AFRRI SR74-10
JUNE 1974

AFRRI
SCIENTIFIC
REPORT

SEQUENTIAL CHANGES OF VASCULAR ULTRASTRUCTURE IN CEREBRAL VASOSPASM: MYONECROSIS OF SUBARACHNOID ARTERIES

J. M. Fein
W. J. Flor
L. J. Parkhurst
J. L. Parker

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

Research was conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care," prepared by the National Academy of Sciences - National Research Council.

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J. M. FEIN
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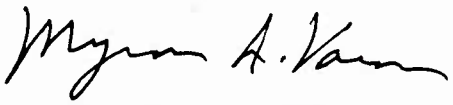
L. J. PARKHURST
J. L. PARKER

D. O. CARPENTER
Chairman
Neurobiology Department



MYRON I. VARON
Captain MC USN
Director

S. J. BAUM
Chairman
Experimental Pathology Department

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

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ABSTRACT

Cerebral vasospasm following subarachnoid hemorrhage may contribute to morbidity and mortality. The inconsistent therapeutic responses to sympatholytic agents described in the literature raise doubts as to the purely functional nature of the vasoconstriction. This study examines the ultrastructural evolution of vacuolar degeneration of the media in subarachnoid arteries which have been in spasm. The striking changes of myoarchitecture are described; however, the relationship of these findings to aberrations in cerebral blood flow remains to be clarified.

I. INTRODUCTION

There are experimental[4] and clinical[17] observations which support the hypothesis that cerebral vasospasm develops in a biphasic fashion related to the particular species and exciting causes. Severe vasoconstriction is noted clinically shortly after spontaneous subarachnoid hemorrhage and may be consistently reproduced in experimental animals by mechanical,[29] chemical,[19] or electrical stimulation.[21] The role of the sympathetic nervous system in acute experimental spasm is suggested by the rich innervation of cerebral arteries,[24,26,27] the depletion of catecholamines from nerve endings after subarachnoid injection of blood,[13] and the resolution of acute spasm in cats by topical and parenteral alpha-adrenergic blockade.[12] Thus, there is a growing consensus[10,12,13] which views cerebral vasospasm in terms of a tonic contractile mechanism probably under sympathetic influence. However, clinical use of adrenergic blocking[9] agents, as well as sympathectomy,[14] yields conflicting results when monitored clinically,[8] radiographically[22] or by cerebral blood flow measurement.[15]

It is possible that after spasm is triggered by sympathetic discharge, irreversible changes may occur in the vasculature so that sympatholytic agents alone no longer effect spasmolysis. Previous studies of autoregulation of blood flow in rhesus monkeys after subarachnoid hemorrhage[11] suggest a loss of vasoelasticity. Whether the luminal narrowing, most commonly seen at angiography in arteries greater than 250 μm diameter, is due to a tonic energy consuming contractile process alone or simply due to reactive changes in the arterial wall is unknown at present. The significant role of these subarachnoid arteries[18] in modulating cerebral blood flow, however, is reflected in the finding that relative pressure drops in arteries upstream from the

large surface arterioles (> 300 μm) vary between 33 and 39 percent depending on systemic blood pressure.[31]

In this series of animals a morphologic approach was used to study the changes in character of these arteries (> 240 μm) after angiographically demonstrable vasospasm.

II. METHODS

The data from 18 rhesus monkeys were analyzed. After induction of anesthesia, blood pressure and arterial blood gases were monitored through a polyethylene catheter inserted transfemorally into the abdominal aorta and maintained chronically with a Luer-Lok cap after heparinization. Arterial blood gases were obtained daily, and animals with a PO_2 lower than 65 mm Hg were removed from the study to insure that morphologic changes were not due to generalized hypoxemia. In 11 monkeys intracisternal injection of 3 ml of fresh isologous arterial blood was utilized to induce spasm, while in seven monkeys a frontotemporal craniectomy was performed and the intradural internal carotid artery punctured with a 30-gauge needle. Twenty minutes to 1 hour after subarachnoid hemorrhage, angiography was performed via the right transbrachial route and repeated at intervals of 1-2 days until euthanasia.

The diameters of the anterior, middle, and posterior cerebral arteries were measured on the radiographs at 4-mm intervals beginning at their origins. The sum of these diameters was recorded as the arterial index (A.I.). Since a variability of up to 23 percent was noted in repeat control angiograms of the same animal, a reduction of A.I. greater than 25 percent was used to define the presence of vasospasm.

Specimens were fixed for histological and ultrastructural study by vascular perfusion.[20,25,28] After thoracotomy, the left ventricle was cannulated, the descending aorta clamped, and blood washed from the head and cervical regions with McEwen's saline followed by one-fourth strength Karnovsky's formaldehyde-glutaraldehyde fixative and then full strength Karnovsky's fixative. After fixation in situ for at least 3 hours, cerebral arteries were dissected free of the brain parenchyma and washed and stored in cacodylate buffered 10 percent sucrose. Segments of interest were further dissected into small blocks, postfixed in cacodylate buffered 1 percent osmium tetroxide, and processed through graded alcohols, uranyl acetate block stain, and propylene oxide for embedding in Epon 812. Thick sections (1.0-1.5 μm) were cut on a Porter-Blum MT2 ultramicrotome and stained with a mixture of methylene blue and azure II for light microscopy. Areas for ultrastructural study were selected, and thin sections (600-1200 Å) were cut on a diamond knife, stained with uranyl acetate and lead citrate, and examined with a Siemens Elmiskop 1A electron microscope.

