen patients had aggravation of edema (increases in low-density area around an infarction, ICH, or contusion caused by surgical manipulation). In only 2 of the 18 patients with aggravation of edema did DID develop within 6 days post-SAH At that time, a massive new infarction was found in 4 and edema in 10 patients. After hypervolemic therapy, the 18 patients with aggravation of edema deteriorated rapidly and 78% died. In 65% of the 68 patients who had no complication from hypervolemia, the DID was manifest on or after day 7 following SAH. Of the 94 patients treated with hypervolemia, 32% died. Of the 18 patients not treated with hypervolemic therapy, 50% died. Of the 68 patients having no complication from hypervolemic therapy, 16% died. For the 18 patients showing aggravation of brain edema with such therapy, 78% died. Of the 8 patients showing hemorrhagic infarction, 63% died. Of 18 patients having complications of surgery, hypervolemic therapy was associated with aggravation of brain edema in 39% of cases and hemorrhagic infarction in 6%. Of 8 patients developing hemorrhagic infarction after hypervolemic therapy, the time to deterioration after institution of the hypervolemic therapy ranged between 12 hr and 7 days. Most of the lesions were subcortical. Fifty-three percent of patients treated with hypervolemia developed ischemic infarction on the CT attributed to VSP. It was suggested that hypervolemic therapy should be discontinued as soon as possible after a DID deficit resolves in order to prevent hemorrhagic infarction. The authors also suggested that hypervolemic therapy could be very harmful in the early phase after SAH prior to the development of brain edema resulting from surgical intervention or primary brain damage (229).

Thirty patients treated at the Mayo Clinic were selected by virtue of initial good neurological grade with subsequent deterioration due to DID and treatment with hypervolemia and induced hypertension. Isoproterenol infusions were used in approximately half of these patients. Significant cardiopulmonary complications presumed related to the hypervolemia occurred in 10% of the patients. Major pulmonary edema occurred in 7%. These patients had CVP monitoring but not pulmonary artery pressure monitoring. One had been given pitressin in an attempt to overcome diuresis in the course of volume expansion. One of these patients died. Perioperative myocardial infarction occurred in 7%. One patient (3%) developed pneumothorax which required a chest tube and complicated an ultimately fatal course due to VSP. One patient also developed hyponatremia. Two cases (7%) in this series had progressive ischemia, cerebral swelling, and ultimately died. Reliable ICP measurements were not available on these cases. Hemorrhagic infarction was encountered in 4 (13%) patients (2 at postmortem and 2 diagnosed by CT) (230).

Two cases of iatrogenic hypertensive encephalopathy occurred during hyperdynamic therapy for VSP post-SAH. CT in one case showed occipital low-density changes which were reversible and which coincided with neurological deterioration and elevation of blood pressure to the range of 200/130 mmHg. The second case had deteriorated with blood pressures recorded in the 200/110-mmHg range. Ventriculomegaly and hypodensity in the left centrum semiovale were seen. Ventriculostomy and CSF drainage did not result in improvement. This patient also showed occipital low-density areas. On lowering the blood pressure to 180/90 mmHg, there was a slow improvement in the patient's level of consciousness. This patient died following a rebleed (231).

### H. Fluids

#### *1. Albumin*

To assist in hypervolemic therapy we chose from albumin (25 or 5%), 1–1.5 g/kg/day divided in four to six doses/day, each administered over 30–60 min; fresh frozen plasma, 1 or 2 units (150–200 ml each) every 4–6 hr; packed RBCs for Hct <30%; and crystalloid solution: 0.9% sodium chloride or Ringer's lactate at 100–150 ml/hr (4).

Ten patients with symptomatic VSP were treated with a large amount of human serum albumin. ICP and cardiopulmonary function were monitored during the treatment and marked improvement in neurologic signs was observed in all patients. Ninety percent of the patients recovered completely without any permanent neurologic deficits. The degree of improvement correlated with the decrease in total peripheral resistance. ICP was not elevated by the infusion of albumin, and it was concluded that hyperdynamic therapy induced by administration of albumin has a beneficial effect on cerebral ischemia from VSP (232). Intensive cardiovascular and ICP monitoring were performed in a series of 72 patients. Elevations of pulmonary capillary wedge pressure and CVP were observed when 300 ml of 10% glycerol was administered over 30 min, whereas administration of the same dose over 60–120 min caused no significant change. Elevations of pulmonary capillary wedge pressure and CVP and decreases in cardiac index were occasionally associated with premature ventricular contractions in some patients when 100 ml of 25% albumin was administered. Slower administration of the same dose of albumin over 120 or 240 min did not cause deterioration in cardiac function (233).

### 2. *Low-Molecular-Weight Dextran*

McMurtry and coworkers used low-molecular-weight dextran in 100 patients undergoing direct craniotomy for aneurysm. They did not demonstrate a statistically significant difference in the morbidity and mortality among the different groups of patients (234). Some patients, however, did, show dramatic responses to low-molecular-weight dextran during critical phases of their clinical course, in which progressing neurological deficits were apparently reversed.

Between 1987 and 1989, of 172 SAH cases, 69 (40%) had symptomatic VSP. These patients were treated with hypervolemic hemodilution consisting of 500 cc of 4.4% albumin and 500 cc of low-molecular-weight dextran in total volumes ranging from 3500 to 5000 cc/day. CVP and pulmonary diastolic pressures were monitored by Swan–Ganz catheter. The target Hct was 30–33% with systolic arterial pressures in the 160 to 200 mmHg range. With this protocol there was a 7.5% death or major neurologic deficit from VSP rate. Complications of hypertension/hypervolemia included 3 cases of pulmonary edema, 1 case of angina, and 1 case of cardiac arrhythmia. These were successfully treated by diuresis, decreasing fluid volumes, and withdrawing the Swan–Ganz catheter. Rebleeding occurred during this therapy in 14% of the 29 patients who had unsecured aneurysms (235). In 19 patients with symptomatic VSP treated by hypervolemia and hyperdynamic therapy, "oncotic" therapy was applied if cerebral infarction was followed by brain edema. In the 53% of patients who developed cerebral edema the serum oncotic pressure was raised higher than 25 mmHg by increasing the amount of albumin and/or low-molecular-weight dextran and administering furosemide. It was possible to maintain serum colloid oncotic pressure of approximately 25–30 mmHg with cardiac indices of approximately 5.0 liters/min/$m^2$. Seventy percent of patients treated with oncotic therapy had a good to moderate outcome (236). Of the 98 patients undergoing early aneurysm clipping, symptomatic VSP developed in 52% of cases. Symptomatic patients were treated with administration of plasma protein fraction at 500 ml/day, low-molecular-weight dextran at 500 ml/day and 10% glycerol at 900 ml/day. Total fluid intake was set at 4 or 5 liters/day. Daily fluid intake was kept below 3 liters/day in patients without symptomatic VSP. Transfusion was performed when Hct levels were <29%. Hypervolemic hemodilution therapy was continued until resolution of symptoms of VSP, usually 5–7 days. Mean Hcts were less in the group developing symptomatic VSP in all time periods after SAH. The difference became greater after the institution of hemodilution therapy. Patients who became symptomatic from VSP had higher RBC aggregation rates up until the institution of hemodilution therapy. With therapy, cardiac output increased from 4.3 to 5.8 liters/min, BP increased from 147 to 165 mmHg, and pulmonary capillary wedge pressure increased from 7.5 to 11 mmHg. In patients who became symptomatic CBF was lower on average in all time periods. Also, in the symptomatic VSP patients the CBF was consistently lower on the operated side after days 2–4. On the day that VSP developed, CBF fell on the operated side to a mean of 46 ml/100 g/min in comparison with that the on non-operated side which was 52 ml/100 g/min. Of the 51 treated patients, 57% were neurologically normal and 18% died. Twelve percent of the patients who became symptomatic died of VSP in the acute stage. The other deaths were from pneumonia in the chronic stage (134).

### 3. *Hetastarch*

Forty-two patients undergoing acute surgery for ruptured aneurysms received hydroxyethyl starch (500 ml/day) postoperatively. Fifty-five percent had prominent SAH on CT scan. These patients were given dobutamine to maintain BP in the normal range. Heart rate was kept <130/min. In one-third of the 24 patients treated with dobutamine, Swan–Ganz catheters were employed. Of the total group of patients, 27% developed DID from VSP. The DID were reversed by the administration of dobutamine at an average dose of 12.4 μg/kg/min (range, 8–25 μg/kg/min). Only 1 (2%) of the patients developed multiple infarcts. There were no cases of pulmonary edema or heart failure due to volume overload. In the 24 patients with prominent SAH, CBF increased by up to 20% following dobutamine administration although BP stayed in the normal range. In 8 patients monitored by Swan–Ganz catheters, the cardiac index increased markedly after treatment with dobutamine but the pulmonary wedge pressure remained below 10 mmHg. There was no significant change in either the stroke volume index or the stroke:CBF ratio (237).

Hespan (6% hetastarch in 0.9% sodium chloride with a weight-averaged molecular weight of 480 kDa) or plasmanate (5% plasma protein fraction; PPF) are commonly used to institute hypervolemia. Hetastarch or PPF were given at a dosage of 50 cc/hr with a mean dose of 0.67 cc/kg/day for hetastarch and 0.71 cc/kg/day for PPF. Hetastarch significantly elevated the partial thromboplastin time and this was clinically evident in an increased blood loss at surgery (224). The use of 1 g of hetastarch per kilogram of patient weight has not been associated with coagulopathy if limited to a single infusion lasting less than 24 hr. High-molecular-weight hetastarch has a half-life of approximately 36 hr. Long half-life is a problem if other side effects occur. Most of the hetastarch is cleared by the kidney after enzymatic degradation by amylase.

Because of the bleeding tendency, most neurosurgeons have abandoned the use of hetastarch (224) and we never use it.

### I. Models of Hypertension and Hypervolemia

A 6-hr temporary occlusion model of the distal internal carotid artery and proximal MCA was created in splenectomized dogs. Isovolemic hemodilution was performed 1 hr after arterial occlusion or sham operation and was accomplished by phlebotomy and infusion of low-molecular-weight dextran. In the hemodilution group initial mean hematocrit was 45%, which following treatment remained steady at approximately 31%. A decrease in viscosity correlated almost linearly with the decrease in Hct (238). Eight hours after arterial occlusion the volume of infarction estimated by the tetrazolium chloride histochemical method was 7.36% of total hemispheric volume in control animals and 1.09% in the hemodiluted animals. For the chronic animals the infarction volumes were 9.84 and 1.26%, respectively (239). In a similar model, hemodilution was demonstrated to reduce viscosity, fibrinogen, and total protein concentrations as well as plasma oncotic pressure. Systemic arterial blood pressure and pulmonary wedge pressures decreased slightly with hemodilution, but CVP and pulmonary artery pressure did not change. Intracranial pressure increased significantly in time with all dogs subject to arterial occlusions. It was more severe in the hemodiluted dogs. The degree of edema in the ischemic hemisphere of hemodiluted dogs was greater than that in controls. A decrease in CBF to the vessel occlusion was almost completely reversed by hemodilution except in the areas of the greatest edema (240). In a chronic canine two-hemorrhage model, hemodilution produced by 10% low-molecular-weight dextran, 0.8 g/kg/day, was compared to 25% albumin in doses of 1, 2-, or 3 g/kg/day. Only the albumin-treated group showed significant increases in plasma osmotic pressure, cardiac output, and velocity of blood flow in the vertebral artery. There was also a decrease in Hct on day 7. Two of 4 animals receiving 3 g/kg/day of albumin showed signs of pulmonary edema by chest X-ray. The infusions did not significantly ameliorate the degree of basilar artery VSP on day 7 (241). In dogs, cerebral infarction was induced by permanent occlusion of the left MCA and azygous anterior cerebral artery. Isovolemic hemodilution was accomplished 1 hr after occlusion of the vessels using blood withdrawal and dextran infusion. Animals were grouped by a target Hct of 25, 30, 35, and 45%. Animals were sacrificed after 6 days and infarct volumes were determined by fluorescein-stained sections. New infarct volumes as a percentage of total hemisphere volumes were as follows: control, 28.3%; 25% Hct, 33.6%; 30% Hct, 17.1%; 35% Hct, 29.2%; and 40% Hct, 29.9%. The Hct of approximately 30% appeared to be optimal in this canine model for protecting the brain (242).

In a rat model of MCA occlusion for 3 hr followed by 2 hr of reperfusion, animals were treated by hemodilution with albumin and hypertension with phenylephrine. Ischemic injury was assessed histochemically. There was more blood extravastation into the ischemic region in the hemodilution/hypertension therapy-treated group but the volume of ischemic tissue was the least in this group (243).

In a monkey unilateral MCA occlusion model during volume expansion with intravenous boluses of 6% hetastarch and subsequent exsanguinations, cardiac output was evaluated by repeat CBF measurements using $H_2$ clearance techniques. Cardiac output was increased by 159% and reduced to −166% by these experimental maneuvers. Local CBF in ischemic brain regions varied directly with cardiac output, whereas CBF in nonischemic brain was not affected by upward or downward manipulations of cardiac output. This important study suggests a profound loss of regulatory control in ischemic brain in response to alterations in cardiac output, indicating that cardiac output monitoring and manipulation are important for patient care following acute cerebral ischemia (244). In the unilateral clot application monkey model, there was an approximately 50% decrease in diameter of the right MCA 7 days after clot application. Mean CBF on the clot side decreased in parallel with a decrease in mean arterial blood pressure from 120 to 40 mmHg, indicating the abolition of autoregulation. In the vasospastic hemisphere the values of phosphocreatine, adenosine triphosphate, and pH decreased significantly at a mean arterial blood pressure <60 mmHg in the involved hemisphere. The ATP showed stepwise decreases during hypotension. The results indicated that during chronic VSP changes in cerebral energy metabolism are coupled to changes in CBF and autoregulation is impaired. A critical level of ischemia exists below which high-energy phosphorus metabolites become markedly depleted (245).

### J. Assessing Cardiac Function

#### *1. Swan–Ganz Catheter*

The Swan–Ganz catheter is frequently used to monitor patients post-SAH; we restrict use to those patients in whom deliberate therapeutic hypertension is being employed and in whom cardiovascular deterioration is an important issue. The catheters are an aid to obtaining optimal pulmonary artery wedge pressure for a particular patient and to help avoid cardiogenic pulmonary edema. The complications of the catheters are vessel and

cardiac injury, pulmonary infarction, cardiac arrhythmias, thromboembolism, balloon rupture and knotting of catheter, infection, and pneumo- or hemothorax (4).

Baek and associates found no correlation between circulating blood volume and CVP or Hct determinations and only minimal correlations between volume and wedge pressure in a large series of postoperative patients. High initial venous pressure was not a reliable index of either hypervolemia or cardiac failure in critically ill patients. In such patients with shock or trauma they found that a trial of volume loading with an oncotic agent such as 5% albumin should be carried out with frequent auscultation of the chest and careful observation of the CVP trends to give maximum therapeutic information. There were occasional instances of pulmonary artery wedge pressure in the normal ranges associated with decreased blood volumes of 20–30% (246).

Although some authorities suggest targets for pulmonary capillary wedge pressure between 16 and 18 mmHg and cardiac outputs of >6–8 liters/min (45), others tend to optimize the individual patient's cardiac output. Most authorities suggest Hct in the 30–33% range as a minimum. Dobutamine (a β-adrenergic agonist) can be used to elevate cardiac output providing there is no evidence of pulmonary edema (45).

Of 113 cases admitted within 2 weeks post-SAH, 37% developed DID and 35% had DID and angiographic VSP. Therapy with crystalloid, plasmanate, hetastarch, dextran, phlebotomy, dopamine infusion—all as appropriate—aimed at the following parameters: Hct, 33–38%; systolic BP, 160–200 mmHg with a clipped aneurysm and 120–150 mmHg with an unclipped one; CVP, 10–12 mmHg; and pulmonary artery wedge pressure, 15–18 mmHg. On this regimen 60% of cases improved, 24% showed no change, and 16% worsened. Ultimately, 19% had a major deficit or died. Adverse cardiovascular developments occurred in 7% of cases, none of whom were being monitored with Swan–Ganz catheters and all of whom improved with diuretics (126).

Balloon-tipped catheters were used to measure indices of cardiac function in 10 patients. Following baseline measurements plasminate was infused at 300 cc/hr. There was poor correlation between pulmonary artery wedge pressure and CVP in the ranges recorded in this study. Pulmonary artery wedge pressure increases did correlate in a statistically significant manner with increases in cardiac index, stroke volume index, and left ventricular stroke work index. There was no statistical correlation between increases in pulmonary wedge pressure above 14 mmHg and improvements in cardiac performance. In previously healthy individuals, their policy was to enhance fluid volume status until pulmonary artery wedge pressure of approximately 14 mmHg was obtained. In patients in whom the target level of pulmonary wedge pressure was associated with the reduction in cardiac index, they employed dobutamine at 10 μg/kg/min. It was used in association with sodium nitroprusside at 85 μg/min and they attempted to increase stroke volume index, sometimes up to 120%. This method of augmenting cardiac response was used in the presence of unsecured aneurysms rather than using dopamine, which was used in postoperative cases (247). In 9 previously healthy patients balloon-tipped central catheters were inserted following SAH. Pulmonary artery wedge pressure, CVP, cardiac index, stroke volume index, and left ventricular stroke work index were measured serially. After baseline measurements hetastarch or plasmanate were infused at 300 cc/hr. Cardiac output was recorded every 15 min. The pulmonary artery wedge pressure did not correlate with the CVP in the ranges recorded. There was a statistically significantly correlation between pulmonary artery wedge pressure and increases in cardiac index, stroke volume index, and left ventricular stroke work index. There was no statistically significant correlation between pulmonary wedge pressure increases above 14 mmHg and improvements in cardiac performance. It was concluded that CVP is an unreliable index of cardiac performance during hypervolemic therapy and that in previously healthy individuals a pulmonary artery wedge pressure of 14 mmHg is associated with maximum cardiac performance (209). Use of a Swan–Ganz catheter is not a guarantee against volume overload. For patients who fail to respond to maximum hyperdynamic therapy in 6–12 hr, this therapy should probably be discontinued since it can exacerbate cerebral complications. Twenty-three patients failed to respond to traditional preload enhancement following aneurysmal SAH. Thirteen percent had a history of cardiac disease. Cardiac parameters were measured with flow-directed, balloon-tipped catheters. Baseline left ventricular stroke work index (47.6 g/min/$m^2$) and cardiac index (3.3 liters/min/$m^2$) were within normal limits. After recording baseline measurements 5% albumin was infused at 300 cc/hr and dobutamine was initiated at a rate of 5–10 μg/kg/hr. Compared to hypervolemia alone, the addition of dobutamine increased heart rate by 20%, cardiac index by 52%, and left ventricular stroke work index by 15% and decreased the total peripheral resistance by 21%. Reversal of symptoms of ischemia due to VSP occurred in 78% of these patients (248).

Invasive BP monitoring requires accurate zeroing of the transducer which is critical for acquiring accurate data. A common complication of an arterial line is thrombosis, which occurs in 10% of 20-gauge catheters left in place for 3 days (249). The risk of thrombosis is increased by the use of larger bore catheters, hemodynamic instability, hypercoagulable states, preexisting atherosclerosis,

and Raynaud's phenomenon. Serious blood loss can result from inadvertent disconnection of an arterial line. Other complications include infection, nerve injury, and distal digital ischemia.

The overall incidence of complications from a pulmonary artery catheter is estimated to be 24%, with a 4.4% serious complication rate (250). The most common complications are arrhythmias, which usually resolve as the end of the catheter is moved out of the heart. Conduction defects may also result from cardiac injury. Pulmonary infarction may be produced by thrombus forming or in areas of endothelial injury. If balloons are left inflated for prolonged periods, large pulmonary infarction can result. The catheter tip may migrate into and occlude branches of the pulmonary artery. Rupture of the pulmonary artery is 0.06–0.2% of all catheterizations but has a high mortality (251). Swan–Ganz catheters have complications: infection, 13%; congestive heart failure, 2%; subclavian venous thrombosis, 1.3%; and pneumothorax, 1%. In addition, life-threatening arrhythmias and perforations can occur (252). Cardiopulmonary complications occur in about one-third of patients receiving aggressive hypervolemic therapy (228). Multiple insertions are usually necessary. Risk of sepsis appears to directly relate to the length of time that the catheter is in place.

Intraaortic balloon pump counterpulsation was used successfully in the management of a patient with concomitant VSP and cardiac failure (253). A patient with cardiac dysfunction and severe VSP refractory to traditional treatments was also given an intraaortic balloon counterpulsation device. Using Xe CT, average global CBF increased from 20.5 to 34.7 ml/100 g/min after counterpulsation. No complications of the device were observed (254).

### *2. Optimal Heart Rate*

Vander Ark and Pomerantz noted that cardiac output could be raised by raising the heart rate, assuming that all other factors remained constant. They described a 60-year-old patient with a ruptured internal carotid aneurysm operated 5 days post-SAH. She awoke from surgery aphasic and hemiparetic. Blood pressure was increased from 112/70 to 160/80 mmHg by using intravenous metaraminol bitartrate. Following this maneuver, the patient began to speak and move her paralyzed side. Aphasia and hemiplegia reoccurred when the systolic blood pressure decreased to 120 mmHg. At that point the pulse varied from 48 to 60 beats per minute and her cardiac output was 4 or 5 liters/min. Her cardiac output was doubled by giving atropine to reverse the bradycardia, which increased the heart rate to 80 beats per minute. At that point it was possible to stop the metaraminol bitartrate despite a drop in blood pressure to 90/60 mmHg since her neurological status did not deteriorate. The patient subsequently markedly worsened whenever her pulse decreased below 80 beats per minute (255).

Bradycardia presumably aggravates the risk of decreased cerebral blood flow in patients with established VSP. Nine patients with bradycardia (<50 beats/min) were treated by temporary pacing. No major side effects were seen. This policy was begun after the authors experienced a fatality from bradycardia during the period of VSP. They employed a demand-type pacemaker which turned on when the patient's heart rate fell below a previously determined level. Of 552 patients post-SAH 18 had documented bradycardia <50 beats per minute before or during cerebral VSP. The patients were treated with hypervolemia but not hypertension. Of the 9 cases treated with pacing, only 1 died and none were severely disabled. The heart rate before pacing ranged between 38 and 47 beats per minute and with pacing the rate was 60–75 beats per minute. It is considered that CBF will be compromised when the heart rate dips below 50 beats per minute. Cardiac output is the product of heart rate and stroke volume. At pathologically slow rates there is a compensatory increase in stroke volume required to maintain cardiac output. If the ability of the ventricles to contract is limited, there will be a decrease in circulating blood volume as a result of the bradycardia. Pacing has been employed for up to 3 weeks post-SAH (256).

Pressors such as dopamine and isoproterenol have frequently been used to treat VSP following SAH. It was originally thought that these agents might act by relaxing spastic cerebral arterial smooth muscle cells, but it was subsequently thought that they were stimulating the myocardium and that the fluid volumes administered with them have probably helped increase cerebral perfusion pressure (248). Dobutamine has been commonly used in the postoperative care of cardiac patients in failure. Dobutamine is a synthetic sympathomimetic amine that functions as a potent inotrope by stimulating $\beta_1$ receptors in the myocardium. It also stimulates peripheral $\beta_2$ receptors resulting in vasodilation and afterload reduction (248). Phenylephrine was selected because it is a pure $\alpha_1$-adrenergic receptor agonist and does not produce tachycardia or tachyarrhythmias as can dopamine or dobutamine. It can be titrated rapidly and produce a sustained hypertension. Cerebral blood vessels have a low density of $\alpha_1$ receptors (257) and are probably relatively insensitive to adrenergic agents (258). It is assumed that phenylephrine does not have significant direct cerebral constrictor effects.

## K. Reducing Intracranial Pressure

ICP is raised following aneurysm rupture in virtually every patient. About one-third of patients will develop

secondary elevation in ICP (6,259). This may be the result of rebleeding, hydrocephalus, cerebral edema, or medical complications. Conscious patients may complain of headache or become increasingly lethargic. In patients with significantly impaired consciousness, the signs of raised ICP include posturing, weakness, pupillary changes, and cardiorespiratory alterations. Ventricular catheters provide the best means of evaluating and treating raised ICP. In critically ill patients this should be performed along with online arterial blood pressure recording and frequent blood gas evaluation. Drainage of spinal fluid is the most rapid and efficacious means of controlling raised ICP. Medical adjuncts include analgesia, sedation, and paralysis, osmotic diuretics (mannitol and furosemide), and hyperventilation. Obstruction to venous drainage in the neck should be avoided. Anemia, if of sufficient degree, should be treated by blood transfusion. Electrolyte abnormalities should be monitored and treated. Hyperventilation should be used as a short-term treatment of raised ICP while other therapies are being instituted. There is no evidence to support the routine use of glucocorticoids post-SAH (45).

External ventricular drainage is the best method of controlling raised ICP and for monitoring it. In patients with significant large-volume SAH in whom the aneurysm has been clipped and a ventricular catheter placed at the time of surgery, we sometimes use this catheter to deliver t-PA postoperatively. The catheter is also used to drain bloody CSF. It is probable but not proven that this reduces the incidence of DID and the requirement for permanent CSF diversion. The risks of ventricular catheters are bleeding and infection (45). In one unit, good-grade patients postoperatively were found to have 50% incidence of at least one episode of raised ICP (260). Ideally, the cerebral perfusion pressure (CPP) should be at least 50–60 mmHg. Severe global ischemia occurs at CPP <30 mmHg. Moderate head elevation is a way of lowering ICP. The optimal angle is considered to be 15–30° (261). Fever can increase ICP and should combated initially with acetaminophen and then cooling blankets. The patient should be observed for shivering, which can adversely affect ICP (261).

ICP can be markedly increased by coughing and straining during mechanical ventilation. Sedation has a beneficial effect on ICP, and we preferentially use short-acting sedatives such as midazolam and propofol because they allow continued periodic clinical exams. Nondepolarizing muscle paralysis can be used to lower ICP in mechanically ventilated patients with intractable intracranial hypertension. Depolarizing neuromuscular blocking agents is avoided since they can induce fasciculations and histamine response, thereby increasing ICP and lowering CPP (261).

Occasionally, excision of infarcted cerebral tissue or decompression with removal of the bone flap and opening of the dura can help a patient through a critical episode (262). Between 10 and 20% of patients will require a chronic CSF shunt. This is more likely in large-volume SAH, in cases of rebleeding, and for those with IVH (263, 264).

Osmotic therapy may utilize mannitol, glycerol, urea, hypertonic saline, or Tris buffer. The normal brain is 76–78% water and effective osmolar therapy decreases $H_2O$ content by 1–3%. Mannitol administered 0.25 k/kg every 4–6 hr induces diuresis within 30–60 min and causes a nadir in ICP within 2–4 hr. Benefits from osmotic agents such as mannitol may be related to other factors independent of brain water content. Plasma osmolarity should not be allowed to rise above 320 mOsm. If ICP is stabilized with continuous hyperosmolar therapy, it is suggested that such therapy be withdrawn slowly (262). In stroke patients it is uncertain that the control of increased ICP using barbiturates produces an improved functional outcome (265).

Mannitol transiently increases cardiac output, which then increases cerebral perfusion. It also decreases blood viscosity, and this induces vasoconstriction which subsequently decreases cerebral blood volume (266). Hypokalemia is a common side effect. Serum osmolarity should be maintained between 300 and 310 mOsm/liter. Normal saline is 309 mOsm/liter (261).

Since it is important to maintain optimal circulating blood volume, agents such as mannitol which ultimately reduce it, have limited usefulness in the management of vasospasm or delayed ischemia. Mannitol is a hyperosmolar solution which does not cross the intact BBB. The normal osmolarities of brain, CSF, and blood are appropriately equal at 275–300 mOsm/kg $H_2O$. When 1 g/kg weight of mannitol is given over 10 min serum osmolarity rises 20–30 mOsm/kg before returning to control levels in 3 hr. ICP, regardless of the cause, usually decreases; this correlates with a decrease in brain $H_2O$. Doses as low as 0.25 g/kg are effective. A hypermolar state can be induced by chronic and repeated administration and an osmolarity >320 mOsm/kg is generally considered a contraindication. Chronic administration can cause reduction in serum $Na^+$. We use it in aneurysmal SAH at the time of surgery, postinduction as well as prior to temporary clipping in a dosage of 0.5 mg/kg. It is seldom employed if VSP is developing or established unless there is CT-demonstrated brain swelling and clinical deterioration despite CSF removal by ventricular drainage. Theoretical uses other than as a dehydrating agent are as a free radical scavenger or to favorably influence blood viscosity, thereby improving CBF in ischemic regions. It may also transiently open the BBB. If the BBB is damaged and mannitol enters the injured area, it may increase harmful brain edema. Other potential

complications are renal insufficiency and acute myocardial insufficiency (267).

Loop diuretics may be used to achieve the desired hyperosmolar state. Their use can be associated with severe hypovolemia and hypotension. It is important to monitor $Na^+$, $K^+$, and osmolarity when using loop diuretics (261).

## L. Respiratory Support

### *1. Blood Gas Evaluation*

Respiratory support is usually provided for patients in poor neurologic grade. In seriously ill patients, one should aim for a $P_aO_2$ of at least 80 mmHg and a $P_aCO_2$ of 30–35 mmHg. These patients require continual monitoring of blood pressure, electrocardiogram, $O_2$ saturation, ICP monitoring, and frequent blood gases (4). Pulse oxymetry measures saturation. At a $P_aO_2$ above 100 mmHg, the oxymeter reading is virtually 100%; a decrease in $P_aO_2$ from 100 to 60 mmHg causes the saturation to decrease from 100 to 90%. The accuracy of the measurement diminishes below this level (250). Jugular venous $O_2$ saturation ($SjvO_2$) measurement may be indicated in certain poor-grade patients. The $SjvO_2$ can be continually assessed, allowing detection of hyperemia, increased $O_2$ extraction, and global cerebral ischemia ($SjvO_2 < 50\%$) (45). This methodology has not received widespread use in the setting of SAH.

Hyperventilation is effective in rapidly lowering ICP. Increased ventilation lowers $P_aCO_2$ and creates a CSF alkalosis with resultant cerebral arterial vasoconstriction. The effect occurs within seconds and is maximal within minutes. Maintained hyperventilation over 6–24 hr leads to a CSF alkalosis which is buffered by the loss of intracerebral bicarbonate. Cerebral vessels relax and cerebral blood volume and ICP increase. The salutary effect of hyperventilation is not sustainable. Prolonged hyperventilation also causes BP instability, arrhythmias, and pulmonary barotrauma. Hyperventilation should not lower $P_aCO_2$ below 25 mmHg. The continuance of hyperventilation may be associated with an exaggerated brain pH fall because of depletion of the intracerebral pool of bicarbonate. Consideration should be made to discontinuing hyperventilation gradually over 24 hr (262). Electronic capnometry expresses the highest concentration of $CO_2$ in expired gas as a number. A physiologically constant difference of approximately 5 mmHg exists between end expiratory $CO_2$ by capnometry and $P_aCO_2$ by blood gas analysis (250).

### *2. Hyperbaric Therapy*

Hyperbaric oxygen was evaluated in 43 patients with symptomatic VSP. It was used as an adjunct to mild hypertensive hypervolemia in 24 patients. Sixty-three percent of patients were considered to respond favorably to hyperbaric oxygenation (268). The technique has not found general acceptance.

## M. Angioplasty

### *1. Clinical Series, Complications, and Outcomes*

In 1984, Zubkov and colleagues astonished the neurosurgical world with a report of balloon angioplasty treatment of VSP (269). They referred in their seminal work to an attempt by Dotter and Judkins (270) to dilate a segmentally stenotic extremity artery using biliary duct dilators. They were also aware of Suzuki *et al.*'s (271) attempted dilation of a constricted segment of the internal carotid artery in its cervical portion by clamping and saline injection under pressure. They also knew that Mullan *et al.* (272) had used a balloon catheter technique to dilate a stenotic internal carotid artery. However, theirs was the first report describing the use of the balloon catheter technique to dilate vasospastic arteries following SAH. They described the dilation of 105 major cerebral arteries in 33 patients. None of the dilations were performed within the first 3 days post-SAH. The most common time was 8–10 days post-SAH, during which 36% of the treatments were performed. Interestingly, 85% of the patients were vasodilated before operation. They noticed that following the dilation, the patients frequently had a decrease in headache and a regression in neurological signs. They used the treatment on patients in grave condition to improve them enough to undergo aneurysmal treatments. The mortality rate was 21%. They concluded that the effect of vasodilation was persistent. In 2 patients with diffuse VSP they visualized the aneurysm only after the vasodilation. They suggested that the procedure was a simple one to be performed immediately after angiography. The time course of improvement was not studied systematically, but in 1 case the focal and general neurologic signs started to regress the day following the treatment. The carotid angioplasties were performed by direct carotid cannulation in the neck and the basilar dilations by retrograde femoral techniques. It was several years before reports began to appear in the American and Japanese literature regarding the efficacy of this procedure (269). A rapid downhill course despite induction or hypertension and hypervolemia in the absence of any potential surgical lesion should lead to the serious consideration of angioplasty (1). Numerous reports suggest that significant improvement occurs in 60–80% of patients, often within hours after dilatation. Normal angiographic caliber is present in nearly all cases without recurrent VSP. CBF seems to be improved by TCD and

single photon emission computed tomography (SPECT) criteria. Complications of rupture have been reported in approximately 5% of cases (273–276).

In 1988, 10 patients at the University of Washington were treated for DID due to VSP unresponsive to hypervolemic/hypertensive therapy. Improvements occurred in 80% following the procedure. TCD velocities diminished in 2 cases when this was measured. Mortality was 25%–1 patient died from the VSP and 1 from an aneurysmal rebleed. One delayed stroke occurred 6 weeks following the procedure (276). Thirteen patients had angioplasty for VSP; 23% of these were not the result of SAH. Of the SAH patients, 2 were treated while in neurological grade II, which was the same neurological grade as their admission grade. Thirty-one percent of the overall series died and 3 of the 4 deaths occurred in patients who were grade V at the time of angioplasty. The authors successfully angioplastied all the major basal conducting arteries (275). Barnwell *et al.* reported a case in which 2 episodes of DID were treated by angioplasty. The first angioplasty of the right MCA and distal carotid arteries took place more than 30 hr after the onset of left hemiplegia. The patient was also ultimately shown to have a small ipsilateral infarction. Despite this, there was marked improvement in left-sided motor function. The second angioplasty occurred when the patient had a decline in level of consciousness. The patient again improved. The second angioplasty was also performed 24 hr after the onset of coma. The craniotomy in this patient had been performed on day 5 post-SAH, the first angioplasty on day 7, and the second angioplasty on day 9. The CT infarction was only demonstrated on day 8 post-SAH, so it may not have been present at the time of initial angioplasty (273).

Between 1981 and 1991, in 95 consecutive patients angioplasty was performed. Angioplasty was used for patients demonstrating angiographic VSP even if they were currently asymptomatic. Major complications occurred in 4.5% of cases. These consisted of balloon detachment requiring open surgery in one case, aneurysmal rupture from a contralateral aneurysm in one case, ipsilateral aneurysmal rupture during attempted concurrent aneurysmal occlusion, and one other unspecified complication. In patients who were Hunt and Hess I and II at the time of angioplasty, all completely recovered. Patients who were a grade III had an 86.5% improvement and there was no change in 13.5%. Of the patients who were "decompensated" Hunt and Hess IV or V, only 40% showed improvement. Most of the patients who were comatose or moribund remained so and died (277). It has been suggested that angioplasty can improve the neurological grade of the patient presenting late, to the point at which operation becomes feasible (278).

By the 1990 cerebral VSP symposium multiple accounts of successful angioplasty began to appear (279–283). It was reported that angioplasty could cause dramatic clinical improvement within a couple of hours and that its use would be followed by marked improvement in vasodilator capacity in the treated MCA territory as judged by SPECT studies (279). Nemoto and colleagues reported on 10 patients treated by angioplasty. Three comatose patients died. Two other comatose patients were unchanged and 1 became alert with a deficit following the angioplasty. Only 4 of 10 patients with significant deficits benefitted from the angioplasty (40%). Adverse prognostic factors were considered to be technical failure to dilate all spastic vessels and the preexistence of infarction as seen by CT scan (281). By the early 1990s, a consensus had arisen among neuroradiologists that the criteria for angioplasty should include a new onset of neurological deficit, the absence of CT evidence of infarction, neurological deficit refractory to hypervolemia and hypertension, and angiographically apparent VSP in an accessible vessel (274).

Angioplasty was performed in 20 cases of aneurysmal SAH. Clinical improvement occurred in 70% of cases shortly after. The mortality rate was 23%. Fourteen patients having angioplasty within 6 hr of onset of symptoms experienced excellent results and 78% had no deficits. Of 8 patients having angioplasty more than 6 hr after onset of symptoms, only 25% had an excellent outcome, 38% were good, and 38% died. Flows as judged by SPECT scans normalized within a day of cerebral artery angioplasty (283). A successful angiographic dilation of vasospastic MCA can be followed by delayed ICH. This is presumed to be due to reperfusion in areas of unrecognized prior infarction (284). The fatal rupture of an intracranial carotid artery during transluminal angioplasty was first reported in 1991. The most likely reason for the carotid rupture was that a small portion of the aneurysmal neck between the clip and the aneurysm-bearing vessel remained. Until the time of this report, the only previous fatality had been as a consequence of hemorrhagic infarction occurring 24 hr after angioplasty (285). The fatal rupture of an MCA cortical branch during angioplasty was described. Brothers *et al.* (286) described the technical factors involved in balloon angioplasty. They suggested that distal angioplasty would require inflation volumes far less than that resulting from the maximum 0.1-ml inflation of a restricted-inflation-diameter balloon. The periprocedural mortality rate for balloon angioplasty was considered to be 2–5% in the late 1990s by the most experience American group (287).

In 1991, Alexander *et al.* reported latex balloon angioplasty of 89 consecutive patients. Fifty-eight percent underwent dilatation with unclipped aneurysms and only

18% were treated postoperatively. Fifty-three percent underwent dilatation in a single vascular tree, whereas 47% had bilateral carotid or carotid–basilar angioplasty. Improvement in neurological deficits was achieved in 78% of patients (288). In a series of 26 patients, 70% were clinically improved. The delay between the onset of neurologic deficits and angioplasty was believed to correlated strongly with outcome. Patients treated within 8 hr were believed to do better postangioplasty (289). Thirteen patients were treated by angioplasty between 1990 and 1992 in Toronto. Thirty-one percent of cases showed neurological improvement immediately after angioplasty. At 6 months, 38% of patients were independent, 15% were severely disabled, and 46% had died. Poor clinical grade at the time of angioplasty was associates with a poor outcome. It was considered that the best results are likely to be obtained if the procedure is performed on patients in poor clinical grade in whom new neurological deficits have not become established (290).

The presentation of a patient with established VSP poses a difficult management problem. Five such patients were treated by acute clipping of their aneurysms followed by immediate postoperative angioplasty between 1988 and 1992 at the University of Washington. Four patients improved clinically and made a good recovery (291).

In a similar series, four cases presented post-SAH with established severe VSP and neurological grades IV. The aneurysms were clipped and the operations were immediately followed by angioplasty. Two cases returned to their previous occupations, one remained bed ridden, and one died (292).

Angioplasty was performed on 36 vasospastic arterial segments in 19 cases. These segments had more than 50% constriction from admission diameters. In 83% of arteries, dilatation to at least 70% of admission diameters was achieved. Follow-up angiography showed no recurrence of VSP nor chronic atherosclerotic changes. Clinical improvement within 24 hr of angioplasty was observed in 63% of cases. There was an 11% mortality rate and 22% morbidity rate (293).

Takis *et al.* performed a study of 10 patients who had angioplasty between 1993 and 1996. Stenosis in all patients was 75% or greater. Eighty percent were technically successful and 20% could not be performed due to inability to traverse stenosed areas. Twenty percent had strokes from VSP. Twenty percent had compromise of perforating vessels or arterial dissection (294). The University of Washington series analyzed their first 50 cases and presented the results in 1998. Ninety-two percent had objective clinical deterioration despite maximal medical therapy. Eight percent were treated on the basis of rapid acceleration in TCD velocities. Sixty-four percent of patients underwent angioplasty within 12 hr and 92% within 18 hr of onset of clinical VSP. Of patients with clinical evidence of VSP-induced ischemia, 61% had sustained neurological improvement within 72 hr of angioplasty, 6% deteriorated within this time interval, and 4% died immediately after angioplasty as a result of vessel rupture. Two additional patients in poor condition at the time of angioplasties subsequently died during hospitalization. Two other patients died as a result of unclipped aneurysms bleeding 4 and 12 days after angioplasty. When improvement resulted from the angioplasty, it was sustained and only 1 patient required repeat angioplasty of a previously dilated segment. Two patients developed VSP in previously undilated segments and had second angioplasties (295).

Bejjani *et al.* (296) performed a study of 31 patients with 43 aneurysms and one atriovenous malformation (AVM) who had angioplasty between 1993 and 1997. Angioplasty was performed on average 6.9 days (1–14 days) post-SAH. It was performed within 24 hr of refractory clinical deterioration in 21 patients. Dramatic clinical improvement occurred in 38% of patients, moderate improvement in 34%, and no improvement in 28%. The mortality rate was 6% and was considered to be unrelated to angioplasty. Twenty-eight percent had good recoveries, 38% moderate disabilities, and 34% severe disabilities (296). Of 466 aneurysmal SAH patients, 20% had endovascular management of clinical VSP which was medically refractory. Ninety percent of these patients were available for 6-month follow-up. All patients had balloon angioplasty and additional papaverine if distal spasm was present. Sixty-one percent of the patients had endovascular management within 2 hr and 39% were treated later. The group with the earliest treatment after the development of symptoms demonstrated a greater level of sustained clinical improvement. Fifty-five percent of early and late-treated patients had sustained overall improvement. Fifteen percent of patients treated after 2 hr showed some degree of "luxury perfusion" and "salt- and-pepper" appearance of small islands of hemorrhage within CT low-density regions. For these patients, particular attention was given to blood pressure control and immediate reversal of anticoagulation upon completion of angiography. Clinical improvement was noted as early as 60 min after the procedure when it was possible to evaluate motor function. Global improvement was observed for up to 48 hr postangioplasty. Mortality rate was 9%: It ranged from 11% in grades I and II to 4% in grade III and 25% in IV. Good recoveries occurred in 78% of grade II patients, 56% of grade III patients, and 38% of grade IV patients. There were no dissections, no ICH requiring surgery, or no SAH related to angioplasty (297).

Balloon angioplasty in 9 patients decreased MCA velocity from 159 to 76 cm/sec on posttreatment day 1, but

CBF did not change significantly (31 to 30 ml/100 g/min). In 19 patients given papaverine infusions MCA TCD velocity also decreased (113 to 86 cm/sec), vessel diameter increased only 37%, but CBF increased from 27 to 37 ml/100 g/min. When both angioplasty and papaverine were employed, velocity decreased from 163 to 114 cm/sec, diameters increased 96%, and flow increased from 36 to 48 ml/100 g/min. The average initial flows in these patients were obtained by $Xe^{133}$ clearance but did not indicate a serious level of ischemia initially, although focal areas of ischemia may have existed (298).

In patients having angiographic VSP but CBF in the normal range, it is not unexpected that angioplasty will dilate the spastic segment and decrease TCD velocity but have little or no effect on outcome.

### *2. Does Angioplasty Make a Difference?*

Between 1994 and 1997, at 70 University Centers in the United States which share clinical and economic data, there was a 16% reduction in risk of in-hospital death when angioplasty was used for vasospasm (relative risk 0.84). Of course, this might only reflect that the most up-to-date units gave better care generally for VSP rather than a specific benefit from angioplasty, this represented a period when endovascular therapy was coming into vogue (299).

In the North American Tirilizad Study, 38 patients underwent angioplasty for symptomatic VSP. Various factors were used to select a group of individuals to provide a case-matched control population. Fifty-three percent of patients undergoing angioplasty showed good recovery or only moderate disability on their 3-month Glasgow Outcome Scale score. Angioplasty, severity and type of VSP, need for papaverine, timing of angioplasty, and dose of study drug were not found to have an effect on outcome in the 38 patients undergoing angioplasty. The neurological examination improved in only 11% of the patients immediately after the procedure. No effect of the procedure could be demonstrated in comparison with the case-matched control population. Angioplasty was very effective in reversing angiographic VSP (300). In the same study, 31 patients had intraarterial papaverine. Twenty-four other patients were treated with a combination of angioplasty and papaverine. TCD studies demonstrated a decrease in flow velocities in the MCA in treated groups. Angioplasty alone produced a decrease in velocity of 26 cm/sec and papaverine produced a decrease of 18 cm/sec. There was no difference between these two groups in clinical improvement on days 1 and 4 postprocedure. Neither of the two treatment approaches showed an effect of timing of therapy on neurological outcome. Neither intraarterial papaverine nor balloon angioplasty correlated with the high percentage of short-term neurological improvement (300). An example of early, angiographically successful angioplasty in a patient who died from fulminant ischemia is shown in Figs. 8.4–8.6. What, then, is the proper role of angioplasty? Clearly, it reverses vasospasm. However, the procedure has significant morbidity and mortality. Many operators have had experience with procedure disasters which never found their way into the literature. There is a significant learning curve and most units, at least in North America, do not provide a sufficient number of cases of refractory, life-threatening VSP to provide interventional neuroradiologists with sufficient personal experience. In our opinion, a technique with a 4 or 5% mortality should not be applied to asymptomatic patients or those whose prognostic factors indicate a reasonable chance of good outcome with medical therapy alone.

### *3. Preemptive Angioplasty*

Based on the canine model of VSP in which *in vivo* angioplasty prevented the development of chronic VSP, Muizelaar *et al.* introduced prophylactic angioplasty in humans. Thirteen patients were treated who had a Fisher grade III SAH on initial CT scan. Prophylactic angioplasty was performed within 3 days of bleeding. Within 2 days of SAH all patients underwent clipping of aneurysms. Postoperatively, 9 patients were allowed to wake up for neurological examination prior to angioplasty, but the other 4 patients in the series remained intubated for angioplasty immediately after operation. Of the 13 patients, 33% were neurological grade II on admission and preoperatively. Sixty-two percent of the patients had anterior communicating artery aneurysms and only 8% (1 patient) had an MCA aneurysm. In 16% of the patients, prophylactic angioplasty could not be performed because of severe atherosclerosis and tortuous arteries. A similar percentage of patients received prophylactic angioplasty in only one part of the intracranial circulation, also because of severe antherosclerosis and vascular abnormalities. Thirty-one percent of the patients developed symptomatic VSP, 23% died, and 1 died as a result of rupture of the posterior inferior cerebellar artery during the angioplasty. Seventy-seven percent of patients had a good recovery or a motor disability. No patient subsequently developed moderate to severe VSP as documented by TCD or developed a DID. The goal of this study was to perform angioplasty on no less than 10 vessels, but this goal was not achieved. The authors concluded that it might be sufficient to dilate prophylactically only the vessel bearing the aneurysm. Because of imbalance in the size of $A_1$ segments, the angioplasty could only be performed on the dominant side. The single mishap occurred during road map misregistration due to patient movement. Subsequently, patients were kept intubated until angioplasty was performed (301).