III. RESULTS

Angiographic spasm. Fourteen of the eighteen animals (70 percent) developed spasm, and in six (33 percent) the spasm persisted for longer than 1 week (Figure 1). Two normal animals as well as animals which did not develop vasospasm after subarachnoid injection of blood (three) or puncture (one) served as controls. Intracisternal injection of fresh arterial blood produced spasm lasting 1-3 days, while all the cases of chronic spasm (> 7 days) resulted from needle puncture of the intradural internal carotid artery. After puncture, spasm was usually confined to the ipsilateral subarachnoid arteries, but the distribution after cisternal injection was more random.

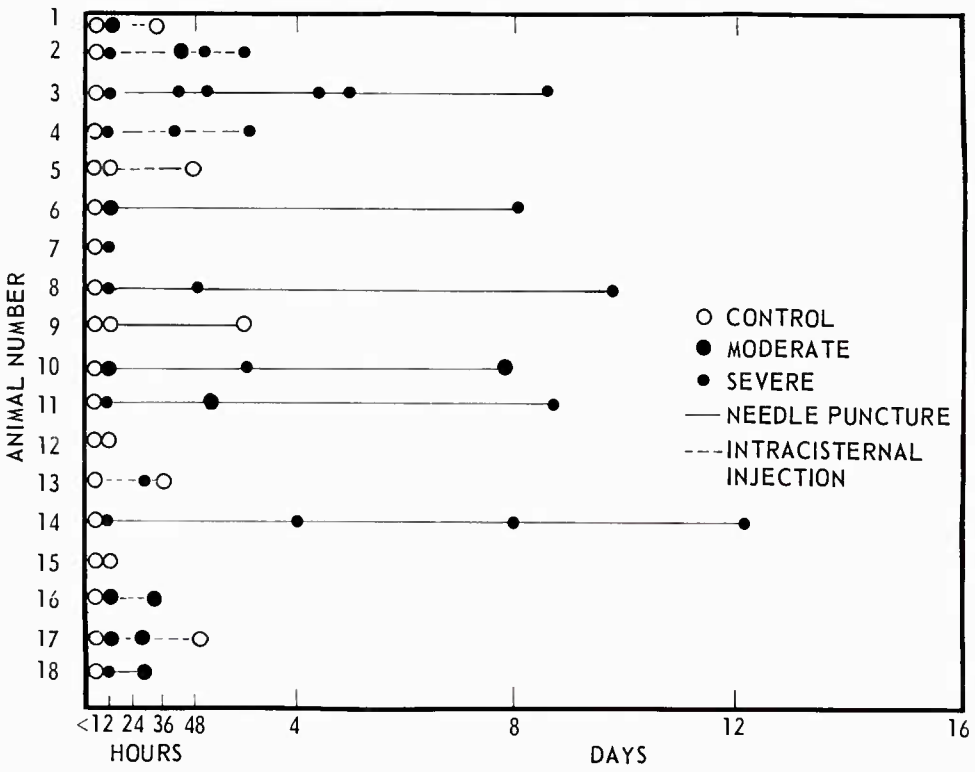

Figure 1. Subarachnoid hemorrhage was induced in 18 animals and resulted in angiographically demonstrable vasospasm in 14.

Ultrastructure of normal cerebral arteries. The subarachnoid arteries were lined by a layer of spindle-shaped endothelium, containing occasional pinocytotic vesicles, mitochondria and membrane bound lysosomal-like structures (Figure 2). The cells were joined by tight junctions showing overlapping or interdigitation. The abluminal aspect of the elastica was of uniformly low electron density, and its luminal aspect was lined with more electron dense material. The media consisted of smooth muscle cells surrounded by basement membranes and intercellular collagen (Figure 3). These fusiform cells contained a central "core" of nucleus plus organelles (mitochondria, endoplasmic reticulum, etc.) surrounded by well organized muscle

filament bundles. Vesicles were prominent just beneath the membranes of the smooth muscle cells. Muscle cells closest to the lumen were inserted into fenestrae of the internal elastic laminae. Occasional nerve fiber bundles in the adventitia contained both myelinated and nonmyelinated fibers. Sections of cortical arteries taken from normal animals and those in which vasospasm could not be induced showed no significant deviation from this pattern.

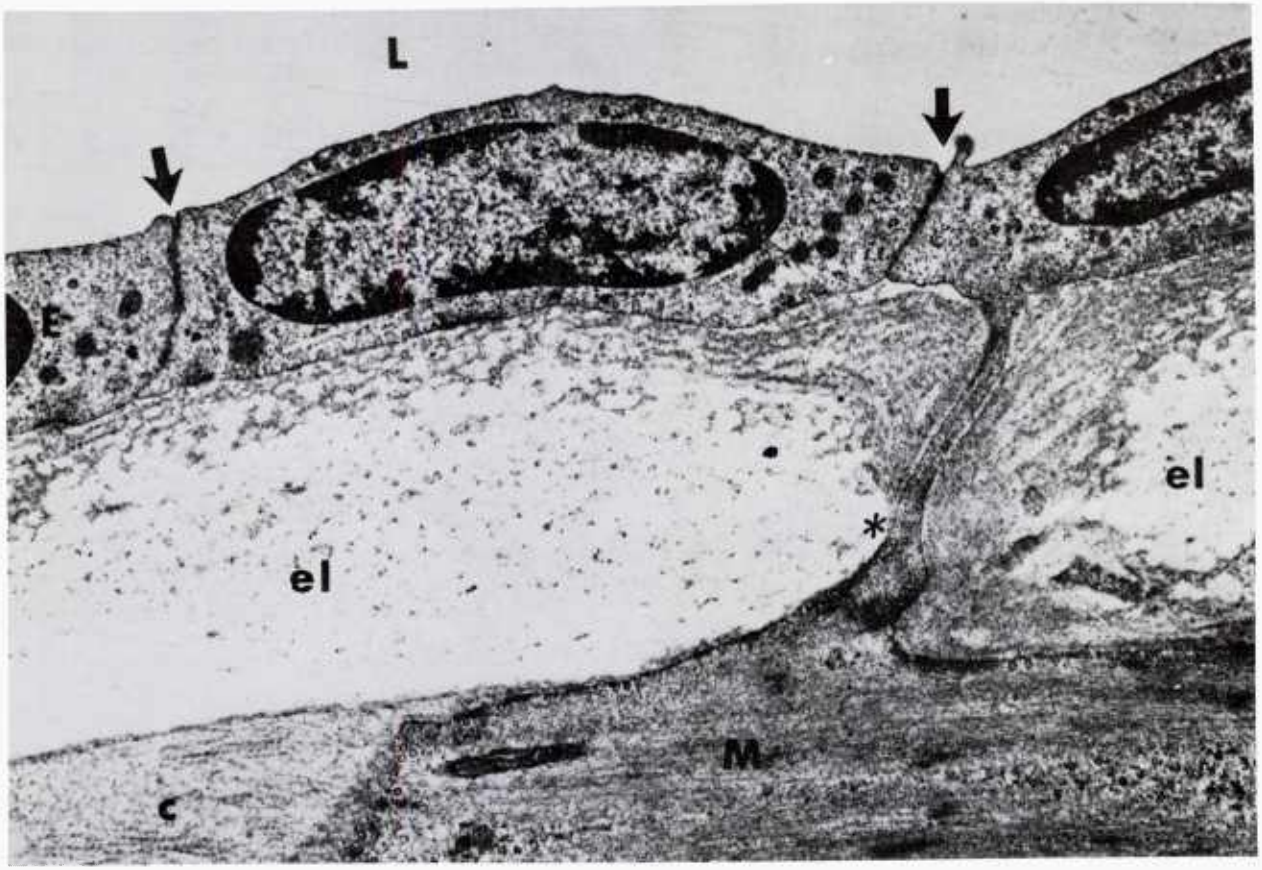

Figure 2. Endothelial surface of normal cerebral artery. Single layer of spindle-shaped endothelial cells (E) lines the lumen (L). These cells are joined by tight junctions (arrows), often with overlapping cytoplasmic flaps, and contain pinocytotic vesicles, mitochondria and occasional lysosome-like bodies, and cytoplasmic filaments. Elastic lamina (el) separates endothelium from smooth muscle cell layer (M). Occasional "insertions" (*) of muscle cell processes through the elastica attaching to the endothelial layer are observed. Intercellular collagen (c). X 18,000.