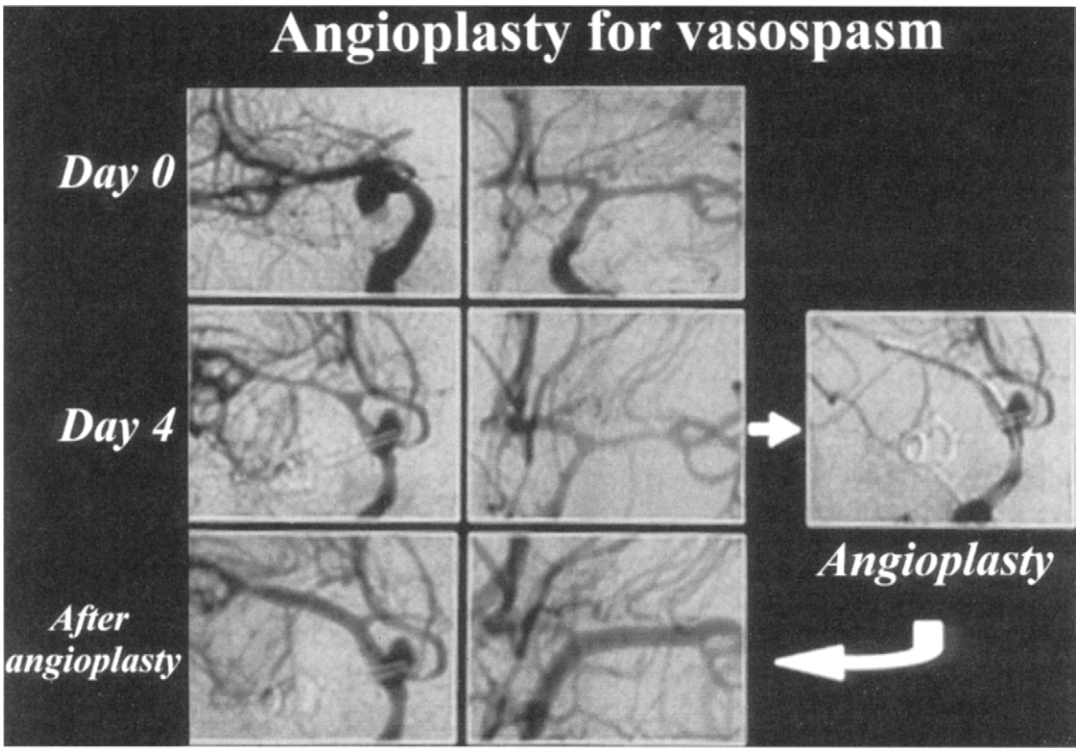


**FIGURE 8.4** The same patient had clipping of the ruptured posterior communicating artery aneurysm on day 0. Because of an immediately adjacent aneurysm that could not be visualized because of a very tight brain; t-PA was not used. Angiographically successful bilateral angioplasty was performed on day 4.

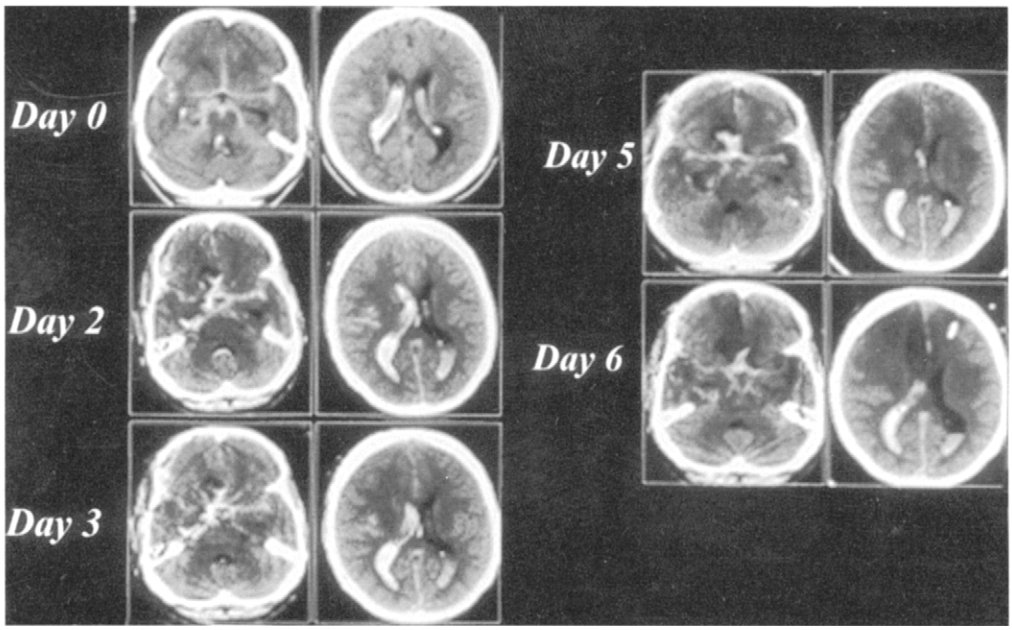


**FIGURE 8.5** Serial CT scans on the same patient show persistent SAH and IVH to day 6 and increasing low-density areas.

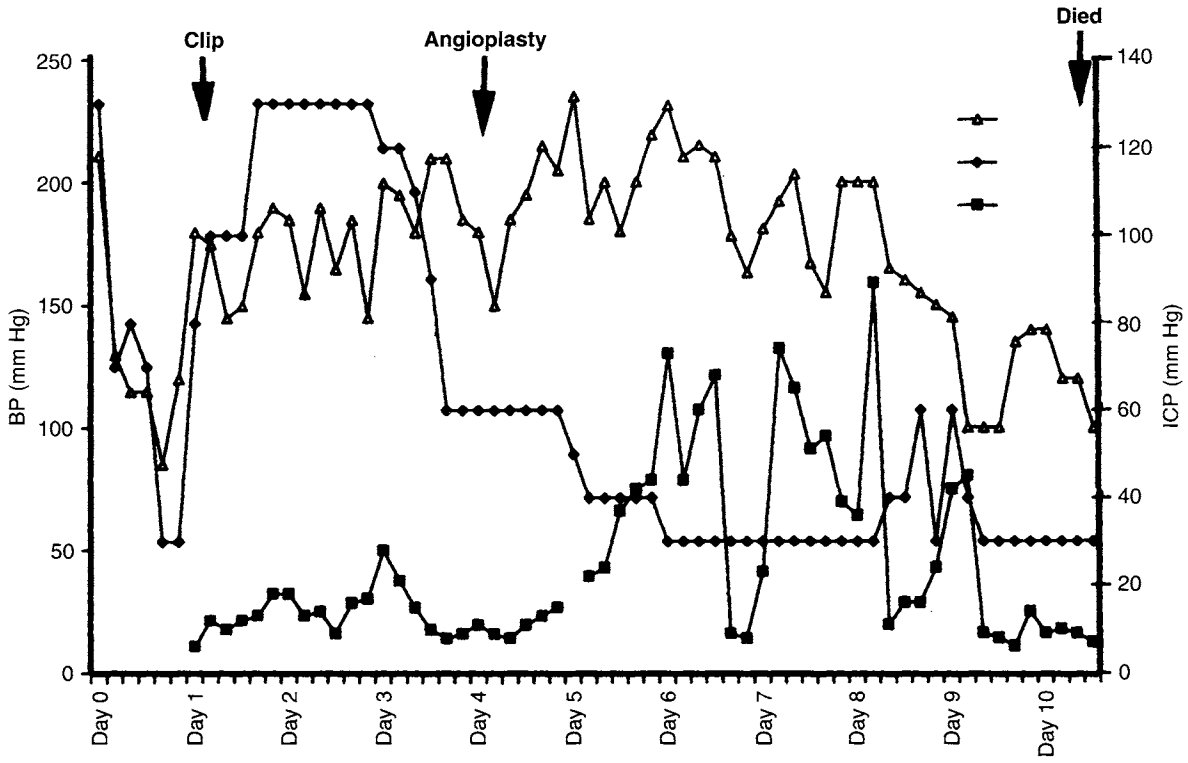


**FIGURE 8.6** The patient's GCS decreased from 13 on day 1 to 6 on day 4, at which point angioplasty was performed. A ventricular drain had kept ICP normal to the point. Despite these means, decline continued steadily to death on day 10.

### *4. Angioplasty and Papaverine*

Fujiwara *et al.* studied 20 patients with symptomatic VSP who had angioplasty and/or papaverine between 1989 and 1996. Nine had only angioplasty, 8 had only papaverine, and 3 had both. Improvement in neurological deficit within 48 hr after treatment occurred in 67% treated by angioplasty, 75% treated by papaverine, and 100% of patients treated by both angioplasty and papaverine (302). Xe CT was used to evaluate the effect of angioplasty on regional CBF. Fourteen patients were treated. Ninety-three percent had technically successful angiography, 92% were neurologically improved following the procedure, and 58% had complete reversal of all DID. Angioplasties significantly decreased the mean number of regions of interest at risk. Fifty percent of the patients no longer had regions of interest showing marginal flows after the angioplasty. At-risk regions of interest had increase in flows from 13 to 44 ml/100 g/min postangioplasty. The addition of papaverine to the angioplasty did not apparently alter the degree of improvement (303). For symptomatic VSP treatment, 3 patients had angioplasty and 12 had intraarterial papaverine because narrowing was in more distal or inaccessible vessels. Both procedures were combined in 10 patients. Sixteen percent of patients were severely disabled and 16% died. Intraarterial papaverine was found to sometimes be ineffective for distal or diffuse VSP (304). Twenty-three vascular territories were dilated in patients. In 3 patients, angioplasty was performed before papaverine infusion. Jugular bulb $O_2$ saturation was measured, with critical values reflecting an improvement in oxygenation after treatment. Lactate concentration in the jugular bulb normalized within 4 hr in all patients who had evidence of brain lactic acidosis before papaverine infusion. One patient had recurrence of abnormal metabolic and oxygenation patterns after treatment when optimal hypertension/hypervolemic therapy could not be achieved (305). Improvement in acute symptoms only occurred in about one-half of the patients treated with intraarterial papaverine. Its use was believed to be more effective when it was combined with angioplasty (306). Between 1989 and 1995, 125 spastic internal carotid and proximal MCA segments were treated in 52 patients. Angioplasty was performed on 81% of vessel segments and 75% of patients. Papaverine infusion alone was used in 19% of segments and 25% of patients. Retreatment was required in one vessel segment after balloon angioplasty but in 42% of segments following

papaverine. Twenty-nine percent of recurrent spastic segments were treated with angioplasty following initial papaverine infusion. The papaverine infusion was associated with a 20% mean decrease in TCD velocities on posttreatment day 1 but no significant difference by posttreatment day 2. Balloon angioplasty, on the other hand, resulted in a 45% mean decrease in velocity which was sustained (307). DID was reported in 242 cases who were treated with transluminal angioplasty. The outcome was as follows: dead, 28%; deficits, 27%; good, 53% (7). Serious or fatal complications have been reported from papaverine infusion: brain stem depression, convulsions, paradoxical aggravation of VSP, cardiopulmonary arrest, elevated ICP, and thrombocytopenia. During intraarterial infusions ICP should be monitored and the dosage of 300 mg per vascular territory and rate of administration adjusted if necessary. Papaverine should not be mixed with a contrast agent or perhaps with heparin to limit precipitation of the drug. Patients at risk of respiratory depression should be intubated beforehand (308).

### *5. Evaluation of the Effect of Angioplasty*

Acute reductions in TCD velocities from mean 242 to mean 76 cm/sec were illustrated in one case (280). Five patients had angioplasty and showed a dramatic reduction in MCA velocities as judged by TCD on the treated side. These patients were treated between 4 and 9 days post-SAH. In all of them, angioplasty was performed more than 24 hr after the onset of neurological symptoms. All the patients improved (309).

Improvements in rCBF were demonstrated in 9 of 10 patients following angioplasty judged by SPECT analysis (310). In the same series the MCA diameter generally increased from 3.5 to 4.5 mm. The hydrogen clearance method of judging CBF showed levels prior to surgery of 56.4 ml/100 g/min. In patients having angioplasty, the predilatation level was 32.8 ml/100 g/min, which increased to 52.7 ml/100 g/min posttreatment (311). Digital subtraction angiography was used to measure transit times before and after angioplasty. Superselective infusion of 0.2% papaverine was used as an adjunct to angioplasty in some cases. Mean transit times in 10 patients showing complete recoveries were 6.92 sec, which compared to a mean time of 7.66 sec for the those without complete recovery (312).

### *6. Angioplasty and Small Arteries*

A new microballoon catheter made of silicone was described which was considered to be suitable for dilating distal vessels such as the M2 portion of the MCA. A case was illustrated (313). In their initial experience, Eskridge and colleagues were able to perform balloon angioplasty in the A1 segment of the anterior cerebral artery in fewer than 10% of patients. With a new Stealth balloon microcatheter/steerable hydrophilic microguidewire system they were able to perform such angioplasties in 10 A1 segments. Following treatment in 7 patients with severe VSP, 4 patients showed no VSP, 2 showed moderate VSP, and 1 showed only mild VSP. The significant complications were a vertebral artery dissection and microcatheter-induced severe spasm (314).

### *7. Angioplasty after Aneurysm Coiling*

Nineteen patients treated by clipping were compared to 18 patients treated via endovascular coiling. Endovascular cases received 48 hr of full heparinization followed by 24 hr of dextran infusion. Twenty-two percent of the endovascular patients developed DID which responded to elevation of blood pressure and did not require either mechanical or chemical angioplasty to reverse their symptomatology. In the surgical group, 74% of the patients developed clinical VSP and were treated with hypertension/hypervolemic/hemodilutional therapy. Sixteen percent of the patients required angioplasty. The data were interpreted to suggest that the frequency and severity of VSP were reduced by endovascular occlusion of aneurysms compared to open surgery (315). (Figures 8.7 and 8.8) illustrate a case with concurrent coiling and angioplasty.

### *8. Angioplasty after Arteriovenous Malformation Surgery*

Intraarterial papaverine and angioplasty were performed in 2 patients with severe, symptomatic VSP who had AVM (316). Nishimura and Hawkins first described angiographic VSP from a ruptured AVM in 1975 (317). Fourteen cases of angiographic VSP from AVM have been reported in the literature (318,319). One of these patients made a good recovery but the other died of pneumonia.

### *9. Pathology of Angioplasty*

The collagen network in human basilar arteries is mainly circumferential in the media and longitudinal in the adventitia. Following angioplasty, circumferential collagen fibers in the media appear to be stretched and some are torn. The internal elastic lamina appears stretched without laceration (282). The structure of collagen fibers in human vessels subjected to dilation with 3 atm was presented by the Mississippi group. They showed stretched and torn collagen fibers. The most commonly used angioplasty balloon in clinical practice only generated pressure of 1 atm, however. In this experiment the polyethylene balloon stealth catheter was used which generates extremely

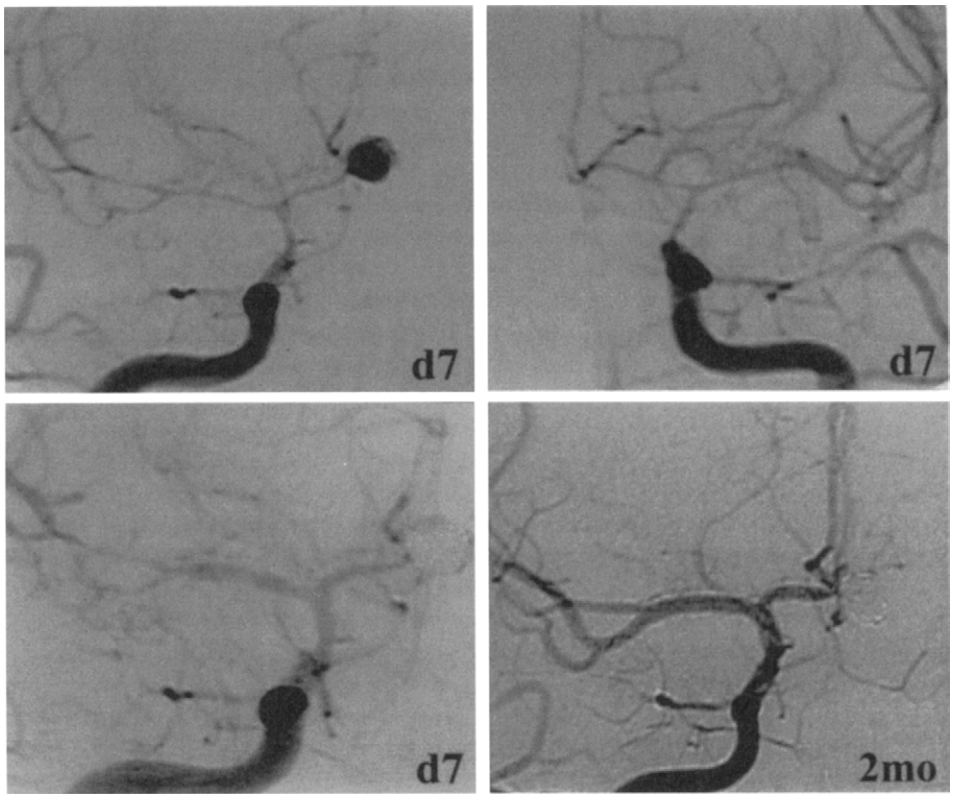


**FIGURE 8.7** A patient presenting on day 7 was treated with ventricular drain insertion, coiling, and angioplasty. Normal vessel diameter was immediately established in the internal carotid, proximal MCA, and proximal anterior cerebral arteries as evidenced by the later follow-up angiogram.

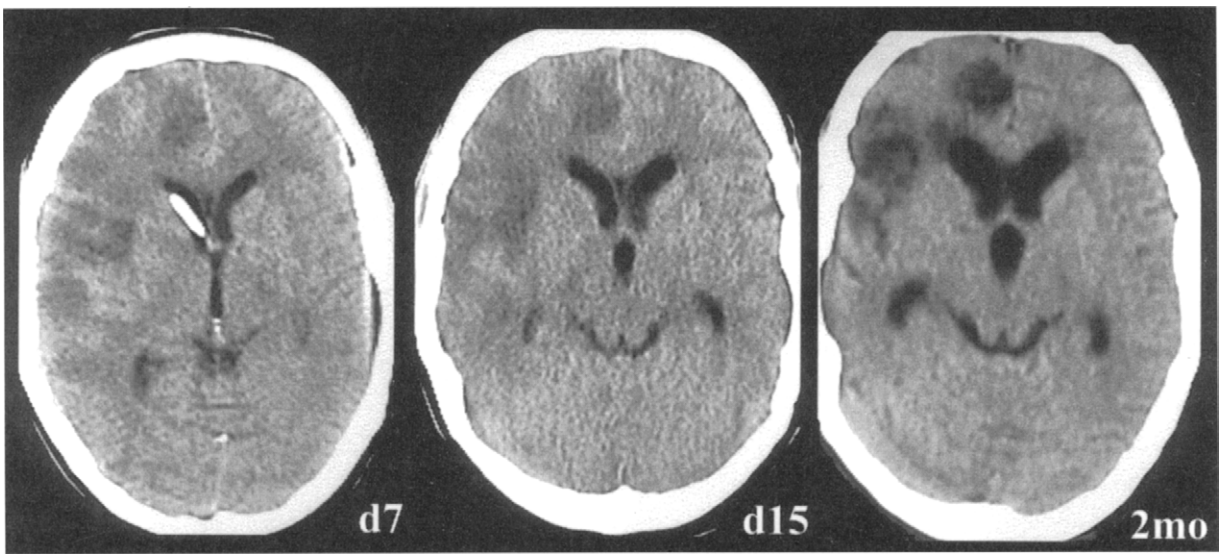


**FIGURE 8.8** The CT scans on the patient discussed in the legend to Fig. 8.7 show initial residual SAH and early low-density areas. Infarction was evident at 2 months.

high pressures compared to the silicon VSP balloon, which generates approximately 0.5 atm during dilatation. One atmosphere of pressure equals 14.7 pounds/in.$^2$ (320). The same group also studied the vessels from 2 patients dying following angioplasty. They observed the connective tissue matrix in the media and intima to be compressed and reorganized with restoration of normal vessel wall thickness and an increase in lumen diameter. There was no evidence of tearing or stretching of smooth muscle in the media due to angioplasty and the endothelium remained virtually intact without tears or openings found between endothelial cells (321). In another study arteries from 2 patients who died following angioplasty were examined by electron microscopy. Collagen was observed to be stretched throughout the vessel wall. There were torn and thinned areas of the wall with intramural hemorrhages caused by the overinflation. One patient died of a gastric hemorrhage and the other died of VSP postangioplasty. There was prominent stretching of the walls at the margins of atherosclerotic plaques. Angioplasty was believed to stretch and disrupt degenerating muscle as well as nonmuscle components (322). In a patient who failed to improve after angioplasty and subsequently died of aneurysm rerupture, examination of brain arteries by light microscopy and scanning and transmission microscopy was performed. The arteries had been dilated by angioplasty and showed compression of connective tissue, stretching of the internal elastic lamina, and compression and stretching of smooth muscle. Small arteries and arterioles that had been treated with infusion of intraarterial papaverine appeared constricted with a thickened intimal layer (323).

### *10. Models of Angioplasty*

An arterial segment from a patient who died from VSP 22 days post-SAH was grafted onto the femoral artery of dogs and subjected to angioplasty five times at 3 atm for 1 min. There was photographic evidence of dilation, and histologically there was evidence of compression of intima and stretching of the internal elastic membrane and muscle layers of the vasospastic artery but no lacerations (324). Progressive stenotic lesions in the internal carotid arteries of dogs were induced by injecting pentobarbital into isolated segments for several minutes. Stenotic lesions formed within 1–6 weeks. Balloon angioplasty revealed depressions in the intima, thrombi and stretching of the internal elastic membrane and media. Angiograms as late as 6 months after angioplasty showed persistent dilatation. Without angioplasty the stenotic vessels went on to complete occlusion (325).

The unilateral clot application model was used in primates. MCA reduction in caliber of 55% had occurred by day 7. A silicon microballoon microcatheter system was used to perform angioplasty which increased the diameter of the MCA to 68% of the baseline. Histologic studies demonstrated that there was no significant endothelial cell damage (326). In rabbit cervical carotid artery clot application models, balloon angioplasty was performed at 2 or 7 days post-clot application. Sacrifice was performed 1, 7, and 21–28 days postangioplasty. Mean reductions in diameter following clot applications were 39% on day 2 and 48% on day 7. Angioplasty on day 2 increased arterial diameters of vasospastic arteries to 50% of control diameters and on day 7 to 47% of control diameters. Arteries remained dilated after angioplasty, although there was significant VSP 7 days after the day 2 angioplasty. Three or 4 weeks after angioplasty there was significant endothelial proliferation and a trend toward thinning of the tunica media. There were no significant changes in the control arteries subjected to angioplasty. Angioplasty was not associated with the significant development of fibrosis as estimated from hydroxyproline content (327). Also, in the rabbit cervical clot application model angioplasty produced significant long-lasting dilation angiographically. Vessels that had angioplasty had significantly reduced contractions to serotonin, KCl, and caffeine compared to arteries not subjected to angioplasty. These pharmacological differences in responsiveness disappeared by 28 days after angioplasty. At all times after angioplasty, VSP significantly decreased ACh induced relaxation of arteries contracted with 5-HT. Arterial wall compliance was significantly decreased in the VSP and control groups at all times after angioplasty, although there were no significant differences between arteries with and without angioplasty. It was suggested that arteries do not reconstrict after angioplasty because angioplasty decreases smooth muscle contractility. There was no evidence of a disruption in arterial wall matrix as judged by a lack of increase in arterial wall compliance after angioplasty (328). In a canine two-hemorrhage model angioplasty was performed on days 1, 4, and 7 post-SAH. Histological evaluation of vessels immediately postangioplasty showed denudation of endothelial cells and stretching of the internal elastic lamina without disruption of the muscle layer. In pharmacological evaluation there was no difference in isotonic constrictive force created by vasoconstrictors between the angioplasty and nonangioplasty segments without SAH. However, there was a statistically significant reduction of isotonic constrictive force in angioplasty segments from arteries subjected to SAH (329).

In a blood clot application to monkeys silicon microballoon dilation was carried out at 1 atm at three times for 10 sec. Angiography demonstrated significant dilatation. Intraarterial papaverine was then infused. Histological examination showed some deformation and denudation of endothelial cells. At the site of denudation platelet

clumps were observed despite generalized heparinization. Stretching of the media was observed and myocytes were deformed and arranged irregularly (330). Thermal balloon angioplasty effects on the vasoconstrictor response on peripheral arteries of pigs were studied. Thermal angioplasty reduced the extent of inducible VSP from 79 to 6% compared to nonthermal control inflations, which reduced the vasoconstrictor responses from 75 to 60%. The extent of myonecrosis was significantly greater in the thermally treated arteries than in the control vessels. Thermal balloon angioplasty at 60°C significantly attenuates peripheral artery VSP induced by mechanical trauma in the porcine model (331). VSP occurred in saline-treated animals following coronary angioplasty. The VSP was markedly reduced by fantofarone, an nondihydropyridine L-$Ca^{2+}$ channel antagonist. It was much more potent than verapamil (332). Also in the two-hemorrhage canine model, arteries taken on day 7 were compared to those of control animals. Immediately after *in vitro* angioplasty both normal and previously vasospastic basilar arteries showed a significant reduction of responses to both vasoconstrictors and vasorelaxants. Microscopy showed that both the normal and the vasospastic vessels dilated with angioplasty showed flattening and patchy denudation of the endothelium, straightening and occasional rupturing of the internal elastic lamina, and decreased surface rippling with mild stretching and straightening of smooth muscle cells associated with mild resultant thinning of the tunica media (333).

In the canine distal cervical internal carotid artery clot model, in both distal internal carotid arteries angioplasty was performed prior to clot application around one of the arteries. Angiography was repeated on day 7 and animals were sacrificed. Contractile responses of the internal carotid arteries to a variety of agonists were assessed. Angioplasty produced angiographic enlargement which was still present 7 days later despite the placement of clotted blood around the artery. Microscopy revealed flattening of the intima and internal elastic lamina associated with patchy losses of endothelial cells. Internal carotid artery exposed to clotted blood without prior angioplasty developed consistent angiographic and morphological VSP. In comparison with control vessels and nondilated spastic vessels, ones predilated by angioplasty prior to clot application showed significantly diminished responses to KCl, NE, and 5-HT but not prostaglandin $F_{2\alpha}$. *In vivo* angioplasty therefore produced functional impairment of vascular smooth muscle contraction which persisted at least 7 days. These results were interpreted to show that normal smooth muscle function is required for the development of VSP. Of considerable potential clinical importance was the conjecture that angioplasty performed before the onset of VSP might prevent it (334). Megyesi *et al.* studied the long-term effects of *in vivo* angioplasty in normal and vasospastic canine carotid arteries. They concluded that the canine high cervical internal carotid artery model produces consistent and reproducible VSP which follows a similar time course to that of human VSP. The functional impairment of vascular smooth muscle responses which immediately follows angioplasty persists for 2 weeks and resolves by 3 weeks. Morphological changes are mostly resolved 3 weeks postangioplasty. In normal vessels, angioplasty causes functional impairment and morphological alterations that are not as severe and long-lasting as those seen in vasospastic arteries (335).

## N. Nitrovasodilator Therapy

### *1. Nitrovasodilators*

*Intravenous*

Spasm of the external carotid artery encountered during embolic procedures of head and neck tumors has been amenable to the application of nitropaste to nonbearing hair skin surfaces. In 10 such cases vascular relaxation was observed within 2 or 3 min. Good responses were reported from both forehead and anterior chest application sites. The duration of action was approximately 8 hr. The contraindication to the use of nitropaste is intolerance to nitrates, but it was not encountered in this series. Heart rates and blood pressures were not significantly changed even after large applications. Adverse reaction in patients having angina have included headaches and hypotension (336).

Fourteen patients were treated with glycerol trinitrate (GTN) intravenously in a dose of 0.05–0.10 mg/kg/hr until day 14 post-SAH. Five of the patients who were subjected to early surgery also received 4 mg of intrathecal nicardipine twice a day for 10 days. Nine of the 10 patients treated with late surgery received intravenous injections of 0.2 or 0.3 mg/kg/hr of diltiazem for 14 days. Only 1 patient in 8 having check angiography showed VSP and it was of a local type (337). Sixty-six patients having continuous iv infusion of GTN (0.5–1.0 μg/kg/min) for 14 days were compared to 68 patients not treated with GTN. Symptomatic VSP was found in 20% of the GTN group and there was no fatal case attributed to VSP; in the control group 49% of patients had symptomatic, severe VSP and 12% of the patients died from VSP. The infusion was carried out for 2 weeks (338). Nitroglycerin was used in prophylaxis of VSP. One hundred and twenty-five patients classified as Fisher's groups II–IV were operated within 72 hr after SAH. Fifty-eight were treated with continuous IV infusions of GTN (0.5–1.0 μg/kg/min) for 2 weeks postoperatively. Another 67 patients did not receive the GTN. In the CT groups III or IV, sympto-

matic VSP occurred in 31% of the GTN group and in 63% of the control group. Seventeen percent of the GTN group had poor result or died as opposed to 33% of the control group. The difference was considered to be significant. In the GTN group there were no deaths due to cerebral VSP (339). Nitroglycerin and suspensions of human vascular smooth muscle cells are without effect singly on platelet aggregation, but specific amounts of these two agents can profoundly inhibit such aggregation when added in combination. The effect was prevented by Hb, a potent inhibitor of NO, which suggested that the inhibition of platelet aggregation by GTN in the presence of vascular smooth muscle is due to its generation of NO. Sublingual nitroglycerin has been used in the treatment of retinal artery thrombosis (340).

*Intracisternal*

Thomas *et al.* reported prompt and substantial reversal of medically refractory VSP post-SAH in humans using intrathecal administration of a NO donor. Clinical, angiographic, and TCD documentation was obtained. All patients showed severe clinical VSP 5–12 days post-SAH. All manifested stupor of new onset and new hemiplegia. The condition was angiographically demonstrated in all cases. SNP was administered via the ventricles. The SNP was mixed with aliquots of 1 ml of the patient's CSF and given in dosages of 4 mg/ml. They considered the reversal of VSP by SNP and delayed segmental reccurrence to be consistent with a mechanism that depends on the presence of a finite quantity of substrate producing VSP that can at least temporarily be overwhelmed. A substantial vasodilation of large-caliber conductance vessels in response to SNP was demonstrated. Smaller resistance vessels may have also been dilated as evidenced by shortening of the circulation time. A brief episode of hypertension occurred on one occasion following a high intraventricular dose (12 mg). The same investigators subsequently reported 21 cases treated with intrathecal SNP. Ruptured aneurysms of patients were clipped and showed CT grades of III or IV. In 57% of cases refractory VSP was diagnosed prior to treatment and the others were treated prophylactically. All patients with established VSP were in poor neurological condition prior to treatment. SNP was delivered by intraventricular or subdural catheter or direct intraoperative suffusion. There were 171 individual injections. Good or excellent outcomes were obtained in 76% of the total number of patients. Of the 5 patients with a less than good outcome, 4 had presented with initial neurological grade IV. Angiography demonstrated reversal or amelioration of VSP in 83% (5 of 6 cases) of established VSP treated by SNP alone. None of the 10 patients treated prophylactically developed clinical VSP. Of the 12 patients treated for established VSP, only 58% had actual DID and 45% were diagnosed on the basis of TCD only. Seventeen percent were diagnosed on the basis of angiographic VSP without clinical symptoms. Of the 9 cases with symptomatic VSP in which check angiography was performed, reversal of the angiographic VSP was seen in 6 cases (75%). In 75% percent of the patients treated with SNP, angioplasty was also performed. In the 10 patients considered at risk of developing VSP who received SNP prophylactically, the CT SAH grade III was present in 60%, and the others were grade IV. The prophylactic dose of SNP was 4 or 8 mg every 8 hr through the ventricular catheter. This usually produced nausea (341). Wolf *et al.* showed reversibility of experimental VSP using an intrathecally administered NO donor (342).

### *2. Papaverine*

*Intraarterial*

Topical application of papaverine in an effort to relieve spasm has been performed by neurosurgeons for many decades. It was generally considered to have a transient beneficial effect. Papaverine was first used for the treatment of the cerebral circulation in 1948 when it was administered orally in the hopes of preventing recurrent cerebral ischemic events of various etiologies (343). Kaku and colleagues performed superselective intraarterial infusion of papaverine hydrochloride in 37 vascular territories of 10 patients with symptomatic VSP. The infusions followed angioplasty of the internal carotid artery and proximal MCA. Papaverine was infused as a 0.2% solution. Thirty-four of 37 vascular territories were successfully dilated and 8 of 10 patients showed improvement in neurological function after the procedure. No serious side effects were observed. Papaverine is thought to act by inhibiting both cAMP and cGMP phosphodiesterase activity, thereby increasing the availability of cAMP and cGMP for active dilation (344). Kassell and colleagues also presented 12 patients who had treatment with intraarterial papaverine. Eight showed marked angiographic reversal of the VSP following infusion, and in 4 there was dramatic reversal of profound neurological deficits. Two patients deteriorated 5 days after the initially successful papaverine infusion. Repeat angiography demonstrated severe recurrent VSP which was partially reversed by a second intraarterial papaverine treatment. Two patients developed focal neurological deficits during papaverine infusion which resolved spontaneously over several hours after its cessation. In several of the patients no appreciable change in diameter was noted during the first 30–60 min of infusion but then fairly dramatic dilation occurred in the last 30 min. The dosage of 300 mg/100 cc infused over 60 min appeared to be adequate and safe (345). The explanation of why some patients

appeared to be resistant to intraarterial papaverine may have been indicated in the experiments of Vorkapic *et al.*, who found that VSP had a papaverine-sensitive phase followed by a resistant one in which the arterial wall had become stiff and unresponsive (346). Two additional early successes to papaverine given intraarterially were reported with angiographic relief of VSP (347). It has been suggested that intraarterial papaverine can be used to facilitate the successful passage of nondetachable angioplasty balloon systems (348).

Twenty-four patients received intraarterial papaverine to treat VSP. Half did not improve clinically after the initial treatment. Of these 12 patients, 9 received second or third infusions on consecutive days. Superselective infusion was performed in all cases. Despite angiographic improvement after the initial or second infusion, all 9 patients showed varying degrees of recurrent VSP at the second or third treatment. Within 24 hr of the second infusion half of the 6 patients showed significant clinical improvement and 1 showed marked improvement following a third infusion (349). For the years 1992 through 1995, it was possible to review 81 treatments in 34 patients. Angiograms obtained at presentation were examined in 26 of the 34 patients. Nine carotid territories were visualized by repeat angiography on the day after infusion. Papaverine produced an increase in average arterial diameter ranging between 3 and 74%, with a mean increase of 27%. The increases in diameter occurred in proximal, intermediate, and distal arteries. The infusions had been performed between days 3 and 19 post-SAH. The vasodilator response did not seem to depend on the time post-SAH. The effect of papaverine did not persist until the day after treatment in the patients in whom repeat angiography was performed. For proximal, intermediate, and distal arteries the degree of dilatation was greater in the vessels showing the greatest vasospasm preinfusion (350).

Fifteen patients with DID and focal cerebral hypoperfusion shown on Xe CT scans as well as angiographic VSP had infusion of papaverine into 32 arteries on 23 occasions. Six patients had multiple infusions between 1 and 8 days apart. In 5 cases, angioplasty was combined with the intraarterial papaverine. There was a reversal of angiographic VSP in 78% of patients; however, this was associated with an increase in small artery CBF in only 46% of the cases analyzed and major clinical improvement occurred in only 26%. There was a poor correlation between angiographic response and clinical response. Of the 6 cases in which neurologic examination improved, two grades of angiographic improvement occurred in 1 case and one grade of angiographic improvement occurred in 4. In 17 cases of no improvement in neurological status, 7 were associated with two grades of angiographic improvement. All cases with improvement in CBF, however, had shown angiographic improvement (351). In Firlik's group there was no uniform correlation between clinical improvement and degree of angiographic VSP, age, days post-SAH, dose of papaverine, infusion rate, or neurological grade on admission. In this series neurologic improvement was seen in 2 cases who in fact had no angiographic response to papaverine. This is because other therapies were concurrent, such as ventilation, fluid replacement, induced hypertension, and angioplasty (351). Chopko *et al.* reported their experience in infusing 190 mg of papaverine (range, 60–450 mg) into 49 vascular territories. In only 6 cases were the initial treatments sufficient. Twenty-four territories had retreatment within 6–48 hr. In 6 territories angioplasty was combined with papaverine. Every territory initially responded. Retreatment was effective in 21 of 24 territories. Two patients succumbed to intractable VSP and 1 developed a hemorrhagic infarct in a previously treated territory (352). Sixty-seven patients undergoing endovascular treatment for symptomatic VSP had the following treatments: intraarterial papaverine (46), papaverine and angioplasty (18), angioplasty alone (3). Sixty-seven percent of patients treated with papaverine alone improved by at least 1 GCS point and 28% improved by at least 2 CCS points within 24 hr after initial treatment. This compared favorably with patients with balloon angioplasty alone or combined with papaverine infusion in whom 43% improved by at least 1 GCS point and 24% improved by 2 GCS points. Thirty-four patients underwent a second treatment and 10 patients underwent a third. SPECT and TCD studies were performed in 44 patients whose VSP was managed by endovascular means. SPECT revealed improvements in CBF in 42% of patients treated with papaverine and 70% treated with angioplasty. TCD correlated with SPECT in 71% of patients in the papaverine group and 73% in the balloon angioplasty group. TCD showed that angioplasty was effective in 93% of segments, whereas this was found in only 43% of papaverine-treated segments. TCD sometimes showed velocity improvements, whereas SPECT demonstrated unchanged or worsening flow imaging. It was concluded that angioplasty was superior to papaverine in the treatment of VSP (353).

Ninety vascular territories were infused with papaverine at the top of the internal carotid artery. Infusions were performed with 0.1–0.2% up to 2% weight/volume. With the 0.4% infusions, 80% of territories dilated and 44% of patients showed a marked reversal of neurological deficits, so the authors concluded that 80 mg/20 ml (0.4% weight/volume) papaverine infused over 10 min was of benefit. Transient deficits attributed to the infusion occurred in 7% of the 0.1–0.2% group but 44% of the 0.8–2% group (354).

Three patients were described who experienced transient neurologic events associated with intraarterial papaverine infusion in the vertebrobasilar system. There were two cases of respiratory depression (355). A patient having infusion of papaverine into the left vertebral and left internal carotid arteries simultaneously had a respiratory arrest with rapid, progressive loss of brain stem function beginning 25 min after the infusion had begun (356). Five cases of ipsilateral pupillary dilatation developing during intraarterial papaverine were reported from one center. The tips of the infusion catheters were in the internal carotid artery close to the ostium of the opthalmic artery. The pupillary dilatation in all patients readily resolved after termination of the infusion (357). Immune-mediated papaverine-induced thrombocytopenia has been reported. On day 8 post-SAH intraarterial papaverine was associated with a fall in platelets from 224 to 20 k/mm$^3$, and when this was repeated on day 16 post-SAH the platelets fell from 290 to 7 k/mm$^3$. Drug-induced thrombocytopenia is often preceded by flushing and chills and is usually self-limited if the offending agent is discontinued. Platelet transfusion may be necessary for stabilization (358).

A series of 21 patients receiving arterial papaverine was reviewed. Seventy-six percent experienced good angiographic results and 52% obtained objective clinical improvement within 48 hr. Significant elevations of ICP, blood pressure, and pulse rate were noted during papaverine infusions. In one elderly patient, infusion into the common carotid artery resulted in profound bradycardia, hypotension with a subsequent significant increase in ICP, and a marked decrease in CPP. The treatment procedure consisted of 300 mg of papaverine hydrochloride diluted in 22–24 ml of normal saline and infused in tiny aliquots over 20–35 min. If the 300-mg dose was tolerated, a repeat angiogram was performed and then additional papaverine was administered to a maximum dose of 500 mg/vessel. Five of the 21 patients died. Deaths possibly attributable to the procedure involved rupture of a left posterior cerebral artery and ICH into the basal ganglia. Two nonfatal complications were bradycardia and a first-degree heart block (359). Twenty-eight patients had 51 sessions of intraarterial papaverine infusions. Baseline ICP ranged from 0 to 34 mmHg. With typical doses of 300 mg/territory given over 5–60 min per vessel, ICP increases ranged from 0 to 60 mmHg. ICP increases were observed even in some patients with low initial ICP, although increases were more likely with high initial ICP. ICP monitoring during infusions was recommended (360). A case was reported in which paradoxical aggravation of cerebral arterial narrowing during selective intraarterial papaverine of infusion occurred. During infusion of papaverine into the left internal carotid artery the patient became aphasic and right hemiplegic. Angiography performed immediately after the infusion demonstrated diffuse exacerbation of VSP in the distal anterior cerebral artery (ACA) and MCA territories. A repeat Xe CT CBF study showed a dramatic reduction in rCBF. Multiple attempts have been made at selective catheterization of the left ACA with various wires and catheters during the procedure. No systemic heparin administration was used and no heparin was infused through the tracker microcatheter, although small amounts of 6000 U/liter heparin flush were slowly infused through the base catheter. Three hundred milligrams of papaverine (0.3%) was infused in 100-cc normal saline at 4 cc/min for 25 min before the patient developed the aphasia and hemiplegia. Vasodilation of the proximal arterial tree was achieved but severe distal MCA VSP developed. The complication may have resulted from precipitation of papaverine from solution. The drug at high concentrations (3%) will crystalized with as little as 2000 U/liter heparin. At lower concentrations, such as those used in the cerebral vascular system (0.3%), crystallization does not occur even in 10,000 U/liter heparin. Papaverine should be infused slowly and at low concentrations to allow adequate dilution in serum and prevent precipitation (361).

*Intracisternal*

In an artery which had become yellowed and atrophic more than 3 months after SAH, porencephaly had developed, there was no dilatation to the intraoperative application of papaverine (362). An earlier account of papaverine being injected into the basal cisterns following aneurysm clipping was that of Kataoka and colleagues who injected the drug into an Ommaya CSF fluid reservoir placed at craniotomy (363). In 3 patients, CBF recordings were done before and after cisternal injections of papaverine post-SAH. One patient showed a prominent increase in CBF in the frontal lobe and basal ganglia but a paradoxical decrease in the parietal lobe and corona radiata. The other 2 patients did not have a significant CBF response. It was considered that the dense clot in the basal cisterns may have prevented diffusion of the drug (364). In a series of 15 patients studied by Segawa and colleagues (365), angiographically confirmed VSP was treated about 6 days post-SAH. Serial angiograms after the initial dosage of papaverine intracisternally were carried out at 30, 60, and 90 min in 7 cases. Papaverine (40 mg, 1%) was injected into the basal cisterns as a bolus through one of two catheters used for irrigation. The irrigation catheters were then clamped for 60 min after the papaverine injection. Catheter tips were in the Sylvian fissure in 5 cases and in the cistern lateral to the carotid syphon in 10 cases. Papaverine was administered twice a day in 15 cases. In the second angiogram

performed on average on day 6 post-SAH, the vessel caliber was reduced by 12–52% (mean, 29%) at five measured points compared to the initial angiogram. Vasodilation up to 88% was observed in either carotid, middle cerebral, or anterior cerebral artery in all 7 cases with serial angiography after the initial dose of papaverine. The response was maximum at 60 min and persisted at 90 min. In 3 patients the vessel calibers increased to even larger diameters than were present on the initial admission early angiograms. Two cases had no response. Improvement in neurological function was observed in 7 patients whose deterioration was attributable to VSP. Five showed improvement in the level of consciousness, 1 in hemiparesis, and 1 in both. Progressive neurological dysfunction was arrested in 2 additional cases. Six cases were not helped. Overall, 60% of patients were considered to have benefitted neurologically. Two cases developed ICH during papaverine administration, 1 occurred in the frontal lobe adjacent to the catheter and the second occurred in the brain stem (this was fatal). Papaverine is a nonspecific smooth muscle relaxant capable of producing arterial dilatation in the systemic, coronary, and cerebral circulations. The duration of action is considered to be about 60 min when administered intrathecally in monkeys (366). In a previous series, Segawa noted that 80 mg of 0.02% papaverine continuously irrigated into the cisterns was without obvious effect on VSP either angiographically or by neurological examination. In Segawa *et al.*'s subsequent study, 40 mg of 1% papaverine was found to be effective. They postulated that the concentration of papaverine is more important than the dosage administered (365).

Oda *et al.* placed silicon pellets loaded with papaverine hydrochloride in cisternal drainage tubes made of silicon. These pellets were placed in 30 patients post-SAH operated days 0–4 post-SAH. Seventy-three percent of the patients had Fisher CT grade III or IV CT scans. The concentration of papaverine hydrochloride in the CSF on the second to third postoperative days reached the same maximum concentration as that obtained by bolus injection and the level on postoperative days 5–10 was similar to that obtained several hours after bolus injection. All patients except 1 showed excellent results and returned to a normal life (367).

### 3. *Models of Nitrovasodilator Therapy*

*Nitrovasodilators*

Intravenously (368) or intraarterially (369) administered SNP has effectively relieved VSP in canine models. Clinical use of SNP has been limited by systemic hypotension (370). Takase and colleagues used a rat basilar artery perfusion system to study the effect of extraluminal oxyHb application and the responses to GTN. Intraluminal application of GTN had a stronger relaxing effect than extraluminal application on the constriction resulting from SAH. The relaxing effect of GTN was significantly more potent in arteries precontracted by endothelin than in arteries precontracted by KCl. Extraluminal oxyHb significantly inhibited the relaxation induced by GTN. It was suggested that extraluminal oxyHb might impair or affect NO in the smooth muscle cells and that SAH changed the sensitivity of smooth muscle cells to NO (371).

Nitroglycerin causes hyperpolarization and cGMP elevation in vascular smooth muscle cells. In a primate unilateral clot application model, 7 days after surgery angiography was repeated. Either GTN (3 μg/kg/hr) or saline was administered intravenously. Both cGMP and cAMP were measured in the cerebral arteries and bilateral cortex. Significant VSP occurred bilaterally but more on the side of clot application. rCBF was significantly decreased on the side of the clot. cGMP levels were significantly lower in the MCA beneath the clot in normal MCA. After the administration of GTN for 3 hr the cerebral vessels were significantly dilated on both sides. rCBF was significantly increased on the side of the clot. Although depressed cGMP levels in the right MCA did not return to normal after the GTN, a significant increase in the cGMP levels was observed in the basilar artery. cGMP levels were also decreased in parietal cortex following SAH. It is possible that GTN hyperpolarizes vascular smooth muscle cells in order to relax them rather than doing so by increasing the cGMP levels (372). Egeman *et al.* (373) provided an early report of efficacy for intrathecally administered SNP in which such administration relieved severe chronic VSP in a canine model. NO is the mediator of a wide range of physiological processes and is the smallest biologically active molecule known. NO donors have been demonstrated by direct observation to reverse endothelin induced cerebral arterial contraction in an animal model in which NO was delivered intrathecally to the adventitial side of the blood vessel wall (374).

*Papaverine*

In a monkey clot model MCAs were found to be narrowed by 50% on day 7. Papaverine intraarterial infusion resulted in vascular dilatation of about 20%. After the infusion the mean blood flow velocity increased by 70% on day 7. The mean blood flow velocity in the MCA decreased by about 30% but increased again after 24 hr to nearly the level before papaverine infusion (375).

In a two-hemorrhage canine model in which blood was injected on days 1 and 3, intraarterial bolus injection of 2 mg/kg papaverine failed to reverse the resulting constriction on eight dogs. The basilar artery luminal diameter ranged between 1.2 and 2 mm in the control angiograms

post-SAH and between 1.0 and 1.5 mm in the angiograms performed postpapaverine injection. Half the animals showed minimal dilation in response to the papaverine which was not statistically significant. Vessels did not return to control diameters (105). In 18 experiments Lende used photographs of exposed arteries subjected to vasoconstriction by electric current and found that 1.6% papaverine produced moderate to marked dilatation and was fairly effective in preventing the arteries from developing acute VSP. It worked in dilutions up to 0.02% (376). In a canine SAH model papaverine (15 mg) was injected into the subarachnoid space in six animals. The vessels were initially constricted to between 25 and 57% of baseline by the SAH. After the subarachnoid injection diameters changed from −7 to 25% of baseline. The average increase in diameter was 41%. The time to maximum change ranged between 10 and 60 min and the effect was durable for between 10 min and more than 2 hr (377).