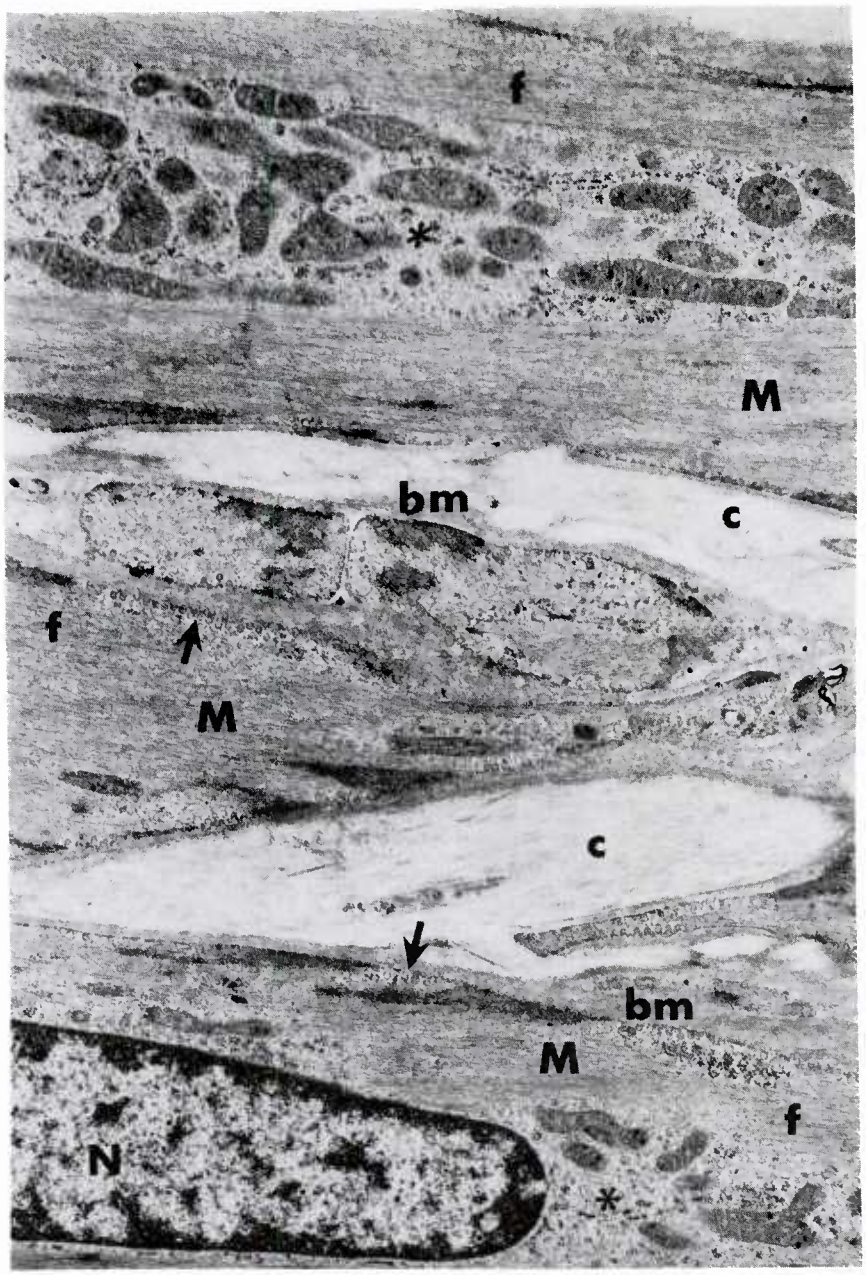

Figure 3. Media of normal cerebral artery. Spindle-shaped muscle cells (M) contain a central core of cellular organelles (*) and an elongated nucleus (N) surrounded by a layer of muscle filaments (f). Vesicles are arrayed beneath the muscle cell membrane (arrows). A basement membrane (bm) coats each of the cells, and the intercellular space contains bundles of collagen fibers (c). X 14,100.

Ultrastructure of cerebral vasospasm.

Early spasm (< 1 day). Specimens from this group (two animals) demonstrated a reduction in lumen size with corrugation of the internal elastica which, on the light microscopic level, was indistinguishable from the appearance of normally constricted arteries. On the ultrastructural level, however, early electron lucent changes in the muscle cells were noted within 8 hours, especially at the crests of the corrugations in the elastica. Condensed lysosomes and degenerating mitochondria were present within occasional muscle cells and lipid figures were observed among the pinocytotic vesicles beneath the sarcolemmal membrane (Figure 4).

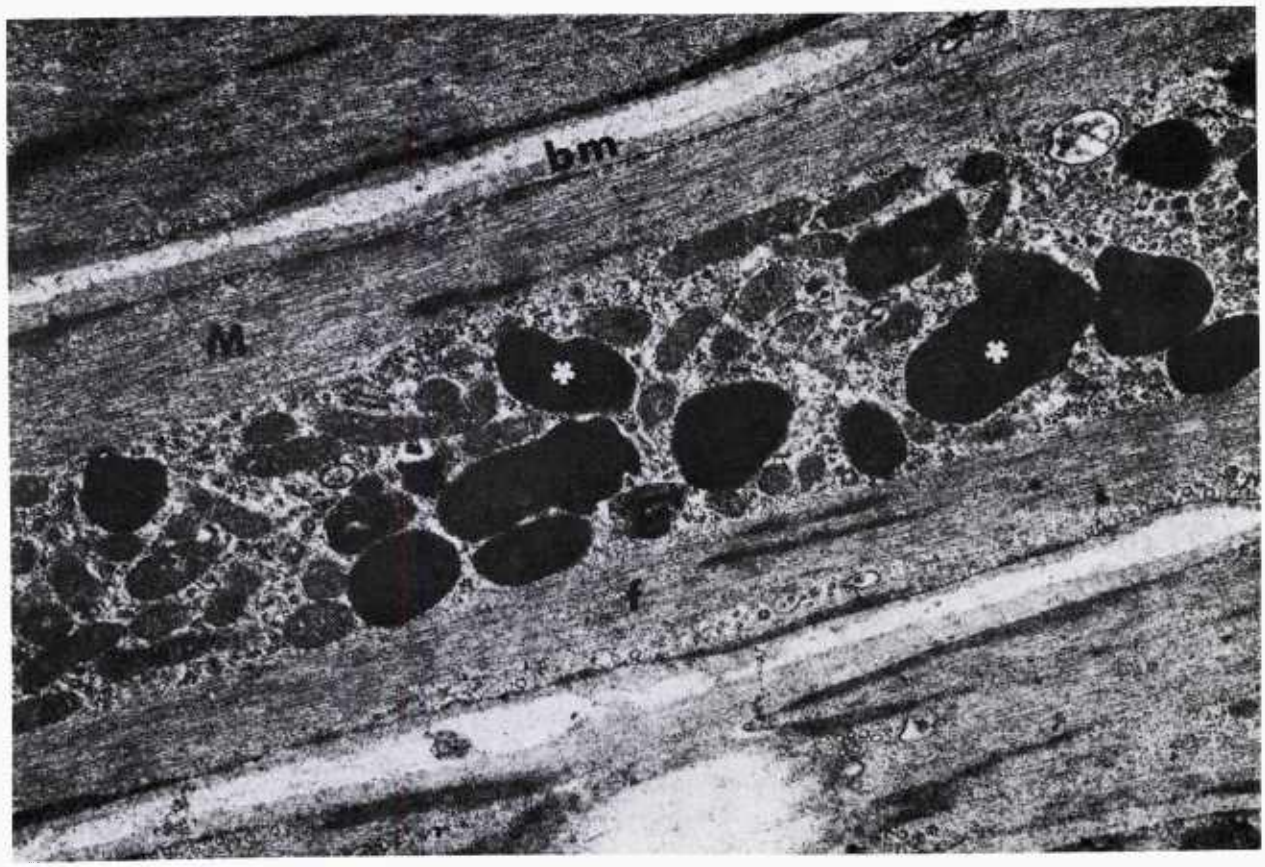

Figure 4. Medial changes in early spasm. Occasional muscle cells (M) are observed with condensed lysosomes and degenerating mitochondria (*) within the central core of organelles. Basement membrane (bm). Muscle filaments (f). X 18,000.

Prolonged spasm (2-7 days). Specimens (six animals) demonstrated rounding of the endothelial cell nuclei and cytoplasm (Figure 5) with cell processes assuming a flattened configuration along the elastica and a prominent loss of tight connections between endothelial cells (Figure 6). Even after fixation by vascular perfusion, platelets were observed adherent to the altered endothelial surface, both near the rounded endothelial cells and along denuded regions of the elastica itself. The elastica remained dense along its luminal edge, and the most visibly affected regions

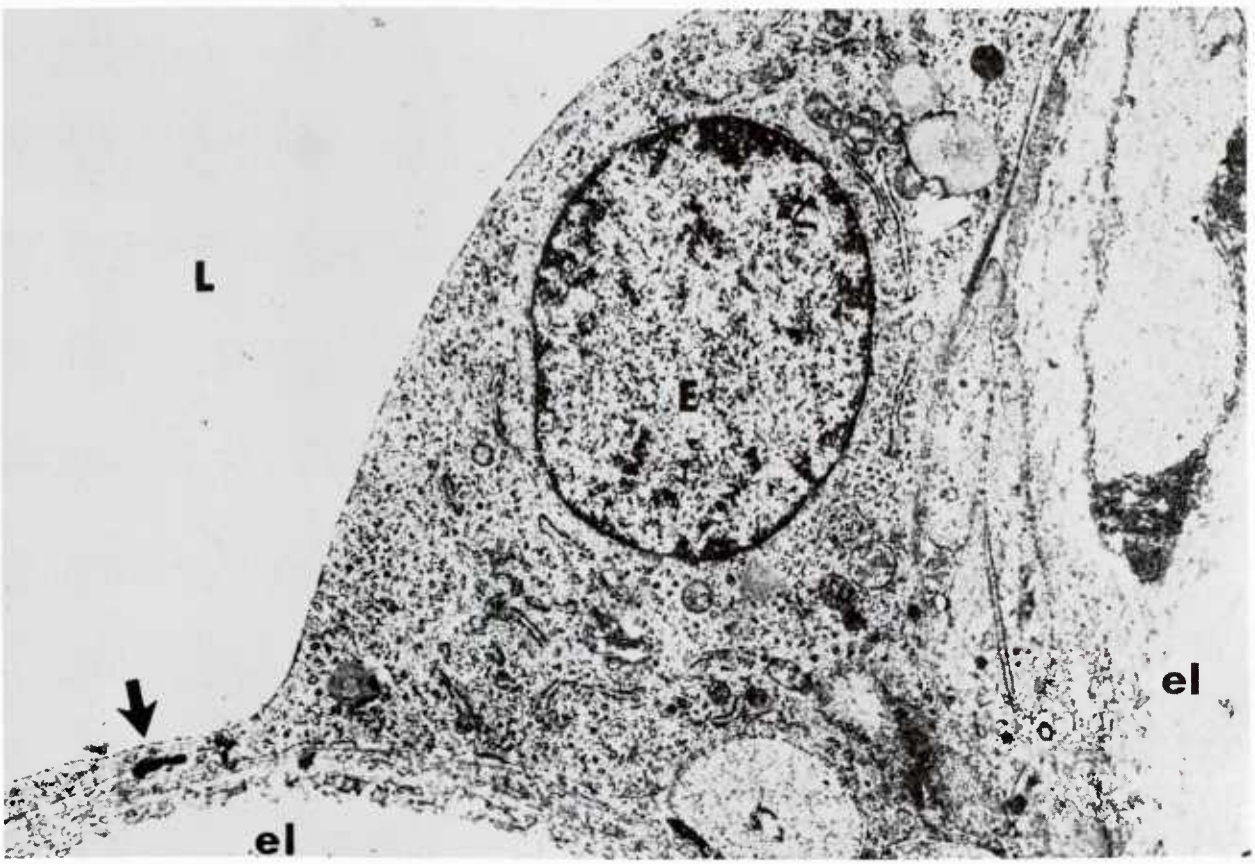

Figure 5. Endothelial surface changes in prolonged spasm. Nucleus and cytoplasm of endothelial cell (E) rounded, with flattening of cytoplasmic process (arrow) along the surface of internal elastic lamina (el). Lumen (L). X 10,800.