An early evaluation of the efficacy of papaverine administered via the intrathecal route was performed by Ogata *et al.* Blood was placed into the basal cisterns of monkeys by an open procedure. On day 8, papaverine was administered directly into the Sylvian fissure through a drainage tube. There was a dose-dependent reversal of VSP at drug concentrations ranging between $8 \times 10^{-3}$ and $8 \times 10^{-5}$ M. The vasodilating effect was transient in all cases and never lasted more than 24 hr beyond termination of the infusion (366). There is a considerable body of experimental work showing that vasodilator drugs can be successful if administered intrathecally despite their ineffectiveness when administered intravenously or intraarterially (105,112,366,377,378). Heffez and Leong devised a papaverine-loaded polymer sheet. Ten millimeter discs placed *in vitro* into baths with comparable volumes to the CSF space showed that steady-state concentrations of drug equal to $1.6 \times 10^{-6}$ M could be achieved for up to 220 hr (379). A rod-shaped implant of papaverine and copoyl(lactic-glycolic acid) was developed and placed in 16 dogs. SAH was induced by placement of clot in the sylvian fissure. Two pellets containing 25 mg of papaverine were used in the active treatment group. Fifty-six percent of the actual papaverine loading was released in the first 4 days and 78% within 8 days. On day 7, angiography was repeated and the animals were killed. An additional experiment using 5 mg of papaverine was conducted on another group of animals. There was a significant difference between papaverine- and placebo-treated groups in the reduction of vessel diameters on the clot side. The mean concentration of papaverine in the clot was $4.5 \times 10^{-4}$ mol/liter. The low-dose pellet did not prevent VSP, although the mean concentration in the clot was $2.3 \times 10^{-5}$ mol/liter. This type of delivery system might be useful for placement at the time of aneurysm clipping. It is possible that abluminal application of papaverine is more effective than intraluminal administration. Sustained release delivery systems would avoid the risk of infection inherent in multiple injections through cisternal drains. In this experimental series the pellets were placed before the application of the blood clot (380).

Using a rabbit model, in seven animals SAH and papaverine infusion via the intracisternal route were performed. A continuous infusion of 0.01 *M* in saline was carried out for 2 hr. Animals were then perfused and fixed. A 9% decrease in mean arterial pressure was observed in the papaverine group compared to non-SAH controls. Morphometric measurements showed a 25% decrease in luminal diameter in SAH animals on day 3 compared to non-SAH controls. Papaverine-treated animals demonstrated a reversal of the arterial narrowing produced by SAH so that the arterial diameters were not different from those of control animals. The saline vehicle with a pH of 5.3 also attenuated the experimental VSP (381). In their rabbit model, Nakagomi *et al.* used morphometric techniques to assess pharmacological reversibility of luminal narrowing after experimental SAH. To induce maximal vasodilation after VSP had been established, animals had intraarterial injection of $10^{-4}$ M papaverine, $2 \times 10^{-4}$ M SNP, and $10^{-5}$ M adenosine. Infusion of these vasodilators was performed 48 hr after injection of blood. The arteries were perfusion fixed at time of sacrifice. The vasodilator drugs used in combination caused significant dilation of rabbit basilar arteries preconstricted by either SAH or $BaCl_2$. This study demonstrated that the thickening of the vessel wall and reduction of the lumen that follows SAH are reversible if a sufficiently potent, high concentration of vasodilating agents is used intraarterially (382). In *in vitro* experiments, papaverine ($10^{-4}$ M) always induced a maximal amount of vasodilation of control and spastic human arteries following SAH, whereas endothelium-dependent vasodilation was markedly disturbed (383).

## VII. Randomized Clinical Trials

We believe that the results of randomized clinical trials of the past two decades lend strong support to the belief that angiographic VSP can result in CT demonstrable infarction and death and disability. Given that the general cause of this phenomenon is the clot which forms in the subarachnoid space shortly after aneurysmal rupture, it has appeared evident to us for years that actions aimed at the expeditious and efficient removal of such clot would inevitably have a favorable effect on outcome in the same way that early clipping of the aneurysm would have a favorable effect on rebleeding morbidity and mortality.

In retrospect, it is clear that many of the practices neurologists and neurosurgeons performed in the past actually contributed to morbidity and mortality. Such practices included the preservation of the intact clot in the basal cisterns by the use of antifibrinolytic agents and the reduction in $O_2$ delivery to the brain by deliberate reduction in circulating blood volume resulting from venesection, deliberate dehydration, and the inappropriate use of osmotic diuretics in the deteriorating patient. In addition, CBF was frequently adversely affected by the prolonged use of antihypertensive agents in the period of greatest risk of narrowed vessels inducing ischemia. The treatment of hyponatremia by fluid restriction that occurred in the 1970s and 1980s is now uncommon as a result of the realization that cerebral salt wasting is much more common than inappropriate secretion of ADH. The avoidance of management errors leaves a relatively small group of patients who will develop a lethal progressive arterial constriction which will cause fatal infarction if untreated. Aggressive management with intubation and central catheterization and endovascular intervention may be indicated in such patients. However, the mortality rates inherent in interventional therapy of all sorts are similar to the mortality rate of delayed ischemic deficits managed conservatively. Meticulous nursing and respiratory therapy care are critical in obtaining a good result for the seriously ill patient.

The randomized clinical trials of the past two decades provide important information on the nature of VSP and the results of its treatment. The landmark international Cooperative Study on the Timing of Aneurysm Surgery entered 3521 patients between 1980 and 1983 into a prospective observational trial. At admission, three-fourths of the patients were in good condition and surgery was performed in 83% (263). However, at 6-month evaluation 26% of the patients had died and only 58% exhibited a complete recovery. At that time, VSP was considered to be the leading cause of morbidity and mortality in these patients, who had been admitted on days 0 and 3 post-SAH. Late referral to neurosurgeons was the norm in the 1960s and 1970s. Interestingly, a history of hypertension was obtained for only 21% of patients, which is about half the rate of recent studies. Patients had a better level of consciousness the later they were admitted to the study (fully alert, day 0, 41%; day 3, 68%). On day 0, 92% showed SAH on the first CT scan compared to 58% on day 5. Thick clot was observed in 33% of the day 0 admissions. Patients whose anterior or MCA lumens were narrowed to 0.5 mm or less and in whom the supraclinoid internal carotid artery had a diameter of 1.5 mm or less were classified as angiographic VSP grade III or IV. The incidence of severe angiographic VSP on days 0–2 was under 2%, and this approximately doubled in succeeding days up until day 6 post-SAH, after which it was stable above 16%. Surgery was performed on days 0–3 post-SAH in 51% of the operated cases, days 4–6 in 12%, days 7–10 in 13%, days 11–14 in 9%, and $\geq$ day 15 in 16%. The morbidity from VSP accounted for 39% of the total 575 disabled cases. VSP was considered to be the cause of death in 7.2% of cases and of disability in 6.3%, for a total death and disability rate of 13.5%. This compares to 10.6% for direct effects of the initial bleeding. The therapeutic practices at that time were markedly different from those of recent years. Forty-four percent of patients were treated with antifibrinolytics, 70% with steroids, and 31% with antihypertensives. Potentially dehydrating agents such as mannitol were used in 33% of patients and diuretics in 12%. Hypervolemia was only used in 22% of patients and vasopressor agents in 9%. Multivariate analysis indicated that the most important factors for outcome were neurological grade, age, and blood pressure. Subarachnoid clot distribution on CT was also important, as was the absence of VSP on the admission angiogram. The incidence of focal neurological deficits overall was 28% of the entire patient group, which has not changed much in the past two decades. Inappropriate ADH secretion was considered to have occurred in 3.6% of the patients. Among the prognostic factors for good recovery in surgical patients was the use of preoperative hypervolemia. In this worldwide multicentered trial the prespecified "planned" surgical interval showed no difference in outcome between days 0–3 selection and days 11–14 selection (384). However, outcome was worse if surgery was performed in the 7–10 days post-SAH interval. We now know that this is the time during which VSP becomes maximal and the brain is highly susceptible to further ischemic insult. The failure to demonstrate a clear advantage to early surgery may have indicated that surgical trauma cancelled out the beneficial effects of reduction in rebleeding and VSP. It is worth stressing that almost half the patients admitted to these centers were not in the timing trial because they were admitted too late and of the patients who were in the trial, only half were operated on in the first 3 days. Death and disability attributable to VSP were highest in the group operated earliest (13.5%) and fell to 7.3% in the group operated on day 11–14 and 9.9% in the group operated on day 15 or later. In our opinion, the explanation for this data is that some death and disability resulting from operative factors were attributed to the DID from VSP. When the patients from North America (22% of the total study population) were analyzed separately, overall outcome in the patients planned for surgery on days 0–3 was equivalent in terms of mortality to outcome when surgery was planned for days 11–32. Earlier operated patients had significantly improved rates of good recovery (71 vs 62%). As in the entire study,

patients planned for surgery on days 7–10 had nearly twice the mortality of patients in the other intervals.

In a Japanese trial of a calcium antagonist AT877, the placebo group had 136 patients with an average age of 55. Seventy-four percent had Fisher grade III CT scan findings on admission and 100% were operated on days 0–3. Forty-four percent had a history of prior hypertension. Moderate to severe angiographic VSP was documented in 61%. Symptomatic VSP occurred in 50%. The time to onset of clinical VSP averaged 7.3 days. Fifty-six percent of patients developed low-density areas attributed to VSP. Good outcomes occurred in 60% of patients and the mortality rate was only 7%. Of the poor outcomes, the most common causes were VSP(21%), directs effects of SAH(7%), Hyc(3%), surgery(3%), and infection (1%, in the placebo group). AT877 was considered to have reduced the incidence of moderate to severe VSP and symptomatic VSP as well as poor outcomes from VSP (385).

Between 1987 and 1989, 906 patients were randomized to receive placebo or 0.15 mg/kg/hr nicardipine intravenously. There was an imbalance in the prior existence of hypertension, with 43% of the placebo group having such a history versus 26% in the treated group. Prophylactic hypertension/hypervolemia was used in 38% of the placebo cases versus 25% of the treated ones. Symptomatic VSP occurred in 46% of the placebo group versus 32% of the treated group. At 3 months the outcomes were similar, with 56% good in placebo and 55% good in the treated group. The death rates were 18 and 17%, respectively. Symptomatic VSP was based on a clinical deterioration between days 5 and 12 characterized by increasing headache, neck stiffness, fever, confusion, disorientation, decreasing level of consciousness, and fluctuating but progressive focal deficits. This occurred in the presence of CT scan negative for other causes and with normal electrolytes, blood gases, and no evidence of seizure activity. Some patients were studied by repeat angiography and TCD. Diffuse clot was present in 40% of the placebo group on admission CT; this was almost identical to the percentage of the treated group. Prior hypertension was present in 41% of both groups, and late surgery was performed in 17% of both groups. Symptomatic VSP was diagnosed in 46% of the placebo group and 32% of the treated one. VSP was considered the primary cause of death in 4.2% of placebo and 3.9% of the treated patients. VSP was believed to have contributed to death in 10% of placebo cases and 5.7% of treated cases. VSP was the primary cause of disability in 7.4% of placebo cases and 4.3% of treatment cases. Angiograms were obtained between days 7 and 11 in 26% of placebo and 23% of treated patients. Fifty-one percent of placebo patients had moderate to severe VSP on days 7–11 angiograms compared to 33% of treated patients. This was believed to be a statistically significant difference. Forty-nine percent of placebo cases examined by TCD between days 7 and 11 showed MCA velocities $\geq$120 cm/sec compared to 23% of treated patients. Antifibrinolytic medication had been employed in 21% of the placebo group patients and 19% of the nicardipine-treated group (95). In a subsequent trial of two different doses of nicardipine carried out between 1989 and 1991, 365 patients were randomized to 0.15 versus 0.075 mg/kg/hr. The incidence of symptomatic VSP was 31% in both groups and good outcomes occurred in almost 60% of both groups. Antifibrinolytic therapy was used in 8% of the high-dose group and 15% of the low-dose group. Forty-two percent of the high-dose group and 47% of the low-dose group had diffuse thick clot on initial CT scan. A history of hypertension was present in 45% of the high-dose group and 34% of the low-dose group. Neurological worsening attributable to VSP occurred in 17% of the high-dose group and 18% of the low-dose group. Antihypertensive drugs were used in 34 and 30% of the high- and low-dose groups. Prophylactic hypertensive/hypervolemic therapy was used in 68% of the high-dose group and 66% of the low-dose group. Therapeutic hypertension/hypervolemia for VSP was employed in one-fourth of the patients in both groups. The employment of angioplasty began to occur in North America during this time period. One patient in the high-dose group and 5 patients in the low-dose group were treated with this modality. More than 90% of the patients in both groups were actually operated on and late surgery occurred in only 4% of the high-dose group and 8% of the low-dose group. DID attributable to VSP occurs in a steadily cumulative fashion, reaching its peak of about 30% of patients by day 10 post-SAH. Because of the systemic vasodilatory effect of nicardipine, 5% of the high-dose group and 6% of the low-dose group patients failed induction of hypertension. There was an incidence of renal dysfunction in 16 and 13% of these two treated groups, respectively. Pulmonary edema was reported in 34% of the high-dose group compared to 20% of the low-dose group. This probably reflected the more aggressive approach to fluid management as well as the specific effect of the nacardipine (96).

The early 1990s witnessed a truly monumental effort to establish efficacy of the drug tirilazad mesylate, a 21-amino steroid free radical scavenger, in improving outcome following SAH and possibly ameliorating the effects of VSP. In the first major safety study, 245 patients were admitted to 12 Canadian centers. The drug was administered in progressive dosage tiers at 0.6, 2, and 6 mg/kg trilazad or vehicle intravenously through day 10. No serious side effects with the drug were encountered. In the four treatment categories, late surgery at day $\geq$7 was

performed in between 6 and 12% of patients. Antifibrinolytic agents were used in between 3 and 7% of patients. Hypertension/hypervolemia was utilized in between 61 and 74% of patients in each group. Diffuse thick clot was present in 47–69% of initial CT scans. Symptomatic VSP occurred in 21% of the group receiving 2 mg/kg of tirilazad and 41% of patients receiving the vehicle. Good outcome was present in 90% of the patients receiving the 2-mg/kg dose versus 70% in the vehicle. The results for the other dosage group were intermediate. Death rates by group were as follows: vehicle, 13%; 0.6 mg/kg, 8%; 2 mg/kg, 5%; and 6 mg/kg, 20%. At 3 months the percentage of patients dying or disabled from VSP as a primary or contributory cause for the four groups ranged between 10 and 24%, for the direct effects of SAH the percentages ranged between 24 and 31%, and for complications of surgery the percentages ranged between 2 and 12%. This study had not been structured to prove efficacy but there was a suggestion that the 2-mg/kg dose was the best (386). In a subsequent study, 1023 patients were randomly assigned to receive 0.6, 2, or 6 mg/kg/day of intravenous tirilazad or placebo at 41 centers in Europe, Australia, and New Zealand. All patients received concurrent nimodipine. The patients receiving 6 mg/kg/day had a reduced mortality and a greater frequency of good recovery. There was a reduction in symptomatic VSP in this group. The benefit of treatment appeared to be predominantly in men. This large group of patients appeared to truly represent the universe of patients with ruptured aneurysms since the average age was about 51 years and two-thirds were women. About one-fourth were neurological grades IV or V on admission and 45–48% showed diffuse thick clot on the initial CT scan. About 90% of each group actually had surgery, and late surgery (days 7–10) occurred in only 4 or 5%. Prophylactic hypertensive/hypervolemic therapy was administered in 45–49% and therapeutic hypertension/hypervolemia in 10–18%. Angioplasty was performed in 1 or 2% of the groups. The median time to surgery was only 1 day. Symptomatic VSP occurred in 12% of the 6 mg/kg/day group versus 18% in the 2 mg/kg/day group, 16% in the 0.6 mg/kg/day group, and 17% in the vehicle controls. Moderate to severe angiographic VSP was present in 28–32% of patients. TCD velocities were >120 cm/sec in 40–48%. The 3-month outcomes were 63% good and 12% dead in the 6-mg group, which was highly significantly different from the 21 and 53% in the vehicle group. However, the other two treatment groups did not differ significantly from the vehicle group. The significance of these differences was even greater when only males were examined, although there was no significance difference between dosage groups in women. The common medical complications in this large study were hypotension (0–3%), renal dysfunction (0–2%), pneumonia (1–3%), and sepsis (1.6–2%) (387).

The tremendously encouraging result of the first large efficacy trial of tirilazad was unfortunately not replicated in the subsequent North American trial in which 902 patients were admitted. There were 300 patients in the vehicle group, 298 patients were in the 2 mg/kg/day, group, and 999 patients were in the 6 mg/kg group. These dosages were administered within 48 hr of SAH and continued for 10 days. As in the other study, all patients received oral nimodipine. During the first 14 days after SAH there were no significant differences between the groups in the incidence or severity of clinically symptomatic or angiographically identifiable VSP. Men with admission neurological grades IV or V had a 33% mortality rate in the vehicle group, 52% in the 2 mg/kg/day tirilazad group, and 5% in the 6 mg/kg/day tirilazad group. The patient groups in this study were comparable in age and sex distribution to those in the prior trial. Diffuse thick CT on admission was present in 46–48% of the groups. Ninety-three percent actually had surgery at a median time of 1 day post-SAH. Hypertension/hypervolemic therapy was performed in 68% of the 6 mg/kg/day group versus 75% of the vehicle group. Moderate to severe angiographic VSP occurred in 24% of the high-dose tirilazad group versus 28% of the control group. Symptomatic VSP occurred in 33% of both groups. By day 14, infarction on CT scan was evident in 24% of the high-dose tirilazad group and 27% of the vehicle group. TCD >120 occurred in 55% of all groups. Moderate to severe angiographic VSP was diagnosed in 24% of the 6 mg/kg group versus 28% of the vehicle group. Complications occurring in all groups included angiographic complications (0.3–1%), surgical complications (0.7–2%), pneumonia (0.3–1.7%), adult respiratory distress syndrome (0.3–2%), and sepsis (0.7–2%) (388). In the belief that women were being underdosed because of unknown biological factors, two additional studies were done with higher doses of tirilazad in women. Between 1995 and 1997, 832 patients were randomized at 65 North American centers. Patients were randomized to vehicle or 15 mg/kg/day tirilazad mesylate groups. During the course of the study the protocol was amended to select mortality at 91 days postdosing as the primary efficacy end point. The analysis at this point favored the study drug in patients who were initially neurological grades IV or V at admission (24.6 compared to 43.4% mortality in the placebo-treated group). When the entire population was considered, however, there was no significant difference in mortality (13% in the tirilazad group and 15.6% in the placebo-treated group). Favorable outcomes occurred in 71% of the tirilazad group and 74% of the placebo group. Symptomatic VSP was diagnosed in 38% of the placebo-

treated group and 35% of the tirilazad-treated group. Severe VSP occurred in 14% of both groups (389).

In a partially concurrent trial in 56 centers in Europe, Australasia, and South Africa, 819 patients were randomized to receive either 15 mg/kg/day of tirilazad or a placebo containing the citrate vehicle. This study included males and females. The groups were evenly matched for all prognostic factors. Mortality rates and overall outcome were not different 3-months post-SAH. Post hoc subgroup analysis suggested a trend toward a lower mortality rate favoring patients who presented in neurological grades IV and V at admission (32 compared to 37%). Symptomatic VSP occurred in 34% of the placebo-treated patients as opposed to 25% of the tirilazad patients. Clinical cerebral infarction from VSP was reduced from 13% in the vehicle-treated group to 8% in the tirilazad-treated group. The primary end point, mortality rate at 3 months post-SAH, was not affected by the study drug. Considering the drug and vehicle groups in these two studies performed in the late 1990s, the following is evident: Symptomatic VSP still occurs in 25–38% of groups, severe symptomatic VSP occurs in 6–14%, and CT infarction from VSP was diagnosed in 8–13% of patient groups (390). Patients are now admitted much earlier after their SAH, with an average time to admission of 7–9 hr post-SAH. Prexisting hypertension was present in between 27 and 44% of each group. Thick SAH on admission CT was present in 64–71%. Neurological grades III–IV comprised 17–30%. The mean GCS ranged from 11 to 12.4. Prophylactic hyperdynamic therapy was employed in between 59 and 74%. Hypervolemia was employed more commonly than hypertension (48–70 vs 24–29%). Hemodilution occurred in between 34 and 43% of the groups. Surgery was actually performed in 87–94%. The mean time to surgery ranged between 26 and 29 hr. Good results occurred in 53–58% and mortality rate ranged between 13 and 18%. VSP was considered to be the primary or contributing cause of neurological worsening in 15–22% of the vehicle groups. In the vehicle group of the North American female study, direct effects of the initial SAH were considered the primary or contributing factor to death and disability in 27% of cases, and VSP was considered the primary or contributing factor in 18%. Other factors contributing toward ischemia occurred in 3%. The rebleeding rate was down to 6%. Surgical and medical complications were being reported more commonly than in prior studies, perhaps due to better data collection, with surgical complications occurring in 8% of cases and medical complications in 12%. Angioplasty was reported in 0.7% of the vehicle group. In this group symptomatic VSP occurred in 34%, severe symptomatic VSP in 11%, and infarction from VSP in 13%. Hyperdynamic therapy was utilized in 24% of cases consisting of deliberate hypervolemia in 21%, hypertension in 13%, and Hct reduction in 16%. In the North American high-dose tirilazad study in women, angioplasty was performed in 5.8% of the vehicle group and 7.1% of the tirilazad group. Various meta-analyses of this huge databank have already been performed that suggest that there was a reduction in the incidence of clinical VSP in poor-grade patients, particularly in males. The Food and Drug Administration has not approved tirilazad mesylate.

In retrospect, one might observe that the preclinical animal studies suggested only a moderate effect on angiographic VSP. In addition, it was never demonstrated that significant quantities of tirilazad passed into the CSF. Although a beneficial effect might have been obtained purely by a salutary action on the endothelium, a drug that passed through the blood–CSF barrier would have greater intrinsic appeal as a antagonist of VSP. The early selection of clinical and angiographic VSP as therapeutic end points failed to recognize that although clinical VSP was probably not decreasing in incidence, its clinical sequelae were decreasing. The mortality rate from VSP had fallen considerable in the decade or so prior to the initiation to these studies. It is sobering to reflect on the fact that two extremely large prospective, randomized clinical trials carried out with impeccable data collection and expert statistical input produced opposite results. The failure of recent drug trials searching for efficacy against VSP may have something in common with the failure of the trials of neuroprotective agents to show convincing efficacy in head injury. Nimodipine is an exception, but it had to cross a much lower hurdle than is currently placed by regulatory agencies. In a thoughtful recent analysis of the trials of neuroprotective agents in head injury (391), it was suggested that the failures to demonstrate efficacy could be attributed to the heterogeneity of the patient population, the importance of baseline diagnostic indicators, and the problems caused by the distribution of outcome and dichotomization of these outcomes in the GOS. In the tirilazad head injury and PEGSOD trials, it was also not recognized initially that these agents penetrated only slightly into the injured brain, that plasma concentrations of tirilazad are lower in women (sometimes below the therapeutic range), and that the metabolism of tirilazad might be increased by concurrent use of phenytoin. In SAH, as in head injuries, the absolute clinical benefit of a treatment can be regarded as a function of the baseline risk. The outcome is determined by prognostic factors, therapeutic effect, and random effect. Populations of patients studied so far frequently include patients with a very poor chance of survival as well as those with a very high chance of favorable outcome. New protective therapies are unlikely to show a benefit in such patients. Of the head injuries studies so far, none has reported cross-

validation of the prognostic equations between different databases and none has taken into consideration that death after head injury usually occurs within the first few days. Prognostic indices may correlate more strongly with early mortality because other factors such as medical complications subsequently become more important. When patients are graded by a 5-point outcome scale, such as the GOS, but efficacy is based on a favorable versus unfavorable categorization, it is possible to miss an important biological effect because of lack of statistically significant difference between the favorable and unfavorable groups. For instance, if 50% of patients in the severe disability category (on the 5-point scale) were improved to the mild disability category, the dichotomized GOS (favorable 1 and 2 vs unfavorable 3–5) would only be favorably increased by 5%. Upgrading 25% of cases in the five categories by three categories increases favorable outcome by only 9%. It is also important in clinical trials that participating medical centers adopt a relatively uniform therapeutic approach. In the SAH trials, for instance, there were radical differences in the use of various medications between the North American centers and those throughout the rest of the world.

It seems unrealistic to expect drug companies to undertake the same massive prospective trials that were done with tirilazad. Intensive pharmacological and physiological investigations should be carried out in preclinical studies. Patients at high risk should be studied using the best contemporary techniques, multiple end points should be selected, and post hoc analysis should not be treated with derision.

Nearly 3500 patients with SAH from various centers have been the subject of various prospective clinical trials. The rate of smoking in the patients enrolled was compared to smoking rates for the general population. In virtually all age and gender subgroups, patients with SAH reported current smoking rates 2.5 times higher than expected based on U.S. and European surveys. Cigarette smoking was also associated with a younger age at onset of SAH and an increased incidence of clinically confirmed VSP. Of 2090 current smokers, 54% developed clinical VSP compared to a rate of 49% in the 1349 nonsmokers ($p = 0.005$) (392).

## VIII. The Art of Treatment

For salvageable patients, the optimal outcome is fixed by biological factors surrounding the aneurysmal rupture. It is very easy to impair that potential level of recovery and virtually impossible to improve upon it. The moments of clipping or coiling, although of simultaneous high drama and risk, are no more vital than the posttreatment care provided by a well-informed and coordinated team of medical, nursing, and ancillary personnel.

We consciously attempt to simplify the care of the patient by reducing intubation, catheterization, use of drains, invasive monitoring, laboratory testing, vital sign testing, and concurrent use of many potent medications with unknown interactions to the minimum levels consistent with safety. There is no substitute for ongoing critical patient evaluation. However, frequent painful stimulation of an exhausted or already uncomfortable patient when no therapeutic decision is likely to flow from the response should be forbidden.

An optimistic approach on the part of the hospital staff helps the patient. A realistic attitude is best for the family; relief of their anxiety should also be a deliberate objective of therapy.

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CHAPTER 9

# NONRUPTURED ANEURYSM VASOSPASM



## I. Postoperative Cases

### A. Pituitary Tumors

Krayenbühl in 1960 recorded the case of a patient with an intrasellar chromophobe tumor who did well for 4 postoperative days following craniotomy and then had the sudden onset of hemiparesis. Angiography demonstrated marked VSP, which interestingly was treated with good effect using intravenous papaverine (1). VSP followed transfrontal craniotomy for three large pituitary tumors. One patient was hemiparetic postoperatively and recovered, a second patient developed confusion and hemiparesis on the fifth postoperative day and eventually died, and the third patient developed hemiplegia on the 10th postoperative day with complete recovery. Angiographic VSP was documented in all 3 cases but immediate postoperative CT scans were not available to judge the presence or absence of SAH from the operations (2). Twenty-six cases of VSP complicating tumor resections have been documented. The majority occurred after removal of pituitary adenomas (2–5). A transsphenoidal removal of a pituitary tumor was associated with significant bleeding and complicated by cerebrospinal fluid (CSF) rhinorrhea necessitating a second transsphenoidal procedure. About 2 weeks after the patient's original operation, angiogram demonstrated marked VSP of the distal internal carotid, proximal anterior cerebral, posterior communicating, and basilar arteries. The patient died a month after a third operation with bilateral cerebral infarction including neuronal loss in the hypothalamus. A yellow, thickened membrane in the region of the pituitary stalk was noted. There was no documentation of SAH by CT scanning (6). Cases of pituitary apoplexy have been associated with angiographically demonstrated VSP and thromboembolic phenomenon (7).

VSP was reported following a transsphenoidal tumor removal and was associated with the use of oral contraception (8). An additional case of VSP following removal of a large pituitary adenoma by craniotomy was reported by Ono *et al.* (9). Some necrotic pituitary tumor material may be extruded into the subarachnoid space and caused VSP. Another possibility is that the tumor mass within the sella compresses the carotid artery significantly within the cavernous sinus causing either occlusion or thrombosis with subsequent embolization (10). Itoyama and colleagues described an acromegalic who had a fall and subsequent pituitary apoplexy. Angiography demonstrated diffuse VSP on the 16th day following the ictus. His onset of hemiparesis occurred on the 14th day. Significant SAH had been demonstrated by CT scan on the day of onset of the weakness (11). There have been other reports of pituitary apoplexy and VSP (12–17).

### B. Other Tumors

Other tumor types associated with VSP include acoustic neuroma (18–19), meningioma (19), craniopharyngioma

(3), and adenocarcinoma (3). The amount of postoperative blood clot was not documented. It is also possible in some cases that there is arterial wall damage from the surgical manipulation or electrocoagulation. In a review of 20 cases of postoperative VSP, blood was documented in the CSF in only 8 of the cases. In 11, lumbar puncture or CT scan were not obtained, and in 1 case the CSF was clear, although there may have been blood in the CSF or evidence of infection at another point in time (20). LeRoux *et al.* reported 2 cases of symptomatic VSP following removal of an acoustic neuroma and a sphenoid wing meningioma. There was significant bleeding into the subarachnoid space during surgery and CSF diversion had been used, perhaps aggravating the effect of the subarachnoid clot (19).

Cervoni *et al* had five cases of postoperative VSP after cerebral tumors had been removed. The time of onset of symptomatic VSP was 3–7 days after surgery. Three of their patients died from VSP. The diagnosis was assumed on the basis of high transcranial Doppler (TCD) flow velocities (21). A 6-year-old girl presenting with diplopia had a third nerve schwannoma. She had a deterioration in mental status a week postoperatively and demonstration of a low-density area in her frontal and temporal lobes. Angiography revealed VSP of the ipsilateral internal carotid artery as well as the middle and posterior cerebral arteries (22). A 45-year-old male had excision of a large supersellar pilocytic astrocytoma. On the fifth postoperative day the patient developed hemiparesis and aphasia, and VSP was documented by angiography (23).

From a series of 470 consecutive cervical base tumors, 8 patients developed clinical VSP 1–30 days postoperatively. Half the patients showed altered mental status and about two-thirds had weakness. The patients who developed VSP, compared to those that did not, had longer operations (12.4 vs 10.1 hr) more vessel encasement by tumor (88 vs 37 %), more preoperative vessel narrowing (66 vs 19%), and more preoperative embolization (66 vs 35%) (3) Symptomatic VSP has also been reported complicating pheochromocytoma (24), electroconvulsive therapy (25), and myelography (26).

## II. Vascular Lesions

### A. Arteriovenous Malformations

VSP from arteriovenous malformations (AVMs) has been reported by several authors (27–29). A case of symptomatic VSP after intraventricular hemorrhage (IVH) from a ruptured AVM was presented. No significant SAH was observed in the basal cisterns at any point in the illness. Presumably, sufficient volume of vasoconstrictor agonist was released into the CSF that significant constriction occurred. The patient ultimately showed not only angiographic VSP but also low-density areas by CT scan indicating infarction, The 13 patients from the literature were reviewed in addition to an additional patient, and it was found that 30% had associated intracranial hemorrhage (ICH), 50% had IVH, the mean day of onset was 10 days post-SAH, and the incidence of VSP in this AVM series was 14% (27, 29, 30–32). A subsequent patient had ICH and IVH from an AVM. Internal carotid artery VSP developed and was treated by angioplasty. On the initial CT scan only traces of blood had been seen in the basal cisterns (33). Two cases of angiographically documented VSP complicating resection of AVM were treated by intraarterial papaverine injection. In one case there was sustained neurological improvement with a normal outcome. In the second there was neurological deterioration and associated cerebral edema (34). Two different cases of severe angiographic VSP were reported following rupture of an AVM. CT scans showed ICH in the thalamus in 1 and in the putamen in another, both were accompanied by IVH distending the ventricles. There was no radiological evidence of SAH. However, the initial angiograms showed severe arterial narrowing of both internal carotid arteries in the supraclinoid portion and failed to initially demonstrate the AVMs and these were subsequently demonstrated on repeat angiograms. The failure to demonstrate the AVMs was presumably due to reduced flow associated with the severe bilateral VSP. The latter may have been due to external vascular compression from raised ICP (35). Zubkov and colleagues performed transluminal angioplasty and intraarterial papaverine injection for the treatment of VSP after AVM rupture (36).

### B. Unruptured Aneurysms

Isolated reports that VSP could complicate surgery of unruptured aneurysms appeared in the 1980s (37,38). Friedman and colleagues documented a patient who presented with a 5-day history of cranial nerve III palsy in whom CSF was clear and CT demonstrated no evidence of blood. By angiography, however, the patient had intense VSP. Unless there was some unique neurogenic reflex at work, it seems likely that there could have been an earlier unrecognized SAH with rapid clearing of blood from the CSF and/or failure to demonstrate it for technical reasons (39). The incidence of symptomatic VSP following microsurgical treatment of unruptured aneurysms was 2 patients in a series of 104. The authors found 14 other such reports in the literature. Their patients experienced this complication, which was life threatening and medically intractable. Treatment with balloon angioplasty was performed (40).

### C. Aneurysms with Very Delayed Onset of Vasospasm

In an analysis of 605 patients with aneurysmal SAH, 33% developed delayed ischemic deficit (DID). Of these patients, 137 had undergone early aneurysm surgery. The date of onset of DID was definitely established in 131 patients. Five percent of these developed DID over 15 days after SAH. In all 6 of these patients asymptomatic angiographic VSP and infections, usually meningitis, preceded the onset of DID. It was suggested that the very late onset of DID was stimulated by the associated hyperdynamic hemodynamic changes due to sepsis superimposed on an underlying angiographic VSP (41). It also seems possible that the meningitis independently worsened the degree of vessel constriction.

### D. Benign Perimesencephalic Hemorrhage

Fourteen patients had benign perimesencephalic hemorrhage and constituted 5% of all SAH patients in one series. These patients were all awake on admission without significant neurological deficit. Patients had initial and repeat angiography. VSP was not or only slightly present, and none of the patients developed DID (42). However, 2 patients have been reported in whom severe angiographic VSP was demonstrated on days 7 and 9 post-SAH. One patient had asymptomatic VSP and the other was dysphasic but recovered with hypervolemia (43).

## III. Head Injury

### A. Clinical Series

Wilkins and Odom assessed the presence or absence of VSP in 4 patients with acute head injury. VSP appeared identical to that which occurs in relation to ruptured aneurysms (44). Wilkins also suggested that hypothalamic dysfunction might be important in the development of VSP since VSP seemed to occur preferentially in the region of the hypothalamus after the rupture of aneurysms. In patients dying of VSP there are sometimes destructive lesions in the anterior hypothalamus. He considered that certain cases of postoperative VSP lent support to this concept (6). Arseni *et al.* demonstrated 3 cases of posttraumatic VSP (45). Three hundred and fifty patients suffering a moderate or severe head injury were studied with cerebral angiography which was repeated in 40 patients. Angiography revealed narrowing of one or more of the intracranial arteries in 19% of cases and narrowing of proximal segments of the intracranial circulation in 5%. In 1 patient delayed hemiparesis developed after head injury. Diffuse VSP occurred in 3% of the patients. In some cases VSP lasted at least 7 days. The supracavernous portion of the internal carotid artery was the most affected segment of the intracranial circulation. Only 40 of the 350 patients studied by Suwanwela and Suwanwela had repeated angiography so that the incidence of VSP they encountered may have been an underestimate because posttraumatic VSP is a delayed phenomenon and a single, early angiogram could miss the VSP which developed later (46).

In another series of 44 head injury patients who developed VSP (34% of the total patient population of head injuries), the VSP was either diffuse or localized. There was no correlation between the type of VSP and prognosis. Localized spasm developed in 20% of the patients and was restricted to the intradural segment of the internal carotid. The onset of VSP was from 1 hr to 13 days after the injury. Follow-up with cerebral angiography did not reveal any continuing VSP or progressive arterial obstruction due to thrombosis after VSP developed (47). Only rarely are basal subarachnoid hematomas the sole evidence of head injury (48). Posttraumatic VSP causing fatal ischemic brain damage usually occurs after severe head injuries in which SAH is associated with obvious cerebral contusions and hematomas (47,49).

Marshall and associates treated six patients who did poorly following head injury because of vertebral–basilar artery VSP. None of the patients had a supratentorial mass or intracranial hypertension (50). A patient with a documented head injury was well for 1 week following treatment but then deteriorated sharply. Carotid angiography demonstrated severe VSP. The patient died on the 15th day after injury and postmortem examination established that there was no vascular abnormality or arterial disruption. The initial CT scan demonstrated a dense SAH in the basal cisterns and no parenchymal lesion (51). The CT scan shows areas of focal ischemia in approximately 10–15% of patients with head injuries (52). Two cases of posttraumatic VSP were documented angiographically on days 12 and 14 after injury. Follow-up angiography at 2 and 4 weeks showed resolution of the VSP (53).

Increased TCD velocities can result from increased CBF and increased perfusion of distal territory as well as VSP. Martin and coworkers estimate that one-fifth of observed elevated flow velocities are associated with absolute hyperemia (54). Previous reports have also shown that decreased TCD velocities may be associated with elevated intracranial pressure (55), hypotension (56), and advanced age (57). A shortcoming in the so called Lindegaard ratio is that it is not a dependable substitute for CBF measurement. Cervical internal carotid artery velocities vary depending on gender and patient size, and

they can be influenced by the TCD probe positioning and angulation (58).

Twenty head injured patients showed SAH. Of the 9 patients with only mild SAH, the blood on CT scan was localized in the Sylvian fissures in 4 patients and was found in the basal subarachnoid cisterns in the other 5. Only 1 of these patients developed delayed VSP. In 11 patients with massive basal SAH, only 1 patient had a good outcome (59). In the European head injury study, 33% of patients with moderate to severe head injuries showed traumatic SAH on an early CT scan and this was associated with a significantly worse outcome (60). Traumatic SAH is an important and independent predictor of unfavorable outcome in head injury. Between 1994 and 1995, 123 patients with traumatic SAH on initial CT scans were entered into a study using nimodipine. Admission into the study was within 12 hr of head injury and treatment with nimodipine or placebo was continued for 3 weeks. TCD velocities were recorded. Patients treated with nimodipine had significantly less death, vegetative survival, or severe disability at 6 months postinjury than placebo-treated patients (25 vs 46%). Extensive SAH was present in 33% of 63 placebo patients and 42% of nimodipine patients (Fisher grade III). On follow-up CT scans 22% of the placebo group showed hypodensities as opposed to 7% of the nimodipine-treated patients. Maximum TCD velocities occurred on day 14 post-head injury in both groups. Twelve percent of the placebo group and 3% of the nimodipine group showed velocities $\geq$160 cm/sec. In the placebo group 71% of the 21 Fisher grade III patients had an unfavorable outcome, whereas only 20% of the 25 Fisher grade III patients receiving nimodipine had an unfavorable outcome (61). Blood was most frequently observed in the first CT scan after injury over the convexity of the hemispheres, and this related to the presence of contusions and subdural hematomas. The basal cisterns were less frequently involved. Similarities between traumatic and aneurysmal SAH have been suggested (62,63).

A study was conducted using 130 patients with head injury who showed SAH on admission CT scans. Eight percent developed DID between days 4 and 16 postinjury. DID developed in 3% of 110 patients with small amounts of SAH and 24% of 29 patients with massive SAH on admission CT scans. In each of the 10 patients with DID, severe VSP was demonstrated by angiography performed soon after development of DID. The main site of SAH correlated with the location of the most severe VSP. Seven of 10 patients with DID had evidence of focal ischemia on follow-up CT scans. Of the 10 patients with DID, 3 died and 2 had a persistent vegetative state. There were only 3 good recoveries. Of 10 patients with thin SAH on admission CT, the first angiograms showed severe VSP in 1 and the second angiogram showed severe VSP in 6. In the 10 thick clot patients, 2 had severe VSP on the initial angiogram done on days 7 and 14 and 7 showed severe VSP on the second angiogram done between days 9 and 18. Patients with DID were treated by means of hypervolemic therapy and dopamine-induced hypertension (64). The investigators were not impressed with the efficacy of this therapy. They considered that hypervolemic therapy could have unwanted side effects considering the coexistence of contusions, diffuse edema, or mass lesions. Traumatic VSP had been treated with papaverine infusion (65), calcium antagonist (66), or balloon angioplasty (67).

Forty-six severely head injured patients (GCS $\leq$ 8) were assessed by daily TCD studies. In those cases with presumed posttraumatic VSP diagnosed by TCD, 22% died. This compared to only 4% of the patients without elevated TCD velocities. In addition, posttraumatic amnesia lasted on average 83 days in the patients with elevated TCD velocities compared to only 51 days for those without presumed VSP (68).

CT scans were obtained from 252 patients with traumatic SAH. A stepwise regression analysis of CT features ranked in descending order of contribution to GOS at time of acute hospital discharge were basal cistern effacement, thickness of traumatic SAH, cortical sulcal effacement, the presence of mass lesion(s), and location of traumatic SAH. The basal cistern effacement was the most significant variable in terms of contribution to GOS (69).

Martin and colleagues found that delayed post traumatic VSP affects the larger basal intracranial arteries in 25–40% of head trauma patients. They suggested that the onset occurred 2 or more days after injury (54). Traumatic arterial VSP has been treated with intraarterial papaverine infusion (70).

Thirty-two GCS 4–8 patients and 11 GCS 9–14 were studied using TCD for 10 days post-head injury. TCDs gradually increased beginning on the second postinjury day and peaked on the fourth or fifth day after injury. The changes were most marked and appeared first in the basilar artery. Velocities > 90 cm/sec in the basilar artery occurred in approximately one-third of all patients. In the patients with higher GCS, only 2 showed the high velocities in the basilar artery (71). VSP post-gunshot wounds was defined as middle cerebral artery (MCA) velocities > 120 cm/sec. On average, TCD studies were begun the day after admission and an average of six studies per patient were done between the first and 33rd day after injury. TCD showed VSP defined in this manner in 42% of patients. VSP was most prominent between days 5 and 11 and occurred in all levels of injury severity. Initial CT scans showed SAH in all of the patients with VSP defined by TCD but only 47% of patients who did not show TCD

VSP. Good outcomes occurred in 36% of those with VSP versus 47% of those without VSP (72).

Martin and colleagues characterized three phases following head injuries based on an analysis of 125 patients who had severe head trauma and measurements of arteriovenous $O_2$ difference and cerebral metabolic rate of oxygen ($CMRO_2$). Phase I was termed the hypoperfusion phase and was characterized by a low CBF, normal TCD velocity, and normal cerebral arterial venous $O_2$ difference. $CMRO_2$ is approximately half normal in the second or hyperemic phase occurring between days 1 and 3, CBF increases, $AVDO_2$ decreases, and TCD velocity increases. In the third or vasospastic phase occurring on days 4–15, CBF decreases, and TCD velocities increase further (73). The same group studied 152 patients and correlated blood flow parameters with 6-month outcome. Stepwise logistic regression analysis showed that hemodynamically significant VSP was a significant predictor of poor outcome, independent of the effects of admission GCS and age. Outcome was significantly impacted by hemodynamically significant VSP producing low CBF. In 64 patients whose MCA velocities exceeded 120 cm/sec, 41% had a poor outcome compared to only 27% poor outcomes in patients whose velocities remained lower (74).

In 17 patients with severe head injury, Doppler imaging was performed. Decreased velocities, increased pulsatility indices, and vessel diameter increases were observed. Increased velocities without vessel area decrease was interpreted to indicate hyperemia rather than VSP (75).

Fukuda *et al.* compared 99 patients with diffuse brain injury and traumatic SAH with 114 patients who had aneurysmal SAH. Traumatic SAH was more likely to extend to supratentorial regions and interhemispheric fissures. In the traumatic SAH group, mean CBF decreased to 85% of normal during the acute phase and increased slightly during the subacute phase. Neurological deterioration and hospital deaths peaked on day 0 in association with traumatic SAH. Traumatic SAH had a significantly lower incidence of low-density areas on the CT scan compared to aneurysmal SAH. All low-density areas on the CT scan of patients with aneurysmal SAH were appropriate to spastic vascular territories. The low-density areas on the CT scan of patients who had traumatic SAH usually corresponded to contusions. Outcomes deteriorated with increases in SAH severity in both traumatic and aneurysmal SAH groups. The death rate in the traumatic SAH group was 77% compared to 35% in the aneurysmal SAH group. In the traumatic group 7 patients deteriorated neurologically on day 0 and almost all deteriorations occurred in the first 2 days. The aneurysmal SAH group showed a twin-peak distribution of day of deterioration with the first peak on day 0 and the second on days 8. The SAH disappeared much more rapidly from the traumatic SAH patients than the aneurysmal ones. By day 4 post-SAH the aneurysmal SAH CT scan still showed blood in 76%. In the traumatic SAH cases 100% showed blood on day 0, and this decreases to 69% on day 1, 9% on day 2, and 2% by day 3. CBF studies showed that in aneurysmal SAH patients there was a tendency for CBF to decrease below normal in the first 4 days, and it then decreased even more between days 5 and 20. On the other hand, in the traumatic SAH patients there tended to be a slight increase in CBF in the subacute phase (days 7–21). Since in the traumatic SAH patients no significant number of DID developed by approximately day 7, Futiuda *et al.* suggested that neurologic deterioration in such patients was seldom due to VSP but more likely caused by cerebral edema or intracranial hematomas. The incidence and spatial distribution of the low density areas seen on late CT scans were believed not to support the occurrence of VSP-induced ischemic brain damage in association with traumatic SAH. It was suggested that VSP occurring after traumatic SAH was unlikely to cause DID or secondary deterioration of outcome (76).

### B. Experimental

Symon used mechanical force from forceps to induce spasm in primate MCAs which he observed to last 20 min from a single application. Spasm reached its maximum in 3.8 min and the average reduction of pulse pressure was 42%. The spasm could not be duplicated by application of fresh blood (77).

## IV. Infections

VSP can follow central nervous system infections (78). A 34-year-old female developed fever, headache, and vomiting associated with neck stiffness and hemiparesis. She became comatose 10 days after onset of illness. CSF showed purulent meningitis. Angiography showed significant VSP in the major arteries with vasodilatation observed in some regions. Angiography was repeated 2 months after the onset of illness and showed diffuse narrowing of the previously dilated arteries. CT scans showed multiple low-density areas bilaterally and in the cerebellar hemispheres. She died of uncal herniation 67 days after the onset of the illness. Autopsy demonstrated infarction in the territory of the spastic arteries. Involved arteries showed atrophy and fibrosis of the media with infiltration of polymorphonuclear leukocytes. Electron microscopy demonstrated degeneration of vascular smooth muscle cells with loss of myofilaments, fine granular

material in cytoplasm, and increases in nuclear inclusion bodies (79).

Twenty patients in septic shock showed increased TCD velocities which were attributed to mild VSP of basal cerebral arteries (80). Eighteen of 22 patients with meningitis showed elevated flow velocities, and in about one-third flow velocities were > 210 cm/sec. Patients with the highest TCD velocities showed poorer GCS scores on admission, more focal ischemic deficits, more seizures, and poorer clinical outcome (81).

## V. Eclampsia

### A. Magnesium Sulfate

Magnesium sulfate ($MgSO_4$) has been used in the treatment of toxemia of pregnancy since 1925 (82). Early studies showed that hypermagnesemia caused skeletal muscle paralysis without causing anesthesia. The antieclamptic effects of $Mg^{2+}$ are attained at serum levels below those that depress neuromuscular transmission. $MgSO_4$ may act in the preeclampsia – eclampsia syndrome by preventing VSP through its action as a calcium antagonist. The transient minor effect of $MgSO_4$ in reducing blood pressure is the opposite from its persistent cerebral antiepileptic effect. There may be a differential sensitivity of cerebral and systemic arteries to calcium blocking. Altura and Altura concluded that this was consistent with the levels of free $Mg^{2+}$ in CSF which were three times higher than those in plasma and with the higher content of $Mg^{2+}$ in the walls of cerebral arteries compared to systemic arteries (83). Sadeh suggested that cerebral VSP is involved in the pathogenesis of eclampsia. $Mg^{2+}$ may have a beneficial effect by opposing the calcium-dependent arterial constriction, thereby relieving VSP. $Mg^{2+}$ may also antagonize the increase in intracellular $Ca^{2+}$ caused by ischemia and prevent cell damage and death (82).

In a rat model of VSP, 3 days following induction the basilar artery was exposed by a transclival approach. A >50% reduction in diameter was observed. Intravenous $MgSO_4$ dilated the spastic artery to approximately 75% of the baseline diameter in control rats. Topical $MgSO_4$ produced dilation of the basilar artery to 150% of the baseline diameter of controls. Hemodynamic effects were mild and immediately reversible upon cessation of $MgSO_4$ administration (84). Isometric tension experiments on human MCA segments were performed and concentration–response curves to 5-HT and $PGF_{2\alpha}$ were constructed. $Mg^{2+}$ inhibited the 5-HT-elicited contractions but was less effective in inhibiting $PGF_{2\alpha}$ contractions (85).