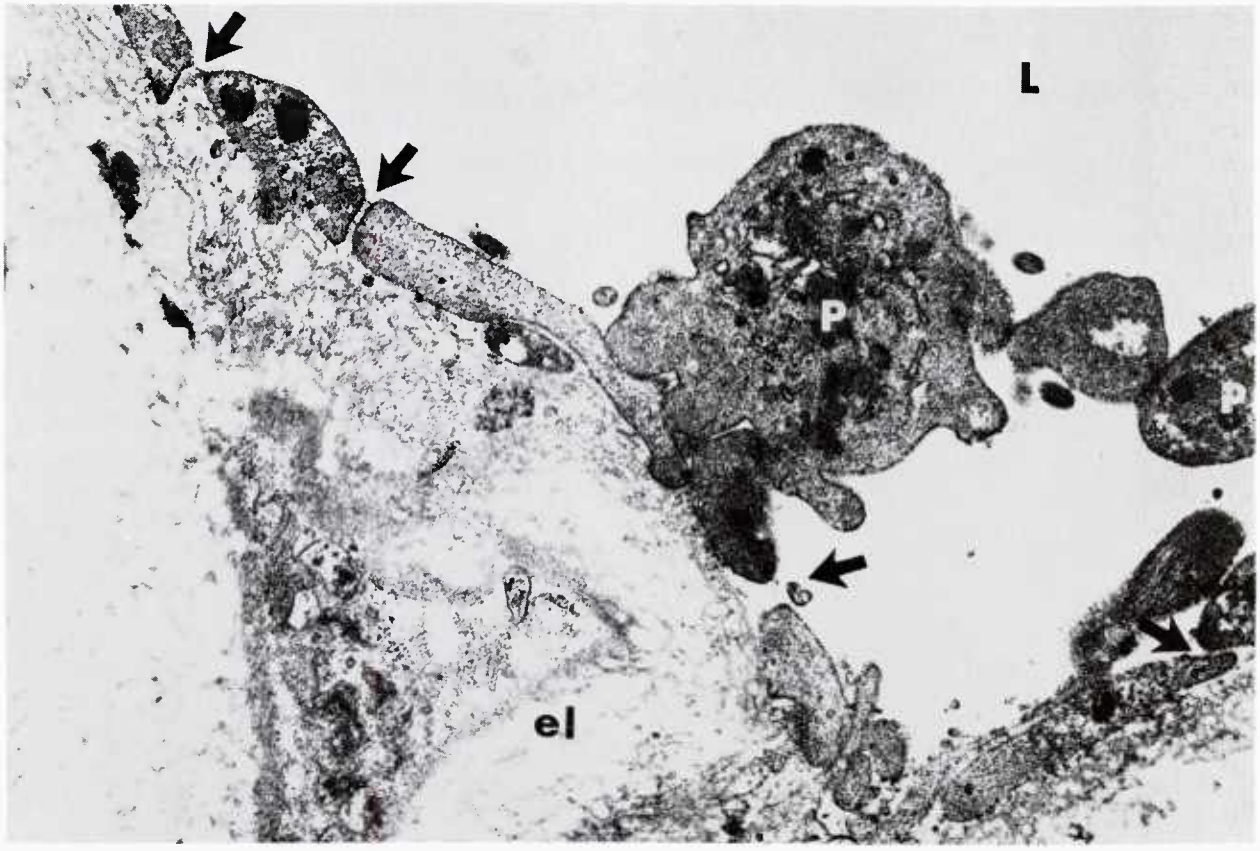

Figure 6. Endothelial surface changes in prolonged spasm. Prominent loss of tight connections (arrows) between endothelial cells formerly completely covering internal elastic lamina (el). Numerous platelets (P) are observed adherent to the denuded elastica and remaining flattened endothelial processes, in spite of fixation by vascular perfusion. Lumen (L). X 18,100.

were most severely corrugated. Smooth muscle cells were more electron dense than normal and those nearest the adventitia contained intracytoplasmic vacuoles of various sizes and content, some appearing "empty" and others containing a light granular or denser more amorphous material (Figure 7). On the other hand smooth muscle cells subjacent to the crests of the elastica contained degenerated organelles and lipid figures. Throughout the media there was a decrease in the number of pinocytotic

vesicles below muscle cell membranes. In the most dramatic sections frankly pyknotic muscle cells were encountered. Occasional changes were observed in the nerve fiber bundles, consisting of degenerating lipid in the cytoplasm of Schwann's cells and lipid figures in axis cylinders.

Chronic vasospasm (> 1 week). The endothelial cells in these arteries (six animals) assumed a more normal spindle-shaped configuration (Figure 8) with

Figure 7. Medial changes in prolonged spasm. Adjacent smooth muscle cells (M), one of normal appearance and one containing large vacuoles (V) with light granular contents. X 9,350.

tight intercellular junctions and an increase in cytoplasmic filaments. The entire internal elastic laminae remained somewhat more electron dense than normal. Numerous smooth muscle cells still contained large vacuoles as previously described. Throughout the media were muscle cell remnants of increased electron density (Figure 9) with loss of complex internal structure, surrounded by increased amounts of intercellular collagen.