Ninety-eight patients with both ischemic and hemorrhagic stroke showed early and significant deficits in serum-ionized $Mg^{2+}$ but not total Mg as measured by a unique $Mg^{2+}$ sensitive, ion-selective electrode. One-fourth of stroke patients exhibited >65% reductions in mean serum ionized $Mg^{2+}$. The stroke patients also demonstrated a significant elevation in serum-ionized $Ca^{2+}$. Exposure of cultured canine cerebral vascular smooth muscle cells to the same low concentration of ionized $Mg^{2+}$ as found in stroke patients (0.30–0.48 m*M*) resulted in rapid and marked elevations in cytosolic $Ca^{2+}$ and spasm of the cells (86).

### B. Pathology

Sheehan and Lynch described the cerebral pathologic findings in eclampsia, which included hemorrhages in cortex and periventricular areas and hematomas in the white matter and basal ganglia, and suggested that such changes could result from VSP. Cerebral angiography performed in toxemia demonstrated severe arterial VSP (87–90).

### C. Angiography

Trommer *et al.* demonstrated VSP of large- and medium-caliber arteries by angiography associated with focal cerebral and brain stem ischemia in the setting of postpartum eclampsia (90). VSP was demonstrated by magnetic resonance angiography (MRA) in a patient with eclampsia who did not show evidence of cerebral ischemia by CT or magnetic resonance imaginy (MRI) (91).

Of 4 patients with eclampsia and low-density CT areas and high $T_2$ intensity area on MRI, there was normalization of the studies within a month in 3 of the patients but the other demonstrated definite cerebral infarction and fixed hemiparesis and aphasia. All cases had shown VSP by cerebral angiography in the acute phase of their illness. In the patient who developed the permanent deficit there was arterial occlusion of the MCA attributed to VSP. Angiography showed the spasm to be diffuse or multifocal and to be peripheral as well as central. Angiographic VSP had partially or completely recovered when angiography was repeated 3 or 4 weeks following the acute phase (92). A 23-year-old woman, gravida 1, para 1, had headaches and seizures 1 week after an uncomplicated delivery. Cerebral angiography showed severe diffuse VSP. She was treated with hyperosmolar hypervolemic therapy and nimodipine. MRA on day 23 showed persistent severe VSP but it had abated by day 33 (93). Sixty-five women with eclampsia were studied within 48 hr postpartum by single photon emission computed tomography (SPECT) and TCD studies. SPECT

revealed perfusion deficits in watershed areas in all women, and 75% showed regional deficits in the parietal occipital areas. Low densities were seen by CT scans in 59%. Eighty-six percent showed increased TCD velocities (94).

### D. Transcranial Doppler Sonography

MCA TCD velocities were significantly higher in eclamptic patients than in preeclamptic or normotensive pregnant patients. Four eclamptic patients had mean MCA velocities of 165 cm/sec (95). In 13 eclamptic patients, TCD velocities were obtained before and after $MgSO_4$ treatment. The treatment apparently significantly reduced the pulsatility index and mean flow velocities in the MCA. Eleven patients treated with dilantin did not show any statistically significant differences in these parameters. Systolic and diastolic blood pressures were reduced by both the $MgSO_4$ and dilantin treatment (96). Keunen *et al.* suggested that TCD velocities could be used to classify eclamptic patients into those with vasodilatory hyperperfusion and vasospastic hypoperfusion. We doubt this. Their patients had blood pressures >200 systolic, Hb below 8.5 mmol/liter and 3+ proteinuria. The TCD MCA mean velocities were below 112 cm/sec, and angiography was not performed (97). There was one report that as the symptoms of eclampsia abated, TCD velocities increased. On the basis of MRA, this was postulated to be due to delayed VSP. MRA as a technique of evaluating VSP has severe limitations (98), as does TCD. A case of severe preeclampsia with reversible cortical blindness was associated with TCD velocities in the MCA and posterior cerebral artery of up to 380 cm/sec which returned to normal within 2 weeks (99).

### E. Magnetic Resonance Studies

VSP was demonstrated by MRA in a patient with eclampsia who did not show evidence of cerebral ischemia by CT or MRI (91). Cerebral blood-flow (CBF) was measured in patients with eclampsia and severe preeclampsia using phase-contrast MRI. These studies were performed after patients had received seizure treatment or prophylaxis. Patients served as their own controls and were studied 4 to 5 weeks postpartum. All 28 women enrolled were studied initially within 24 hr of delivery or of their most recent seizure. CBF estimated in this fashion did not show differences between patients with eclampsia or preeclampsia, and there were no differences between the initial studies and those done more than 1 month later. There were $T_2$-weighted abnormalities in all 8 women with eclampsia and mild abnormalities in 20% of those with severe eclampsia. It is possible that pretreatment with magnesium could have treated VSP and normalized CBF prior to the study being initiated (100).

## VI. Migraine Headaches as a Vasoconstrictor Phenomenon

It is not yet established whether migraine is a vascular or neurogenic disease and whether sumatriptan acts by constriction of dilated arteries or other mechanisms. Significant increases in TCD velocities were observed in MCA and basilar artery after administration of sumatriptan. The majority of patients showed no change in TCD following sumatriptan. There were no velocity differences between headache and nonheadaches side or between migraine and headache-free periods (101). Infarction occurring during migraine crisis was shown in four young patients. Angiography ruled out dissection, fibromuscular dysplasia, and other causes of ischemic lesions. Segmental changes of vasculitis were demonstrated (102).

## VII. Coronary Artery Vasospasm

Recent evidence suggests that the basic abnormality of coronary VSP may be hypercontractility of the arterial wall associated with the atherosclerotic process. Endothelial injury may be associated with mitogenic factors and generation of leukotrienes by platelets and macrophages.There may also be changes in histamine and 5-HT receptor density of vascular smooth muscle and neovascularization of the plaque (103).The endothelium of coronary arteries normally inhibits platelet aggregation by prostacyclin formation and release. Any thrombin formed causes endothelium-mediated relaxation. On the contrary, when endothelial damage occurs these protective mechanisms disappear. Patients with coronary artery spasm usually have morphologic changes at the site of the spasm (104). In only a minority of patients with Prinzmetal's angina is there decisive evidence that coronary vasodilation induced by calcium channel antagonist plays a specific therapeutic role. There appears to be a minor role for VSP in the overall spectrum of angina. Long term prognosis may be different among different categories of calcium channel antagonists (105). A patient was reported who had coronary artery spasm during migraine attacks. These two conditions have been associated in prior reports (106). Coronary spasm is an abnormal contraction of epicardial coronary arteries resulting in myocardial ischemia. There is a relatively high incidence in Japanese people. It occurs mostly from midnight to early morning when patients are at rest. It may be induced by acetylcholine and endothelium – dependent vasodilators which

cause vasodilation of normal coronary artery. The spastic artery is hyperresponsive to the vasodilator effects of nitroglycerin, a NO donor, and is deficient in NO activity. Cigarette smoking is a major risk factor for coronary spasm (107).

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CHAPTER 10

# SURGICAL ASPECTS OF VASOSPASM



## I. Surgical Trauma Mimicking or Aggravating Vasospasm

There is no doubt that operative intervention may result in damage to the brain from retractor pressure or brain laceration; sacrifice of veins; injury to arteries; major vessel or perforator occlusions; intraoperative aneurysmal rerupture with additional SAH, intracranial hemorrhage (ICH), or intraventricular hemorrhage (IVH); trauma to the pial banks during dissection or clot removal; bleeding and tissue damage from ventricular catheter placement; and postoperative hematomas and deliberate brain resection. With surgery now being performed early in the majority of cases, it is evident that postoperative swelling, bleeding, and ischemia may induce neurological deficits occurring in a "delayed" fashion in the time period of VSP (4–14 days). Such problems may aggravate delayed ischemic deficit (DID) from VSP and complicate differential diagnosis and treatment.

Somewhat of a sensation was created by the neurologist Millikan in 1975 when he attacked the concept of DID from VSP and adverse influence of VSP on mortality rates. On the basis of a study of 198 cases from the Mayo Clinic, he asserted that there was no relationship between the frequency and severity of the complications from surgical or conservative treatment and the presence of absence of VSP. His views (1) were not espoused by his neurosurgical confreres—then or now. However, intellectual honesty demands caution in ascribing all delayed deficits to VSP, particularly if they follow surgery. Not all deficits due to operation are evident in the immediate postoperative period.

Positron emission tomography studies were performed 1 day before and 6–17 days after operations performed via right frontotemporal craniotomy for ruptured anterior circulation aneurysms. No patient had clinical VSP. Areas subject to surgical retractions were compared to contralateral regions. The retracted areas only showed a 45% reduction in the cerebral metabolic rate for oxygen ($CMRO_2$) (1.87 ml/100 g/min) and a 32% reduction in rOEF (0.41–0.28 ml/100 g/min). There were no changes in regional cerebral blood flow (rCBF). Patients with angiographic and clinical VSP have much more diffuse changes. The retractor injury apparently uncouples flow and metabolism, resulting is relatively excessive flow (luxury perfusion) (2). Twenty patients underwent transsylvian amygdalohippocampectomy for epilepsy. Transcranial Doppler ultrasonography (TCD) was done pre- and post operatively to assess surgically induced TCD changes (velocities >50% of baseline). Twenty percent showed no change, 70% showed increased velocities ipsilaterally or bilaterally, and 10% showed contralateral increased and ipsilateral decreases. There were no associated symptoms or morbidity (3).

## II. Clot Removal at Surgery

### A. Clinical Series

It is highly likely that in the past there were iatrogenic factors contributing to the poor outcome consequent to VSP. These factors included the preferential performance of surgery at approximately 1 week post-SAH when VSP was becoming maximal, the routine use of intraoperative hypotension, deliberate dehydration "therapy", and the employment of antifibrinolytic drugs which increased the temporal and physical exposure of the conducting arteries

to blood clot (4). The first efforts to deliberately remove blood clot during surgery for aneurysmal clipping were made in Japan. In the early 1970s, early surgery with attempted clot removal was systematically applied with good results by Suzuki and colleagues. Surgery within 24 hr was considered to decrease the possibility of postoperative VSP by removal of subarachnoid clot (5).

Saito *et al.* analyzed 96 cases in which VSP followed SAH. Twelve percent of patients with VSP died and half the survivors had neurologic deficits. There were no deaths among 20 patients operated on within the first 3 days post-SAH. VSP was always seen to some degree postoperatively in patients in whom the basal cisterns were observed at surgery to be filled with blood clot. On the contrary, postoperative VSP was not observed in cases in which the cisterns were free of clot. Four to 11 days elapsed between the last bleed and the onset of postoperative VSP, regardless of the time of surgery (6).

In 1978, Takemae and colleagues establish definitively a relationship between VSP and blood clot in the basal cisterns on CT scans. They concluded (7)

> Early operation is recommended for the cases without high density on CT-scan performed within 4 days after the onset. For the cases with high density on CT-scan, it seems to be reasonable to remove subarachnoid clot as much as possible at surgery to prevent or minimize the future development of vasospasm.

Another group found that all the patients in whom CT showed high-density areas in the basal cisterns within 3 days post-SAH developed symptomatic VSP. Morbidity and mortality were less in patients who were operated on within 24 hr following their hemorrhage than in those operated on between days 2 and 7 thereafter (8).

Sixty-four patients operated on within 4 days post-SAH were studied. Two-thirds of patients had high-density subarachnoid blood clot on preoperative CT scans. The postoperative studies showed that if it had been possible to remove the majority of blood clot there was no spasm or only mild spasm at any site. It was more difficult to achieve removal of clot from the frontal interhemispheric fissure, the posterior insular cistern ipsilaterally, and the insular cistern contralaterally. DID occurred only in those cases in which SAH remained in the cisterns (9).

The effect of subarachnoid clot removal in 239 consecutive patients hospitalized within 24 hr of SAH was studied. In more than 100 patients the aneurysm was obliterated and aggressive clot removal was performed within 24 hr of SAH. Symptomatic VSP occurred in only 10% of cases. The rate for patients operated on after 10 days was about 25%. In almost half the patients operated within the first 2 days who did not have aggressive clot removal, the infarct was on the side contralateral to the operative approach suggesting that there was more residual clot left there. More than half the patients operated on early with aggressive clot removal who still had infarction experienced it in the hemisphere contralateral to the surgery. This study was retrospective and unrandomized (10).

A patient was illustrated in whom delayed infarction and VSP occurred on the side contralateral to aneurysmal clipping. The preoperative CT showed that most of the clot was in the contralateral subarachnoid space. This case unequivocally demonstrated that it was subarachnoid clot and not operative trauma that produced the infarction (Fig. 10.1). (11).

In a series of 177 patients, 10% of the early operated cases and 21% of the delayed group developed DID. The amount of Hb in the cerebrospinal fluid (CSF) obtained by cisternal drainage averaged 6.4 g, corresponding to about 40 ml whole blood, during the 12-day period after SAH in which drainage was performed. The amount of Hb removed was higher in patients with larger clots (12).

Operative misadventure can result in a larger rather than smaller SAH at the end of the case. Notwithstanding, we are impressed at how often mechanical and other measures can covert an angry, red, swollen brain into a much more normal-appearing organ by the end of the craniotomy. The totality of evidence supports the operative aim of clot removal, and we routinely perform intraoperative drainage of CSF using copious irrigation and meticulous clot removal from accessible cisterns. We do not think it is justifiable to coil a ruptured aneurysm during the period of "vasospasm risk" simply to avoid craniotomy.

## B. Models of Clot Removal

### *1. Fibrinolytic Therapy*

Kennady investigated the removal of injected subarachnoid blood from dogs. Red blood cells (RBCs), were tagged with radioactive tracer. Factors involved in the fate of the injected blood included the initial volume of injectate, rate of injection, and the increase in subarachnoid fluid pressure. The addition of "fibrinolysin" to the irrigating fluid apparently increased the efficiency of the removal of injected blood. He concluded that irrigation of the subarachnoid space in dogs was an efficient means of removing whole blood and that the addition of fibrinolytic agents in the irrigating fluid facilitated the blood removal (13). Peterson and coworkers suggested that dissolving the fibrin clot might make possible the modification of the effects of SAH and allow dispersal of foreign blood elements, thereby restoring the space to its normal condition with greater speed and completeness. They used

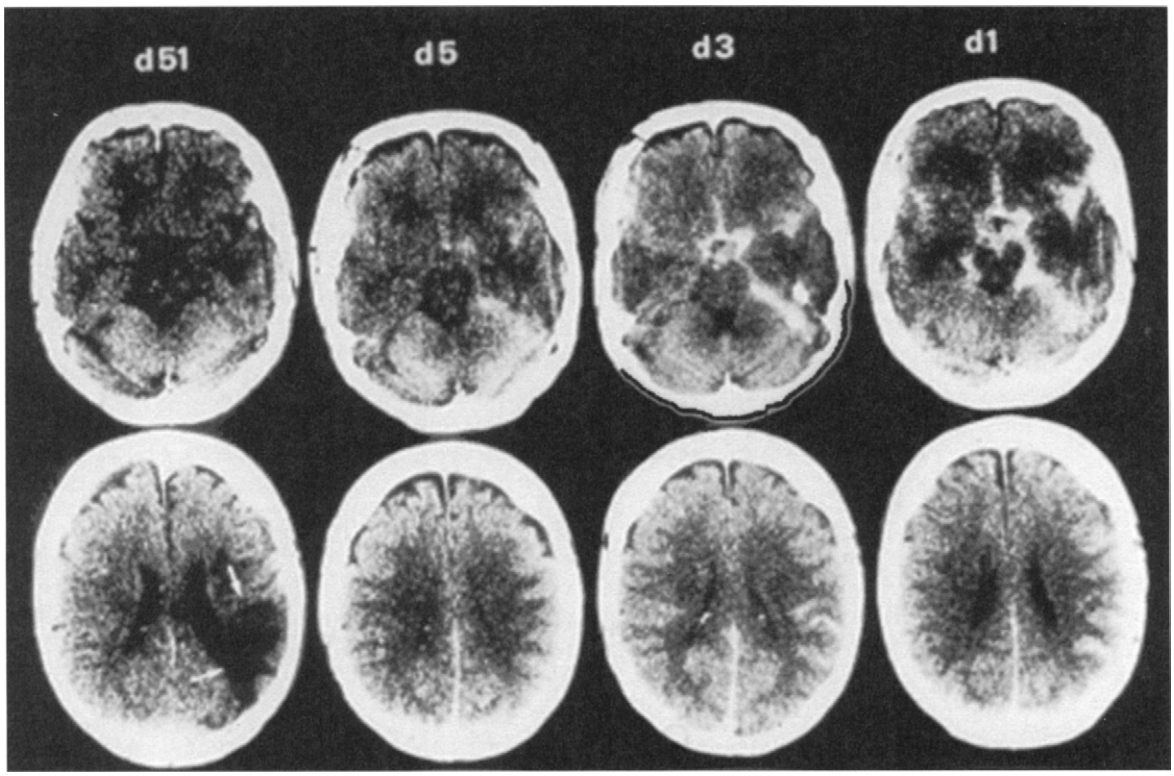


**FIGURE 10.1** On postop day 1 the CT scan showed that the ruptured posterior communicating artery aneurysm on the right side had directed its blood jet mainly into the left subarachnoid space. At surgery on day 3, via a right pterional craniotomy, the ruptured and two other right-sided aneurysms were clipped. The immediate postop CT on day 3 CT scans showed continued significant left-sided clot. When the patient developed aphasia and hemiplegia on day 5 post-SAH, it was refractory to treatment. Only minimal SAH was still evident. Late CT on day 51 showed a left parietal infarct. The focal neurological deficits were due to the clot and VSP developing on the contralateral side to the craniotomy.

a cat model and for the fibrinolytic enzymes selected a mixture of streptokinase and streptodornase. Unfortunately the substance chosen produced a meningoencephalitis in the cats (14) In a double-injection blood pig model, plasmin was injected 1 hr after the second blood injection with an apparent reduction in vasculopathy as seen in these animals at sacrifice (15). Two years later, the same group, using the same model, injected either plasmin or saline 2, 4, and 6 hr after the double-SAH injection. Angiography was not done. There was a progressive increase in the extent and severity of intimal proliferation the longer the plasmin injection was delayed after SAH. The severity of medial necrosis did not apparently correlate with the delay (16).

Using the primate clot model developed at the University of Alberta, Findlay and coworkers investigated the concept of intrathecal fibrinolytic treatment post-SAH as a means of augmenting the normally limited fibrinolytic activity of CSF. It was postulated that dissolution of the fibrin clot plus the normal pulsatile movement of the brain would permit clearance of enmeshed RBCs by bulk CSF circulation or drainage prior to their hemolysis. This presumably would prevent the resultant release of oxyHb in high concentrations adjacent to the adventitia of the conducting arteries (10,11,17–21).

In 1988, a blinded, randomized, placebo-controlled trial of intrathecal t-PA were performed in this model. Twenty-four monkeys underwent placement of clot against the right middle cerebral artery (MCA). Twelve placebo animals were compared to 12 receiving three t-PA injections of 0.5 mg through an Ommaya reservoir and subarachnoid catheter. All placebo-treated animals had residual subarachnoid clot 7 days later and all developed more than 30% reduction in vessel caliber. In contrast, 11 animals treated with t-PA had complete clot clearance by day 7 after SAH, and VSP did not develop to a significant degree except in the anterior cerebral artery, where a 14% vessel caliber reduction was noted. Evidence of systemic fibrinolysis was not found. There was no evidence of meningoencephalitis or intracranial bleeding

complications. The dose of 1 mg t-PA completely lysed 5 ml of whole clotted monkey blood (19). Subsequently, the safety of intrathecal thrombolytic therapy was tested in the monkey model by administering a relatively high dose of 10 mg of t-PA after clot placement. Seven days later, clot clearance was complete and VSP absent. No abnormalities in systemic coagulation parameters were detected. Two animals developed minor wound hemorrhages persisting for 24 hr (20). The efficacy of t-PA injections seems to be related to the proximity of t-PA administration after clot application. In five groups of 6 animals, 0.7 5 mg of intrathecal t-PA was injected 0, 24, 48, 72 hr or not at all after SAH clot placement. VSP was prevented by t-PA injections prior to 72 hr. When t-PA was not used until 72 hr VSP still occurred (21). The efficacy of unilateral administration of 0.5 mg of t-PA in lysing bilateral subarachnoid clot was analyzed in two groups of 8 monkeys. In one of the placebo group a DID developed on day 5. In the placebo group significant VSP occurred in all major right- and left-sided anterior cerebral vessels but no VSP occurred in t-PA treated animals. Clot at sacrifice averaged 1.13 g in all animals in the placebo group, whereas only a small fragment of clot was found in a single t-PA-treated animal. The t-PA in these animals was given in a slow-released gel t-PA formulation (20). In another study 16 monkeys were divided into four groups of 4 and received different doses of sustained-release gel t-PA. No VSP occurred in the groups receiving 0.5 or 0.75 mg t-PA, and mild to moderate VSP occurred in the groups receiving 0.15 or 0.25 mg t-PA. Gross subarachnoid clot remained in all of the animals in the 0.125- and 0.25 mg dose groups, in 2 of the animals in the 0.5-mg dose group, and in none of the animals in the 0.75-mg dose group. It was therefore concluded that 0.75 mg of gel t-PA is sufficient to completely lyse 4.25 ml of subarachnoid clot and prevent VSP in this model (20).

Urokinase (UK) is purified from human urine. The specific activity is 147,000 IU/mg and the molecular weight is 54 kDa. t-PA is produced by recombinant molecular biological techniques and has a specific activity of 600,000 IU/mg. The molecular weight is approximately 62 kDa. Lysine plasminogen is found in human plasma and has a specific activity of 24 casein units and a molecular weight of 84 kDa (22).

Twenty monkeys were assigned to one of five groups of four. Bilateral clot placement was employed. Animals received via an Ommaya reservoir placed unilaterally one of the following treatments: normal saline, 100,000 IU UK, 200,000 IU UK, 1 mg t-PA, or 2 mg t-PA. Animals receiving the higher dosages of UK and t-PA showed excellent clot removal by 7 days, whereas the other groups had gross clot remaining. VSP, however, occurred in all groups. The plasminogen activators had been injected 24 hr after initial clot placement. It was presumed that larger doses given earlier or more often would be required to completely lyse the bilateral clots and prevent VSP (23).

In a rat model of ICH, t-PA and UK given in conjunction with thrombin appeared to aggravate the brain edema caused by thrombin (24).

In a canine model, treatment was with a single intracisternal bolus injection of 25 μg of t-PA into the cisterna magna 48 hr after the first and 6 hr after the second injection of blood. Basilar artery VSP was prevented in all animals so treated. In the control group, severe VSP occurred. Subarachnoid clots were removed by the intracisternal t-PA application (25). Espinosa *et al.* thought that the inflammation in Peterson's experiment might have been due to the streptodornase and so conducted a trial of intracisternal streptokinase to lyse subarachnoid clot and prevent VSP. This treatment was not found to be effective because significant VSP developed in both streptokinase- and saline-treated dogs (26). Single intracisternal bolus injection of t-PA was studied in a double-hemorrhage canine model. In 2 groups of animals, three hours after the second hemorrhage cisterna magna injections of 250, 25, or 25 μg of t-PA with 50 IU of plasminogen were given. The treated animals had significant inhibition of VSP and clot lysis compared to controls. Hb and fibrin degradation products decreased earlier in the CSF of treated animals. The clearance was most remarkable in the higher dosages of t-PA. The circulation of CSF was considered to be important for the removal of clot and spasmogenic metabolites (27). A single bolus injection of t-PA after induced SAH in cats was sufficient to inhibit intimal platelet accumulation which in control animals occurs at the site of adventitial blood. The t-PA was injected within 10 min of the SAH induction (28). Also in a feline model, elevation of CSF outflow resistance was shown to follow experimental SAH. Intrathecally administrered t-PA (2 mg) was injected 30 min after the SAH induction. This resulted in normalization of CSF outflow resistance 30 min after the t-PA injection (29).

Monkey and human blood was allowed to clot *in vitro* at room temperature. At frequent time intervals thereafter, 50,000 IU t-PA or 1 ml saline was added to each tube and the rate of clot lysis was calculated. Residual clots were studied by electron microscopy. The clots of varying agents were added to baths in which canine basilar arteries were studied pharmacologically. t-PA was found to dissolve monkey clots less effectively than human clots but was still able to do so for up to 96 hr. Electron microscopy showed morphological changes in RBCs beginning at 48 hr and showed severe disruption

or hemolysis by 96 hr. Clots prepared *in vitro* were able to contract canine basilar arteries (30).

In the canine single-hemorrhage model, blood was injected into the cisterna magna. A single 250-μg injection of t-PA in 1 ml of fluid was injected into the lateral ventricle 3 hr following SAH. In the control group, the basilar artery was 60% of its original diameter on day 4 whereas the t-PA group had a residual diameter of 84% of the original diameter. In the untreated animals there was a significant residual subarachnoid clot around the basilar arteries at sacrifice. Marked lysis of subarachnoid clot was observed in the animals receiving t-PA intraventricularly. One animal of six demonstrated bleeding along the needle tract, and two animals showed subcutaneous hematomas (31). In a rabbit model of a single SAH injection, angiography was performed on days 1, 3, and 5 post-SAH. The experimental groups were as follows: untreated, saline, and t-PA ($1 \times 10^4$ M) plus antithrombin-III (AT-III) (25 units). The animals receiving the t-PA had less residual subarachnoid clot on day 5. There was significantly less VSP on days 1 and 3 post-SAH in animals receiving t-PA, AT-III, or both. The rationale for this experiment was that fibrinolysis might release thrombin into the subarachnoid space, which could have a secondary pro-coagulant effect. The AT-III was to prevent this from occuring (32).

In a canine model four groups were studied: controls, vehicle, 50 μg/kg t-PA, and 100 μg/kg t-PA. A double-hemorrhage was induced. The ratios of final to initial basilar diameters for the four groups were: 75,72,86, and 87%, respectively. Differences between controls and either groups of t-PA were statistically significant. The subarachnoid space of the animals receiving t-PA was relatively clean. No ICH occurred (33). In an acute model subarachnoid clot lysis rates were studied over 24 hr during continuous intrathecal irrigation with t-PA or UK in a canine SAH model. The rate of clot lysis caused by either drug dose dependently increased up to 3000 IU/ml for UK and 125,000 IU/ml for t-PA. With the same molar concentrations, the effective dose of t-PA was higher than that of UK. The slopes of the dose–response curves were similar for both agents. Clot lysis with the combined use of UK and lysine–plasminogen was higher than with the single dose of UK. The administration of lysine-plasminogen markedly enhanced clot lysis by UK in the early stages of irrigation. Since the concentration of plasminogen in CSF may be only one-10th that of blood after SAH (34), the investigators considered that low plasminogen concentrations might be a limiting factor for clot lysis, hence the addition of exogenous plasminogen with the t-PA to improve the rate of clot lysis. The intrathecal half-life of t-PA is 2 or 3 hr, much longer than its intravascular half-life of approximately 5 min (35).

#### *2. Mechanical Clot Removal*

With the development of the primate unilateral clot model the opportunity was afforded to evaluate clot removal and its effect on the subsequent development of chronic VSP in primates. In 1987, Nosko *et al.* demonstrated the efficacy of clot removal 24 hr after SAH in the prevention of chronic VSP. Significant VSP was present on day 7 in 100% of the animals in which the clot was left *in situ* following its placement. There was no significant VSP on day 7 in either the sham-operated or 24-hr clot removal groups. A large volume of clot placed bilaterally resulted in a 25% incidence of delayed ischemia in this series. Evacuation of the clot 24 hr after its initial placement completely prevented the development of VSP and DID (36). Following up on this observation, Handa and colleagues performed a similar study in which the applied clot was removed at 48, 72, and 96 hr post-placement. All animals were sacrificed on day 7 post-clot placement. Angiography showed that the longer the clot was left *in situ* the greater was the amount of chronic angiographic VSP. Significant VSP was found in all animals in which the clot was left in place for longer than 48 hr. No significant neurologic deficits occurred in the sham-operated, 48-hr or 72-hr removal groups. Two animals in each of the 96-hr and no clot removal groups showed deterioration in the level of consciousness developing on days 4 or 5 after SAH induction (37). *In vitro* studies of primate MCA exposed to subarachnoid clot for varying time intervals suggested that the attenuation of cerebral vascular contractile responses to various agonists 7 days after SAH is pharmacologically inevitable, even if the clot is removed as early as 48 hr after the SAH. The maximum responses to 5-HT and ATP decreased in animals having clot removal as early as 48 hr after SAH (38).

#### *3. Cerebrospinal Fluid Lavage*

In a two-hemorrhage canine model, lavage with 120 cc of artificial CSF performed 24 hr after each of the two hemorrhages did not appear to affect the degree of angiographic, neurologic, or morphologic sequelae of the hemorrhage in this model despite evidence of removal of clot as seen at sacrifice (39).

## III. Timing of Surgery and Vasospasm

### A. Optimal Timing

One hundred and fifty patients admitted by day 2 post SAH were studied, with repeat angiography performed between days 7 and 9. Of patients operated on by day 3, angiographic VSP was observed in 95%, whereas in those operated on after day 20 or not operated on, angiographic

VSP was observed in 88%. The incidence of symptomatic VSP in the early surgery group was 18%, which was significantly lower than the 44% in late or nonoperated groups. In early operated patients, the incidence of symptomatic VSP was 13% and that of CT low-density areas 10%. The respective percentages for patients operated on in a delayed fashion were 50 and 36%. The differences in mortality rates appeared to correlate more closely with the amount of SAH and the initial clinical grade (40).

The effect of the diminution of the SAH in early CT scans was assessed systematically. The more rapidly the blood decreased, the lower was the incidence of angiographic VSP, DID, and low-density areas on CT scan. Permanent deficits and CT infarcts were found in 5 of 9 patients with no diminution, whereas they occurred in only 5 of 27 patients with diminution. The first CT scans in this series were performed within 24 hr post-SAH and the second scans were performed within 3 day post-SAH. The second CT scans were performed postoperatively. The diminution rate for SAH increased with increasing age, although there was no correlation observed with neurological grade or the site of the aneurysm. It was concluded that marked spontaneous diminution of SAH in the acute stage after SAH and surgery seemed to alleviate VSP (41). The same surgeon reported on 103 patients in whom initial CT scans were performed within 24 hr post-SAH and repeated within 72 hr. The effect of diminution of SAH by cistern on subsequent angiographic VSP was evaluated. Of the total 1642 cisterns, SAH was found in 83%, of which 40% had a subsequent decrease. The highest diminution rate was 64% in the quadrigeminal cistern and the lowest was 27% in the frontal interhemispheric fissure. The greater diminution of blood in the frontal interhemispheric fissure and the Sylvian stems was associated with a lower incidence of VSP in the contained arteries. A marked diminution in SAH occurred in 13% of paired CT scans done within 6 hr or less and 33% of paired scans done within 72 hr. In this series subarachnoid blood was washed spontaneously by CSF, whereas in the previous series blood was removed surgically by Inagawa. In addition, in the surgical cases in this series diminution of SAH was evaluated by comparing the preoperative CT with the CT done on admission, whereas in Inagawa's previous study the postoperative CT was compared with the preoperative CT. This study was remarkable in that many CT scans were performed within 6 hr after SAH (41).

The time of surgery had no apparent effect on angiographic VSP. Cerebral infarction due to VSP developed in 15% of 34 patients operated on within the first 3 days post-SAH and in 20% of 20 operated on between days 4 and 12 post-SAH. Good outcomes in these two groups were achieved in 88 and 85%, respectively (42).

### B. Treatment in the Presence of Established Vasospasm

When a patient presents to the neurosurgeon in a delayed fashion and angiographic VSP is present the course of action should depend on the severity of the VSP and the neurological condition of the patient. If the patient has become comatose and VSP is severe and diffuse, we recommend (i) immediate clipping, without hypotension and with careful volume maintenance, with immediate postoperative angioplasty under the same anesthetic or (ii) immediate coiling of the aneurysm with angioplasty (or vice versa if necessary) under the same anesthetic. General medical and anatomical factors influence the choice of therapies.

If the patient is fully conscious, without any evidence of DID, the presence of VSP should not delay the obliterations of the aneurysm by whatever means is chosen. The risk of rebleeding outweighs the risk of asymptomatic VSP in such circumstances. An example of the abatement of VSP with delayed operation is given in Fig. 10.2.

## IV. Cisternal Drainage

The removal of bloody CSF using catheters in the cranial or lumbar subarachnoid space, or ventricle, has the advantage of reducing the dosage of spasmogen. A potential disadvantage is disuse collapse of CSF drainage pathways with increased potential for their fibrotic obliteration. The maintenance of a normal CSF pressure sometimes mandates that bloody CSF be removed. In the presence of significant clot (either SAH or IVH), frequent catheter blocks can be overcome by instillation of t-PA.

One hundred and forty patients with SAH were classified according to the total amount of CSF removed by cisternal irrigation as follows <500 ml, 500–3000 ml, and 3000–9500 ml. Angiographic VSP was less severe in the groups with the larger amounts of CSF removed. Also, the incidences of permanent DID and low-density areas on CT were lower in the groups having the larger amounts drained. Mortality rates for the medium and high CSF volumes removed were 22 and 19%, respectively, compared to 33% in the group with the lowest volume of CSF removed. The more CSF that was removed, the higher was the recovery rate. However, the patients with the larger volumes of the CSF removed had a higher requirement for permanent CSF shunting. By small, medium, and high volume of CSF removed, the percentages of permanent shunts were 37, 61, and 49%. These rates of shunting are extremely high compared to those of most series (43). Ninety-two patients operated on within 2 days

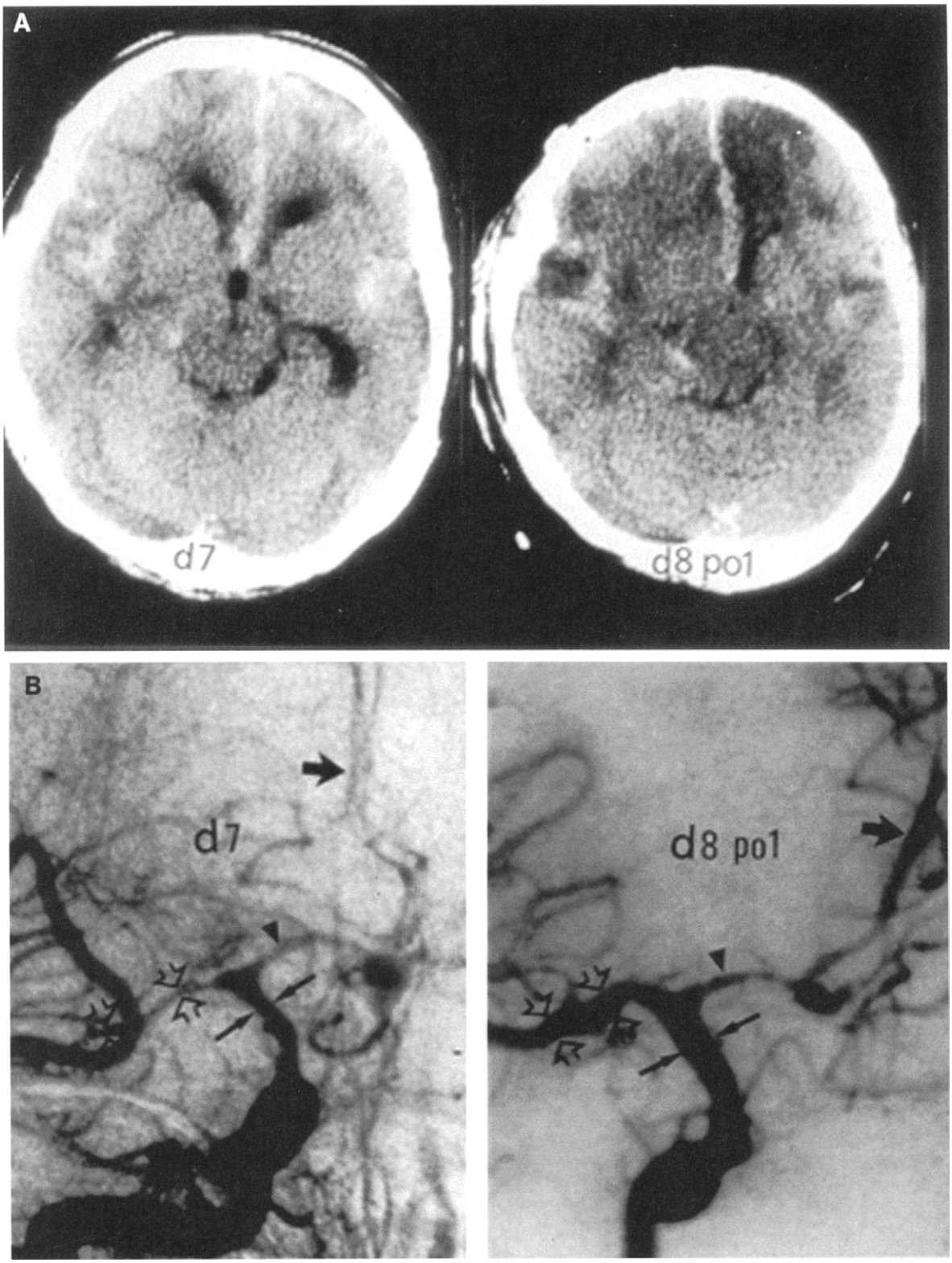


**FIGURE 10.2** (A) CT scan on day 7 post-SAH showed residual SAH, mild ventriculomegaly, and no obvious low-density areas. Following surgery on day 8 there was an increase in brain swelling, ventricular compression, and the appearance of a left frontal low density. (B) Surgery was performed in the presence of known VSP. Angiography immediately post operatively showed a marked diminution in the degree of angiographic VSP.

post-SAH who had CSF drainage performed were analyzed. The average duration of drainage was 10 days and the average daily volume removed was 190 ml. The average total volume removed was 2 liters. Patients with greater drainage volumes at a lower height of drainage in the early period after SAH developed more cerebral infarction. Patients with larger volumes of CSF removed had a higher requirement for shunting. Cerebral infarction and Hyc post-SAH were also found to be statistically associated (44).

In a series of 185 patients studied by Sonobe and coworkers, the incidence of VSP in 150 patients operated on who had CSF drainage was 11% compared to 29% in 35 patients without drainage (45). Kodama and his group added urokinase and ascorbic acid to the irrigating fluid used in conjunction with CSF drainage. Only 3 of 50 patients judged to be at high risk for DID and VSP had a permanent deficit. This type of therapy requires strict antiseptic technique, intensive nursing observation, and a prolonged period of bed rest (46). Bifrontal craniotomy was employed in 185 patients who had surgery within 1 week post-SAH. Both ventriculocisternal irrigation and bilateral cisternal drainage were performed after clipping. This kind of drainage was performed in 81% of this series, and of this group 90% were operated on in the first 3 days. The incidence of VSP was 11% in the patients treated with drainage and 29% in the patients not so treated. Mortality rate was 16% with drainage and 20% without. Symptomatic VSP was 11% with drainage and 29% without (45). One hundred and thirty-six patients who had cisternal drainage within 3 days post-SAH were compared to 69 patients without drainage in a retrospective analysis. Patients in a high Fisher grade or poor clinical condition did better with drainage. Symptomatic VSP occurred in the same percentage of patients with or without drainage (approximately 40%). Persistent VSP was less common in the group with drainage (12%) compared to patients without drainage (26%) (47). Continuous cisternal drainage was performed in 47 of 205 patients undergoing early aneurysm surgery. Symptomatic VSP occurred in 4% of the 24 Fisher grade III patients who had continuous cisternal drainage compared to 23% of the 53 patients in Fisher grade III who were not treated with drainage. It was considered that continuous cisternal drainage significantly reduced the incidence of symptomatic VSP (48). Ventriculocisternal drainage was performed in 245 patients treated between 1979 and 1989 by Yamatani and colleagues. Patients were operated on within 7 days of SAH. Results were compared to those of 22 patients in whom drainage was not performed. Ventriculocisternal drainage was apparently associated with a reduction in DID from 23 to 9% and of death rate from 32 to 16% (49).

## V. Fibrinolytic Therapy and Surgery

Various series involving the intraoperative instillation of t-PA are reviewed elsewhere. The initial clinical trials usually employed the instillation of approximately 10 mg of t-PA which was allowed to settle for 20–30 min before the instillation of irrigating fluid to remove any unbound t-PA. This was the method tested in the only randomized, placebo-controlled study. We no longer employ this method for several reasons: (i) We are often successful in removing the majority of clot by suction and irrigation so that t-PA is unnecessary, (ii) we wish to perform an immediate postoperative CT scan to establish that enough

**TABLE 10.1 Procedures for the Surgeon to Reduce Vasospasm and Infarction**

1. Operate as soon as possible.
2. Maintain close liason with anesthesia in the perioperative period and during the surgery. Ensure that blood pressure does not drift below adequate levels, avoid nondeliberate hypotension; avoid excessive hypocapnia, maintain $p_aCO_2$ in low normal range; administer routine mannitol and furosemide; replace significant blood losses; replace fluid volumes during closure; ensure adequate anesthesia before intubation, placement of head pins, or other painful stimulation; prior to temporary clipping give additional mannitol, and during it raise blood pressure and administer propofol; be alert to modifying medical conditions and allergies.
3. Institute CSF drainage (we prefer ventricular drain placed after opening the dura, the drain is left open until the aneurysm is clipped).
4. Sharp arachnoid dissection with gentle suctioning of clot; frequent irrigations throughout operation; preservation of all perforators and bridging veins unless sacrifice is imperative to achieve operative aim; as many cisterns are opened as can be approached without excessive retraction via a unilateral craniotomy.
5. Perform CT scan in early postopertive period. *If* diffuse thick clot is still present, especially if there is considerable IVH, *if* there has not been a lot of brain parenchymal disruption or dissection, *if* there has not been any new significant, unexpected bleeding, and *if* the ruptured aneurysm is known to have been definitively treated then consider intraventricular instillation of t-PA via the ventricular drain placed at surgery. Instillations of 2 mg t-PA every 8–12 hr with CT control before each dose can be given as long as dangerous amounts of clot are found to be present.
6. Maintain CSF pressure < 15–20 mmHg by ventricular drainage in the postoperative period. We try to remove drains as soon as period of maximum risk for VSP is passed and do not use them in good-grade patients postoperatively.

subarachnoid clot remains to place the patient at significant risk of VSP and DID, and (iii) we wish to establish that no intraoperative bleeding has occurred and that our delivery catheter is in good position (i.e. the tip is not intra-parenchymal). Assuming that we believe t-PA is still indicated, we instill it slowly via a ventricular catheter in multiple doses of 2 mg t-PA dissolved in 1 ml of water every 12 hr until follow-up CT scans show substantial disappearance of the subarachnoid clot and/or intraventricular clots.

Dorsch reviewed reports comprising 268 patients who received intracisternal t-PA. The incidence of DID was 10%. Forty-three percent were considered to have VSP by angiographic or TCD criteria. Four of the patients had extradural hematomas requiring operation. There were four reports of fatal bleeding, but all of these came from a single center in which only 19 patients were treated and report of this specific series did not appear in a peer-reviewed publication (50). The published complication rates from experienced aneurysm surgeons have been much lower than suggested by this review.

A constant infusion system of saline containing urokinase and gentamycin was used between 1974 and 1983 in 98 cases of ruptured aneurysms operated on within 3 days post-SAH. Thirty-five percent of these patients showed symptomatic VSP, which was less than the 45% incidence in 120 historical controls (51). Tips for the surgeon which might reduce vasospasm and infarction are given in Table 10.1.

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CHAPTER 11

# ANIMAL MODELS



## I. Introduction

Cerebral aneurysms and spontaneous SAH have not been reported in dogs or cats, although various species have been affected by various central nervous system vascular malformations (1–3). The modeling of a condition such as VSP that develops 4–12 days after SAH requires the use of animal models. Wellum *et al.* noted that experiments suggested to be of relevance to VSP have been carried out using isolated arterial segments from normal animals *in vitro* (4). Whether or not these experiments can be related to VSP that occurs more than 3 days after SAH is not certain. It may not be appropriate to equate acute studies of contractile activities *in vitro* (5) with VSP. Arteries *in vitro* are denervated, not subject to pulsative pressure and flow, and may not have normal endothelial cell function. Changes in gene expression can occur within hours and may influence the responses (6). In most but not all monitoring systems *in vitro* the intra- and extraluminal surfaces are exposed to vasoactive substances. This probably does not occur *in vivo*. Ohta *et al.* demonstrated that KCl applied extraluminally to rabbit basilar artery caused only minimal contraction, whereas intraluminal application caused strong contraction (7). Pathological changes are reported to occur in vasospastic arteries. These are not known to occur in experiments *in vitro*, which may be important assuming the pathological changes are important and are not simply an epiphenomenon.

## II. Models *in Vitro*

The cerebral arteries of many species have been suspended in tissue baths *in vitro* or smooth muscle or endothelial cells from them cultured *in vitro* and effects of various putative spasmogens, blood products, and antagonists of many different compounds assessed (5). Wilkins *et al.* were among the first to assay the vasoactivity of cerebrospinal fluid (CSF) samples from patients with SAH, normal blood plasma, serum and hemolyzed blood on strips of rabbit aorta suspended under isotonic (8), or intracranial arteries of various species under isometric tension *in vitro* (9–13). Many experiments isolated the vasoactive component of fresh blood and found that activity is due in part to Hb (14). The relevance of these experiments to VSP has been questioned (4). Clot removal experiments and clinical experience suggest that processes are not set in motion in the first days after SAH that lead to VSP 5 or 7 days later independent of the subarachnoid clot. The reverse is not true, however, because it has not been determined if the delay in onset of vasospasm is due simply to time taken for release of a particular spasmogen or whether reversible reactions occur in the first days after SAH that are necessary for the delayed phase of vasospasm. On the other hand, experimental VSP begins as active smooth muscle contraction that appears to be in response to subarachnoid clot since removing the clot early prevents the spasm. These experiments have not been conducted in models of VSP that utilize aneurysm rupture, so theoretically it cannot be stated that such vessel injuries do not contribute. Models of SAH that rupture an artery more closely mimic the acute effects of SAH but are no closer to true aneurysm rupture than most injection or clot placement models since VSP usually follows rupture of an aneurysm and not a normal artery. With this and the limitations mentioned previously in mind, numerous studies utilizing

vascular preparations *in vitro* reviewed elsewhere in this book support a role for Hb and/or a breakdown product in the genesis of VSP and have provided the background for additional studies utilizing the models discussed later.

Excising a cerebral artery, coagulating or ligating its branches, and perfusing it under physiological pressure *in vitro* overcomes some of the limitations of traditional isometric tension studies, although the vessel is still denervated (15–17). Tsuji *et al.* developed such a system using dog basilar artery and contributed to the understanding of how Hb contracts cerebral arteries (16,17). Experimental systems investigating VSP by studying arteries exposed *in situ* are reviewed later.

Recent investigations have utilized cultured or freshly isolated smooth muscle, cultured endothelial cells, or segments of artery in organ culture. Cultured smooth muscle and endothelial cells do not retain characteristics identical to those of smooth muscle cells in the arterial wall (18). It is unlikely that pieces of artery placed in organ culture remain unaltered (19). Application of erythrocyte hemolysate or of some clot or hemorrhagic CSF component to these cells (20–22) in order to model VSP assumes that this compound has access to these cells *in vivo*, an assumption that is difficult to verify.

## III. Acute Effects of SAH versus VSP

Acute brain damage contributes substantially to morbidity and mortality in patients with aneurysmal SAH. Attempts have been made to reproduce these changes in rats, dogs, cats, and monkeys and there are various theories regarding the cause of the brain injury. One possibility is acute VSP, a phenomenon that is well described in animal models of SAH but for which there is little evidence in man. In many cases, acute spasm in animal models is due to inadvertent injection of hemolyzed blood which is known to be a vasoconstrictor (23). If the acute events are independent of "chronic" VSP, then there is no reason to require an acute increase in intracranial pressure (ICP) to occur in an animal model at the time of hemorrhage. If the acute events influence VSP, then some models, such as clot placement models, would miss this interaction. A review of the literature on the various nonhuman primate models shows that VSP produced by clot placement is more severe than that due to arterial rupture or blood injection in animals with a closed cranium. It is also true, however, that a rigorous scientific comparison of VSP occurring with an equal volume of blood with or without arterial rupture and/or acute alterations in ICP and CBF has never been carried out and would be difficult to do because of the problems of quantifying the volume of hemorrhage with different models of SAH.

## IV. Species Differences

There are important differences in the anatomy of the cerebral circulations of the species that have been used in SAH models. These need to be considered if one is attempting to produce delayed cerebral ischemia in addition to VSP of the larger cerebral arteries. The importance of brain ischemia in the pathogenesis of VSP has never been established. Brain ischemia may not have anything to do with the pathogenesis of VSP other than being the final common pathway of poor outcome after SAH. Pharmacological and molecular biological differences between the species may influence the results obtained with regard to VSP and SAH. Serotonin is a potent constrictor of cat and dog cerebral arteries but only a weak agonist of rabbit basilar artery (10,24). Rats do not have myointimal cells, which may be important in the intimal hyperplasia that follows vascular injury (18).

VSP after a single SAH in rats tends to have a rapid onset and a shorter duration than VSP in man. This may be because the erythrocytes are cleared rapidly from the subarachnoid space and there is only minimal hemolysis. The CSF may be cleared more rapidly because of the lack of compartmentation of the subarachnoid space. Therefore, a disadvantage of these models is that they may not produce as much hemolysis as occurs after SAH in man. On the other hand, this is only a disadvantage if hemolysis is an important phenomenon in the pathogenesis of VSP. Furthermore, if the spasm that occurs is due to hemolysis, then even if it is mild, treatment effects might be overestimated, the mechanisms might be the same as in man, and important information could still be obtained from these models. In rats, dogs, and rabbits, the problem of rapid clearance of subarachnoid blood, which results in the development of relatively mild, short-lived VSP, can be partially overcome by giving a second injection of blood into the CSF 48 hr after the first injection. This increases the duration and severity of VSP but has the disadvantage of making it more difficult to study the time course of events as they might relate to VSP after a single SAH in man.

Clot placement models have been developed in dogs (25,26), pigs (27), and monkeys (28). Allowing blood to clot before placement in the subarachnoid space overcomes the difficulty of rapid washout of the subarachnoid blood. It has been argued that these models are technically difficult, but these are routine technical exercises for the modern microneurosurgeon. As discussed previously, there is no evidence to support the hypothesis that vasospasm after clot placement is not the same as vasospasm after aneurysm rupture because there is no rupture of an aneurysm (or, in most models, a different phenomenon such as rupture of a cerebral

artery) or acute ICP changes that are associated with many aneurysm ruptures.

## V. Considerations in the Conduct of Animal Studies

If a treatment is tested and found effective in an animal model, the essential aspects of the animal study, such as the drug levels and the location where they are obtained and the timing of administration, should be replicated in human studies if there is to be any chance of demonstrating efficacy. Lack of attention to such details may have limited the success of some agents that worked in animal models of, for example, ischemic stroke but not in man. The treatment timing should be clinically relevant. Pretreatment with a drug, however, is not unreasonable when one is trying to prevent VSP or to gain insight into disease mechanism. Physiological variables that can affect angiographic arterial diameters or CBF, such as blood pressure, heart rate, arterial oxygen and carbon dioxide content, types of anesthetic agents, temperature, and hematocrit, must be monitored carefully. Randomization and blinding should be employed as much as possible.

The only appropriate end point is arterial diameter measured by standardized angiography or morphometric measurement of perfusion-fixed arterial cross sections (29). Cerebral ischemia is not produced reliably in animal models, probably because of abundant collateral supply to the brain in most lower species. Clinical end points have not been very helpful in animal models of SAH and VSP (30).

There is increasing concern over the discrepancies between results obtained in animal models of stroke and those obtained in humans. Criteria for assessing whether a neuroprotective therapy may be efficacious in animals have been discussed (31); however, it is difficult to apply these to the study of VSP. The most important evidence would be prevention of chronic VSP in a large animal model of SAH in a blinded, randomized study accompanied by measurements of drug level and assays to show drug effect. The key features of VSP in man, as mentioned later, should be reproduced in the model that is used.

A common criticism is the use of young, healthy animals in stroke models. This may be of importance particularly with respect to the response of the brain, although there is no strong evidence that age affects angiographic VSP. Furthermore, SAH and VSP affects a younger population than ischemic stroke. Calcified, atherosclerotic arteries may be resistant to VSP, suggesting that use of animals with healthy arteries is not inappropriate. Review of the literature shows that different blood preparations have been injected into the subarachnoid space (32,33). In many studies, arterial or venous blood has been used or the source was not mentioned and absolutely no evidence for differences in spasm produced was presented (33,34). Heparinization of the blood may allow it to clear more rapidly and thereby produce less VSP (35), although VSP has been reported in such models (36). Only one report suggested that arterial but not venous blood caused VSP (32).

## VI. Creation of SAH

SAH has been created in animals by

1. Injection of autologous blood into the subarachnoid space
2. Deliberate rupture of an intracranial artery
3. Placement of a blood clot in the subarachnoid space

According to Schwartz *et al.*, the ideal model of SAH and VSP would have consistent deposition of a constant amount of blood in the subarachnoid space in the same distribution as occurs after aneurysm rupture by a mechanism that simulates aneurysm rupture (37). It would be easy to perform and inexpensive. The following features must be replicated: The major cerebral arteries must become narrowed in a delayed fashion days after the SAH and remain narrowed for days; there must be histopathological signs of smooth muscle and endothelial cell damage, often associated with intimal hyperplasia toward the end of and following the phase of arterial narrowing (38); and the vasospastic arteries must be resistant to dilation with vasodilatory drugs such as oral or intravenous nimodipine and papaverine (39). Currently, there is no model that fulfills all these criteria. The best model, therefore, will depend in part on the question one desires to answer. Placement of foreign substances such as latex beads (40) and talc particles (41) into the subarachnoid space reliably narrows the cerebral arteries but these are probably far enough removed from the human disease to render them inappropriate for the study of human VSP. The most important factor in the production of severe, prolonged VSP seems to be the ability to surround an intracranial artery with thick blood clot that remains for days.

Injection of blood into the subarachnoid space may be made by inserting a needle percutaneously into the cisterna magna (Table 11.1). A needle may also be placed through the orbit into the chiasmatic cistern. A catheter can be permanently implanted into the subarachnoid space for repeated injections. The advantages of these methods are that they are simple and relatively less expensive. The cranium is closed and these methods are appropriate for studies of acute ICP, CBF and cerebral ischemia.

**TABLE 11.1 Angiographic, Pathologic, and Pharmacological Features and Advantages and Disadvantages of Common Animal Models of VSP**

| Species | Model | Advantages | Disadvantages |
|---|---|---|---|
| Mouse | Endovascular arterial perforation | Inexpensive, possible to use transgenic and knockout animals, extensive knowledge of genome, large number of immunological products available | VSP is mild, time course, the presence of pathological and pharmacological changes incompletely described, small size |
| Rat | Cisterna magna injection | Inexpensive and easy to perform, extensive availability of immunological reagents | VSP is mild and short-lived, pathological changes may be mild or nonexistent, pharmacological changes incompletely described |
| Rat | Endovascular arterial perforation | Inexpensive, may more accurately replicate the acute effects of SAH | VSP time course, the presence of pathological and pharmacological changes not studied |
| Rat | Chiasmatic cistern injection | Inexpensive, unilateral SAH may allow use of contralateral side as relatively "normal" control | Single report, time course, the presence of pathological and pharmacological changes not studied |
| Rabbit | Cisterna magna injection | Inexpensive, time course of VSP established, pathological and pharmacological changes develop | VSP is mild and generally of shorter duration than in man |
| Dog | Cisterna magna injection | Time course of VSP well characterized, pathological and pharmacological changes occur | Expensive, no arterial rupture, may not reproduce acute effects of SAH, no internal standard control, little knowledge of genome |
| Dog | Clot placement | May produce more severe VSP | Expensive, single report, time course, the presence of pathological and pharmacological changes not studied |
| Monkey | Clot placement | Time course and severity of VSP, the presence of pathological and pharmacological changes very similar to human VSP, may produce cerebral ischemia | Expensive |

Also, cardiovascular changes are of interest, although these changes are generally not as severe as those produced by arterial rupture techniques. Disadvantages are that relatively small volumes of blood can be injected and this limits the volume of clot that forms around the cerebral arteries.

SAH with the cranium closed has been produced by placing a needle through a cerebral artery and withdrawing it when the head is closed, by placing a tourniquet around an artery such as the posterior communicating artery and avulsing it, or by advancing a pointed suture endovascularly through the carotid bifurcation. Advantages of these methods include the ability to closely mimic the ICP and acute pathophysiological changes of SAH. They have disadvantages, however, including high mortality, variable degree of SAH, and difficulty with forming enough clot around the arteries to produce severe, prolonged VSP.

Clot placement in the subarachnoid space is best described in monkeys (28) but has been reported in dogs (25,26) and pigs (27). Advantages include reliable production of severe VSP, ability to use the contralateral, unaffected artery as an internal control, and occasional development of cerebral ischemia. Disadvantages are higher cost and lack of production of acute changes of SAH.

Some investigations have utilized subarachnoid injections of norepinephrine, serotonin, PGs, Hb, lipid peroxides, and various other substances to attempt to reproduce VSP as it occurs after SAH (10,42–44). It probably is not possible to reproduce the exact time course and concentration from release of such substances as it occurs after SAH. This makes such experiments difficult to interpret.

## VII. Specific Animal Models

### A. Mouse

The basilar artery is 150–200 μm in diameter and has two to four layers of smooth muscle cells. Kamii *et al.* advanced a 5–0 monofilament nylon suture with a blunt end up the internal carotid artery (ICA) and anterior cerebral arteries of the mouse until resistance was felt (45). It was advanced 5 mm further to perforate the anterior cerebral artery and then withdrawn, resulting in SAH. Acute mortality was 28%. Animals were perfused

with 10% formalin and then carbon black mixed with 10% gelatin. VSP was measured by the diameter of the middle cerebral artery (MCA) under a microscope. There was significant arterial narrowing 3 days after the hemorrhage but not 1 or 7 days after. MCA diameter was reduced from 139 ± 15 μm in sham-operated mice to 111 ± 21 μm 3 days after SAH (approximately 20% reduction in diameter). The model was used to demonstrate a protective effect of overexpression of superoxide dismutase in transgenic mice. Pathological and pharmacological changes and resistance of VSP to vasodilators were not assessed. A model of SAH in mice would have great potential because of the ability to use transgenic and knockout mice.

Matz *et al.* injected 50 μl of autologous erythrocyte hemolysate over the cortex of mice (46). This model was used to investigate DNA fragmentation and changes in gene expression in the brain. The arteries in the subarachnoid space were not assessed for VSP and hemolysate did not reach the basal cisterns, rendering this an inadequate model of cerebral VSP. The acute mortality rate was 16%. Injection of a similar volume of hemolysate into the cisterna magna was associated with a 50% mortality and with substantial reflux of blood around the needle.

### B. Rat

The ICA supplies much of the brain via five branches (anterior choroidal, optic nerve, middle cerebral, anterior cerebral, and posterior communicating arteries) (47). There are few extraintracranial anastamoses. As in other rodents, the basilar artery supplies the brain stem and cerebellum but its supply may be compensated for by the anterior circulation. The rat basilar artery is approximately 300–400 μm in diameter and has four layers of smooth muscle cells.

Methods to produce SAH include transclival exposure of the basilar artery and needle puncture followed by withdrawal, injection into the cisterna magna, injection into the chiasmatic cistern via transorbital approach, cortical injections through a burr hole over the cerebral convexity, and endovascular perforation of the ICA or anterior cerebral artery near the bifurcation. Injection of blood through a burr hole over the cerebral convexity produces local SAH over the hemisphere and is not a model of VSP of the basal arteries of the circle of Willis (48).

Ohta and colleagues studied topical applications of barium chloride to the basilar artery of the rat via transclival exposure almost 30 years ago (49), and the acute effects of cisternal blood injections on regional cerebral energy metabolism were studied in rats by Fein in 1975 (50). Barry and coworkers introduced the rat as a possible model of VSP (51). The basilar artery was exposed transclivally and punctured with a microelectrode. The diameter of the artery was measured by direct measurement. Overall mortality was 26%. Most of the hemorrhage dissipated within 72 hr. Spasm was maximal (approximately 20% reduction in diameter) 1 hr after SAH. Significant narrowing was present 2 days later (15% reduction) and this was followed by significant dilation on days 5 and 8. Cerebral blood flow (CBF) was reduced for days in this model (52). Transclival puncture of the basilar artery was used to measure changes in electrolyte concentrations in the basilar artery and surrounding clot (53) and was combined with MCA occlusion in an attempt to produce delayed cerebral ischemia (52).

Changes in basilar artery diameter have been measured acutely after SAH (54) or after applications of various candidate spasmogenic solutions such as erythrocyte hemolysate (55). The changes in these studies may not be representative of VSP in man because of the short time course, because clot removal within 2 days of SAH prevents VSP in man suggesting that irreversible processes important in VSP do not occur in the first days after SAH, and because the contents of freshly lysed erythrocytes are not necessarily those that are released days after SAH during VSP.

Lacy and Earle injected heparinized blood into the chiasmatic cistern of rats via a catheter that was placed through a frontal burr hole and then advanced around the hemisphere to the cistern (56). Acute electrocardiographic changes were studied but not VSP. A modification of this model was the injection of blood while the animal was awake (57). VSP was not an end point but the authors did demonstrate that SAH increased plasma renin activity and pretreatment with losartan, an inhibitor of angiotensin II, reduced blood pressure, and increased mortality.

Solomon *et al.* inserted a cannula into the cisterna magna of rats through a parietal burr hole at least 5 days before subsequent testing (58). Rats were injected with 0.3 ml fresh autologous arterial blood, mock CSF, or not injected. CBF was measured with labeled microspheres for up to 1 hr after injections and there was a 40% decrease in CBF with SAH, whereas saline injection was associated with a 15% decrease (58). The model was modified in that no permanent cannula was implanted and blood (0.37 ml fresh arterial nonheparinized) was injected via a needle advanced into the cisterna magna from a parietal burr hole (59). Forty-eight hours later, glucose metabolism was decreased in four metabolically active brain regions compared to saline-injected controls. The degree of VSP was quantified in this model and the basilar artery was reduced to 54% of control size at 10 min and 26% at 2 days (60). These and other (61) authors commented that VSP was less in Wistar compared to

Sprague–Dawley rats but the data provided to support the claim were sparse. The model was used for a variety of biochemical and pharmacological studies (62–69) and to demonstrate antivasospastic effects of a hydroxyl free radical scavenger (70).

Swift and Solomon induced SAH in rats by the same method using an even greater volume of blood (0.6 ml) and found CBF was decreased 3 hr after SAH but then returned to normal 1, 2, 3, 7, and 14 days afterward (71). Electron microscopy of basilar arteries was normal 3 days after SAH. The mortality rate was not reported. This volume of blood corresponds to massive SAH according to Ram *et al.*, who injected an average of 0.57 ml fresh arterial blood directly into the cisterna magna of rats and noted a 50% reduction in basilar artery diameter when observed through a burr hole in the clivus 3 days later (72). VSP in this model was reversed by magnesium (73).

In a cisternal blood injection model in rats, rCBF was decreased for up to 24 hr post-SAH although it returned to normal by 48 hr (74). Myonecrosis was reported in smooth muscle cells in the basilar artery at 48 hr, although there were no significant changes in the intima or perivascular nerves.

Delgado and colleagues injected 0.07 or 0.3 ml of fresh arterial blood into the cisterna magna of rats using a previously implanted catheter (61). VSP was assessed by angiography performed through catheters inserted into both axillary arteries. Injection of 0.3 ml was associated with maximal reductions in basilar artery diameter of 40% at 10 min and 27% at 2 days. Mortality was 12% and SAH was absent by day 3. Histochemical staining 2 days post-SAH showed loss of sympathetic nerve terminals. CBF was reduced 20% and glucose utilization, in contrast to other findings (59), increased 30% during the late phase of spasm 2 days after SAH (75). The model, generally with injection of only 0.07 ml blood, was used for extensive investigations into the role of neural pathways originating in the brain stem that when lesioned prevented VSP (76–79). Other investigators could not detect a reduction in CBF in this model (80). Autoregulation was normal 2 days post-SAH in one study (81) but was impaired 2 and 5 days after SAH in another (82) and this was prevented by nimodipine (83). A positron emission tomography study found reduced CBF and focal areas of impaired autoregulation 2 days after SAH in this model (84) and that these changes were reduced by treatment with a free radical scavenger (85). Histopathological changes were absent 1–3 days after SAH (80). In an effort to standardize the acute ICP changes associated with SAH, Klinge and colleagues used computer-controlled intracisternal blood injection into the cisterna magna and olfactory cistern of rats (86). Expression of heat shock protein 70 in the hippocampus was present for up to 5 days after SAH. VSP was not assessed. It would be an excellent model in which to definitively answer the question of whether acute ICP changes affect chronic VSP.

SAH was induced in rats by exposing the posterior atlantooccipital membrane, inserting a 25-gauge needle, aspirating 0.1 ml CSF, and then mixing with 0.4 ml venous blood and injecting 0.1 ml of the mixture (34). The injection was repeated the following day. Eight days later extracellular $K^+$ and $Ca^{2+}$ were found to be elevated in the cortex and CBF decreased by about 5%. Corrosion casts of the cerebral arteries showed VSP that was not qualitatively altered by a short, therapeutic infusion of nimodipine. This may be the first report of a double-injection rat model. Quantitation of VSP 8 days after the first injection showed that 11 of 15 rats developed VSP that on average reduced the MCA diameter by 25% and that was associated with immunohistochemical evidence of inducible NOS in the brain and cerebral arteries (87). Ryba *et al.* reproduced in rats a model established in rabbits by Endo and coworkers (88,89). Both common carotid arteries were ligated in Wistar rats. Two weeks later, SAH was induced by two injections of 0.1 ml autologous arterial nonheparinized blood 2 days apart. Mortality was 25% and myonecrosis was not observed in the basilar arteries. No measurements of the degree of arterial narrowing were reported.

Okada and colleagues placed blood and blood fractions in silicone cuffs around the femoral arteries of rats (90). Whole blood produced spasm with a time course that was similar to that described in man. VSP, assessed by morphometric measurement, was maximal at 7 days. There was a good correlation in one animal between angiographic and morphometric measurements of arterial lumen diameter. Pathological changes were present in the vasospastic arteries. This model, like other clot placement models, has the advantage that usually there is a contralateral artery available to serve as an internal control. The disadvantages include lack of production of diffuse SAH since a preformed clot is placed only focally and, in the case of this model, use of a systemic rather than cerebral artery. It was used to study changes in collagen gene expression (91) and to show that cilazapril (92), antisense oligonucleotides to collagen (93), and various anti-inflammatory agents (94,95) prevent VSP. The degree of inflammation that occurs in this model is much greater than that usually observed in intracranial models, although the differences have not been studied quantitatively.

The Sheffield model of SAH in Wistar (23) and Sprague–Dawley (96) rats was described in 1995. SAH was created by inserting a pointed 3–0 monofilament nylon suture into the carotid artery in the neck and advancing it up the ICA until it perforated the right anterior cerebral

artery. Bederson and colleagues reported SAH in 89% and intracerebral hemorrhage in 11% of rats. Fifty percent died within 24 hr of SAH and the remainder were euthanized at 24 hr. The key finding was that CBF remained depressed immediately after SAH despite recovery of cerebral perfusion pressure, leading the investigators to perform a series of studies investigating this phenomenon (97,98) and other acute responses to SAH that have no known relationship to VSP (99–103). According to Veelken and colleagues, SAH resulted in 95% of animals (23). Three groups were studied. After perforation, the thread was removed and the common carotid artery unclamped in the neck or the carotid remained occluded with or without removal of the thread. None of animals that had the carotid artery reperfused survived the 3 hr experiment, whereas survival with the other methods was 57–67% at 3 hr. The model produced an acute rise in ICP and reduction in CBF on the side of the hemorrhage that persisted and became global with reperfusion, persisted if the common carotid artery remained occluded, and recovered if the thread was left in place and the carotid artery occluded. The degree of SAH produced may be quite variable with this model, pathological changes in the cerebral arteries are not reported, pharmacological features have been investigated only 3 hr after SAH (104), and the time course and severity of VSP are not addressed. Schwartz and colleagues compared injection of 0.3 ml autologous arterial blood into the cisterna magna via a needle placed through a parietal burr hole with SAH produced by the advancement of a 4–0 or 3–0 nylon suture up the ICA (37). Animals were monitored for 1 hr. Production of SAH using a 4–0 suture resulted in lower peak ICP compared to that of the other groups, whereas plateau ICP values after this were higher in the 3–0 suture group. CBF reductions were similar between the models, with the injection model resulting in more rapid CBF recovery. The 4–0 suture model tended to produce less SAH.

An anterior circulation model of SAH in Wistar rats was also described in 1995 (105). SAH was produced by transorbital injection of 0.3 ml fresh, autologous, nonheparinized blood into the chiasmatic cistern. Mortality was not reported. Acute increases in ICP and reductions in CBF were noted. Angiography before and 2 days after SAH showed an approximately 18% reduction in right MCA diameter.

Injection of autologous erythrocyte hemolysate into the cisterna magna of rats reduced the basilar artery diameter to about 60% of normal after 10 min (106). The narrowing could be alleviated by antisense oligodeoxynucleotides to preproET-1 (107) and increased HO-1 expression in the brain that did not occur after injection of other agents such as ET-1 that caused a similar degree of VSP (55). In this model there is induction of stress genes in the brain 24 hr after hemolysate injection that can be prevented with tirilazad-like antioxidants (108).

Zhao and colleagues placed a shunt from the common carotid artery of the rat to the chiasmatic cistern and produced SAH by allowing flow through the shunt for 30–90 sec (109). Acute increases in ICP, decreases in CBF, and death rates were studied for 2 hr.

One limitation of rat models is that severe (usually defined as >50% reduction in arterial diameter), prolonged VSP has never been reported. There may be no histological changes in the cerebral arteries. It has not been determined whether other accepted measures of VSP occur, such as resistance to vasodilators such as intravenous nimodipine. The latter has only been studied in the acute phase (110) or in response to single doses of nimodipine (34) or acid fibroblast growth factor (111). The advantages are simplicity of some of the models, they are economical, and there is substantial knowledge of the genome and availability of biological reagents such as antibodies for rat studies. An example of transclival clot placement and angiography over ensuing days in rats is provided in Fig. 11.1. The time course of VSP in a rat injection model is shown in Fig. 11.2.

### C. Cat

The ICA is an insignificant source of flow to the brain (47). Various external carotid artery branches form an external rete mirabile in the orbit and superior orbital fissure that may have a smaller, intracranial portion to which the ICA may or may not contribute. The rete mirabile gives rise to an anastamotic artery that contributes to the circle of Willis. This and the vertebral arteries supply the brain. The basilar artery contains six to eight layers of smooth muscle cells.

The earliest record of mechanical and electrical stimuli causing local spasm of cerebral arteries is that of Florey, (112), who studied cats. Additional early investigations in cats demonstrated the ability of a variety of autonomic blocking agents and vasodilators, such as chlorpromazine and papaverine, to prevent and reverse acute mechanically or electrically induced arterial spasm (113–115). Mechanical spasm was noted to reverse within 15 min, whereas spasm induced in the basilar artery but cutting it and producing SAH lasted at least 100 min (114). Kapp et al. characterized the substance in blood that caused acute spasm. Fractionation of blood showed that most of the activity resides in lysed platelets. The activity was not lost by boiling for 5 min, centrifugation, or incubation at 37°C for 18 hr but was removed by heating to 950°C for 30 min or passing the sample through a cation exchange resin. The cat basilar artery is constricted by

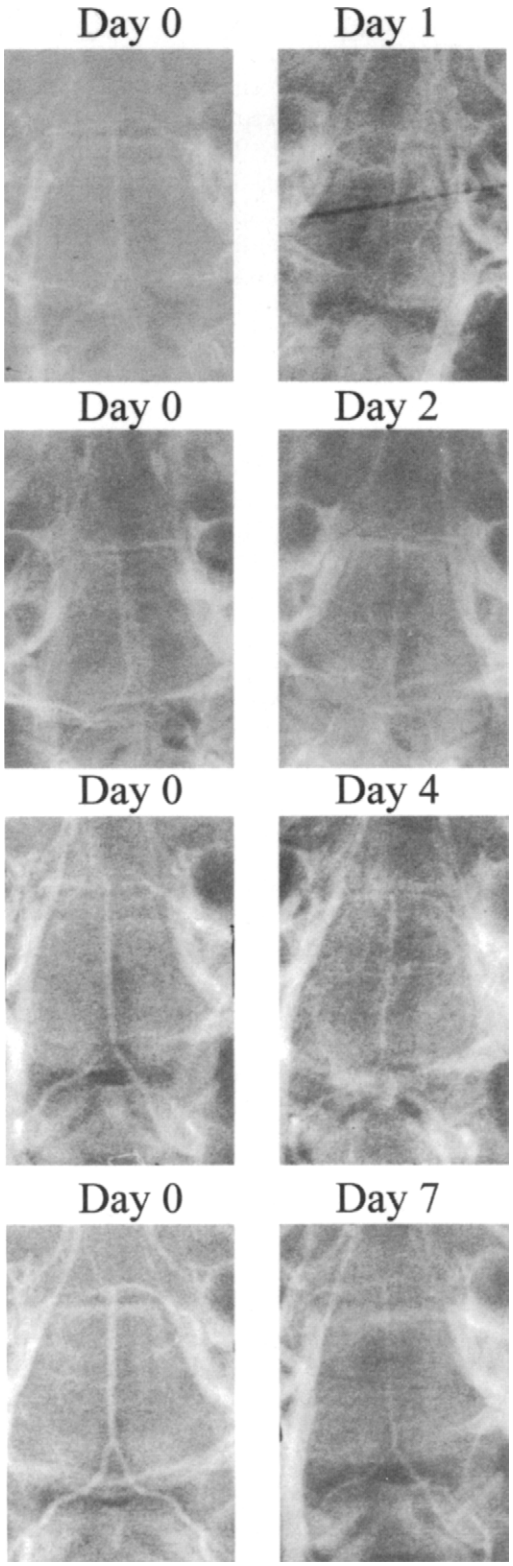


**FIGURE 11.1** Angiograms of rat basilar artery on day 0 (left) and 1, 2, 4, and 7 days after creation of SAH by placement of blood clot (200μl) against the basilar artery exposed transclivally. There is vasopasm at each time after SAH.

topical application of serotonin, angiotensin, norepinephrine, and whole blood and erythrocyte hemolysate (114–117).

Simmonds injected autologous arterial blood into the cisterna magna of cats and found that it was cleared rapidly (35). More extensive studies were reported in rabbits. SAH models used injection of blood over the cerebral cortex (118), percutaneously into the cisterna magna (119,120), or by implanted catheter, rupture of the MCA by incision or withdrawal of a surgically implanted needle, and topical applications of blood and blood products to the basilar artery exposed by removal of the clivus (115,121). Levitt and colleagues injected whole blood, hemolyzed whole blood, hemolyzed erythrocytes with or without their membranes, or fresh serum into the subarachnoid space over the cerebral hemispheres of cats (118). Suppression of electrocorticogram was most marked with solutions containing lysed erythrocytes, which is consistent with findings of other studies showing that lysed erythrocytes affect the cerebral cortex (46). Endo and Suzuki recognized that VSP is a delayed phenomenon after SAH (121). They applied fresh and incubated mixtures of blood and CSF directly to the basilar artery of cats. Acute spasm was noted in response to samples that were incubated for 3–15 days, corresponding to the time when VSP occurs in man. The spasmogenic substance in samples incubated for 7 days had similar properties on heat coagulation, ultrafiltration, Sephadex G-100 gel chromatography, disc electrophoresis, and spectrophotometry to oxyHb (122).

Mayberg *et al.* described creation of SAH in cats by injection of autogenous arterial blood (2 ml) percutaneously into the cisterna magna (119). Scanning electron microscopy showed endothelial cell furrowing that corresponded to angiographically visible VSP 4 hr and 1–7 days after SAH, but histopathological changes in the smooth muscle were not observed. Increased contractile responses of cerebral arteries to noradrenaline and serotonin were observed in this model (123). Subarachnoid injection of impure oxyHb produced ultrastructural changes in the tunica intima and myonecrosis in the tunica media (124). Trojanowski placed a catheter in the abdominal aorta of cats and connected it to a needle inserted transorbitally and percutaneously into the chiasmatic cistern (125). The author noted that in prior models, 2–10 ml was injected in dogs and 3–10 ml in monkeys and that injection of less than 2 ml failed to produce VSP. Bleeding on average lasted for 2 min and then ceased spontaneously within 25–60 sec of the peak in ICP, which ranged from 50 to 110 mmHg. The parameters were similar to those observed in a similar model in dogs (126). The average volume of SAH was calculated to be 2.4 ml. The animals were euthanized immediately.

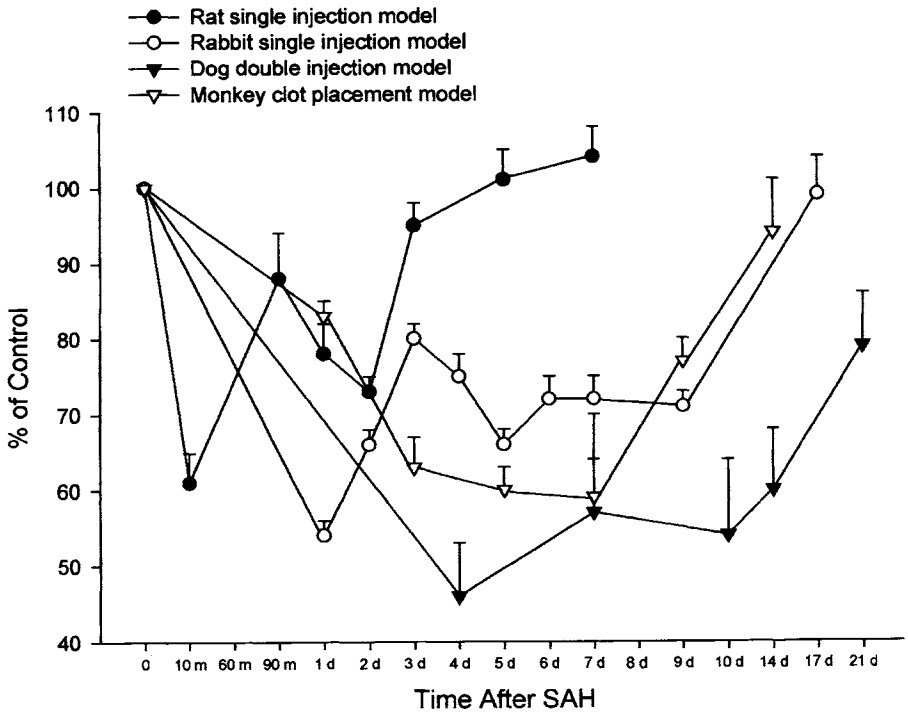


**FIGURE 11.2** Angiographic time course of VSP after SAH (m; minutes) in various species with data replotted from cited sources. Arterial diameter at each time is expressed as a percentage of the control artery diameter on day 0. In rats (•), after injection of 0.3 ml autologous blood into the cisterna magna there is acute spasm at 10 min that reverses to some extent at 90 min and then recurs and is maximal at 2 days (61). In rabbits (0), 6 ml autologous blood was injected into the cisterna magna (39). VSP was maximal 1 day later but persisted for 9 days. Day 14 time is from Edvinsson and colleagues (145). The double-hemorrhage dog model (▼), utilizing two injections of autologous blood (6–8 ml), 48 hr apart, produces significant VSP that lasts for 14 days (306). Clot placement against the middle cerebral artery of cynomolgus monkeys (▽) produces significant VSP starting on day 3 that persists until day 7 (307). All data are means ± standard errors.

Yamaguchi and Waltz briefly described exposure of the cat MCA extradurally and incision of it with a knife to cause SAH (127). The effects on CBF were studied for 2 hr. Clower and colleagues believed that arterial rupture was an important cause of pathological changes in cerebral arteries after SAH. This was based on their comparison of two SAH models in cats. Three injections of 2 ml autologous nonheparinized arterial blood on days 1, 6, and 11 did not cause light or electron microscopic changes in the cerebral arteries on day 16. On the other hand, when they exposed the cat MCA through a transorbital approach, placed a needle through it, and then avulsed the artery 7 days later, platelets and erythrocytes adhered to the endothelium, peaking 24 hr after SAH; this effect, but not VSP itself, could be prevented by pretreatment with the $TXA_2$ synthase inhibitor, OKY-1581 (128). This model had a 41% mortality and in survivors it produced intimal proliferation 16 days after SAH. Also, in keeping with its known pharmacological actions, heparin reduced the proliferation (129). The model was used to study acute effects of fibrinolytic agents on intimal platelet accumulation (130). Transorbital surgical exposure of the circle of Willis was used to insert needles into one or both ICAs of cats (131). These were subsequently removed and the resulting SAH was studied for its effects on the electroencephalogram, ICP, and CBF, which was generally reduced within the first few hours. VSP was not assessed.

Diringer *et al.* injected 2 or 3 ml (0.57–1 ml/kg) of autologous arterial blood percutaneously into the cisterna magna of cats on days 1, 2, 5, and 6 for a total of 9–12 ml (2.6–4 ml/kg) (132). Basilar artery VSP was noted in two of three animals studied and although there was no difference in CBF, reactivity to hypercarbia was blunted. Two injections of autologous fresh nonheparinized arterial blood, 3 ml (1 ml/kg), were administered 48 hr apart to the cisterna magna of cats. Angiography was not carried out but there was some endothelial convolution evident on scanning electron microscopy for 7

days after SAH (133). The effect of intrathecal fibrinolysis on CSF absorption (134), the effect of SAH on ICP pulse waves (135), the role of free radicals (136), the acute membrane changes (137), and the effects on CBF (120) were studied in the acute setting in cats by injection of blood into the cisterna magna.

A cat model of VSP was developed by Duff and colleagues (138). The basilar artery was exposed transclivally and blood or blood fractions were injected into the subarachnoid space (139). A variety of characteristic pathological changes developed 7 days after SAH and were shown to be due to the erythrocyte component of blood. Angiographic or morphometric assessment of the severity of VSP was not carried out.

The severity and time course of VSP and pharmacological responses are not characterized well and histopathological changes are not well described. Other disadvantages are that cats are not used routinely for other experiments so that less is known about their arteries and genome, and overall the models are used less frequently and are thus less well characterized.

### D. Rabbit

The rabbit does not have a rete mirabile (47). The ICA and vertebral arteries contribute to the brain blood supply, and occlusion of both ICAs or both vertebral arteries does not produce ischemia because the other arteries can compensate via the posterior communicating arteries. Like most lower species, there is a common A2 trunk with no anterior communicating artery. The extent of extraintracerebral anastamoses is uncertain. The CSF volume is believed to be relatively large in relation to brain and body size in comparison to some other species. The basilar artery is 700–800 μm in diameter and has five or six layers of smooth muscle cells.

Some models involve single or multiple percutaneous injections into the cisterna magna, transorbital injection, arterial rupture via craniotomy, injection through the exposed dura, and placement of blood in silicone cuffs around the cervical common carotid arteries. The most common end point is angiographic VSP, although morphometric measurements of perfusion-fixed basilar artery cross sections correlate very well with angiographic measurements (29).

Early investigators injected heparinized blood (0.5 ml/kg) into the cisterna magna of the rabbit in order to gain insight into mechanisms of clearance of blood from the CSF (140). Heparinization might have influenced the results; experiments in dogs suggested that modifying the double-hemorrhage model by injecting heparinized blood produced no spasm (32). Nevertheless, an important finding was that only about 20% of the blood remained in the CSF 5 hr later and most was cleared in 48 hr. Even repeated injections of blood every 2 or 3 days did not produce persistent intracranial blood. This is consistent with other reports that found that three injections of fresh nonheparinized blood (1 ml each; 0.33–0.5 ml/kg) into the cisterna magna of rabbits on days 1, 4, and 8 followed by euthanasia on day 15 resulted in little remaining subarachnoid clot at that time and no evidence of ultrastructural damage to the basilar artery endothelium or smooth muscle cells, unlike findings in dogs (141,142).

Mechanical causes of VSP were usually discounted since mechanically induced spasm of cerebral arteries generally lasted less than 30 min (113,143). Duckles *et al.* reported that stretching the basilar artery of rabbits to twice its initial length produced short, localized constrictions of the artery that lasted for at least 72 hr (144). The constrictions corresponded to areas of rupture of the internal elastic lamina. It does not seem likely that such extreme mechanical distortions occur after SAH.

Svendgaard *et al.* were the first to create SAH in rabbits by transorbital injection of 1 ml of autologous blood (0.3–0.5 ml/kg) into the chiasmatic cistern (24,145). Angiographic diameter of the basilar artery was reduced 27% 3–5 days later, after which time it slowly returned to normal size by 26 days. Sensitivity and maximal contraction of the basilar artery to norepinephrine and serotonin were increased by SAH. Logothetis *et al.* performed repeated measurements for 72 hr of cerebral pial arterial diameters by videomicroscopy and CBF measurements in control rabbits and those subjected to SAH by puncture of the MCA and superior sagittal sinus (146). SAH reduced the MCA diameter from $0.84 \pm 0.02$ to $0.46 \pm 0.02$ mm at 72 hr. CBF decreased to a similar degree. The model is complex, requiring two vascular punctures, continuous anesthesia for 72 hr, and rotating teams of researchers to work continuously during this time.

SAH was created in rabbits by injecting 1.25 ml/kg of autologous, heparinized, fresh arterial blood into the cisterna magna, followed by suspending the animals in a head-down position at 30° for 15 min (36). Angiography 3 days after SAH showed reduction of the basilar artery diameter to 45% of baseline in association with reduced CBF 4 days post-SAH. Reductions in both parameters were somewhat reversed by treatment with prostacyclin or carbacyclin and completely prevented by OKY-1581 or nutralipid. In contrast to the results of others (141), histopathological abnormalities occurred in the vasospastic arteries. Kassell and coworkers popularized the rabbit model of SAH (147). Five milliliters of autologous nonheparinized arterial blood was injected percutaneously into the cisterna magna. Subsequent experiments usually

used about 1 ml/kg. Angiography was utilized initially, but a highly significant correlation was found between arterial diameters measured by angiography and those by morphometric measurements of perfusion-fixed basilar artery diameters (29). Endothelium-dependent relaxation to ATP but not acetylcholine was impaired and contraction to serotonin was augmented 2 days but not 4 and 6 days after SAH. Two injections of blood (148,149) produced impaired relaxation responses to ATP and acetycholine and disruption of the blood–arterial wall barrier that persisted for 3 weeks. Spallone and Pastore injected 1 ml (0.5 ml/kg) of fresh arterial blood into the cisterna magna of rabbits on two occasions, 24 hr apart (150). Mortality was 20% when a single follow-up angiogram was obtained, whereas it was almost 50% when angiography was performed before SAH and on day 3. VSP was most severe on day 3 and corresponded to a 15–20% reduction in basilar artery diameter. The authors questioned the necessity of two blood injections in this model since VSP did not seem to be substantially more severe than after one SAH, although more substantial and prolonged VSP is usually produced by repeated blood injections in rabbits (151) as in other species. Numerous publications from the laboratory of Kassell and colleagues as well as others have demonstrated 20–54% reductions in basilar artery diameter 48 hr after single SAH and that this can be attenuated by pretreatment of animals with U74006F (152,153), antithrombin-III (154,155), nicardipine (156), deferoxamine (157), CGRP (158,159), U88999E (a tropolone derivative that inhibits lipid peroxidation and is a $Ca^{2+}$ antagonist) (160), an ET receptor antagonist (ETant) (161), intracisternal vasoactive intestinal peptide (159), ramacemide (an excitatory amino acid receptor antagonist) (162), histidine (163), bosentan (164), CGS 26303 (an inhibitor of endothelin-converting enzyme) (165), tissue plasminogen activator (166), deferiprone (167), antibodies to ICAM-1 and CD18 (168), cromokalin (169), nontoxic endotoxin analog (170), antisense oligodeoxynucleotides to NF-κB (171), and $ET_A$ receptor antagonist TBC 11251 (172). VSP 48 hr after a single blood injection was completely reversed by an intracardiac infusion of papaverine ($10^{-4}$ M), sodium nitro prusside (SNP) ($2 \times 10^{-4}$ M), and adenosine ($10^{-5}$ M), suggesting that an irreversible component of VSP does not develop in this model, at least at this time (173). The resistant component may develop later. Experiments were conducted in rabbits in which 3 ml/kg autologous arterial blood was injected over 4 hr into the cisterna magna (39,174). Serial angiography and pharmacological studies *in vitro* showed a papaverine-insensitive component of arterial narrowing developing 3–5 days after SAH and progressing to comprise 63% of arterial narrowing by 9 days post-SAH. This coincided with increasing stiffness and decreasing contractility of the basilar arteries. The arterial narrowing was prevented by continuous treatment with a $Ca^{2+}$ antagonist, 8-chlorodiltiazem (175). These investigators injected more blood that produced a 54% reduction in basilar artery diameter after 24 hr, which was greater than that observed in many other experiments using rabbits. In some cases, cisternal saline injection prevented VSP in rabbits, possibly by promoting clot clearance (158).

A double-hemorrhage model in rabbits was associated with significant reductions in activities of protein phosphatases 1 and 2a, 2 and 4 days after SAH (176). VSP was reduced in this model by oral treatment with ET antagonists, bosentan, or PD155080 started after SAH (151). Controls developed severe VSP with reduction of basilar artery diameter to 34% of normal. The injection of more blood was associated with findings similar to those of Vorkapic *et al.* (177) in that VSP was not completely reversible pharmacologically (151).

The time course of changes in TCD flow velocity of the basilar artery was monitored after injection of 0.5 ml/kg blood percutaneously into the rabbit cisterna magna (178). Flow velocities were 22 ± 2 cm/sec prior to SAH, reduced to 13 ± 1 30 cm/sec after injection of saline, increased to 47 ± 7 cm/sec, and returned to normal within 5 min after saline injection. After SAH, they decreased to 12 ± 2 cm/sec at 30 sec and then slowly increased to 39 ± 7 cm/sec at 5 min and returned to control values at 15 min. By day 3, there was a 41% increase in mean flow velocity to 31 ± 4 cm/sec and values declined but remained elevated until day 6. Autoregulation was impaired after SAH in this model.

Endo and colleagues opined in 1988 that available SAH models did not replicate human VSP because they did not reliably produce cerebral ischemia during VSP (88). There is no reduction in CBF after single SAH in rabbits (179). Bilateral common carotid artery ligation was performed, followed 2 weeks later by SAH induced by two injections of autologous blood (2.5 and 1.5 mls in 3-to 3.5-kg rabbits) 48 hr apart. The basilar artery was constricted 23% on Days 3–6 on average and cerebral infarction was observed pathologically in 2 of 13 (15%) animals. Subsequent experiments showed reduction in VSP and prevention of ischemic deficits by treatment with E5880, a platelet-activating factor antagonist (180). Cerebral ischemia could be produced in rabbits by injection of concentrated erythrocyte hemolysate into the cisterna magna (181). There was a good correlation between neurological condition of the animals and CBF but not angiographic spasm.

The interpeduncular cistern of 2- to 2.5-kg rabbits can be accessed by inserting a needle from the ventral surface of the skull through the synchondrosis between the

basal portions of the sphenoid and occipital bones (182). Injection of $PGF_{2\alpha}$ produced electrocardiographic changes that were reported to mimic those occurring after SAH. Egemen *et al.* exploited this route by drilling away the clivus to expose the basilar artery (183). SAH was created by puncturing the basilar artery with a 22-gauge needle. A 68% reduction in basilar artery diameter was noted on angiography 5 days later. A therapeutic effect of iloprost, a stable analog of prostacyclin, was demonstrated in the model (184).

The single-injection rabbit model produces mild to moderate VSP that lasts for days. There are conflicting reports as to whether histopathological changes occur, although there seems to be development of a pharmacologically resistant phase. The model is simple and economical, but there is a paucity of genetic knowledge in comparison to that for other rodents. The time course in one version of this model is shown in Fig. 11.2.

Pickard *et al.* placed autologous blood in polyvinyl chloride cuffs around the common carotid arteries of rabbits (185). VSP was not studied but the arteries that were exposed to blood had increased eicosanoid production compared to control vessels. Macfarlane and colleagues found that this method constricted the artery when a silicone sheath was placed around the artery and it was filled with clotted human blood (186). VSP developed 24–48 hr later and persisted for up to 6 days. The model was used to demonstrate that laser treatment dilated spastic arteries and prevented VSP when used prophylactically. The relatively large size of the carotid arteries allowed study of the effects of transluminal angioplasty (187).

### E. Dog

The vertebral arteries make a relatively greater contribution to total CBF in dogs than in primates (47). The ICAs are important, but less so, and there are extensive anastamoses between the intracranial ICA and branches of the external carotid artery. There is a single A2. The volume of the CSF space of the mongrel dog used in most VSP research was estimated to be 11 ml (141). The diameter of the basilar artery is about 1.5 mm and there are six to eight layers of smooth muscle.

Injections into the cisterna magna and chiasmatic cistern, rupture of arteries, and placement of clot against the MCA have been reported. In 1928, Bagley studied the histopathology of clearance of venous blood injected into the cisterna magna of dogs (188). VSP had not been described and the purpose of this and other early investigations in which blood was injected percutaneously into the cisterna magna of dogs was to study mechanisms of clearance of SAH (189,190). Jackson injected various fresh and incubated blood fractions into the cisterna magna of dogs (191). Hemogenic meningitis appeared to be due to Hb, heme, bilirubin, or a related pigment.

Topical applications of putative spasmogens such as Hb and iron solutions to the dog basilar artery showed that ferric iron caused a contraction, whereas Hb and ferrous iron did not (192). This technique, which avoids some of the limitations of studies of arteries *in vitro*, has been used more frequently in lower species.

Lougheed and Tom attempted induction of SAH in 50 dogs by the transoral, transclival route (193). Five milliliters of arterial blood was injected and SAH was produced in only 42%, with technical failures in the remainder that were due to IVH, trauma to the third ventricle, meningitis, subdural hematoma, and failure to produce any ICH. A percutaneous approach to the chiasmatic cistern via the optic foramen was reported the following year (194). The model was used to investigate ICP changes acutely after SAH and acute responses to blood and PG injection rather than delayed VSP (195). Brawley and colleagues provided the initial and now classic description of experimental VSP (196). A strain gauge was placed around the intracranial ICA, and a thread was placed around the anterior cerebral artery via craniectomy. The strain gauge monitored the diameter of this artery after SAH which was created by avulsion of the anterior cerebral artery by pulling on the thread. The ICA decreased in size by 6% 5 min after SAH, returned to normal at 1 hr, and then reconstricted about 20% on days 4–6. A role for serotonin was excluded even at this time since repeated intraarterial injections did not produce chronic VSP and the chronic phase could not be prevented by methysergide. Nagai and colleagues modified this model by placing the thread into the posterior communicating artery and by assessing VSP by angiography (197). There was a 25–40% reduction in arterial diameters within 30 min of SAH; the diameters returned to normal by 2 hr. Spasm was again present 24 hr later. Similar effects seemed to be produced by injection of 5 ml blood (0.33–0.5 ml/kg) into the cisterna magna. The model was used to demonstrate decreased contractility of vasospastic arteries during chronic VSP 7 days after SAH (198) and reversal of chronic spasm with papaverine or isoxsuprine (199). In another approach, 2–10 ml fresh blood was injected into the chiasmatic cistern via a needle inserted percutaneously through the optic foramen (200–202). In only one study was VSP assessed by serial angiography in a small number of dogs undergoing injections of different volumes of blood (200). Some animals had VSP days after injection, but an exact analysis of these results cannot be performed. CBF was reduced acutely but autoregulation remained intact. Ultrastructural changes in the

vasospastic arteries are reported to occur after injection of large volumes of blood (>6 ml) (203,204).