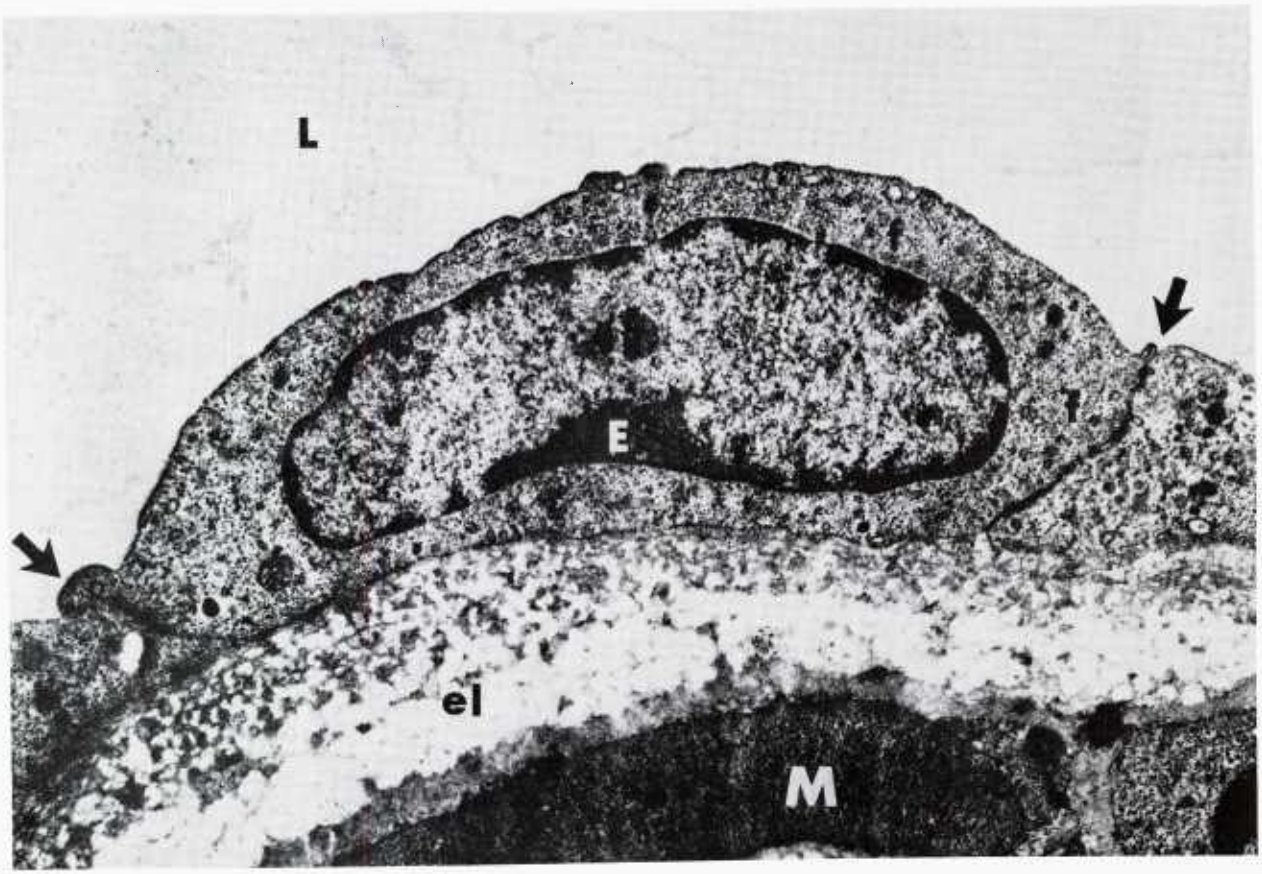

Figure 8. Endothelial surface in chronic spasm. Endothelial cells (E) resume a more normal spindle-shaped configuration, with tight junctions (arrows) between cells, and with an increase in cytoplasmic filaments (f). Elastic lamina (el) remains somewhat more electron dense along its luminal side and muscle cells and muscle cell remnants of increased electron density (M) are observed. Lumen (L). X 14,000.

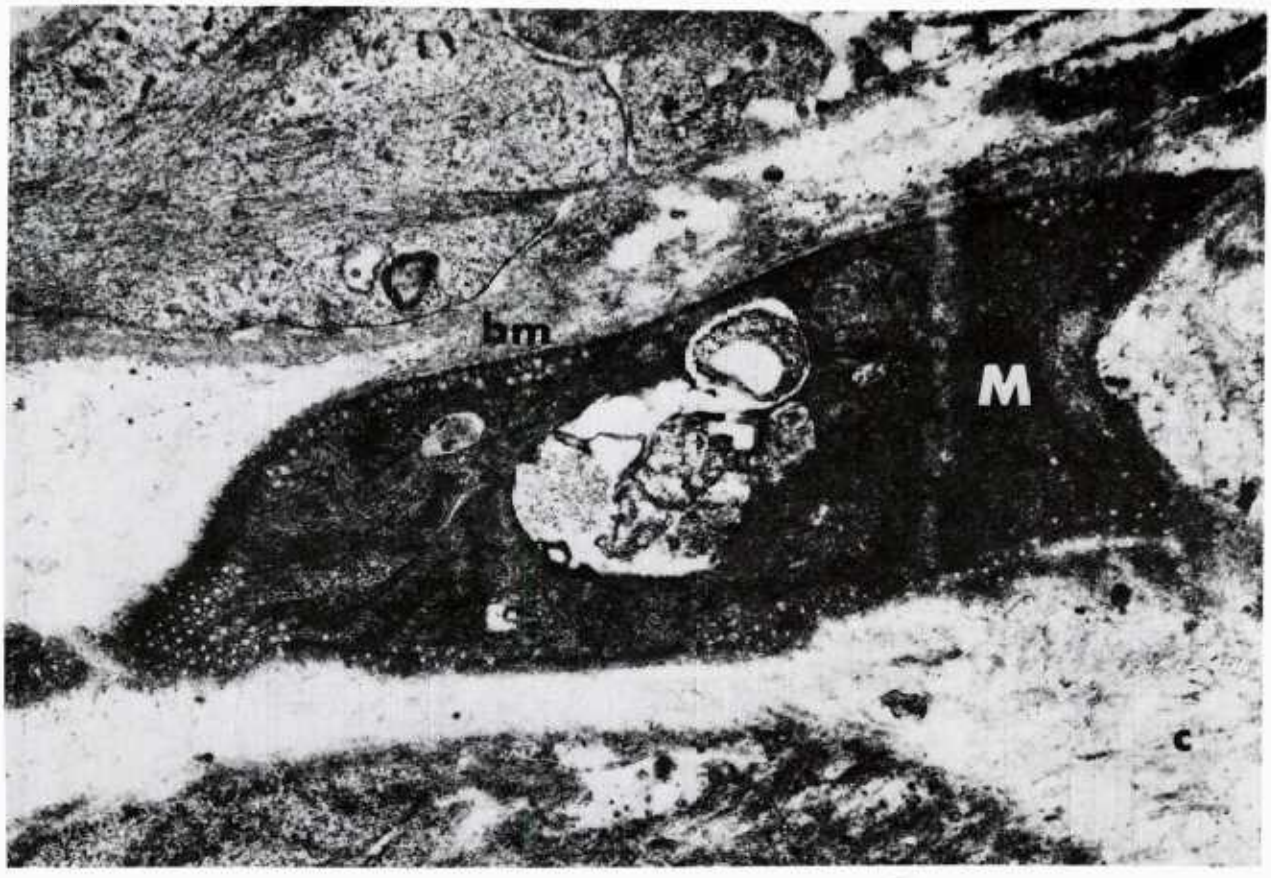

Figure 9. Medial changes in chronic spasm. Pyknotic muscle cell remnant (M) with increased electron density and loss of complex internal structure. Basement membrane (bm). Intercellular collagen (c). X 20,600.