Kuwayama *et al.* injected 2 ml fresh arterial blood (0.13–0.29 ml/kg) into the cisterna magna of dogs (205). Angiography showed acute spasm that reduced the basilar artery diameter an average of 37% at 30 min. There was slight dilation and then recurrence of an identical degree of narrowing 2 days after the SAH. Important modifications made by two groups were careful standardization of angiography and maintenance of Trendelenberg position for 15 min after injection to promote pooling of blood in the basal cisterns (206,207). This model was used with little modification for numerous studies that documented at 2-day post-SAH a relatively constant 25–35% reduction in basilar artery diameter (30) and, for example, reversal of VSP at this time with $Ca^{2+}$ channel antagonists (206,208), nitrates (204), angiotensin-converting enzyme inhibitor (209), a TX synthase inhibitor OKY-1581 (210), and nonsteroidal anti-inflammatory agents (211). Numerous biochemical measurements were carried out, upon which theories of the pathogenesis of VSP were based (212,213). This model does not produce severe VSP. Pathological changes in the spastic arteries do not occur (214) or at least are not marked (210) after single blood injections, and other markers of VSP, such as pharmacological changes of decreased contractility and compliance and resistance to vasodilator drugs, are not found (215). Injections of blood into the chiasmatic cistern were reported to cause pathological changes, although the volume of blood injected in these experiments was greater (203,204). Interpretation of many of these early studies is difficult because they often utilized small numbers of animals and qualitative or unstandardized assessment of angiographic VSP, and they assessed drug effect on acute but not delayed vasospasm. In addition, most studies did not measure drug levels or perform additional testing to confirm that the drug effect was associated with alteration in the specific biochemical pathway which the drug was proposed to exert its action.

Simplistically, in order for a spasmogen to fulfill criteria for a cause of spasm, it should produce VSP when administered into the CSF at the concentration and for the time that occurs naturally after SAH. This is difficult to model since concentrations of substances at their site of action in the arterial wall may be difficult to measure. Researchers have tried to model VSP by injecting various spasmogens and blood components into dogs (216). Sasaki *et al.* studied the effects of intracisternal injection of the lipid peroxide, 15-hydroperoxy arachidonic acid, on the angiographic diameter of the basilar artery of dogs (43). Two milligrams of compound produced mild constriction associated with ultrastructural damage to smooth muscle and endothelial cells that lasted for 7 days, which was the last time examined. Possible mechanisms included free radical-mediated damage and detergent effects. Injection of ET (217), dextran or latex beads (216), and isolated erythrocytes (213) produced some degree of VSP in dogs.

A dog model of SAH was created in which blood was shunted from the femoral artery into the chiasmatic cistern (126). Spontaneous arrest of the hemorrhage occurred when the ICP reached blood pressure. By measuring flow through the shunt and its duration, it was determined that 18 ml (1 ml/kg) was lethal in the chiasmatic cistern and 30 ml was lethal in the cisterna magna (218).

In an attempt to elicit reliable production of more severe, prolonged VSP associated with histopathological changes in the affected arteries, Eldevik and coinvestigators gave multiple injections of blood into the cisterna magna of dogs (214). A variety of different protocols were used, and they produced VSP and Hyc but not histopathological or ultrastructural changes in the basilar artery. The commonly employed double-hemorrhage model of SAH was reported 2 years later by Liszczak *et al.* (30,141,219). They injected 4 ml fresh nonheparinized blood into the cisterna magna of dogs on days 1 and 3 and then studied the animals pharmacologically and pathologically on day 5. Angiographic VSP was present in 82% of animals on day 5, and in 50% there was >30% narrowing with an average diameter of 70% of baseline. Histopathological changes were observed in the vasospastic basilar arteries and they could not be dilated by modest doses of intraarterial papaverine. This model has been used extensively to study VSP and the degree of spasm produced and accompanying pathological changes extensively documented. The second injection of blood has been administered 2 or 3 days and VSP assessed 5 (220) to 7 days (221) after the first injection. The standard procedure is to use percutaneous injections, but implantation of catheters into the prepontine cistern for administration of blood and drugs was described (222). VSP was effectively treated with corticosteroids, ibuprofen (223), nimodipine (208), numerous ET antagonists (164,224–226), HA1077 (227), a leukotriene antagonist (228), cyclosporine A (216), lipopolysaccharide (229), various types of phosphodiesterase inhibitors (230), inhibitors of protein and nucleic acid synthesis (231), and ebselen (232). FK506 failed to produce an effect (233,234). Disappointingly few studies have carefully documented whether pharmacological effects were achieved *in vivo* and what drug levels were obtained. One exemplary study found that trifluoperazine could not reverse established VSP but massive doses had some prophylactic effect (220). The production of more severe spasm with two injections and/or a greater volume of blood is consistent with experiments in which increas-

ing volumes of blood delivered intracisternally within 24 hr led to increasingly severe spasm and addition of another injection 3 days after the first two led to even more intense VSP (235). Injecting 7 ml (0.5–0.7 ml/kg) and 3 ml 2 days later reduced the basilar artery diameter to 54% of normal 8 days after the first injection and resulted in persistent narrowing for as long as 3 weeks (221). Only a few studies have utilized three or more injections to evaluate a drug treatment (236). Two-injection models have been used to support a role for erythrocytes and platelet-rich plasma (237), ET (238), protein kinase C (239), NO (240), metabolic failure (241), preservation of cAMP-dependent relaxation mechanisms (242), and other biochemical and molecular biological changes (243–245).

Regional CBF was measured in multiple areas in the double-hemorrhage dog model with or without prior bilateral common carotid artery ligation (246). There were no differences in CBF 7 days after SAH. Pressure autoregulation was intact. The only abnormality demonstrated was poor $CO_2$ reactivity in some regions in the group with common carotid artery ligation. There are reports that CBF is reduced 7 days after SAH, but the vast majority of the data suggest that this model does not alter CBF by itself. Ischemic stroke does not develop, and the animals appear healthy with both single- and double-hemorrhage dog models (30).

Jin and colleagues compared the double-hemorrhage dog model with clot placement against the MCA and found that severe narrowing of cortical arteries could be produced by clot placement (32). Clot placement in dogs was described in an abstract (25). Shiokawa *et al.* replicated in dogs the clot placement method described later that had been used in monkeys (26). A 50% reduction in MCA diameter occurred 7 days later and could be prevented by papaverine.

The dog double-hemorrhage model is well characterized. The time course of VSP is similar to that in man (Figs. 11.2 and 11.3), severe VSP can occur, and there are histopathological changes in the cerebral arteries and resistance to pharmacological vasodilators. The disadvantages include cost, the need for two injections of blood which leads to some uncertainty as to the time after SAH, and lack of genetic knowledge of the dog.

Percutaneous transluminal angioplasty of the vasospastic dog basilar artery was performed with a 1-French catheter and 0.4 μH 4-mm silicone balloon (247). Megyesi placed blood-filled silicone cuffs around the extracranial ICAs of dogs after microsurgically removing some of the arterial adventitia (248). Angiographic VSP developed 7 days later and was associated with the characteristic morphological and pharmacological features of VSP.

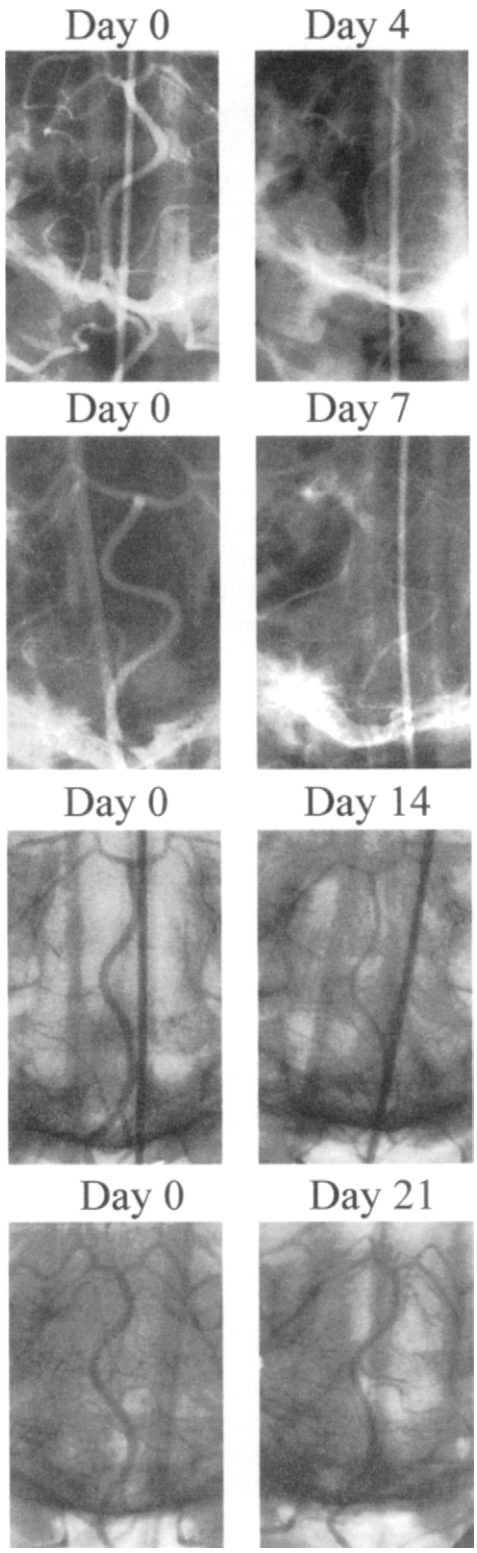


**FIGURE 11.3** Representative angiograms on day 0 prior to SAH and then 4, 7, 14, and 21 days after the first of two cisterna magna injections of 6–8 ml autologous blood 48 hr apart. There is significant VSP 4, 7, and 14 days after SAH.

### F. Pig

Takemae *et al.* noted that the pig spontaneously develops atherosclerosis with advancing age, which distinguishes it from many animals that are used as models of human vascular disease (249). They inserted silicone catheters into the prepontine cistern of pigs and then gave two injections of autologous nonheparinized arterial blood (12 ml each, 0.5–0.75 ml/kg) 48 hr apart. Of six animals, angiography 48 hr after the second injection showed VSP in four, although the severity was not stated. Histopathological examination of the cerebral arteries 7–24 days after the first SAH showed a variety of changes, including intimal proliferation in 75% of animals, splitting and fragmentation of the internal elastic lamina, and necrosis of medial smooth muscle cells in 92%.

A clot placement model was developed in immature pigs (250). The left MCA was exposed surgically and clotted autologous whole blood placed against the artery. The clot was covered with a silicone cup and VSP was assessed by morphometric measurements 10 days later. Whole blood caused a 55% reduction in lumen area that was associated with pathological changes in the MCA that were characteristic of VSP. The model was utilized to show that only blood fractions containing Hb caused VSP in this model. Three milliliters (0.33–1.2 ml/kg) of autologous nonheparinized venous blood was injected over the cortex of newborn piglets (251). Four days later, vasodilator of pial arterioles was impaired to iloprost, $PGE_2$, and histamine but not to SNP. Constrictions to leukotriene $C_4$ and ET-1 were potentiated. Many changes in pharmacological reactivity have been reported after SAH. Direct comparison of adult and newborn animals of the same species was not reported so as to allow one to determine the effect of postnatal development on the response to SAH.

The pig remains a little used species for VSP research.

### G. Nonhuman Primate

The arterial circulation of the brain of nonhuman primates used in SAH models is very similar to that in man (47). The main difference is that the precommunicating segments of the anterior cerebral arteries fuse to form a single A2 segment. There is no anterior communicating artery. Seventy percent of CBF is derived from the carotid circulation and 30% from the vertebral arteries. The similarities to man include the paucity of extraintracranial anastamoses. The volume of CSF in *Macaca fascicularis* is about 10 ml and in *Macaca mulatta* about 13–15 ml. The MCA in the former is approximately 1 mm in diameter and contains six to eight layers of smooth muscle cells. SAH has been induced in nonhuman primates by percutaneous injection of blood into the cisterna magna, percutaneous or open transorbital injection into the chiasmatic cistern, arterial rupture by removal of a suture placed via craniotomy into an intracranial artery, and craniotomy and clot placement.

Monkey pial arteries were less responsive to mechanical stimuli than cat and dog arteries (252). Topical applications of blood or rupture of a branch of the basilar artery caused acute spasm of the basilar artery of monkeys that generally lasted minutes (252,253). It was immediately recognized that spasm did not occur unless the arachnoid was completely opened and blood was introduced into the subarachnoid space. Acute spasm could be reversed by an α-adrenergic blocker, similar to the effects reported in cats (143). Simeone *et al.* attempted to produce more prolonged spasm by injecting blood into the subarachnoid space of rhesus monkeys (*M. mulatta*) or by avulsing a needle placed previously into the ICA (254). Sixty-five percent of animals developed VSP that lasted over 1 hr and one animal survived and was found to have VSP after 4 days. Spasm was worse with vessel rupture. Large monkeys were chosen in order to allow repeated angiography. Size is not necessarily an important criteria; a model of Parkinson's disease was developed in the fruit fly (255). Landau and Ransohoff injected 2 or 3 ml blood into the subarachnoid space of African green monkeys via a catheter implanted in the cisterna magna and compared angiographic VSP over time with that resulting from squeezing the ICA, MCA, and anterior cerebral arteries with forceps, squeezing plus blood injection, and squeezing and rupture of the artery and additional subarachnoid blood injection into the cisterna magna (256). Late spasm occurred in 29% of animals in the last group and fewer than 13% in the other groups, which supported the hypothesis that arterial rupture was important in the pathogenesis of VSP; however, differences in volume of blood released and/or injected are another possible explanation. For example, Echlin injected 2–5 ml fresh autologous arterial blood into the subarachnoid space of monkeys exposed ventrally through the vertebral bodies of the lower cervical spine. Angiographic VSP was present at 24–72 hr in 16 of 18 monkeys and in 7 of 9 followed for 7 days. Transfixing an intracranial artery with a needle without producing hemorrhage caused spasm lasting less than 2 hr. Clear serum was not spasmogenic (257). Clower *et al.* (258) are also proponents of the theory that arterial wall rupture is required to produce structural changes in the arteries and to have some role in VSP. In rhesus monkeys, SAH was created by cisternal injection of 3 ml autologous nonheparinized blood and the effects were compared to those produced by avulsing the MCA by pulling on a ligature that had been placed on it 7 days earlier. It seems inconceivable that this would result in a

similar degree of SAH as that of the cisternal injection; indeed, more hemosiderin staining was seen pathologically in the avulsion model. These animals also developed more morphological damage to the cerebral arteries.

Roy *et al.* produced SAH by inserting a needle into the ICA of the squirrel monkey (*Saimiri sciurus*) and then withdrawing it (259). Some animals were made hypertensive, but this did not seem to affect the clinical condition after SAH. In some animals, the hemorrhage was repeated 7 days later. None showed evidence of clinical spasm, although pathological examination showed formation of saccular aneurysms at the site of needle puncture.

Weir *et al.* gave weekly injections of 4 ml (0.6–1.5 ml/kg) of autologous blood into the subfrontal subarachnoid space of 16 rhesus monkeys via a needle placed through a low frontal burr hole (260). Mortality with successive injections was 6% after the first injection and 37% after the fourth. VSP was short-lived and mild, amounting to $-24 \pm 19\%$ at 15 min, $-2 \pm 18\%$ at 24 hr and $6 \pm 16\%$ at 7 days. There was no significant difference in the degree of spasm with each successive injection. Electrocardiographic changes were noted to be related to the height of the ICP increase. A series of experiments utilizing this model of acute spasm demonstrated acute decreases in CBF associated with spasm (261,262), impaired CBF responses to changes in $p_aCO_2$ (263) and blood pressure (264), and exclusion of a role for serotonin in acute spasm (265). Subsequent experiments used cynomolgus monkeys (*M. fascicularis*) and demonstrated increasingly severe reductions in CBF and increased death rate with increasing volumes of blood injected (266). Since the acute pathophysiological responses were volume related, Weir's laboratory studied the relationship of volume of subarachnoid blood injected to chronic vasospasm. Larger volumes of blood were injected ($1.5 \pm 0.2$ ml/kg total in three injections over 5–7 min) and this resulted in severe, >50% VSP after 7 days in 50% of animals that was seen mainly in animals with thick clots detected on CT scans (267). The unilateral clot onlay method was devised at the University of Alberta (28) in order to maximize this effect and provide a control artery. Serial angiography can be done by repeated retrograde catheterization of the right brachial and axillary arteries. A frontotemporal craniectomy was performed and an arachnoid membrane opened widely over the ICA and anterior and middle cerebral arteries. Arterial blood was drawn from the angiogram catheter and allowed to clot. It was cut into pieces and placed against the exposed cerebral arteries. Acute mortality was 10%. All animals developed some spasm 7 days after SAH and severe spasm (>50% reduction in arterial caliber) occurred in one fourth. The time course of VSP has been characterized (Figs. 11.2 and 11.4). An advantage is the ability to produce prolonged, severe VSP with all of the features of human VSP. Pathological changes are observed in the vasospastic arteries, CBF and autoregulation are impaired, the vasospastic arteries are resistant to dilation with intravenous nimodipine, and they show other pharmacological characteristics of VSP such as decreased compliance, contractility, and endothelium-dependent relaxation (28,268–270). An extensive series of investigations in this laboratory and in those of Yuji Handa and Richard Pluta, among others, have documented effects of clot removal by surgery or intrathecal fibrinolytic therapy, prophylactic effects of U74006F, iron chelators, calcitonin gene-related peptide, and ET antagonists; and reversal of VSP by NO donors. These investigations have also provided insights into the pathogenesis of VSP that are reviewed elsewhere in this text (271–285). The arteries are large enough to accommodate balloon angioplasty catheters (286). The contralateral arteries serve as internal, normal controls, although for molecular biological studies it is possible that surgical stress alters gene expression as it has been shown to do in the vessels of stressed animals (6). Disadvantages are cost and risks of transmission of simian herpesvirus to the investigators, which are currently minimized by antibody testing of monkeys.

Other investigators reported acute 8–44% spasm developing for up to approximately 1 hr after cisterna magna injection of fresh, autologous blood in baboons (*Papio cynocephalus*) or cynomolgus monkeys with complete resolution of spasm by 7 days in 50–75% of cases (287,288). Kim *et al.* injected 4–8 ml (0.5–2 ml/kg) arterial blood intracisternally (the exact site was not provided) in rhesus monkeys (289). Severe VSP, which was not defined, was observed 1 or 2 weeks after the SAH and treatment with aminophylline or isoproterenol had no statistically significant effect on the incidence of VSP. Norwood and colleagues described an average of 30% reduction in intracranial artery diameters in one-third of animals 1 week after cisterna magna injection of 5 ml (0.6–0.8 ml/kg) of fresh arterial blood (290).

Simeone *et al.* injected 3 ml of fresh autologous arterial blood into the subarachnoid space of 14 rhesus monkeys or punctured the intradural ICA with a 30-gauge needle in 15 (5–12 kg) (291). The patterns of CBF reduction that developed were different between methods. Acute VSP of approximately 50% reduction in arterial diameter was observed in both groups, although quantitative statistical assessment of all animals was not provided. The cranium was open, so ICP was not markedly elevated in either group. Similar experiments whereby the MCA or ICA of rhesus or other monkeys were punctured with needles of various sizes reduced arterial diameters on angiograms done 5 days later by 30% (292–294). Jakubowski *et al.* created SAH in baboons by avulsing the posterior

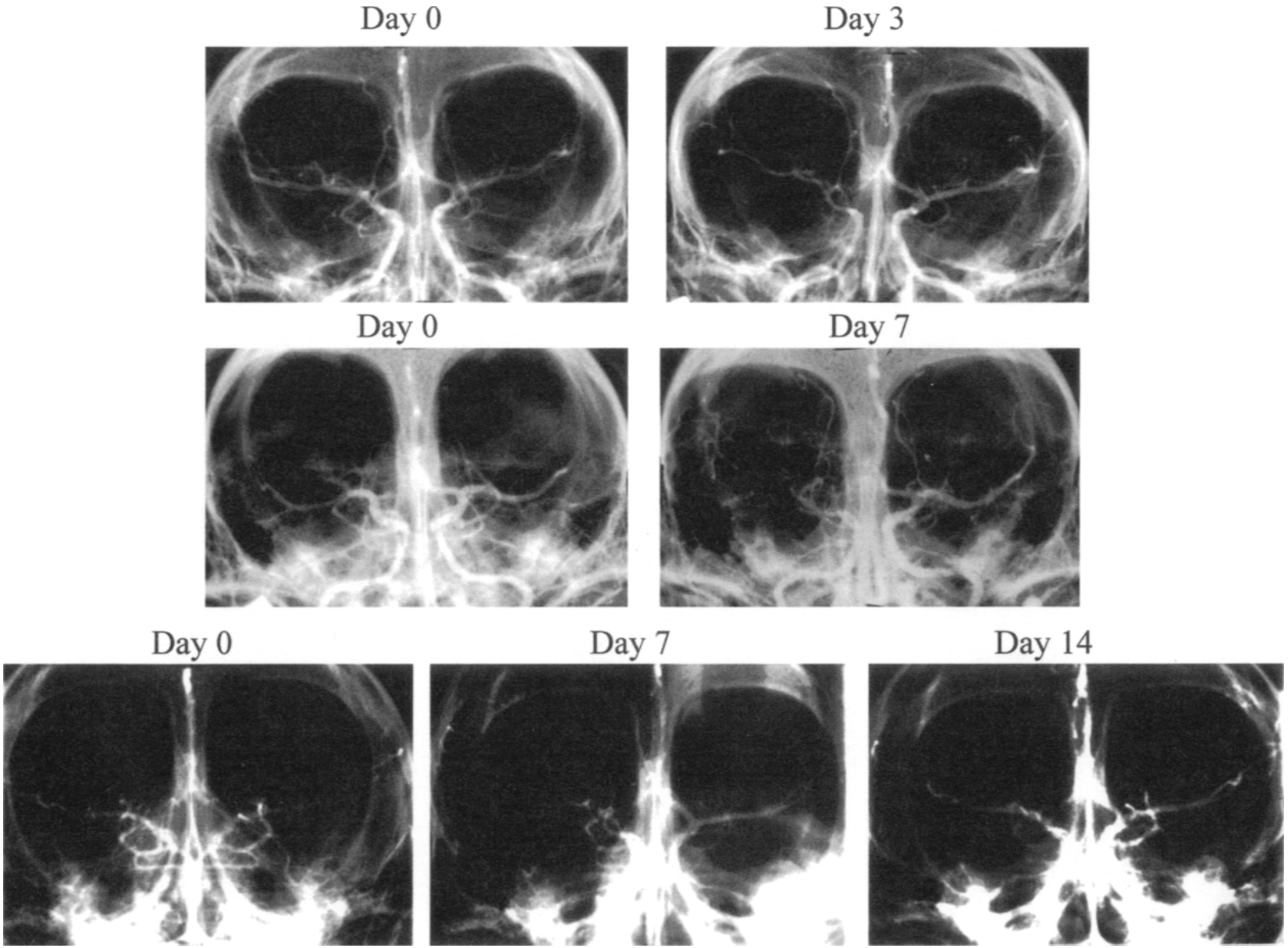


**FIGURE 11.4** Angiography in a clot placement model in cynomolgus monkeys. Comparison on baseline (day 0) angiograms and angiograms taken 3, 7, and 14 days after placement of clot against the right ICA, MCA, and anterior cerebral arteries shows VSP of these arteries on days 3 and 7 that resolves by day 14.

communicating artery and by pulling on a suture that was brought out through the skin and that had been placed around it 3 days previously (295). ICP increased rapidly and CBF decreased and then recovered in good-grade animals or remained depressed. Autoregulation and blood flow responses to changes in $p_aCO_2$ were impaired transiently. The model seems to reproduce the acute effects of SAH, but since angiography was not done it is unknown whether VSP occurred days later.

SAH was created in rhesus monkeys by transorbital exposure of the ICA, puncture with a threaded needle, and removal of the needle after the wound was closed (296). Duckles *et al.*'s publication is the only one to report that this method caused moderate to severe VSP lasting for 10–14 days.

Autologous blood (0.3–5 ml), was injected into the lumbar subarachnoid space of nine rhesus monkeys and serial spinal angiography was performed frequently over 24 hr (297). No immediate or delayed spasm of the anterior spinal artery was seen, which was a different finding from their results with intracranial arteries. Alksne *et al.* studied 16 stump tail monkeys (*Macaca arctoides*) weighing 6–11 kg (298). SAH was created by performing an upper cervical laminectomy and inserting a silastic catheter into the prepontine cistern. After 5 days, 4 ml of nonheparinized autologous arterial blood was injected. Half the animals were treated with phthalazinol, a phosphodiesterase inhibitor. This prevented VSP on angiograms done immediately after SAH and vasonecrosis that was seen pathologically at autopsy 10 days later.

Few of the aforementioned monkey models produced severe, prolonged VSP. One method to overcome this may be to inject more blood. Svendgaard *et al.* used two injections of blood 24 hr apart into the cisterna magna of baboons and a third 24 hr later into the chiasmatic cistern via a transorbital approach for a total of 14–33 ml of blood (1.6 ml/kg) (299). Mortality was 32%, average reductions in arterial diameter were 10–20% 7 days later, and severe VSP was not seen. Sensitivity to and maximum contractions of several agonists were increased in pial arteries removed 7 days after SAH (300), in contrast to the decreased contractions noted in other models (270). Another complex model was developed by this group in squirrel monkeys (*S. sciureus*), in which blood was injected in multiple aliquots via catheters that had been implanted days before into the interpeduncular and cistern and cisterna magna (average of 3.6 ml/kg) (301). Six days after SAH, there was 44–80% constriction of the most severely affected artery, which was different in each animal. Mortality was 17%. There wasreduced CBF and increased deoxyglucose uptake during VSP 6 days after SAH (302).

### H. Other

SAH was induced in goats by placement of a silicone catheter through a parietotemporal burr hole into the basal cistern (303). Blood, 10 ml (0.25–0.30 ml/kg), was injected after the animals had recovered and were awake. VSP was not measured but CBF was found to be reduced by 28% 3 days after SAH. It was normal by 5 days. The model is like many of those previously discussed in that VSP has not been adequately assessed, and morphological and pharmacological changes in the arteries are incompletely described.

## VIII. Ethical Considerations

Some individuals have ethical concern about use of animals in research. Hsu suggested that researchers adhere to the same criteria in conducting animal studies as those used by the clinician for clinical trials, randomization is appropriate for most studies, and blinding should be employed as much as possible (31). Placebo controls and pretrial assessment of sample sizes should be employed, and mortality and morbidity rates of procedures should be reported.

Can useful information about human VSP be obtained from animal models? If not, then they should not be used. The first step, therefore, is to characterize the human disease and then assume that a model that reproduces most or all of the identified characteristics will have the same mechanisms and that effective treatments in the animal model will be effective in man. Such an approach has proved useful for other human diseases. Many animal studies of ischemic stroke, however, show drug efficacy using dose schedules that are not applicable to human stroke, such as pretreatment or immediate treatment. Although these may allow insight into mechanism, they do not necessarily translate immediately into useful human therapy. It has been asserted that advances will occur only if advanced technologies are applied to humans (304,305). The balance between animal and human studies has varied over the years and research efforts will be determined to some extent by the varying societal standards and rules applied by regulatory agencies. This textbook, however, shows that important information about SAH and VSP has been obtained from animal models. The challenge is to continue to characterize models carefully and modify or abandon them and the theories on which they are based if necessary on the basis of continuing advances in knowledge.

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CHAPTER 12

# MOLECULAR BIOLOGY AND GENETICS



## I. Introduction

Studies have demonstrated that experimental SAH is associated with changes in gene expression in cerebral arteries and brain tissue. There is evidence in a broad sense that such changes are in some way important in VSP. For example, Shigeno *et al.* administered the RNA synthesis inhibitor, actinomycin D, to dogs for 5 days after SAH and found that this completely prevented VSP (1). In subsequent experiments, however, more complex results were obtained in that low doses of another RNA synthesis inhibitor, dactinomycin, aggravated VSP, whereas higher doses prevented it in dogs (2). Human studies were carried out, but many different protocols were used in small numbers of patients so that definitive conclusions cannot be drawn. In addition, the nonspecific and broad pharmacological effects of these drugs make it impossible to make any statements about etiology or pathogenesis of VSP.

In general, eukaryotic cells in many tissues, including artery and brain, alter their pattern of gene expression in response to nonlethal injuries, such as ischemia, metabolic toxins, trauma, and heat (3,4). After such stresses, protein synthesis may be reduced for many nonessential proteins and increased for a small subset of stress proteins that include stress, immediate early, heat shock, growth factor, and growth factor receptor genes. Which changes are critical to the pathogenesis of the injury has only begun to be determined.

In some experiments, protein levels are assessed by semiquantitative measurements such as immunoblotting or immunohistochemistry. It cannot be assumed that a change in gene expression accounts for this since changes in the level of protein in a cell may reflect alterations in transcription, translation, mRNA stability, protein degradation, or all of these. It is also difficult to estimate the importance of some changes since, for example, a 50% alteration in the concentration of an enzyme may have no effect on reactions catalyzed by it depending on the concentrations of substrate, products, and necessary cofactors, if any.

The process of ischemic tolerance supports a role for changes in gene expression in ischemia. There is no evidence to support the role of a preconditioning response in VSP. In fact, repeated injections of blood 2–21 days after the first injection make VSP worse in dogs (5,6) and had no effect on VSP in monkeys (7).

## II. Genetic Predisposition to Vasospasm

Studies of prognostic factors for outcome after SAH have focused on clinical and radiological factors and found that the amount of SAH, clinical grade, age, preexisting hypertension, aneurysm size and location, and VSP influence outcome (8). One mechanism by which genetic factors predispose to VSP is by being associated with intracranial aneurysm formation. Aneurysms may be familial or found in association with inherited diseases, such as autosomal dominant polycystic kidney disease, fibromuscular dysplasia, aortic coarctation, and possibly Marfan's syndrome (9). Autosomal-dominant polycystic kidney disease consists of type 1, which is associated with mutations in the polycystin gene on chromosome 16, and type 2, which has been linked to chromosome 4. Marfan's syndrome is due to mutations in the fibrillin gene on chromosome 15. The genes involved in the other disorders are not known. Ehlers Danlos type 4 is associated with fusiform intracranial arterial dilations and carotid cavernous fistula but generally not with intracranial saccular aneurysms. Associations of intracranial aneurysms and HLA antigens have been reported, although conflicting results have been presented apart from a repeated association of aneurysms with HLA B7, Cw2, and DR2. One study suggested that HLA B7 was associated with an increased risk of nonhemorrhagic deterioration after SAH and that HLA DR3 was protective (10). Candidate genes for intracranial aneurysms include type 3 collagen, elastin, and α-1-antitrypsin. Mutations in type 3 collagen do not seem to be associated with intracranial aneurysms, but the other two genes have not been investigated in detail. There is little evidence, however, that these factors increase the risk of VSP above that produced by the SAH.

Certain genetically determined variations may affect outcome after ischemic stroke and other brain injuries. Apolipoprotein E is an important lipid that is involved in cholesterol, cholesterol ester, and lipid transport in the brain. Patients with the ε4 allele appear to be overrepresented among those with ischemic stroke and recurrent primary intracerebral hemorrhage (11,12) and they have worse outcome after head injury than those without the allele (13). Studies on the effect of apolipoprotein alleles on outcome after SAH have not been performed.

## III. Molecular Biological Techniques

### A. Transgenic and Knockout Mice

Transgenic mice are animals in which a new gene [called a transgene(s)] has been introduced into the genome of the mouse (14). The protein product of the gene, which may be any type of protein such as an enzyme, ion channel, receptor, hormone, neurotransmitter, signal transduction pathway component, or cytoskeletal or contractile protein, is overexpressed. Methods to study loss of a specific gene include "knockdown" experiments in which gene expression is reduced, usually by administering a oligodeoxynucleotide that has a sequence complimentary to some part of the sequence of the gene in question (15). Oligodeoxynucleotides are hypothesized to inhibit gene expression by binding to the gene and reducing transcription, by binding to the mRNA and reducing translation, and by increasing mRNA degradation. The limitations of this antisense technology include incomplete target specificity and partial knockdown. A second method is to completely knock out the function of the gene in embryonic stem cells, usually from mice, and then generate mice from mixtures of these cells and those of genetically similar mice. Hetero- and homozygous knockout mice can then be produced. Some factors to be kept in mind are that some knockout phenotypes may be lethal *in utero* or shortly after birth because of alteration in the gene or in genes whose expression is affected secondarily. Changes in the expression of other genes can compensate and obscure the effects of gene knockout, and phenotypes can be different in different strains of mice. Transgenic and knockout mice have been used to investigate the role of specific genes in many diseases including cerebral ischemia.

Kamii *et al.* studied transgenic mice overexpressing CuZn–SOD (16). They created SAH by a method previously described in rats in which a thread is advanced endovascularly up the ICA and used to perforate the anterior cerebral artery. Transgenic mice overexpressing CuZn–SOD developed less VSP than did wild-type littermates.

### B. Screening for Changes in Gene Expression

It is estimated that there are 75,000 genes in the human genome and that 15,000–40,000 are expressed specifically in any given cell. Techniques are becoming available that allow screening of cells or tissues to determine which of hundreds or thousands of genes are expressed and the changes that occur in response to a stimulus or a disease. These include differential display and cDNA array analysis. Suzuki *et al.* used fluorescent differential display to screen for changes in gene expression after SAH in rats (17). Two hundred eighty (3%) of 9542 bands, each band corresponding to a specific mRNA, were altered in the basilar artery at some time after SAH. Onda and colleagues identified 18 genes whose mRNA was increased and 2 which were decreased in the double-hemorrhage

dog model (18). Forty-nine of 588 genes examined (8%) using a cDNA array were expressed in dog basilar artery. In addition to cDNA array, they used differential display. Genes that were upregulated and that could be identified (11) were vascular endothelial growth factor, BiP protein, glucose-regulated protein 78, growth-arrest and DNA damage-inducible protein (gadd45), neuromodulin, protein disulfide isomerase-related protein P5, acid sphingomyelinase-like phosphodiesterase, and 5 genes related to inflammation (monocyte chemotactic protein 1, cystatin B, inter-α-trypsin inhibitor family heavy chain-related protein, serum amyloid A protein, and glycoprotein 130). Frizzled-6 was downregulated. An additional 7 unidentified genes were upregulated and 1 was downregulated. Gene expression was examined 2 days after a single blood injection and 7 days after the first of two injections. The changes in expression of each gene were progressive in one direction over time.

We used cDNA array analysis to study gene expression after unilateral SAH in three monkeys. Right (clot-side) and left (control-side) MCAs were collected 3, 7, or 14 days after clot placement. VSP was assessed by angiography performed on day 0 and at tissue harvest. The cDNA arrays contained 5184 genes and changes in gene expression were made in each animal by comparing the right and left MCAs in order to eliminate nonspecific changes induced by stress. There was significant (more than 5-fold expression of mRNA compared to internal standard control) expression of 537 genes (10%). Of these 164 (31%) did not change significantly and 373 genes (69%) were differentially expressed at 3, 7, or 14 days after SAH. These 373 genes changed from 1.2- to 7-fold compared to control arteries. The most common pattern was a progressive increase in mRNA with increased time after SAH. The functions of differentially expressed genes included regulation of gene expression, cell proliferation, inflammation, membrane proteins and receptors, and various kinases and phosphatases. Whether the differentially expressed mRNAs are derived from endothelial, smooth muscle, and/or inflammatory cells in the vascular wall and what their contribution is to the onset and resolution of VSP need to be further evaluated.

## IV. Changes in Regulatory Mechanisms of Smooth Muscle Contraction

### A. Smooth Muscle Contraction

Smooth muscle contracts in response to electrical depolarization of the membrane (electromechanical coupling) or by pharmacomechanical coupling in which an extracellular agonist acts on a cell membrane receptor. Depolarization may be due to transmission of action potentials from perivascular nerves or from neighboring cells via gap or other junctions. Innervation of blood vessels might also cause contraction by neurotransmitter release with pharmacomechanical coupling. The classic intracellular process involved in contraction is the increase in intracellular ionized or free $Ca^{2+}$ by $Ca^{2+}$ influx and/or release from intracellular stores. These pathways involve cell membrane $Ca^{2+}$ channels (voltage gated and possibly receptor operated) and the inositol triphosphate pathway that releases $Ca^{2+}$ from intracellular stores. $Ca^{2+}$ binds to CaM and activates MLCK, which phosphorylates MLC and increases actin-activated myosin ATPase activity, cross-bridge cycling velocity, and contractile force. Contraction results in all cases from an interaction between actin and myosin. Contraction or force development may persist with lower rates of cross-bridge cycling. This is associated with return of intracellular $Ca^{2+}$ to basal or near basal levels, reduced MLC phosphorylation, and decreased cross-bridge cycling velocity. The persistence of contraction in the absence of the processes that seemed to mediate it initially has been attributed to a latch bridge state or to changes in $Ca^{2+}$ sensitivity of the contractile apparatus. A postulated mechanism that decreases $Ca^{2+}$ sensitivity is the agonist-induced inhibition of $Ca^{2+}$-mediated activation of $Ca^{2+}$/CaM-dependent protein kinase II. This kinase normally phosphorylates MLCK and decreases its activity, potentially leading to $Ca^{2+}$ desensitization. Sensitivity may be increased by phosphorylation of caldesmon and/or calponin, by activation of MLC phosphatase by Rho A and Rho kinase (Figs. 12.1 and 12.2), and/or by changes in intracellular CaM concentration.

External stimuli (hormones, peptide growth factors, stretch, cytokines, various injuries, etc.) are sensed by cells and lead to changes in cellular function through a process termed signal transduction. This involves detecting the signal at the cell membrane, converting it inside the cell to a signal that can be sensed by the intracellular molecular machinery, conducting the signal through the cell and modifying it as necessary for physiological control, if any, and effecting some response. Pharmacomechanical coupling by definition involves an intracellular signal transduction pathway—classically the inositol trisphosphate pathway to transduce agonist stimulation to contraction. In this pathway, receptor stimulation initiates hydrolysis of $PIP_2$, producing $IP_3$ and DAG. A G protein usually couples receptor binding to activation of phosphoinositidase C (PLC) that cleaves the $PIP_2$. $IP_3$ releases $Ca^{2+}$ from intracellular stores leading to contraction, and DAG participates in activation of PKC (Fig. 12.3). There are other signal transduction pathways and some may be involved in smooth muscle contraction.

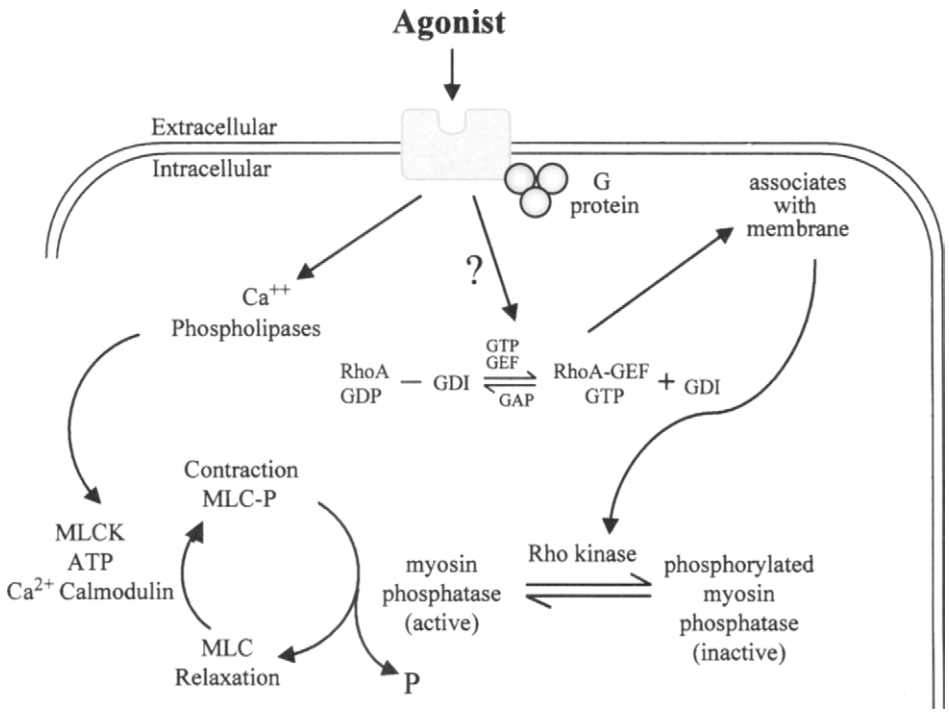


**FIGURE 12.1** Proposed signal transduction pathway involving Rho kinase. Agonist binding to a receptor leads to activation of $PLA_2$ and/or PLC and to increased intracellular $Ca^{2+}$. This leads to contraction by the classic pathway of activation of MLCK and phosphorylation of MLC. In addition, aganist binding may activated RhoA by mechanisms that may or may not involve components of heterotrimeric G proteins. This leads to binding of GTP and, by uncertain mechanisms, activation of RhoA by release of guanine nucleotide dissociation inhibitors (GDI). This is assisted by putative guanine nucleotide exchange factors (GEFs) and inhibited by guanine nucleotide activating protein (GAPs). Activated RhoA associates with the cell membrane and activates Rho kinases, including a Rho kinase that may induce smooth muscle contraction by phosphorylating MLCP and inactivating it and/or by directly phosphorylating MLC.

Contraction may in some cases develop in the absence of a substantial or any increase in intracellular $Ca^{2+}$ and possibly MLC phosphorylation, and this may be mediated by mitogen-activated protein (MAP) kinases (Fig. 12.4) (19, 20). Signal transduction pathways involving tyrosine kinases, MAP kinases, and PKC may be important regulators of contraction (Figs. 12.3–12.5).

A protein kinase is an enzyme that phosphorylates a protein. In general, they are classified as those that phosphorylate serine/threonine residues or those that phosphorylate tyrosine residues (tyrosine kinases) (21). There may be 1100 kinases in the genome. Phosphatases remove phosphates from proteins, reversing the effects of kinases, and are estimated to number about 300 in the human genome. Expression and function of many proteins, including MAP kinases, is different in contractile and synthetic phenotypes of vascular smooth muscle. Phosphatases, like kinases, tend to have specificity for tyrosine residues (protein tyrosine phosphatases) or for serine/threonine residues. The latter have been classified as PP1, PP2A, PP2B, or PP2C on the basis of divalent cation requirement, sensitivity to heat-stable inhibitors, and dephosphorylation specificity.

Activation of a signal transduction pathway may end in the cytoplasm with production of a mediator molecule, an increase in intracellular $Ca^{2+}$, or phosphorylation of a protein (22). Some pathways, however, are a series of kinases that eventually lead to phosphorylation of intranuclear proteins, many of which are transcription factors, and to changes in gene expression.

### B. Contractile Proteins

Measurements of levels of contractile proteins in vasospastic VSMC have been carried out and compared to levels occurring after acute contraction of the same artery to agonists or depolarization (23). VSP in dogs was associated with a reduction in actin, desmin, filamin, myosin, talin, α-actinin, vinculin, and metavinculin as measured by immunoblotting. Contraction of dog basilar artery

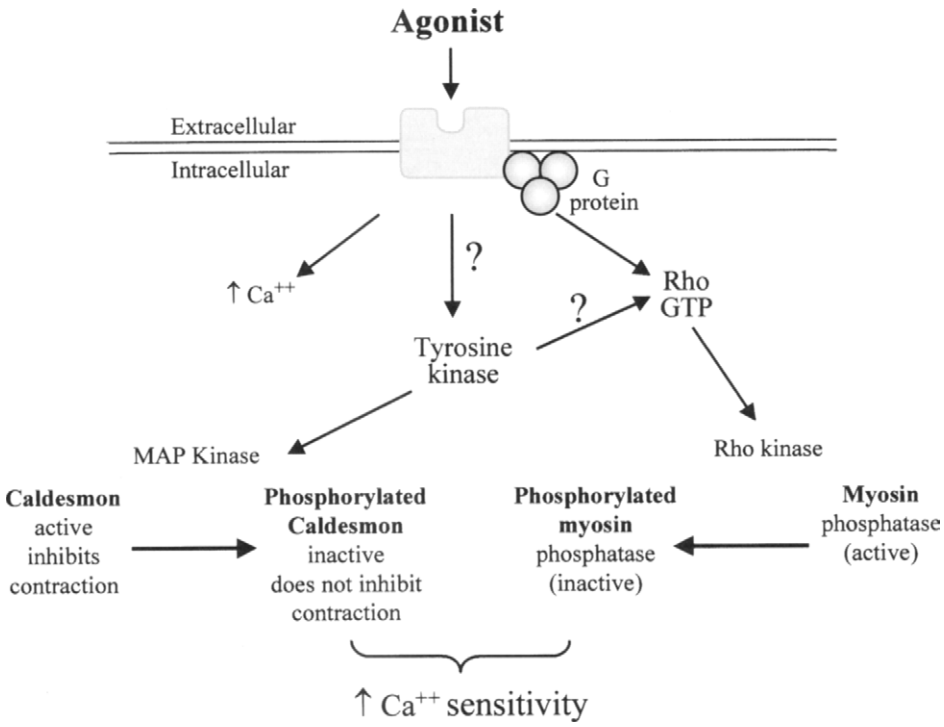


**FIGURE 12.2** Possible mechanisms of increased $Ca^{2+}$ sensitivity of smooth muscle. Agonist binding to a receptor may activate several signal transduction pathways, including increased intracellular $Ca^{2+}$, tyrosine kinases, and Rho GTPases. Tyrosine kinases may affect contraction by activation of MAP kinase cascades, one consequence of which may be phosphorylation of caldesmon that renders it unable to inhibit contraction. Activation of Rho such as RhoA may activate Rho kinase, which may induce smooth muscle contraction by phosphorylating MLCP and inactivating it and/or by directly phosphorylating MLC.

with KCl or serotonin produced similar decreases in filamin, myosin, and talin. Degradation of cytoskeletal and contractile proteins was suggested to result from proteolytic activation of calpains by increased intracellular $Ca^{2+}$ associated with contraction and VSP. The degradation of parts of the cytoskeleton uncouples the contractile and cytoskeletal domains of smooth muscle and results in loss of relaxation and decreased compliance as observed in uterine muscle after treatment with calpain. Immunoblots of rabbit basilar artery were performed 2 days after single SAH in rabbits (24). There were significant reductions in low-molecular-weight caldesmon and MLC and there was no change in tropomyosin and high-molecular-weight caldesmon.

Sakaki *et al.* estimated $Ca^{2+}$ by a cytochemical method and CaM by radioimmunoassay and phosphodiesterase assay in dog basilar arteries in the double-hemorrhage model (25). $Ca^{2+}$ staining and CaM content were reduced during chronic VSP in association with reduced ability of intrathecal nicardipine to reverse spasm and reduced overall arterial contractility of the basilar artery when removed and studied under isometric tension.

During the early stage of VSP, 2–5 days after placement of blood around the rat femoral artery, there was increased mono- and diphosphorylation of MLC (26). After 5 days, however, total MLC was reduced and phosphorylated forms were undetectable. Data are conflicting on the state of MLC phosphorylation after SAH in dogs. Zervas's group reported a significant increase in the anterior spinal arteries 6 days after SAH (27), whereas other groups found no change in the extent of phosphorylation of the 20-kDa MLC on 4 days or 7 in the double-hemorrhage dog model (28,29). Sun and colleagues, however, reported that the ability of spastic basilar artery to increase MLC phosphorylation in response to agonist stimulation was attenuated after SAH (29). Furthermore, immunoreactivity to actin, high-molecular-weight caldesmon, and calponin was reduced 7–14 days after SAH during VSP but returned to normal on day 21 when VSP resolved. Calponin and high-molecular-weight caldesmon but not α-actin immunoreactivities were reduced 4–14 days after SAH in the double-hemorrhage dog model (30). Calponin protein was reduced 2 and 7 days after SAH in the same model, and this reduction

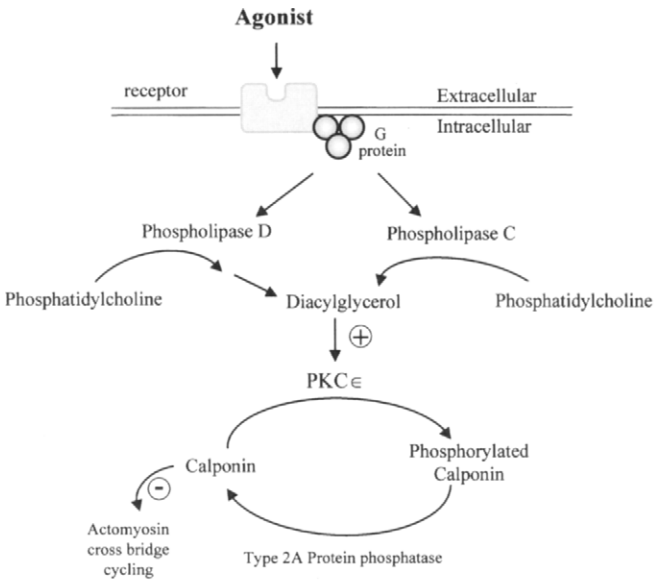


**FIGURE 12.3** Possible role of PKC in smooth muscle contraction. Agonist binding to a receptor may lead to activation of PLC and D via heterotrimeric G proteins. Both PLs may generate DAG, which activates PKC. PKCε, which may be specifically involved in contraction, phosphorylates calponin and renders it unable to inhibit actomyosin cross-bridge cycling and contraction.