Relationship between angiographic and electron microscopic vasospasm. The areas of most severe angiographic spasm were identified in early, prolonged and chronic vasospasm. It was noted that spasm in the first few hours after puncture is very evanescent and variable with little correlation between the location of microscopic and angiographic sites. In prolonged and chronic vasospasm there was more consistency in the location and severity of the maximal spasm and a better correlation with areas of microscopic spasm.

IV. DISCUSSION

Pathologic studies of the intracranial arteries following vasospasm are incomplete. Crompton[6,7] noted frank necrosis of cortical arteries and veins in the vicinity of sylvian hematomas after rupture of middle cerebral artery aneurysms. Conway and McDonald[5] described subendothelial granulation in the intradural arteries of 12 patients surviving 4 weeks or more after subarachnoid hemorrhage. Six of these cases had actual luminal narrowing due to subendothelial thickening while the media and adventitia appeared histologically normal.

Despite the clearly abnormal appearance of the larger (> 250 μm diameter) subarachnoid arteries seen at angiography no systematic effort has been made to study the fine structure of these vessels in vasospasm as a result of the requirement for immediate perfusion fixation. Such studies might demonstrate the locus of pathology within the arterial wall and differentiate changes specific for vasospasm from the secondary effects of anoxia.

It is apparent from the present study that vessels in early spasm are indistinguishable from normally constricting arteries using light microscopy. At the ultrastructural level, changes are noted in the muscularis and internal elastica at 8 hours. In early spasm, however, the structural changes bore little topographic relationship to the angiogram. When spasm remained localized to the same segment for over 1 week corresponding histologic changes were invariably found. Smooth muscle cells were most consistently and severely affected, but there were secondary changes in the intima and nerve fiber bundles. These muscular changes may reflect a cycle of increased mechanical and metabolic work in the presence of decreased cerebral blood

flow. This produces sarcolemmal breakdown, and potassium release which further potentiates norepinephrine-induced tonic contraction,[23] and eventually death of the muscle cell in diastole. In this connection Alksne and Greenhoot[1] have recently demonstrated myonecrosis of vascular smooth muscle after subarachnoid injection of norepinephrine in the rhesus monkey. We doubt that the preponderance of changes in the media is a peculiarity of the experimental animal but may be found if carefully looked for in human material. The subendothelial fibrous reaction noted in Conway and McDonald's report[5] may be related to the much longer time course in the latter studies.

Platelet-like bodies were found adherent to endothelial cells and insinuated into the open "junctions" between cells. These are reminiscent of the small granular aggregates noted after traumatic spasm by Symon[32] and are probably nonspecific indicators of vascular injury rather than of etiologic importance.

The significance of vasospasm is questionable in any particular case, although it is generally considered to be an unfavorable sign, placing the patient with subarachnoid hemorrhage at greater risk.[2] Abnormalities of cerebral blood flow have been noted in clinical cases.[3] A biphasic decrease in flow was noted by Simeone et al.,[30] while Fein and Boulos[11] found a biphasic disturbance of autoregulation in rhesus monkeys. In early spasm autoregulation was impaired during hypertensive stimuli, while in the presence of infarction it was lost to hypotensive stimuli. Whether the disturbance in flow is at all related to the "spasm" visible in the larger arteries has been questioned by some[16] and the microcirculatory disturbances engendered have been largely unexplored. As a first step it seemed important to identify what, if any,

structural aberrations occur in vessels with angiographically demonstrable pathology. The presumed changes in elasticity secondary to the morphologic changes found may partially explain the loss of vasoreactivity to hypotensive stimuli found in prolonged vasospasm; however, they do not account for the autoregulatory loss during early spasm. Further studies of the microcirculation are warranted and may elucidate the role of the perforating arteries, and the responses of the capillary network to the metabolic and hemodynamic alterations in cerebral vasospasm.

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UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
Armed Forces Radiobiology Research Institute
Defense Nuclear Agency
Bethesda, Maryland 20014

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP
N/A

3. REPORT TITLE
SEQUENTIAL CHANGES OF VASCULAR ULTRASTRUCTURE IN CEREBRAL VASOSPASM: MYONECROSIS OF SUBARACHNOID ARTERIES

4. OESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5. AUTHOR(S) *(First name, middle initial, last name)*
J. M. Fein, W. J. Flor, L. J. Parkhurst and J. L. Parker

6. REPORT OATE
June 1974

7a. TOTAL NO. OF PAGES
20

7b. NO. OF REFS
32

8a. CONTRACT OR GRANT NO.

b. PROJECT NO. NWED QAXM

c. Task and Subtask C 912

d. Work Unit 02

9a. ORIGINATOR'S REPORT NUMBER(S)
AFRRI SR74-10

9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*

10. OISTRIBUTION STATEMENT
Approved for public release; distribution unlimited

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
Director
Defense Nuclear Agency
Washington, D. C. 20305

13. ABSTRACT

Cerebral vasospasm following subarachnoid hemorrhage may contribute to morbidity and mortality. The inconsistent therapeutic responses to sympatholytic agents described in the literature raise doubts as to the purely functional nature of the vasoconstriction. This study examines the ultrastructural evolution of vacuolar degeneration of the media in subarachnoid arteries which have been in spasm. The striking changes of myoarchitecture are described; however, the relationship of these findings to aberrations in cerebral blood flow remains to be clarified.

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