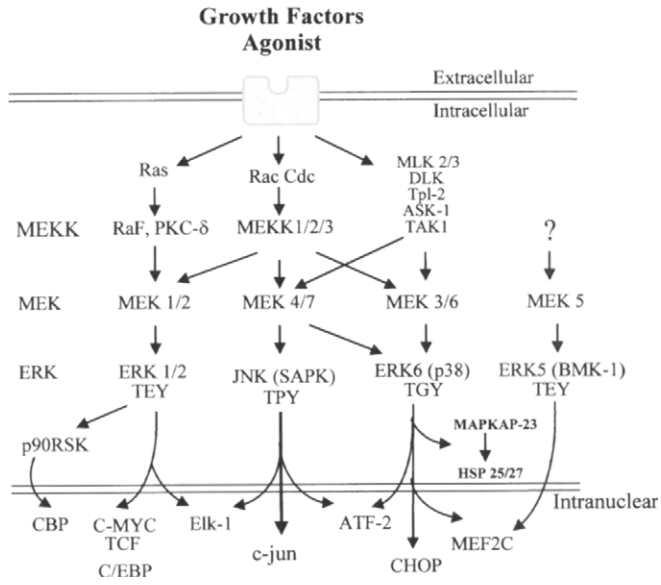


**FIGURE 12.4** Diagram of the main MAP kinase pathways. Growth factors and agonists may activate MEK kinases (MEKK) via pathways involving various intermediary proteins, including Ras, Rac, Cdc42, MLK 2/3, DLK, Tpl-2, ASK1, and TAK1. Activation of MEKKs can then activate one or more MEKs. There is more specificity in activation of MAP kinases (ERK) by MEKs. MAP kinases are less specific in the proteins that are activated downstream, which include multiple possible different transcription factors (modified with permission from Takahashi [21a]).

correlated with angiographic VSP (31). Oka *et al.* also noted that there was reduced polyribosome formation in the basilar arteries when protein levels were reduced, suggesting that a primary defect in protein synthesis might underlie these reductions (30). Finally, reduced calponin immunoreactivity and an increase in its phosphorylation were described during VSP in the double-hemorrhage dog model (32).

What do these changes mean? This is a matter of speculation. Some changes, such as reduced calponin and caldesmon, might contribute to maintenance of contraction. These proteins are believed by some investigators to be involved in physiological smooth muscle contraction but their role is regarded by some as not proven (Figs. 12.2 and 12.3). How reduction in contractile proteins contributes to sustained, resistant contraction is unclear. It could also be speculated that such changes reflect phenotypic change of VSMC which are known to have reduced content of such proteins in the proliferative state (33). The role of calpain activation is reviewed later and has been incompletely investigated. The lack of effect of VSP on levels of MLC phosphorylation has been suggested to reflect the maintenance of contraction in VSP based on other contractile mechanisms variously postulated to be a latch state (29), activation of PKC (34) and/or Rho kinase (35), or alterations in proteins associated with thin filaments such as caldesmon and/or calponin (24,31). In summary, all postulated mechanisms of tonic smooth muscle contraction have been invoked to explain VSP.

### C. Protein Kinase C

PKC is a family of at least 11 isoforms with differing requirements for $Ca^{2+}$, phospholipids, and DAG for activation (Fig. 12.3) (19). Various isoforms may perform different functions in regulation of smooth muscle contraction. The involvement of PKC in contraction was based mainly on the observation that activators of PKC such as phorbol esters, contract smooth muscle, and pharmacological inhibitors of PKC may prevent or reduce contraction. PKC was postulated to inhibit MLC phosphatase and phosphorylate calponin, both leading to increased force development. PKC activity may also activate the MAP kinase pathway that may be involved in smooth muscle contraction (Fig. 12.4). There are numerous conflicting experimental results and it was not universally accepted that PKC is involved in smooth muscle contraction. However, a role for PKC has been postulated in VSP based mainly on pharmacological and not molecular biological approaches. The pharmacological studies are reviewed in Chapter 7 and have shown reversal of established VSP with drugs that inhibit PKC such as

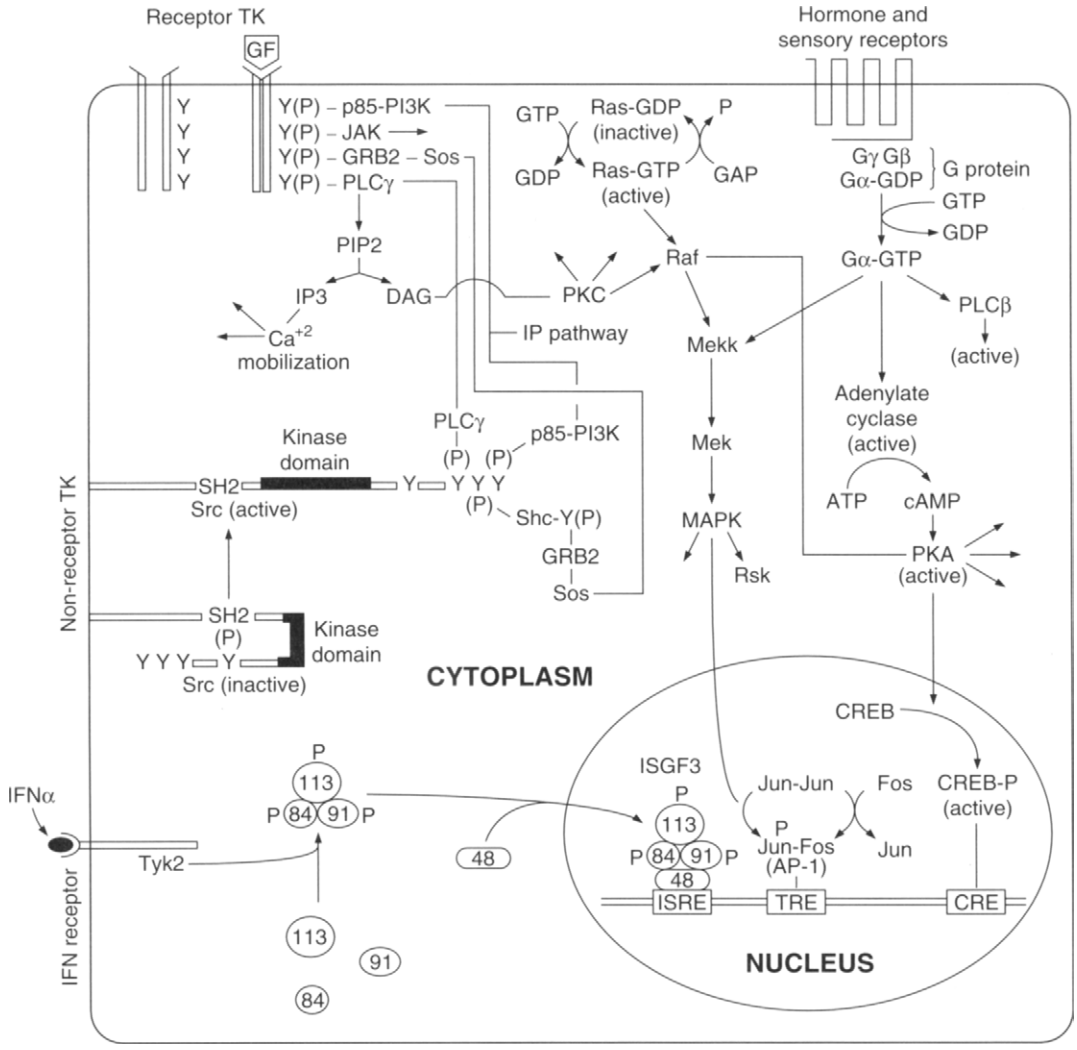


**FIGURE 12.5** Signal transduction pathways involving tyrosine kinases. There are three general types of tyrosine kinases: nonreceptor, membrane-bound forms such as $pp60^{c\text{-}src}$ (Src), receptor tyrosine kinases that span the cytoplasmic membrane such as insulin-, platelet-derived growth factor and epidermal growth factor receptors, and cytosolic tyrosine kinases such as c-abl and -fes. Activation of tyrosine kinases by growth factor receptors involves the heterotrimeric G proteins and is involved in activation of other signal transduction pathways such as the ERK/MAP kinase pathway. The extension of this pathway to activation of nuclear transcription factors such as Jun and Fos is shown. Some receptor tyrosine kinases may activate PLC, leading to $IP_3$ generation and $Ca^{2+}$ release as well as generation of DAG with activation of PKC (reproduced with permission from Barik [22]).

H-7 and staurosporine, increased DAG (an endogenous activator of PKC) in vasospastic arteries, and increased PKC activity in vasospastic arteries. The drugs and methods used are so nonspecific (36) that they cannot be used to draw meaningful conclusions about the role of PKC in VSP. Only very limited examination of PKC isoforms in VSP has been conducted, mainly at the level of protein concentrations.

An early theory of involvement of PKC in VSP was put forth by Matsui *et al.* (37). In the double-hemorrhage dog model, VSP was reversible by topical application of PKC inhibitors such as H-7 and staurosporine (34). VSP was associated with an increase in DAG in the basilar arteries, consistent with the basis for the arterial narrowing days after SAH as being due to activation of PKC. Prolonged elevation of DAG was suggested to result from increased phosphatidylcholine turnover triggered by lipid peroxidation. Other investigators documented increased PKC activity associated with translocation of PKC from the cytosol to the membrane fraction in dog basilar artery during VSP (38,39). Although an increase in membrane-bound PKC activity occurred in the study by Sako and

colleagues, they concluded that PKC activation was not solely responsible for VSP since PKC activity returned to normal days before VSP resolved (39). These findings could not be replicated in the same model; Zervas's group found that DAG was not elevated and PKC activity was only statistically insignificantly elevated in vasospastic dog basilar artery (40). They reviewed the previously published studies and concluded that there is a decrease in PKC with no change in activity after one SAH in dogs and that, after the second injection 2 or 3 days later, PKC content and activity show a small, transient increase that resolves before VSP resolves. Although the studies in the double-hemorrhage dog model showed no or only small increases in PKC activity, studies *in vitro* suggested that changes of this magnitude augmented contractions of dog basilar artery in response to red blood cell (RBC) hemolysate and therefore might be of significance in VSP (41).

Changes in PKC activity and membrane lipid metabolism were examined in the basilar arteries of beagles in the two-hemorrhage model of SAH (28). Cytosolic PKC activity decreased significantly 4 and 7 days post-SAH and then returned to normal by day 14. There was no significant change in membrane PKC activity. PKC isoforms were quantified by immunoblotting. The predominant forms were PKCα, PKCε, and PKCζ. Only a trace amount of PKCβ was detected and PKCγ and PCKδ were not detected. PKCα and PKCε but not PKCζ were decreased in the cytosol fraction 7 days after SAH during VSP. The theory that increased PKC activity contributes to VSP was supported by the demonstration of increased turnover of phosphatidylcholine and phosphatidylethanolamine but not of phosphoinositides, supporting prior data showing increased DAG in VSP dog arteries. The reduction in PKC activity, however, seemed inconsistent with activation of PKC as a cause of VSP and various explanations were given. That PKC is decreased was confirmed in the same model (42). Immunoreactivities of PKCα, PKCβ, and PKCγ were decreased in the basilar artery and increased in the pons and hippocampus in reactive astrocytes most markedly 7 days after SAH during the time of VSP (42).

Nishizawa and coworkers hypothesized that NO inhibits PKC activation, and since cerebral arteries normally release NO continuously, a reduction in the action of NO after SAH may contribute to VSP (43). A series of experiments sought to confirm this theory. Differing severities of VSP were induced in dogs by one or two injections of blood into the cisterna magna (43). After single SAH, there was mild VSP, a slight decrease in cGMP in the basilar artery, and a slight increase in PKC activity on day 4 with return to baseline of arterial diameter and cGMP but persistent increase in PKC on day 7. More severe, sustained VSP developed in the double-injection model and was associated with a more sustained decrease in cGMP and increase in PKC activity. A correlation therefore was shown between VSP, reduced cGMP (a surrogate marker for NO effect), and increased PKC activity. Additional pharmacological evidence was the PKC-dependent increase in baseline tension noted in vasospastic arteries studied under isometric tension *in vitro* (44). NOS inhibitors increased tension in normal but not vasospastic arteries, implying that VSP abrogated the effect of NO. That PKC activity accounted for the increase in tension in normal arteries in response to NOS inhibitors was supported by the ability of PKC inhibitors to suppress the contractions due to NOS inhibitors and by the demonstration that such arteries had increased PKC activity (45). Furthermore, vasospastic arteries exhibited increased PKC activity and this was not increased further by NOS inhibitors. It is known that prolonged stimulation of arterial smooth muscle with phorbol esters, which are known to activate PKC, leads to downregulation of PKC. Nishizawa and colleagues went on to show that when dog basilar arteries were exposed to the phorbol ester phorbol 12-myristate 13-acetate for 24 hr, PKC activity was downregulated and arteries no longer contracted upon application of a NOS inhibitor (46).

Fujikawa *et al.* demonstrated MLC phosphorylation patterns in VSP that were consistent with phosphorylation by MLCK but not PKC (32). PKC could mediate contraction via mechanisms that might not directly phosphorylate MLC. These investigators found evidence for PKC activation by calpain and suggested that the PKC contribution to VSP might be related to phosphorylation of calponin which was demonstrated to occur in VSP in the double-hemorrhage dog model.

Iwabuchi *et al.* reported that application of fresh dog RBC lysate to rat basilar artery VSMC increased intracellular $Ca^{2+}$, producing a characteristic peak increase followed by a sustained plateau (47). Staurosporine, a PKC inhibitor, inhibited the plateau but not the peak.

### D. Calpains

Calpains are neutral proteases that, when activated by various stimuli, can break down cytoskeletal and contractile proteins and protein kinases (48). Calpains are composed of 80-kDa catalytic subunits that are products of separate genes and a common 30-kDa small subunit. The catalytic subunits confer biochemical differences on calpains; the main types are μ calpain (type 1) and m calpain (type 2). Type 1 is active at micromolar and type 2 at millimolar concentrations of $Ca^{2+}$. All cells that contain calpains contain a naturally occurring protein inhibitor, calpastatin. An important stimulus for calpain activation

is believed to be increased intracellular $Ca^{2+}$, and both are activated in a variety of conditions associated with necrotic and apoptotic cell death. Calpain activation may then lead to activation of intracellular kinases, possibly leading to abnormally regulated smooth muscle contraction (Fig. 12.6). Some proteolysis by calpains may mediate physiological functions. Kinases suggested to be substrates of calpain include mitogen-activated protein kinases, PKC, and MLCK. Other proteins may be cleaved by calpains, including some growth factor and other agonist receptors and other enzymes, the classic protein being spectrin. The physiological role of calpains is not known. Their most well-defined function is modifying the cytoskeleton of aggregating platelets. They are believed to be physiologically important since they can partly degrade proteins in a manner that may be designed to activate or inactivate a particular protein.

Minami *et al.* reported that VSP on day 7 in the double-hemorrhage dog model could be reversed by topical application of the calpain inhibitor, calpeptin, and by inhibitors of PKC such as H-7 and calphostin C (49). The effects of these drugs were of different magnitudes against VSP and against acute contraction of the basilar artery with KCl or 5-HT, and the authors explained these results on the basis that VSP activated calpain which dissociated PKC into catalytic (M) and regulatory domains. This rendered the catalytic domain constitutively active in the absence of factors normally required for activation, such as $Ca^{2+}$ and phospholipid. This would lead to sustained contraction by PKC-mediated mechanisms. These investigators demonstrated that μ calpain was continuously activated in the spastic basilar artery and that calpastatin activity was reduced at the same time, setting the stage for excessive calpain activity (50). A single blood injection into the cisterna magna of rabbits activated calpains 2–4 days later since native calpain was reduced and spectrin degradation products increased on immunoblots (51). Reduction in native calpain was suggested to be due to the fact that activation of calpains is associated with proteolytic cleavage of the native protein.

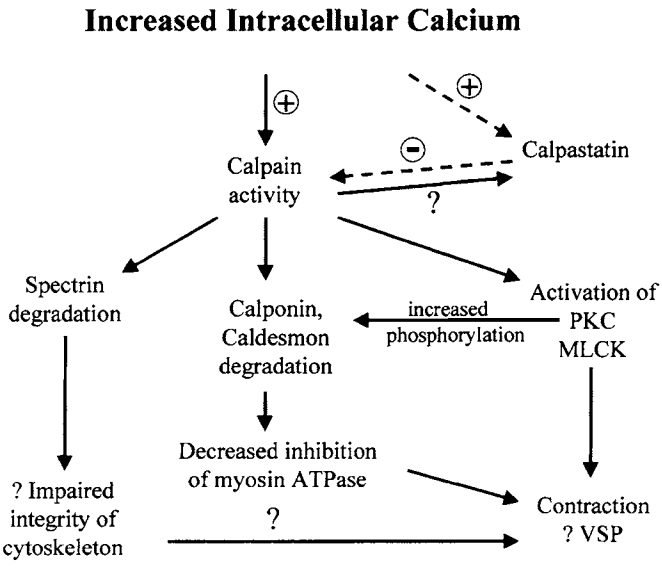


**FIGURE 12.6** Diagram of possible involvement of calpains in smooth muscle contraction and VSP. Increased intracellular $Ca^{2+}$ can activate calpain and possibly an endogenous inhibitor of calpain, calpastatin. Calpains are postulated to proteolytically activate PKC and MLCK and to degrade calponin and caldesmon. All these phenomena may promote contraction. The classic substrate of calpain, spectrin, is a component of the cytoskeleton and degradation of it may also contribute to contraction (modified with permission from Lee [51]).

Cappalletto and colleagues studied the effects of a calpain inhibitor, zLF (*z*-Leu-Phe-CONH-morpholene) on the rabbit basilar artery (52). Contractions of the rabbit basilar artery after topical application of oxyHb, prepared by reduction of impure Hb, were prevented by pretreatment with topical zLF. Topical application of zLF reversed VSP that was present 3 or 4 days after two blood injections into the cisterna magna of rabbits. The authors noted that the ability of RBC hemolysate to increase intracellular $Ca^{2+}$ in VSMC might satisfy a prerequisite for calpain activation and PKC activation (23). A theory of VSP was then expounded. Increased intracellular $Ca^{2+}$ in VSMC occurs as a result of exposure to RBC hemolysate. Calpain activation occurs, which proteolytically cleaves PKC to catalytic and regulatory domains. The separated catalytic unit has less dependence on $Ca^{2+}$ for activation and thus is constitutively or abnormally overactive in the smooth muscle, causing contraction by several possible mechanisms including phosphorylation of calponin and removing its intrinsic inhibition of the myosin ATPase. The role for PKC is supported by at least some findings, such as the efficacy of PKC inhibitors against Hb and subarachnoid blood-induced cerebral arterial narrowing. Concerns about the role of PKC in VSP as discussed previously were noted. Another potential substrate of calpain is MLCK, whose partial proteolysis by calpain reduces its requirement for $Ca^{2+}$ and increases its activity. This suggests that VSP should be associated with increased levels of MLC phosphorylation, which may or may not be the case. Calpain-mediated proteolysis of calponin and caldesmon was postulated to possibly be involved. The antivasospastic effects of other protease inhibitors such as FUT-175 might be related to inhibitory effects on calpain, although this has not been demonstrated and could be due to anti-inflammatory or other effects of these drugs.

Fujikawa *et al.* suggested that PKM, the catalytic fragment of PKCα that is produced by calpain activation, is involved in VSP in the dog double-hemorrhage model

since this fragment was increased in the basilar artery (32). Generation of the fragment was inhibited by the tyrosine kinase inhibitor genistein. PKM also activates the extracellular signal-regulated kinases (ERK) which could also contribute to VSP since MAP kinase may phosphorylate caldesmon.

### E. Tyrosine Kinases

Tyrosine kinases have been postulated to be important in vascular smooth muscle contraction (53). They may be important in coupling receptor activation to increases in intracellular $Ca^{2+}$ and may do so by regulating both the release of $Ca^{2+}$ from intracellular stores and the influx of $Ca^{2+}$ from the extracellular space. Tyrosine kinases may also be activated by increased intracellular $Ca^{2+}$ and this activation may be involved in altering the $Ca^{2+}$ sensitivity of the contractile apparatus. These theories are based on observations that agents, including some growth factors, that activate tyrosine kinases have been shown to increase intracellular $Ca^{2+}$ and inhibitors of tyrosine kinases, such as genistein and tyrphostin, inhibit agonist-induced contractions in some smooth muscles. There are at least three families of tyrosine kinases: nonreceptor, membrane-bound forms, such as $pp60^{c\text{-}src}$; receptor tyrosine kinases that span the cytoplasmic membrane, such as insulin, platelet-derived growth factor, and epidermal growth factor receptors; and cytosolic tyrosine kinases, such as c-abl and fes (Fig. 12.5). Activation of tyrosine kinases by growth factor receptors is involved in activation of other signal transduction pathways such as the ERK/MAP kinase pathway.

RBC hemolysate, a proposed source of spasmogens that contribute to VSP, contracts rabbit basilar artery and these contractions can be inhibited by tyrosine kinase inhibitors such as tyrphostin A23 and genistein (54). It should be kept in mind that this and most other similar experiments use fresh RBC hemolysate that contains substantial amounts of ATP. Since VSP does not occur for days after SAH, at a time when the RBCs no longer contain much ATP, there may be differences in effects of fresh and incubated hemolysates. Hemolysate also enhances tyrosine phosphorylation of proteins in cultured dog basilar artery and human dermal fibroblast cells, and the effect can be inhibited by tyrosine kinase inhibitors genistein and tyrphostin A51 (55). Collagen lattices are contracted by the fibroblasts when exposed to hemolysate, an effect that is also blocked by tyrosine kinase inhibitors.

Iwabuchi *et al.* reported that application of fresh dog RBC lysate induced tyrosine phosphorylation of 100- and 70-kDa proteins in rat basilar artery VSMC (47). RBC lysate also increased intracellular $Ca^{2+}$, producing a characteristic peak increase followed by a sustained plateau. Tyrosine kinase inhibitors such as genistein and tyrphostin A51 inhibited both phases of the $Ca^{2+}$ increase. Most of the $Ca^{2+}$ increase was due to the fraction of RBC lysate with a molecular weight less than 10 kDa. A similar but not identical response occurred in bovine endothelial cells exposed to RBC lysate, although only the plateau increase in $Ca^{2+}$ was blocked by tyrosine kinase inhibitors in these cells (56).

Involvement of tyrosine kinase signal transduction pathways in VSP in the dog double-hemorrhage model was investigated by Fujikawa *et al.* (32). VSP was associated with activation of tyrosine kinases as evidenced by phosphorylation of their intracellular substrates, such as Shc, Rafl, and MAP kinases. Topical application of the tyrosine kinase inhibitor genistein reversed established VSP. The activation of tyrosine kinases was more closely related to the early stage of VSP that to VSP 7 days post-SAH.

### F. Mitogen-Activated Protein Kinases

MAP kinases are present in VSMCs. Contractile VSMCs tend to express p42 and p44, whereas proliferative smooth muscle also contains p34 (21,21a). The function of MAP kinases in smooth muscle is not fully known (Fig. 12.4). MAP kinases are a family of kinases identified and named based on the increase in activity they develop in response to stimulation of cells by mitogens. Growth factors (epidermal, platelet-derived, fibroblast, nerve, insulin, and insulin-like growth factors) and other agonists, including vasopressin, angiotensin II, platelet-activating factor, ET-1, ILs, and muscarinic agonists acting on cell membrane receptors, may activate them in many different cell types. They are generally thought of as comprising three interacting pathways consisting of MAP kinase, a kinase that activates the MAP kinase (MAP kinase kinase or MEK), and a kinase that activates the MEK (MEK kinase; Fig. 12.4). One pathway includes ERK-1/2, which are members of the TEY family of MAP kinases and are activated by most growth factors (epidermal and platelet-derived growth factors), ET-1, vasopressin, and angiotensin II. The activation of ERK-type MAP kinases involves activation of tyrosine kinases in some cases since, for example, some growth factor receptors possess or are associated with tyrosine kinases and activate MAP kinases. ERK-1 is p44 and ERK-2 is p42. The coupling of agonist–receptor binding to activation of MAP kinases is believed to involve G proteins, but the precise proteins involved are unknown. In general, however, the upstream activators of MAP kinases are MAP kinase kinases (MAPKK and MEK). These in turn are activated by upstream kinases such as MEK kinases and

the protooncogene *raf*. *Ras* is involved in activation of *raf*, possibly by localizing *raf* to the cell membrane. Other proteins are involved, including the 14-3-3 proteins. The other MAP kinase pathways include a cascade involving the p54 MAPK stress-activated protein kinase/c-jun NH2 terminal kinase pathway that is involved in responses to oxidative stress, ET-1, cell stretch, shear stress, and inflammatory cytokines and a second stress response pathway involving p38 that has similar activators and may be involved in growth inhibition and apoptosis. One postulated function of MAP kinase in contractile smooth muscle is to phosphorylate caldesmon. Caldesmon is phosphorylated in resting smooth muscle and its phosphorylation, possibly by MAP kinase, increases with pharmacological stimulation, and removes the inhibitory effect of caldesmon on contraction (Fig. 12.2). MAP kinase activity is increased in vascular smooth muscle in response to pharmacological agonists. In proliferative smooth muscle, MAP kinases appear to be involved in progression of cells through the cell cycle. MAP kinase activity is increased in response to growth factors and agonists that induce smooth muscle proliferation and is reduced by agents such as heparin and drugs that increase intracellular cAMP and that inhibit proliferation. Drugs that increase cAMP also inhibit contraction, so there tends to be a direct relationship between agents that cause contraction and proliferation and increased MAP kinase activity.

The role of MAP kinase has been investigated in hemolysate-induced signal transduction and contraction in rabbit basilar artery (57). PD-98059, an inhibitor of the MAP kinase pathway, reduced hemolysate-induced contractions when added before or after the onset of contraction, whereas the Janus tyrosine kinase 2 inhibitor, AG-490, only reduced contractions when preincubated with arterial rings. A Src-tyrosine kinase inhibitor, damnacanthal, did not reduce contractions in either situation. Further evidence of the involvement of MAP kinases was the rapid increase in MAP kinase immunoreactivity observed after exposure of rabbit basilar artery to hemolysate that was prevented by PD-98059. The ERK pathway was shown to be activated in VSP in the dog double-hemorrhage model (32).

### G. Other Mechanisms

Dysfunction of other proteins involved in regulating intracellular $Ca^{2+}$ in VSMC could affect contractility and contribute to VSP. Few have been investigated on a molecular level, but functional studies may be important since protein function may be altered in the absence of changes in transcription and translation. Changes that have been noted to occur during VSP in the smooth muscle of the dog basilar artery but whose molecular basis is unclear include the following: Smooth muscle $Ca^{2+}$ regulation may be abnormally impaired during VSP (58), the contractile apparatus may exhibit $Ca^{2+}$ sensitization (59,60), and there may be reduced function of the $Ca^{2+}$-ATPase, a membrane pump that contributes to reducing intracellular $Ca^{2+}$ (61).

The activities of protein phosphatase-1 and -2a were significantly reduced 2 and 4 days after SAH in a double-hemorrhage rabbit model (62). Acute contraction of the rabbit basilar artery with KCl or serotonin did not alter levels of the phosphatases. Protein phosphatase-1 may catalyze dephosphorylation of MLC and calponin; the phosphorylation of both proteins is suggested to promote contraction. Similarly, protein phosphatase-2a may dephosphorylate calponin and caldesmon, leading to the ability of these proteins to inhibit contraction. Therefore, reductions in the phosphatases was suggested to promote uninterrupted contraction of smooth muscle after SAH.

The Rho GTPases are small, low-molecular-weight G proteins that are a subgroup of the Ras superfamily of 20- to 30-kDa GTP-binding proteins and have become of interest in the field of smooth muscle contraction (Figs. 12.1 and 12.2) (63). The other main class of G proteins comprises the heterotrimeric G proteins that contain $\alpha$, $\beta$ and $\gamma$ subunits, where the $\alpha$ subunit is a GTPase (64). Heterotrimeric G protein function is controlled by binding of agonists to heptahelical receptors (G protein-coupled receptors). Mammalian Rho-like GTPases comprise at least 10 proteins that include Rho A–E, Rac 1 and 2, RacE, Cdc42Hs, and TC10. They are involved in cytoskeletal organization, membrane trafficking, regulation of transcription, development, control of cell growth, and probably smooth muscle contraction. They all cycle between inactive GDP-bound cytoplasmic and active forms that are bound to GTP and probably the cell membrane. The ratio of these two forms is regulated by guanine nucleotide exchange factors (GEFs) which increase exchange of bound GDP for GTP and are therefore involved in activation, GTPase-activating proteins which increase GTP hydrolysis and therefore accelerate Rho inactivation, and guanine nucleotide dissociation inhibitors (GDIs) which inhibit both GTP exchange and hydrolysis of bound GTP and therefore tend to keep unstimulated Rho in a cytosolic, GDP-bound form. Normally, Rho is cytosolic and bound to a GDI that may be relatively specific for the type of Rho. Activation is associated with membrane translocation and GEF binding. Rho activation can in turn activate Rho kinases such as p160ROCK (ROCKII or ROK$\beta$). Rho kinase can phosphorylate the myosin-binding subunit of MLCP. This reduces the phosphatase activity and maintains MLC phosphorylation and contraction. Rho kinase also

phosphorylates MLC at the same sites as MLCK. Ras has been shown to control activation of the p42/44 MAP kinase cascade. Some Rho-type GTPases such as Rac and Cdc42 appear to be activators of JNK and p38 kinase pathways in some cell types.

Fasudil (HA1077 or AT877) was reported to decrease VSP and improve outcome in patients with aneurysmal SAH (65). It is approved for use in Japan but its exact mechanism of action is unknown. Interestingly, it inhibits protein serine–threonine kinases with order of potency p160ROCK > cAMP-dependent protein kinase > PKC > MLCK. Sato *et al*, measured RhoA, RhoGDI (suppressor protein of RhoA), RhoGAP (inactivates RhoA), and Rho kinase in cytosolic and membrane fractions and MLC phosphorylation in basilar arteries of dogs after SAH (35). Membrane translocation of Rho kinase suggests activation of the enzyme. Y-27632, a relatively specific inhibitor of Rho kinase, reversed established VSP by topical application to the spastic dog basilar artery 7 days after SAH. RhoA and Rho kinase were activated in VSP, whereas RhoGDI was reduced. Another finding consistent with a role for Rho kinase in VSP is that MLC phosphorylation was increased, which simplistically should occur if Rho kinase is activated. Interestingly, other investigators have not obtained this result, nor have they found that fasudil reduces MLC phosphorylation, which might be expected if Rho kinase is activated in VSP since fasudil is a potent Rho kinase inhibitor (66,67). RhoGAP was decreased on day 0 but recovered on days 2 and 7, suggesting that it is present to inhibit RhoA during VSP. There was a decrease in RhoGDI early after SAH in the membrane fraction suggesting more GTP-bound active RhoA is present in the membrane.

Kim *et al.* confirmed the results of earlier studies by showing that fasudil inhibited VSP development after SAH in the double-hemorrhage dog model (67). VSP was not associated with increased MLC phosphorylation, which is consistent with findings in other types of tonic smooth muscle contraction. Fasudil treatment prevented what was suspected to be calponin breakdown as observed on immunoblots. This newly described effect of fasudil could contribute to its antivasospastic action and represents a novel mechanism for this drug.

## V. Changes in Smooth Muscle Relaxation Mechanisms

Smooth muscle relaxation is mediated by removal of the inciting stimulus which reduces intracellular $Ca^{2+}$ and also by dephosphorylation of MLC by MLCP. Relaxation to stimuli that activate the cAMP–protein kinase A pathway probably utilizes this mechanism. Relaxation by some mechanisms is more than expected for the degree of reduction in intracellular $Ca^{2+}$ or dephosphorylation of MLCs and tends to be mediated by the guanylate cyclase–cGMP–protein kinase G system. The third pathway for relaxation involves activation of potassium channels. These pathways may interact.

There are two forms of guanylate cyclase in smooth muscle—particulate and soluble. Soluble guanylate cyclase (SGC) is activated by NO and nitrovasodilators, whereas particulate guanylate cyclase is activated by atrial natriuretic peptides. Cyclic GMP relaxes smooth muscle by activating cGMP-dependent protein kinase (protein kinase G) that then phosphorylates other regulators of contraction such as MLCK, which reduces its activity and leads to dephosphorylation of MLC. Both A and G kinases may contribute to relaxation by lowering intracellular $Ca^{2+}$ by phosphorylating voltage-gated channels, by reducing $Ca^{2+}$ influx, and by enhancing $Ca^{2+}$-ATPase activity and the $Na^{+}$–$Ca^{2+}$ exchanger to export $Ca^{2+}$ and sequester it in the sarcoplasmic reticulum (68).

The effect of experimental SAH on cerebral vasodilation has been studied and found to be impaired (69). A fundamental abnormality in $K^{+}$ channel function is suggested by the finding that vascular smooth muscle is depolarized during VSP in dogs and that $K^{+}$ channel openers may reverse VSP (70). Vasodilation of the rat basilar artery 2 days after SAH was impaired in response to ACh and sodium nitroprusside (SNP), agents that produce relaxation by the cGMP pathway. On the other hand, relaxation to papaverine, 8-bromoguanosine 3′, 5′-cyclic monophosphate, and brain natriuretic peptide (an activator of particulate guanylate cyclase) was not affected and relaxation to aprikalim and CGRP, which activate ATP-sensitive $K^{+}$ channels, was augmented. In dog basilar arteries 7 days after double SAH, relaxation to forskolin, a direct activator of adenylate cyclase that relaxes in part by activation of $Ca^{2+}$-activated and delayed rectifier $K^{+}$ channels, was not reduced after SAH but inhibitors of these types of $K^{+}$ channels inhibited contractions to forskolin to a greater degree in arteries from animals with SAH, suggesting that $K^{+}$ channels are relatively more important in relaxation of VSP arteries (71). Increases in cAMP in response to forskolin were also normal after VSP. Other studies have supported a relative preservation of cAMP relaxation after SAH compared to cGMP-mediated mechanisms (72).

Todo *et al.* caused VSP in dogs after one or two cisternal blood injections (68). They note that papaverine induces smooth muscle relaxation by inhibition of the phosphodiesterase enzymes that hydrolyze the cyclic nucleotides cAMP and cGMP. Papaverine also inhibits voltage-dependent $Ca^{2+}$ current and inhibits the $IP_3$ cascade from releasing $Ca^{2+}$ from the intracellular $Ca^{2+}$ store. They measured cGMP and cAMP by

radioimmunoassay 1 hr and 2, 4, 7, and 14 days after single SAH and 1 hr after second injection (48 hr after first injection) and then 4, 7, and 14 days after the first injection in the double-injection dogs. Adenylate and guanylate cyclase were measured by histochemical enzymatic assay on cross sections of basilar artery 4 days after the first injection in the double-hemorrhage model and cAMP-dependent protein kinase (A kinase) and cGMP-dependent protein kinase (G kinase) were measured at the same time. VSP was present on day 2 and then reversed by day 7 after a single SAH but persisted until day 14 in the double-SAH model. SNP and isoproterenol dilated normal but not spastic basilar artery 4 days after double SAH. cAMP decreased significantly 2 days post-SAH in both groups and then recovered with single SAH but remained reduced with double SAH. Furthermore, isoproterenol failed to increase cAMP 4 days after double SAH. For cGMP there were smaller changes, with no change after single SAH and a significant decrease from days 4 to 14 in double SAH. Electron microscopy for adenylate and guanylate cyclases showed qualitative changes consisting of a reduction in both compared to control and less of an increase compared to control when dogs were treated with isoproterenol and SNP, respectively. Finally, SAH reduced soluble and particulate A and G kinase activities and prevented increases in response to isuproterenol and SNP. It was concluded that SAH decreases the amounts of cAMP and cGMP in cerebral arteries. The drugs failed to increase levels of their respective cyclic nucleotides, which seemed to be due to decreased cyclase activity, although other studies have suggested that failure of high-energy phosphate synthesis may also be responsible (73). The following is a key question: What is the mechanism of inhibition of these enzymes? The authors suggest that a free radical reaction in the subarachnoid clot leads to lipid peroxidation, loss of membrane fluidity, changes in membrane potential, conformational changes in membrane proteins, and inactivation of enzymes in cerebral arteries (61,74). Reduction in activities of the non-membrane-bound particulate forms of adenylate and guanylate cyclase suggests a direct effect on these proteins.

## VI. Changes in Endothelium-Regulated Mechanisms

The endothelium of cerebral arteries synthesizes vasoconstrictors such as ET and eicosanoids (such as $PGF_{2\alpha}$) and vasodilators such as NO and/or a related nitroso compound, prostacyclin ($PGI_2$), superoxide anion radical, and endothelium-derived hyperpolarizing factor (75). Some of these compounds may also be released by perivascular neurons and astrocytes. NO is synthesized in endothelial and other cells by the five-electron oxidation of a guanidino nitrogen of L-arginine, producing NO and L-citrulline. There are three isoforms of NOS; endothelial and neuronal isoforms are constitutively expressed but are maximally active in the presence of increased intracellular $Ca^{2+}$, and inducible NOS is induced in response to a variety of stimuli, does not require $Ca^{2+}$, and produces 100- to 1000-fold more NO than the constitutive isoforms. Cofactors for NOS include molecular oxygen, NADPH, tetrahydrobiopterin, FAD, and FMN. The $Ca^{2+}$ mediated activation of constitutive NOS's involves $Ca^{2+}$ binding to CaM.

Endothelial NOS is localized primarily to vascular endothelium (76). The promoter for eNOS contains a shear stress response element. Other factors that may upregulate eNOS expression are estrogen, cGMP, basic fibroblast growth factor, transforming growth factor-$\beta_1$, atherosclerosis, cirrhosis, pregnancy, and cerebral ischemia (76). It may be downregulated by hypoxia, tumor necrosis factor-$\alpha$ (TNF-$\alpha$), lipopolysaccharide, heart failure, and possibly NO. The eNOS protein is myristoylated and palmitoylated and these modifications appear to localize the enzyme to caveolae in the plasma membrane and may be necessary for optimal enzyme activity, although binding of eNOS to caveolin-1 decreases its activity. Caveolin-3 is one of several proteins (in addition to caveolin-1 and -2) localized to caveolae which are invaginations of the plasma membrane in which signal transduction molecules seem to congregate. The enzyme is constitutively active in cerebral arteries so that loss of NO production or effect will lead to arterial constriction and reduced CBF. Relaxation of cerebral arteries in response to ACh, BK, arginine vasopressin, oxytocin, SP, histamine, ET, ATP, UTP, and $PGF_{2\alpha}$ may involve endothelial release of NO. NO relaxes vascular smooth muscle by activating SGC. This increases the concentration of cGMP and leads to relaxation by reducing intracellular $Ca^{2+}$, inactivating MLCK, and dephosphorylating MLC (77). Relaxation may also be mediated by direct action of NO to open $K^+$ channels. NO was suggested to have a half-life *in vivo* of milliseconds, although it may form adducts and be stable for seconds, especially in the absence of Hb, which is the major sink for NO *in vivo* (78). NO can be inactivated by reacting with $O_2^{-\bullet}$ to form peroxynitrite ($ONOO^-$). The biology of reactions of NO is complex and depends on relative concentrations of substrates and various enzymes and may generate vasoconstrictors or vasodilators and increase toxic compounds or remove them. All the NOS isoforms can generate $O_2^{-\bullet}$, especially in the absence of L-arginine and tetrahydrobiopterin (79). NO also inhibits platelet and neutrophil aggregation, adhesion of leukocytes to the endothelium,

and smooth muscle proliferation. NO derived from eNOS may be important in vascular remodeling. NO may mediate biological effects via nitrosation of tyrosine residues on proteins which may modify protein function, nitrosylation of metals which may alter enzyme function, and deamination of DNA that may cause DNA mutation and strand breakage and activation of poly-(ADP-ribose) polymerase (78). Most data in ischemic stroke models indicate that NO generated by eNOS is protective, whereas that generated by nNOS is detrimental.

The tone of cerebral arteries is subject to modulation by NO produced by neurons in the brain and in perivascular nerves (78). Production of this NO by nNOS may mediate the increase in CBF in response to hypercapnia. Although nNOS may be located primarily in neurons and perivascular nerves, catalytically active nNOS may occur in vascular smooth muscle and other tissues (76). The nNOS promoter contains multiple transcription binding sites in the promoter/enhancer region, including putative sites for AP-2, TEF-1/MCBF, CREB/ATF/c-fos, NRF-1, ETs, NF-1, and NF-κB while physiologically and pathologically important, these are not well worked out. The role of nNOS in the regulation of tone in the major cerebral arteries has not been defined clearly; some investigations of changes in nNOS in VSP are reviewed later.

Endothelium-dependent relaxation is impaired after SAH and various pharmacological studies reviewed in Chapter 6 have concluded that the impairment is at any of the possible sites, including impaired synthesis and release of NO, scavenging and destruction of NO before it reaches the smooth muscle, and impaired ability of the smooth muscle to generate cGMP and relax in response to NO (80). Changes in expression of genes involved in the NOS–NO–SGC system have been investigated after SAH. Kasuya *et al.* measured the levels of eNOS and SGC protein in the double-hemorrhage dog model of SAH (81). A small but statistically insignificant decrease in eNOS and a significant decrease in guanylate cyclase were noted 7 days after SAH, suggesting that the reduction in endothelium-dependent relaxation after SAH might be related to impaired smooth muscle response to NO. In a primate model of clot placement against the right cerebral arteries to simulate SAH, mRNA levels of SGC and endothelial and neuronal NOS were determined during VSP 7 days after SAH (82). There was a significant decrease in eNOS in the vasospastic cerebral arteries and a significant increase in the brain perfused by these arteries. No changes in nNOS or SGC were detected. Changes in NO-mediated relaxation occur after SAH but, as mentioned previously, conflicting data have been published and further work is necessary. For example, immunoreactivity for nNOS was lost during VSP of monkey cerebral arteries, although changes in mRNA for this gene were not reported in the same model (82,83).

The inducible form of NOS is not usually detectable in most cells but is increased in many cell types including those in the blood vessel wall in response to inflammatory mediators such as lipopolysaccharide, cytokines (interferon-γ, IL-1β, and TNF-α) and cerebral ischemia and decreased by IL-4, IL-10, transforming growth factor-β, basic fibroblast growth factor, aldosterone, HSP70, insulin-like growth factor, dexamethasone, and NO. Expression is regulated mainly at the transcriptional level and requires binding of NF-κB to the iNOS promoter. Like the other NOS forms, the promoter contains a shear stress response element. The mRNA contains a "AUUUA" sequence that may destabilize it and shorten its half-life to hours (76). Increased iNOS expression occurs after a variety of vascular injuries, such as endothelial removal and balloon angioplasty. The higher levels of NO produced may be detrimental but iNOS may be protective in these circumstances by reducing platelet and leukocyte adherence, decreasing smooth muscle proliferation, and acting as a compensatory mechanism in response to endothelial dysfunction or loss (84). The production of NO by iNOS in inflammatory cells probably has physiologically important antimicrobial and antitumor effects. Inducible NOS mRNA was detected in the basal pia mater of rats 1 day after cisternal blood injection and immunohistochemistry showed immunoreactivity to iNOS in inflammatory cells in the subarachnoid space 1 or 2 days after SAH (85). When SAH was induced by endovascular perforation of the anterior cerebral artery, the increase in iNOS mRNA was more prolonged, being detectable 1–7 days after SAH (86). Administration of the relatively selective iNOS inhibitor aminoguanidine prevented VSP. Tanazawa and colleagues concluded that iNOS was not important in VSP based on indirect pharmacological evidence (87). Injection of lipopolysaccharide into the CSF of dogs dilated the basilar artery and was associated with induction of proinflammatory cytokines in CSF. The basilar artery, when denuded of endothelium and studied *in vitro*, dilated in response to L-arginine, which is characteristic of a response to iNOS. The basilar artery subjected to SAH, however, did not dilate to L-arginine unless pretreated with lipopolysaccharide, suggesting that there is no iNOS induced in the media and/or adventitia of dog basilar artery after SAH.

$PGI_2$ (prostacyclin) is a metabolite of arachidonic acid synthesized in endothelial cells by cyclooxygenase (PGH synthase) (80). There are constitutive (cyclooxygenase-1) and inducible (cyclooxygenase-2) forms of the enzyme. Like eNOS, the cyclooxygenase-1 promoter contains a shear stress response element and enzyme activation

depends on increased intracellular $Ca^{2+}$ in the endothelial cell. Cyclooxygenase-2 is induced in macrophages, fibroblasts, endothelial cells, and VSMCs in response to similar stimuli as for iNOS, including lipopolysaccharide, cAMP, hypoxia, cytokines, growth factors, and hormones. These factors are consistent with its role in inflammation. $PGI_2$ also inhibits platelet aggregation and induces smooth muscle relaxation by multiple mechanisms that probably include reducing intracellular $Ca^{2+}$, dephosphorylating MLC, inactivating MLCK, activating adenylate cyclase and increasing cAMP, activating $K^+$ channels, and increasing NO. Although it was identified long before NO, less appears to be known about the regulation of $PGI_2$ production. The cyclooxygenases may also produce endothelium-derived contracting substances that are not ETs and that may or may not be oxygen-derived free radicals.

Early experiments suggesting that cAMP might effectively reverse VSP were limited to acute spasm (72). Subsequent investigations did not convincingly show that administration of stable $PGI_2$ analogs or pharmacological manipulation of eicosanoid production affected VSP. Recent investigations have begun to examine eicosanoid production at a molecular level. In the double-hemorrhage dog model, cyclooxygenase-2 protein was detected by immunoblotting in the basilar artery 2–7 days but not 9 days after SAH (88). There was no cyclooxygenase-2 in normal basilar artery.

Endothelial cells may also mediate relaxation by production of endothelium-derived hyperpolarizing factor (80). Harder and colleagues suggested that there is a substance released by endothelial cells in response to many of the same factors that release NO, and that this substance diffuses to the VSMC and induces relaxation by activation of $K^+$ channels and hyperpolarization of the VSMC (89). This reduces vascular smooth muscle tone, although the mechanisms are unclear. One mechanism is that membrane potential is reduced, voltage-gated $Ca^{2+}$ channels close, there is a reduction in $Ca^{2+}$ influx, and tone is reduced. Small changes in membrane potential (e.g., 3 mV) can increase or decrease $Ca^{2+}$ by up to twofold. However, other mechanisms may be involved. There is a general tendency for eNOS to be more important in larger cerebral arteries and endothelium-derived hyperpolarization to show the reverse trend with more activity in small cerebral arteries (90). Several studies show that endothelium-derived hyperpolarizing factor is a diffusable substance released by endothelial cells that diffuses to VSMC. Favored candidates in coronary arteries are the cytochrome P450 metabolites produced from metabolism of arachidonic acid such as epoxides (e.g., epoxyeicosatrienoic acid) formed by the epoxygenase pathway. Little is known about endothelium-derived hyperpolarizing factor and the cerebral circulation, and it would be premature to speculate on a role in VSP. It has been suggested that of the three relaxation mechanisms, perhaps $K^+$ channel function, mediated by cAMP, is best preserved after SAH and may be a useful therapeutic target (71,91). The relatively greater effect of SAH on cGMP-mediated relaxation with preservation of cAMP and $K^+$ channel-related mechanisms is consistent with results reported in rabbit (92) and rat models (91) of SAH.

There are three ET peptides (ET-1, ET-2, and ET-3) that are 21 amino acids long and are produced by separate genes (80). The ETs show sequence similarity to sarafotoxin S6c, the venom of the snake *Atraclaspis engadensis*. They are synthesized as preproETs of approximately 200 amino acids and then cleaved by endopeptidases to big ETs and then to ETs by ECEs (80). ECEs (ECE-1 and ECE-2) are metalloproteinases that are found in the membrane fraction of cells. ET-1 is the predominate peptide produced in cerebral arteries and its production is increased by thrombin, transforming growth factor-β, TNF-α, and possibly Hb. Shear stress, NO, and cGMP reduce ET-1 production. ET acts on $ET_A$ receptors found predominately on smooth muscle, leading to contraction. $ET_B$ receptors are located on smooth muscle, on which may mediate contraction ($ET_{B1}$), and on endothelial cells ($ET_{B2}$), on which they mediate relaxation by release of NO and/or $PGI_2$. The mechanism of contraction in smooth muscle appears to involve influx of extracellular $Ca^{2+}$ and possibly PKC.

Evidence that the ET system is involved in VSP includes studies showing that ET concentrations in CSF after SAH may be elevated in experimental animals and humans (93–96) and that antagonists of ET receptors prevent or reduce the severity of VSP in animals (97–101). Both of these findings are not without exception (102–106), and although an ET receptor antagonist prevented VSP in a monkey model, ET concentration in CSF was not elevated (107); when it was found to be elevated, cerebral ischemia seemed to be the cause rather than the reverse (108). On a molecular level, ET immunoreactivity, in addition to free ET in serum and/or CSF, was searched for in the basilar arteries of dogs with SAH (109). ET immunoreactivity was increased in the endothelium of the vasospastic basilar artery. Other investigators documented that ET-1 immunoreactivity was increased 2 days after a single cisternal blood injection in dogs but not 7 days after two injections in the double-hemorrhage model, suggesting that ET-1 might contribute to the early phases of VSP (110). Concentrations of immunoreactive ET-1 averaged 113 pg/mg protein prior to VSP, 180 pg/mg on day 2, and 115 pg/mg on day 7. Hirose *et al.* reported sustained increases in ET-1 immunoreactivity

on histological cross sections of the basilar artery of dogs after 3 and 7 days in the double-injection model (111).

Studies have suggested that Hb may increase ET-1 release from endothelial cells (112,113), but the results of these studies and their significance must be interpreted with the knowledge that pure Hb was not used, that some investigators could not confirm this result (114), and that the concentration of Hb that reaches the endothelial cells may not be high enough to stimulate ET-1 synthesis (115).

Expression of $ET_A$ receptor mRNA was markedly increased on day 3 and slightly on day 7 in a two-hemorrhage SAH model in dogs (116). VSP was prevented by continuous intrathecal administration of the $ET_A$ receptor antagonist BQ-123, supporting a role for alteration in the ET system in VSP.

In a primate model of clot placement against the right cerebral arteries to simulate SAH, mRNA levels of preproET-1 and preproET-3 and $ET_A$ and $ET_B$ receptor mRNAs were determined during VSP 7 days after SAH (117). There was a significant increase in $ET_B$ receptors in the vasospastic right MCA, an increase in $ET_A$ and $ET_B$ receptor mRNAs in the right cerebral cortex, and a decrease in ppET-3 mRNA in the right cortex. ET-1 peptide was not elevated in CSF on day 7. Specific agonist binding to $ET_A$ and $ET_B$ receptors in both MCAs and in surrounding brain cortex measured by autoradiographic binding assays supported the findings of changes in receptor mRNAs. The simple hypothesis that overexpression of ET-1 causes VSP was not supported. More complex changes in the ET system occurred that may contribute to VSP since, in this same model, an ET receptor antagonist did reduce VSP (107). These findings are consistent, however, with those of Roux *et al.*, who performed studies in the rabbit single-hemorrhage and dog double-hemorrhage models (118). In both models, VSP was associated with a shift from $ET_A$ to $ET_B$ receptor expression in the cerebral arteries, a change which also occurs when VSMCs change phenotype from contractile to synthetic type when cultured *in vitro*. There was an increase in big ET-1 in the VSP basilar arteries and an increase in ECE activity. Ohkuma *et al.* were also unable to detect an increase in preproET-1 mRNA in the basilar artery of dogs 7 days after double SAH although, in contrast to the results of Hino *et al.*, there was an increase in ET-1 protein as assessed by immunohistochemistry in the basilar arteries 4 days after SAH (101).

Pluta *et al.* suggested that the increases in ET-1 in CSF that were reported after clinical and experimental SAH might be a consequence of brain ischemia rather than a pathogenetically important initial effect of SAH (108). Any increase in ET-1 protein would be expected to be detectable as an increase in ET-1 gene transcription or mRNA stability. ET-1 mRNA was not increased in one study, although gene transcription assays were not done (117). In rats, antisense oligoDNA directed at preproET-1 was incorporated into all layers of the basilar artery after intracisternal injection. This prevented contractions of the basilar artery exposed *in situ* to whole blood hemolysate for up to 72 hr after treatment (119). Intracisternal antisense oligoDNA to preproET-1 with or without concomitant recombinant tPA was administered intracisternally 20 min after the second blood injection in the double-hemorrhage dog model of SAH (101). Injection of antisense oligoDNA alone did not significantly reverse VSP (basilar artery diameter 64 ± 13% compared to 51 ± 8% for placebo) but tPA alone (81 ± 5%) or combined with antisense oligoDNA (95 ± 6%) did so.

Juvela published a summary of the data on ET, SAH, and VSP (96). Six findings supported a role for ETs in VSP. Intracisternal injections of ET-1 produce long-lasting constriction of cerebral arteries that is associated with histological changes in the arteries. ET-1 acts more potently from the adventitia side than the luminal side of cerebral arteries. Reports of CSF levels and effects of drugs antagonizing the actions of ET-1 are conflicting but are consistent with increases in ET-1 occurring as a result of the cerebral ischemia and/or endothelial damage that may complicate the early stages of SAH or during VSP and with the fact that ET antagonists prevent experimental VSP. Contractions to other agonists are potentiated by subthreshold concentrations of ET-1. Thrombin and Hb may increase ET-1 production in endothelial cells and VSMCs. Various interactions of ET may contribute to VSP even in the absence of major increases in concentration. This is important since CSF levels are generally in the picomolar range after SAH and would not be capable of causing large contractions. Receptor sensitivity may be increased. ET-1 may stimulate its own synthesis by acting on $ET_{B1}$ receptors on endothelial cells but its synthesis may be inhibited by NO and $PGI_2$, which both may be increased by ET-1 acting on $ET_{B1}$ receptors. ET-1 levels are highest generally right after SAH. Its synthesis is also stimulated by cytokines and growth factors that may be increased after SAH. It is mitogenic and could contribute to remodeling and theoretically to the papaverine-resistant late phase of VSP.

Reactive oxygen species may modulate vascular tone. Their possible role in VSP is interwoven in a complex series of reactions involving other mediators released from brain, inflammatory cells, cerebral arteries and leading to production of other substances, that may depend on time, location, and concentration and may lead to constriction or relaxation. Carbon monoxide relaxes some arteries and may relax cerebral arteries (120).

## VII. Changes in Genes That May Alter Vasospasm

### A. Heme Oxygenases

Heme oxygenases (HOs) are enzymes that metabolize the heme groups of various proteins, including Hb, to biliverdin, free iron, and CO. There are at least three types. HO-2 is constitutively expressed in many tissues, whereas HO-1 is an inducible enzyme that is increased in response to heavy metals, oxidative stress, ultraviolet light, heme, and numerous other stimuli. The abundance of Hb in the subarachnoid space and its postulated role in the genesis of VSP have led to investigation of changes in HO-1 in cerebral arteries and brain tissue after SAH. HO-1 mRNA was increased in the basilar artery of rats with SAH, and preventing this increase with antisense oligodeoxynucleotides to HO-1 aggravated VSP and delayed the clearance of Hb from the subarachnoid space (17).

SAH was created in monkeys by placement of blood clot in the subarachnoid space on the right side (121). There was significant VSP 3 and 7 but not 14 days after SAH. There were no significant changes in mRNA for HO-1 or ferritin in cerebral arteries or brain tissue at any time. HO-1 and ferritin proteins were significantly increased in arteries exposed to SAH 3, 7, and 14 days after SAH. The increase in HO-1 protein was maximal at 3 days, whereas the increase in ferritin protein was maximal at 7 days. Most of the increase was in cells in the adventitia of the cerebral arteries. There was no increase in HO-1 or ferritin protein in the brain.

### B. Immediate Early Genes

Regulation of gene expression in eukaryotes in response to a variety of stimuli is often mediated by changes in the activity of sequence-specific transcription factors. The transcription factors bind to specific sites in the promoter regions of the DNA of genes that they regulate. These genes may be other transcription factors, immediate early genes, or others. Eventually, expression of long-term response genes is affected and biological effects occur. One category of genes whose expression is increased early in response to cell stress are the protooncogenes, including the c-*fos* and c-*jun* family of transcription factors. The protein products of these genes interact via leucine zipper motifs to form homo- or heterodimeric transcription factors that bind activator protein-1 (AP-1) binding sites or 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-responsive elements of selected genes and initiate a delayed genetic response. Transcription factors can be modulated in many ways, such as by extracellular signals through activation of one or more of three MAP kinase signaling pathways. Changes in immediate early gene expression were studied in rat aortic VSMCs in response to RBC hemolysate (122). The mRNAs for c-fos, jun B, and c-jun were increased by hemolysate, whereas jun D mRNA was unaffected. ATP and Hb alone or in combination did not reproduce the pattern of immediate early gene introduction. Furthermore, multiple substances in hemolysate seemed to cause the response since molecular size fractionation showed that all fractions of weight greater than 6 kDa were required to produce the full response. The results are consistent with the requirement of multiple RBC cytosol components to increase intracellular $Ca^{2+}$ in isolated VSMCs (123).

### C. Inflammation

Inflammation is the response of living tissue to injury. The pathological insult of SAH results in inflammation in the subarachnoid space, although the exact contribution of this process to VSP remains unclear. Inflammation may have beneficial and detrimental effects. For example, administration of proinflammatory cytokines early after spinal cord injury in mice exacerbated injury, whereas administration later reduced injury (124). Activated macrophages and microglia have been reported to promote axonal regrowth after spinal cord injury in rats (125). Global inhibition of inflammation with broad-spectrum drugs may not produce any overall benefit and may be associated with substantial toxicity in humans. The situation is complicated after SAH because inflammatory processes may be involved in VSP and in cerebral ischemia and the mechanisms may be different and/or have different time courses. The hypothesis that VSP is due primarily to inflammation in the subarachnoid space must also be reconciled with the observation that the pattern of infarction in VSP tends to be cortical and is not the same as that after any of the types of infectious meningitis which tend to be perforator territory.

Repeated blood injections 1 week apart into monkeys did not result in worsening of VSP, suggesting that a delayed-type hypersensitivity reaction did not contribute to VSP (7). On the other hand, Kubota *et al.* studied the kinetics of lymphocyte subsets in the CSF of rats after SAH and found macrophages and T cells 2–5 days post-SAH with a peak at 2 days (126). The pattern resembled a delayed-type hypersensitivity or chronic allergic reaction.

The theory that inflammatory mechanisms might contribute to VSP was supported by the association of many findings with VSP. Immunoglobulin G and the third complement component were found in the walls of vasospastic arteries of animals (127) and man (128), and patients with SAH had elevated levels of circulating immune complexes and activated complement (129–132). The CSF of patients

with poor outcome after SAH contains more IL-1 receptor antagonist and TNF-α than does that of patients who do well (133). Both of these cytokines are proinflammatory and were hypothesized to contribute to brain damage. Ryba and colleagues found immunoglobulin M and the third component of complement in the arteries of patients with SAH but did not detect immunoglobulin G (134). Drugs that reduce inflammation such as corticosteroids improved outcome after SAH in phase 2 (135,136) and 3 trials in humans (137) and reduced VSP in animal models (138–140). Cyclosporine A reduced VSP after SAH in dogs (141) and monkeys (Fig. 12.7) (142). FK506, however, did not reduce VSP in dogs (143,144). Finally, the most common class of gene overexpressed after SAH in dogs was inflammation related (18).

Hirashima *et al.* administered antagonists of platelet-activating factor to rabbits with SAH and found disparate effects in that anti-platelet-activating factor immunoglobulin prevented neurological deterioration and VSP but synthetic platelet-activating factor antagonists prevented neurological deterioration but not VSP (145). Nonrandomized clinical studies of cyclosporine A have shown no convincing evidence for efficacy (146,147). It is axiomatic that these agents, and indeed any pharmacological agent, especially in high doses, will exert effects other than those intended, which makes it difficult to draw conclusions about mechanism of disease. Furthermore, several studies failed to find any effect of cyclosporine A (144) and FK506 (144) and some of the investigators who thought the drugs might work commented on the major problem of toxicity associated with use of these drugs and that the antivasospastic effect was mild and might not translate into a clinically useful effect (Fig. 12.7) (141,146). Patients with antiphospholipid antibodies may do worse if they develop VSP but there is no evidence that this is due to increased severity of VSP (148,149). The increased risk of thromboembolism in such patients and evidence that acetylsalicylic acid reduces cerebral ischemia rates after SAH argues for a role for platelet aggregation and possibly inflammation in the development of ischemia after SAH but not necessarily in VSP (150).

Injection of talc (151) or latex beads (152) into the subarachnoid space of animals causes severe inflammation associated with arterial narrowing. Various other immunological changes of uncertain significance have been found in patients with SAH (153,154).

The work of Peterson in Zervas's group may have delineated an important role for inflammation in VSP (155). The experiments were conducted under the assumption that hemolysis is an important factor in releasing spasmogens that cause VSP. RBCs lysed only very slowly *in vitro* under conditions simulating SAH. The rate of lysis

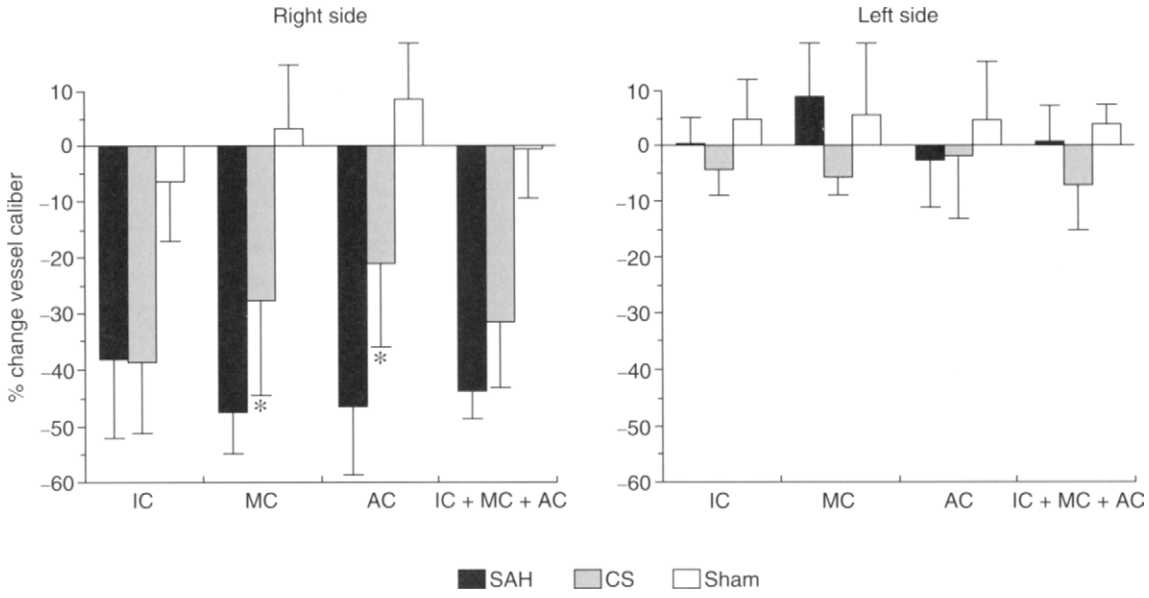


**FIGURE 12.7** Bar graph of percentage reduction in right and left-sided cerebral arteries of monkeys with SAH, sham surgery, or SAH plus treatment with cyclosporine (CS). There is VSP of the right internal carotid (IC), MCA (MC), and anterior cerebral (AC) arteries in animals with SAH which is significantly reduced in the MC and AC after treatment with cyclosporine. Even under conditions of adequate immunosuppression with cyclosporine, however, there is arterial narrowing in animals treated with cyclosporine. There is no VSP of the left cerebral arteries (reproduced with permission from Handa *et al.* (1991), *Neurosurgery* **28**, 380–385).

was markedly increased in the presence of plasma proteins but only if the RBCs had aged more than 2 days. The increase in hemolysis was complement dependent. The role of complement in accelerating RBC lysis could underlie the beneficial effects of systemic complement depletion with cobra venom factor that were noted in a single-hemorrhage rabbit model (156). Similarly, FUT-175 (nafamostat mesilate), a nonspecific serine protease inhibitor, decreased VSP after SAH in rabbits and humans (157,158). Mechanistic data were not presented. Park and colleagues presented another mechanism by which complement might produce VSP (159). Complement activation by aged RBCs resulted in formation of membrane attack complexes that inserted into membranes of freshly isolated rat cerebral artery VSMC *in vitro*. This resulted in large increases in membrane conductance. The authors hypothesized that this could lead to cell damage and perhaps contraction.

Handa's group presented early evidence on a molecular level for immunological reactions in VSP (160). Immunoreactivity to ICAM-1 was studied histologically in rat basilar arteries after SAH. There was VSP 2 days and increased ICAM-1 immunoreactivity 2–5 days after SAH. Sills *et al.* reported that immunohistochemical staining for ICAM-1 was increased 3–24 hr after placement of clot around the femoral arteries of rats (161). This adhesion molecule mediates leukocyte migration from the bloodstream through the arterial wall as wall as cell–matrix interactions that occur during tissue repair. CSF samples were taken from 17 patients with aneurysmal SAH and 16 control patients (162). Immunoreactivity to ICAM-1, VCAM-1, and L-selection was elevated in SAH patients, which seems to support the results of the animal studies.

The function of a protein could be blocked by administration of antibodies that bind to specific domains of the protein. Oshiro *et al.* administered monoclonal antibodies directed at ICAM-1 to rats (163). VSP and inflammation around the rat femoral artery in response to perivascular blood clot placement were reduced, suggesting that inflammation mediated by ICAM-1 plays a role in VSP in this model. Ibuprofen placed around the femoral artery within 6 hr of blood clot placement also reduced VSP in this model (164). Limitations include the use of a systemic artery to study VSP and difficulty in documenting specific effects with such an approach. Bavbek and colleagues administered antibodies to ICAM-1 and/or CD18 into the CSF of rabbits at the time of injection of 3 ml of autologous arterial blood (165). VSP 48 hr later was significantly attenuated.

Ono *et al.* evaluated the effect of antisense oligodeoxynucleotides to NF-κB administered intrathecally on VSP after SAH in rabbits (166). The oligodeoxynucleotides were complexed with cationic liposomes prior to injection. This treatment prevented VSP and morphological changes in the arteries and decreased activity of NF-κB as measured by gel-shift assay. An interesting approach to VSP was taken by investigators in the laboratory of Kassell (167). They administered mono- or diphosphoryl lipid derivatives of lipopolysaccharide intrathecally to rabbits immediately before and 24 hr after single SAH. VSP was reduced in rabbits treated with monophosphoryl lipid A and this was associated with preservation of SOD dismutase activity in the basilar arteries—activity which was lost in animals with VSP. The authors acknowledged that further experiments are needed to define the mechanism of the effect, but it raises the possibility that alteration in the expression of endogenous antioxidant enzymes in the basilar artery contributes to VSP.

A double-hemorrhage model was created in rats by injection of 100 μl of autologous blood into the cisterna magna followed by a second injection 24 hr later (168). VSP was documented by angiography and occurred in 11 of 15 animals. In animals with VSP, iNOS immunoreactivity was observed 7 days after SAH in cells in the tunica intima, media, and most markedly in the adventitia of the basilar artery. Immunohistochemical staining of iNOS was also observed in microglia, astrocytes, and neurons in the brain.

### D. Remodeling, Fibrosis, Proliferation, and Phenotype Change

The observations that VSP arteries develop reduced contractility and compliance after SAH and that there are histopathological changes in the arteries are well-known and were discussed in Chapter 4. These studies show that histopathological changes that occur in cerebral arteries during and after VSP are consistent with those known to occur when an artery is contracted by any stimulus (169). Although contraction may cause the damage and indeed some of the pharmacological changes, the exact cause of these changes after SAH is not known. VSP arteries exhibit a progressive decrease in contractility and compliance with increased time after SAH (170,171). The characteristic response of any artery to many types of injury is for the medial VSMCs to migrate and proliferate in the tunica intima (172). This does occur after SAH but generally toward the end or after the angiographic phase of VSP. The very presence of the phenomenon, however, indicates that several events must be occurring. These include some form of remodeling (173) and of phenotype change in the vascular smooth muscle. There are relatively few studies of changes in gene expression that may mediate some of these processes in VSP and that may represent and/or produce arterial wall remodeling, fibrosis,

and proliferation of and phenotype change in smooth muscle.

The classic remodeling response was the permanent alteration in arterial diameter in response to changes in blood flow. It must involve changes in VSMCs including contraction and relaxation, at least acutely, since these are the only cells in the arterial wall capable of such processes (174). Langille and O'Donnell noted that removal of the endothelium prevented the chronic reduction in arterial diameter of rabbit carotid arteries in response to reduced flow (174). Rudic and colleagues implicated eNOS in the remodeling response by showing that it was absent in mice lacking the eNOS gene (175). The experiments previously reviewed suggest that production of NO by eNOS is reduced in VSP (82). This would theoretically reduce any remodeling after SAH rather than permit or produce it.

Takenaka *et al.* applied CSF from patients 2 days after SAH to cultured pig cerebral artery VSMCs (176). This resulted in a transient peak increase in intracellular $Ca^{2+}$ and an increase in [$^3$H]thymidine incorporation into the cells. The authors suggested that an unidentified factor in the CSF caused these responses and could theoretically produce some of the morphological changes noted in cerebral arteries after SAH such as endothelial proliferation. Pluta *et al.* noted that in monkeys with SAH, there was a small increase in proliferation of cells in all layers of arteries with VSP. This was discounted as being unimportant as a source of cells to physically narrow the artery, although it could be an indication of phenotype change (177).

The stiffening and thickening of the arterial wall after SAH may reflect increased connective tissue (178,179). Nagasawa and colleagues studied pharmacological properties and connective tissue composition of dog basilar arteries after a single injection of blood into the cisterna magna (179). Their pharmacological findings are different from those of others in that there was reduced stiffness of the arterial wall 2 days after SAH, after which time it became stiffer but not more so than a normal artery. The increase in stiffness corresponded with the time of VSP. They measured increased elastic tissue in the arterial wall within 2 days of SAH that persisted for 28 days and an increase in collagen starting 14 days after SAH. Nakamura *et al.* created SAH in cats by intracisternal injection of 2 ml/kg of autologous fresh blood into the cisterna magna (178). The animals were euthanized and fixed by perfusion 3 and 7 days after SAH. Collagen protein was quantified by morphometric measurements and immunohistochemistry. Three days after SAH there was a slight increase in collagen fibrils in the tunica media which became marked 7 days post-SAH. Changes consistent with VSP were also noted at the later time. The volume of collagen increased as early as 12 hr after SAH. The predominant isoform was collagen III. These responses were similar in the basilar and pial arteries. In the rat femoral artery model of VSP, there was increased procollagen I and III mRNA 7 and 14 days after clot placement but not at earlier times (180). The mRNA for transforming growth factor-β, a regulator of collagen synthesis, was increased 3 days after clot placement, after which it declined, suggesting that it could mediate the subsequent increase in collagen expression.

Other studies were unable to document an increase in collagen in arteries during VSP. The angiographic time course of VSP in the monkey clot placement model is for significant VSP to be present 7 days post-SAH followed by substantial reversal by 14 days and complete return to normal diameter by 28 days. Immunohistochemistry of arteries at these times did not demonstrate changes in protein levels of collagens (types I and III–V), desmin, myosin, laminin, or α-actin in the tunica intima or media (181). Fibronectin immunoreactivity increased 14 days after SAH and fibrinogen increased in the media at 7 days, Twenty-eight days after SAH there was intimal thickening which contained immunoreactivity to α-actin, myosin, vimentin, desmin, fibronectin, laminin, and each type of collagen. Hydroxyproline content of cerebral arteries, which would reflect collagen content, was not altered at any time, suggesting that the overall magnitude of increase in collagen is very small.

A role for increased collagen synthesis in VSP was suggested by Onoda and colleagues (182). Inhibition of procollagen type I gene expression with antisense oligodeoxynucleotides reduced VSP in a rat femoral artery model.

Collagen synthesis also occurs in the subarachnoid space after SAH. In rats with SAH, the activity of an enzyme involved in collagen synthesis, prolyl-4-hydoxylase, was increased in the dura mater starting 1 week after SAH and continued for up to 1 month (183). Collagen synthesis was measured *in vitro* with [$^3$H]proline and was increased 1 and 2 weeks after SAH. After 3 weeks, type I collagen could be detected immunohistochemically in the meninges. In humans, CSF concentrations of type I and type III procollagen propeptides, as measured by radioimmunoassay, were increased in a time-dependent manner in patients with recent SAH (183). They peaked about 2 weeks after SAH but remained elevated for up to 10 weeks. Four patients who developed late Hyc had levels higher than those of matched control SAH patients. The authors suggested a role for meningeal fibrosis in posthemorrhagic Hyc.

The VSMCs in the media of arteries of adults are highly differentiated cells that proliferate at very low rates and synthesize only small amounts of extracellular matrix proteins (172). There may be other populations of VSMCs but the majority are mature cells of the so-called

contractile phenotype (172). The contractile VSMCs classically alter their phenotype to a more synthetic and/or proliferative state when placed in culture *in vitro* and during angiogenesis, remodeling, and in response to arterial injury such as in atherosclerosis and restenosis after angioplasty. The synthetic or proliferative phenotype is characterized by increased proliferative rate and synthesis of extracellular matrix. No protein is expressed only in smooth muscle, but proteins that are expressed relatively specifically in smooth muscle include α-actin, myosin heavy chain isoforms SM-1 and SM-2, calponin, SM-22α, and high-molecular-weight caldesmon. Synthetic VSMCs usually have decreased expression of all these proteins as well as vinculin/metavinculin. Although there tends to be an association between the appearance of the synthetic phenotype and proliferation, the two are not always associated. A synthetic phenotype can exist in the absence of proliferation.

Several events occur after SAH that could alter smooth muscle phenotype. Studies of this event in vasospastic arteries have not been as extensive and convincing as those in other diseases. Phenotypic changes occur in atherosclerosis, but it is not known if they are the cause or consequence of the disease. The same could be said for VSP. Exposure of VSMCs in culture to contractile agonists such as angiotensin II and arginine vasopressin increases the synthesis of contractile proteins in the cells; in other words, it causes cell hypertrophy (185). Endothelial cells and heparin tend to promote or maintain VSMCs in the contractile phenotype, whereas denervation, growth factors, and changes in the extracellular matrix favor the synthetic phenotype. Shear stress and mechanical stress are also important modulators.

Smith's group in Mississippi originated the idea that myofibroblasts contribute to VSP (186). This idea was based on immunohistochemical characterization of cells derived from vasospastic human arteries (187,188). These were shown immunohistochemically to express less α-actin than VSMCs and to express types I, III, and V collagen and fibronectin but little type IV collagen or laminin. These were taken to be characteristic of myofibroblasts rather than VSMCs, although the identification of cells is difficult because VSMCs change their expression of α-actin and other proteins when cultured or exposed to conditions that may induce phenotype change and protein expression can vary greatly depending on the culture conditions. In any case, the central hypothesis that some type of structural change in the arterial wall contributes to the persistence of VSP seems justified. The contribution of myofibroblasts remains uncertain since it is difficult to differentiate them from proliferating VSMCs. The hypothesis was investigated further by studies demonstrating increased ability of the cells to contract collagen lattices upon exposure to CSF from patients with SAH (189,190). Lattice compaction was inhibited by verapamil and heparin but not by other $Ca^{2+}$ channel blockers (187). The responsible factor in hemorrhagic CSF had a molecular weight less than 6 kDa (189). Hemolyzed whole blood or pure RBCs did not increase mRNA for the collagen $\alpha_1$ (III) and $\alpha_2$(I) chains in cultured human foreskin fibroblasts or myofibroblasts (191).

Proliferation of VSMCs, which would be associated with substantial changes in gene expression as cells pass through the cell cycle and migrate into the tunica intima, occurs in cerebral arteries after SAH but there is a great discrepancy between results in the literature in this area. In some experimental models and clinical reports, marked intimal proliferation is observed (192,193). On the other hand, detailed studies in a primate model of SAH failed to document enough proliferation of cells in the arterial wall or development of intimal thickening to produce any of the luminal narrowing that is observed during angiographic VSP (177,181). Intimal proliferation tends to develop after angiographic VSP, in keeping with the time course of its development after other vascular injuries in higher species. Higher proliferation rates may be observed in rodents and other species and differences in the kinetics of the response to injury may occur, rendering generalizations to man based on some animal models hazardous (172). There is no question that proliferation occurs; there were more dividing cells in arteries with VSP than in those without even during VSP in monkeys (177). The significance of this phenomenon, however, is unclear. Kasuya *et al.* studied the role of insulin-like growth factor in the intimal proliferation known to be associated with some cases of SAH and VSP (194). Placement of blood clot around the femoral arteries of rats produced luminal narrowing 7 days later and intimal proliferation 14 and 21 days later. There was a four-fold increased expression of insulin-like growth factor-1 mRNA 3–7 days after clot placement, after which levels returned to baseline. Insulin-like growth factor-1 receptor binding assay showed increased receptors at these same times. It was postulated that local synthesis of insulin-like growth factor-1 and its receptor might be a stimulus for intimal proliferation.

### E. Apoptosis

Necrosis of VSMCs of the tunica media and endothelial cells of the tunica intima is well described in vasospastic cerebral arteries. Cells may die by necrosis or apoptosis (195). Necrosis is cell death characterized by early mitochondrial energy failure. There is cell swelling and mitochondrial damage and nuclear disintegration leading to early cell lysis. This type of cell death is associated with a local inflammatory reaction and all the characteristic

pathological sequelae that this may entail, and there may be injury to the surrounding tissue. The process does not require energy and is unregulated. Apoptosis is an active, energy-requiring process that may require maintenance of mitochondrial energy and integrity, at least in the early stages. Loss of mitochondrial membrane potential may activate an apoptotic pathway that involves activation of proteases such as caspases. Apoptotic cell death occurs during development and in some pathological processes. There is cell shrinkage, chromatin condensation within the nucleus, formation of apoptotic bodies, DNA laddering, and phagocytosis of the cell remnants with no inflammation. A general hallmark is degradation of cell components, such as the DNA into multiple short fragments, prior to death of the cell. In some situations, a subtype of apoptosis, programmed cell death, occurs. Apoptosis and necrosis may represent the ends of a spectrum of types of cell death. In many situations, both occur either simultaneously or separated by time, space, and/or severity of insult.

Ogihara *et al.* studied the effects of an impure solution of oxyHb on cultured bovine aortic endothelial cells (196). It was suggested that the oxyHb solution caused apoptosis of the endothelial cells. Apoptosis was detected by DNA fragmentation on gel electrophoresis, apoptotic body formation on transmission electron microscopy, and cleavage of poly-(ADP-ribose) polymerase on immunoblotting. D'Agnillo and Alayash thought that this was incorrect and that the use of impure Hb led to apoptosis (197). Nevertheless, the cerebral arteries of a patient who died 18 days after SAH were found to have endothelial cells that stained positively by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate–biotin nick end-labeling reaction which is suggested to be a marker for apoptosis (198). Characteristic changes of apoptosis such as condensation in the nuclei, pyknosis, vacuolated cytoplasm, and disappearance of the Golgi complex and smooth endoplasmic reticulum were also observed on transmission electron microscopy. Control arteries from one patient who died of head injury did not show such changes. This is the only reported case of apoptosis occurring after SAH. The inherent problems of prolonged time from SAH to examination, assorted medical interventions such as transluminal angioplasty, and the possibility of postmortem artifacts need to be kept in mind.

## VIII. Microvascular Spasm

Nihei *et al.* compared the diameters of brain stem pial arteries of rabbits subjected to two injections of subarachnoid blood into the cisterna magna 2 days apart to those of control rabbits that did not undergo injections and to those of rabbits injected with $BaCl_2$ and euthanized 10 min later (199). The time after SAH that was studied was not stated. The basilar artery diameter was 691 ± 17 μm in the controls, 319 ± 51 μm in the SAH group, and 191 ± 8 μm in the $BaCl_2$ group. There was constriction of all small vessels in the $BaCl_2$ group but not in those subjected to SAH, except in arteries arising from the basilar artery that were >80 μm in diameter when contracted or an estimated 120 μm when not contracted. They concluded that small arteries in the subarachnoid space are not contracted after SAH. It could also be argued that the $BaCl_2$ produced more narrowing of the basilar artery, and it is possible that the smaller arteries would contract after SAH if more severe VSP occurred. They reviewed studies in the literature that supported their demonstration of differential reactivity of large and small cerebral arteries.

A pharmacological study of the basilar artery and its secondary branches in the subarachnoid space was carried out in the double-hemorrhage dog model (200). SAH abolished relaxation of the small arteries to vasopressin, which is mediated through NO, whereas relaxation to BK and A23187 was preserved. The authors concluded that SAH selectively inhibits relaxation by the endothelial NO pathway and that preservation of endothelium-dependent relaxations to BK and A23187 is consistent with small arteries being resistant to VSP after SAH.

Direct morphometric measurements of penetrating arterioles were obtained in the double-hemorrhage dog model of SAH (201). Microvascular corrosion casts were measured and it was found that the penetrating arterioles were decreased in diameter 3 and 7 but not 14 days after SAH. These findings seem to contradict the clinical studies reviewed below that demonstrate increased CBV in patients with SAH. Ohkuma and colleagues hypothesize that there is pooling of blood in intraparenchymal venules which contributes to increased CBV and that there is constriction of the arterioles. The results do not contradict prior results suggesting that small arteries do not undergo VSP since Nihei *et al.* studied branches of the basilar artery of rabbits prior to their entrance into the brain stem (199). Ohkuma and Suzuki found that these arteries were in fact not narrowed 7–14 days after SAH in dogs, whereas the intraparenchymal arterioles were narrowed (202).

Bevan *et al.* studied small human pial arteries removed from patients with SAH and compared them to similar vessels removed from patients without SAH (203). The diameters were 300–900 μm. Arteries from patients who had SAH within the preceeding 48 hr displayed increased sensitivity to contractile agents and developed spontaneous contractile activity. These findings are consistent

with prior studies showing similar changes in large cerebral arteries early after SAH, suggesting that similar processes occur in both large and small arteries including VSP, although the pharmacological studies cannot assess arterial diameter reduction which is the sine qua non of VSP.

Clinical studies have usually been consistent with regard to dilation of small arteries during VSP. The classic study is that of Grubb *et al.*, who performed 45 PET studies of regional CBV, rCBF, and regional $CMRO_2$ in 30 patients with SAH (204). There was a progressive decrease in CBF and $CMRO_2$ as the clinical grade of the patient worsened. VSP added another incremental reduction in these parameters. CBV showed the reverse trend, with marked increases in patients with severe neurological deficits and severe VSP. The authors concluded that the constriction of the large extraparenchymal arteries of the circle of Willis that occurs in VSP is associated with dilation of intraparenchymal vessels which were thought to be small arteries, arterioles, and/or possibly capillaries. An increase in CBV with SAH and VSP was supported by other studies (205). Reduced ability of CBF to increase in response to acetazolamide was taken as evidence that the intraaparenchymal vessels were maximally dilated already and could not dilate further in response to acetazolamide (206). Ohkuma and coworkers challenged these findings (207). They studied 24 patients with aneurysmal SAH using regional CBF measured by single-photon emission CT and digital subtraction angiography. Patients were studied on the same day between 5 and 7 days after SAH and/or within 4 hr of the onset of a delayed ischemic neurological deficit. Cerebral circulation time was measured by assessing the density of contrast in the vessels on digital subtraction angiograms and was divided into the proximal and peripheral circulation times. Proximal cerebral circulation time was defined as the circulation time through the extraparenchymal arteries and peripheral circulation time as that through the intraparenchymal small vessels. Severe angiographic VSP was associated with reduced CBF and a relationship between severity of VSP and reduction in CBF was demonstrated. Peripheral cerebral circulation time correlated inversely with regional CBF. In addition, prolonged peripheral cerebral circulation time was noted in association with reduced regional CBF even in patients with none, mild, or moderate angiographic VSP. The authors concluded that in addition to the well-known effects of VSP on regional CBF, there was evidence for microcirculatory disturbance after SAH. They also suggested that impaired autoregulatory vasodilation or decreased luminal diameter of intraparenchymal vessels may be an important component of cerebral ischemia after SAH. More recent PET studies have shown reduced CBV in patients with VSP (208,209), suggesting that vasodilatory responses of intraparenchymal vessels are impaired after SAH.

## IX. Changes in the Brain

There are several possible reasons why gene expression may be altered in the brain after SAH. Both SAH and VSP may cause cerebral ischemia which is known to alter transcription and translation of many genes in the brain. Subarachnoid blood may have direct effects independent of cerebral ischemia.

Changes in gene expression during and after global and permanent or transient focal cerebral ischemia have been investigated. Global ischemia in gerbils and rats increases mRNA for some immediate early genes and for *hsp72* in the hippocampus, and this is translated into protein in neurons that survive (210,211).

After permanent focal ischemia, *hsp72* mRNA is expressed in neurons and glia in the area of persistent flow around the core of the infarct but not the central region, probably because the cells in the core of the infact are too damaged to be able to synthesize protein. The core eventually expresses some protein, mainly in surviving endothelial cells. Transient focal ischemia produces marked increases in *hsp72* mRNA and protein in the injured area and the pattern progressively shifts to the pattern of that with permanent ischemia with increasing duration of ischemia. After transient focal ischemia, PG endoperoxide synthase-1 mRNA increased and peaked after 30 min of ischemia, whereas c-*fos* mRNA peaked after 60 min (212). There may be increased transcription and translation of some growth factor and growth factor receptor genes (3). Many other genes are also altered, including the growth arrest and DNA damage-inducible gene, microtubule-associated protein 2, and various cytokines and their receptors (213).

Evidence that changes in gene expression play a role in cerebral ischemia is supported by studies showing that a brief episode of nonlethal ischemia renders the brain more resistant to a second, lethal ischemic insult administered 1–7 days later (214). This has been called ischemic tolerance or preconditioning. It is likely that some of the previously noted changes in gene expression mediate this phenomenon, although more experiments are required to delineate precisely the mechanisms involved.

Expression of c-Fos was studied by immunohistochemistry in the brain stem of rats after injection of autologous arterial blood (215). The c-Fos immunoreactivity peaked 2 hr after SAH and was noted in the trigeminal nucleus caudalis, nucleous of tractus solitarius, area postrema, ependyma, pia mater, and arachnoid. If the unmyelinated

c fibers were destroyed by neonatal capsaicin treatment, the resulting adult rats showed reduced c-Fos in the trigeminal nuclear complex, parabrachial nucleus, and medullary lateral reticular nucleus, but not in nucleous of tractus solitarius, area postrema, ependyma, pia mater, and arachnoid. Sectioning of meningeal afferents at the ethmoid foramen produced the same result. The authors concluded that SAH-induced brain stem c-Fos expression was mediated by both direct effects of blood and by trigeminovascular nerve fibers. The pattern of c-*fos*, c-*jun*, and *hsp70* mRNA increase was different when SAH was produced by endovascular perforation of the anterior cerebral artery in rats (216). After this SAH, c-*fos* and c-*jun* were increased in the ipsilateral cerebral cortex, hippocampus, and dentate gyrus, and for *hsp70* they were increased in these areas plus the thalamus, hypothalamus, and caudoputamen. The increases in immediate early genes but not *hsp70* mRNA were blocked by the glutamate receptor antagonist MK-801.

Yufu and colleagues studied rat brain tissue after SAH (217). At 30 min after SAH, some rats were treated with 1 atm of hyperbaric $O_2$ for 1 hr. They were euthanized 2 hr after SAH. SAH was associated with reduced $Na^+$, $K^+$-ATPase activity, membrane fluidity, and protein mobility in the membrane. These changes were alleviated by hyperbaric $O_2$ treatment.

Injection of blood, RBC hemolysate, or oxyHb into the cisterna magna of rats induced expression of HO-1 mainly in microglia throughout the brain (218). Focal areas of expression of HO-1 and Hsp70 and of DNA fragmentation that persisted for days were noted only after injection of hemolysate (219,220). It was suggested that since Hsp70 was not induced in general, there was no general stress response in the rat, and therefore the HO-1 increase was likely secondary to heme that must enter the brain. Hb injected into the cisterna magna of rats did penetrate into the brain within hours (221). Systemic administration of the tirilazad-like antioxidants (U101033E and U74389G) blocked induction of the stress proteins Hsp70, Hsp47, and HO-1 in focal brain regions normally produced by cisternal injections of lysed blood (222). It was hypothesized that the focal areas were areas of ischemia due to acute VSP or injury secondary to direct toxic effects of lysed blood. Kuroki *et al.* injected ET, hemolysate, or hemolysate saturated with CO or saline into the cisterna magna of rats (223). ET and both hemolysate solutions caused a similar degree of VSP as assessed by angiography, although only hemolysate solutions increased HO-1, as assessed by immunohistochemistry. HO-1 appeared to be increased mainly in glial cells throughout the brain as well as the dentate gyrus and molecular layer of the cerebellum. This suggests that focal areas could be due to effects of blood and not ischemia. The same investigators noted increases in HO-1 mRNA in the brains of rats after SAH (17).

Unlike cisternal blood injection, SAH produced by endovascular perforation did induce Hsp70 protein in multiple brain areas bilaterally for up to 5 days (224). Since Hsp70 is known to be induced by ischemia, it was suggested that acute and delayed ischemia induced Hsp70, although vascular diameters or CBF were not measured. In the same model, however, Bederson *et al.* showed that there is acute vasoconstriction that is associated with reduced CBF (225). Whether or not this occurs in man is uncertain. Aneurysm rupture during coiling should demonstrate acute spasm, although this has seldom been reported. Acute spasm can be an artifact of hemolysis that occurs when blood is injected through needles. Whether this occurs after SAH from the endovascular perforation model is uncertain. The reduction in CBF in the rat model could be prevented by NO donors, suggesting that decreased NO availability contributes (226).

Matz *et al.* studied whether apoptosis was present after injection of autologous RBC hemolysate into the cortical subarachnoid space of mice (227). DNA fragmentation and laddering and terminal deoxyuridine nick end-labeled cells were noted in a significant number of hemolysate-injected mice compared to those injected with saline. The changes were consistent with apoptotic cell death in the cortex after SAH.

Widenka *et al.* found induction of iNOS by immunohistochemistry after 7 days in a double-SAH model in rats. Immunoreactivity was observed mainly in animals with angiographically documented VSP and was present in all three layers of the basilar artery as well as in activated microglia, astrocytes, and neurons in the brain (168). Klinge and colleagues studied computer-controlled intracisternal blood injection into the cisterna magna and olfactory cistern of rats (228). Expression of Hsp70 in the hippocampus was present for up to 5 days after SAH. VSP was not assessed.

## X. Gene Therapy

Gene therapy is the insertion of a gene into a somatic cell to correct an underlying dysfunction of the cell or to give the cell an additional function (229). The inserted gene (nucleic acid) does so by using the cell's intrinsic transcription and translation proteins to make protein from the nucleic acid. Proteins make up ion channels, receptors, hormones, neurotransmitters, parts of the cytoskeleton, and enzymes. Enzymes make other proteins as well as other biologically-active substances that might remain intracellularly or be secreted and act in an autocrine, paracrine, or endocrine fashion. Pure plasmid DNA

does not enter cells efficiently so a vector must be used to transport the nucleic acid into the target cell. Vectors include viruses, liposomes, and physical methods such as electroporation and bolistic therapy. Small oligodeoxynucleotides can enter cells more readily but are too small to code for proteins. They are usually constructed to bind to the target gene to inhibit its transcription and possibly translation (antisense therapy). Ribonucleic acid can also be used for gene therapy (230). Numerous approaches are imaginable for VSP after SAH.

Gene therapy might be directed at preventing or treating cerebral ischemia. The delayed onset of VSP could allow administration of the gene and synthesis of protein product before ischemia. This might avoid the problem that protein synthesis often is reduced after ischemia and possibly in arteries with VSP (30). There are several reports of the use of gene therapy for ischemia. The glucose transporter gene was placed in a herpes simplex virus vector and injected into the striatum of rats (231). Transient focal ischemia was induced 6 hr later and there was decreased death of striatal neurons in treated animals compared to those injected with control virus. Since abnormally increased intracellular $Ca^{2+}$ may contribute to neuronal death during ischemia, preventing or buffering the increase may reduce injury. Overexpression of the $Ca^{2+}$-binding protein calbindin D28K using a replication-deficient, amplicon-based herpes simplex virus decreased neuron death in the striatum of rats subjected to focal cerebral ischemia (232). Overexpression of SOD protected hippocampal neurons from global ischemia (233).

The expression of HSPs is increased in areas of cerebral ischemia. HSPs may assist in removing denatured proteins from cells and promote correct folding of newly synthesized proteins. These functions are believed to protect cells from various insults. Injection of herpes simplex virus vector containing the *hsp72* gene into the striatum of rats protected neurons from transient focal ischemia (234). Some neurons may die by apoptosis after cerebral ischemia. The gene for a protein that inhibits apoptosis, *bcl-2*, has been inserted into a herpes simplex virus vector and the construct has been shown to protect rat cerebral cortical neurons from death after permanent focal ischemia (235), to protect striatal neurons after transient focal ischemia (236), and to protect hippocampal neurons after transient global ischemia (237). In the studies of focal ischemia *in vivo*, only focal transgene expression was achieved and neurons were rescued in these areas but reduction in infarct size was usually not achieved. Few studies have examined when the vector must be expressed in order to protect, but for vectors expressing *bcl-2*, neuronal protection was possible with injection 1.5 hr after onset of cerebral ischemia *in vivo* (238) and 8 hr after excitotoxic insult to cultured neurons *in vitro* (239).

Adenovirus expressing the IL-1 receptor antagonist gene was injected into the ventricular system of mice and rats and shown to increase protein levels of this gene in the brain and CSF (240–242). Permanent or transient focal ischemia was induced 5 days later and animals receiving this vector had reduced infarct size compared to those injected with virus expressing a reporter gene. The studies previously reviewed used intracerebral injections of vector, although injection into the CSF may be useful for secreted proteins as demonstrated by these investigators. Vectors expressing the glucose transporter, calbindin D28K, *hsp* 72, and *bcl-2* have been shown to reduce neuronal death due to a variety of other toxic insults *in vitro* and in some cases *in vivo* (243,244). Another gene therapy strategy might attempt to regenerate brain injured by ischemia (245).

The following are features of VSP that make it an attractive disease to treat with gene therapy: it is transient and it may circumvent a limiting factor in systemic treatment with vasodilators—that is, large enough doses cannot be given to dilate the cerebral arteries without causing substantial hypotension that obviates any benefits gained by cerebrovascular dilation. Gene therapy might stimulate collateral flow in the brain, synthesize a vasodilator in or near the VSMCs or inhibit a constricting process (101,229). Adenoviruses injected into the cisterna magna of mice (246), rats (247,248), and dogs (249) transfect cells in the leptomeninges and adventitia of large blood vessels. SAH does not seem to affect gene transfer *in vivo* (250) and may enhance expression *in vitro* (251). Injection of adenovirus expressing NOS into the cisterna magna of dogs will transfect the adventitial fibroblasts of arteries in the subarachnoid space *in vivo* and augment NO-induced vasodilation when the arteries are studied *in vitro*, although this had no effect on angiographic VSP, possibly because of inadequate production of NO *in vivo* to overcome destruction by Hb (249,252). Adenovirus expressing prepro-CGRP, a potent cerebral artery dilator, was injected into the cisterna magna of rabbits (253). Approximately one-third of adventitial fibroblasts in the basilar artery were transfected 5 days later. CSF levels of CGRP increased 93-fold at this time and study of the basilar arteries *ex vivo* showed decreased contractility and increased relaxation to an inhibitor of cyclic nucleotide phosphodiesterase. Experiments utilizing antisense techniques to investigate VSP were previously discussed (101,166,182,254).

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