Elastic Elements in the Media and Adventitia of Human Intracranial Extracerebral Arteries

F. T. Mérei, M.D., F. Gallyas, Ph.D., and Z. Horváth, M.D.

SUMMARY We find that the media and adventitia of adult human cerebral arteries contain elastic fibers forming a dense, coherent network, similar to that found in muscular arteries of the same size in other organs. The external elastic layer in the adult human is masked for the currently employed staining methods. By treatment with 90% formic acid before fixation, the original staining characteristic of elastic tissue can be restored. The light microscopic and scanning electron microscopic features of this network of elastic fibers are presented.

Material and Methods

The circle of Willis and its main branches, together with short sections of their cortical branches, were dissected before fixation from the base of the brain in 40 unselected patients at autopsy. Their ages ranged from 7 to 80 years with 5 collected for each decade. Arteries with atheromatous changes visible to the naked eye were not studied.

The following portions of the arteries were dissected and reserved for the usual histologic procedures: 1) the right internal carotid artery with its bifurcation into the anterior and middle cerebral arteries, 2) the right middle cerebral artery and its main branches, 3) the right anterior cerebral artery and the origin of its first main branch, 4) the right posterior cerebral artery together with the posterior communicating artery, 5) the right vertebral artery, and 6) a 20 mm long segment of the basilar artery.

The remaining areas were subjected to one of the following procedures capable of dissolving all components of the vessel wall except the elastic fibers and membranes which are resistant to extreme chemical and physical measures: 1) treatment with 0.1 N sodium hydroxide at boiling point for 30 min; 2) incubation with 90% formic acid at 45°C for 48 h; 3) enzymatic digestion. The remnants were washed with 3 changes of distilled water for about 10 min each. Despite the laceration and crushing during these procedures, the original arrangement of the arteries was easily recognized, and short segments which suffered no damage were excised. These arterial segments were incised longitudinally using an operating microscope and microsurgical instruments. The external elastic layer was separated from the internal elastic lamina and then air-dried on glass slides in an unfolded state. Some of the preparations were processed for scanning electronmicroscopy, and others were stained by a silver technique developed by one of us. To avoid mechanical damage some arterial segments were mounted without incising. In such preparations both the internal and the external elastic laminae could be studied under the microscope.

Five mm lengths were cut from the arterial segments reserved for common histologic procedures, then fixed in 4% formalin for one day, embedded in...
paraffin, cut at 6 μm and finally stained by one of the following methods: 1) resorcin-fuchsin, 2) orcein, 3) Verhoeff’s hematoxylin method, 4) toluidine blue, topo-optical staining,11 5) silver techniques for the demonstration of elastic elements10 and smooth muscle cells.12 The remaining arterial segments were exposed to 90% formic acid at room temperature for 24 hours, before fixation and subsequent processing. Digestion by formic acid disorganizes the structure of collagen and smooth muscle cells without totally removing them from the arterial wall (“partly-digested” materials). Although the arteries shrink markedly under the effect of formic acid, making the internal elastic lamina fold to an unusual extent, the main layers of the blood vessels (intima, media, adventitia) can easily be recognized.

The main arteries of the brain from 10 more patients at autopsy were incubated in 90% formic acid at 45°C for 48 h without having been cut into segments. The external elastic layer was separated from the internal elastic lamina and the intimal elastic layer. The pooled external elastic material was purified, then processed for amino acid analysis as described by Ross and Bornstein.14

Results

The procedures used for the elimination of “unwanted” components of the arterial wall were all equally effective in isolating elastic fibers and membranes. After digestion by any of them, 3 concentric layers of elastic elements were detected in each of the arterial segments examined, independently of their origin and age (fig. 1). After being cut open longitudinally, the outer layer became separated from the adjoining one without any external force. The middle layer looked like a thick, bright, gleaming sheet under the operating microscope and could be identified as the internal elastic lamina, based on its fenestration.13 For this reason, the inner layer must have been situated in the intima and the outer layer in the media and/or the adventitia of the arterial wall. The outer layer appeared under the operating microscope as an opalescent, loose sheet. This could be stretched repeatedly to about twice its original length without apparent damage to its structure.

The light and scanning electronmicroscopic images of the totally digested arterial segments dried onto glass plates revealed that the outer layer consisted of a dense network of fibers (fig. 2). The thicker fibers had, on their surface, furrows running parallel to their longitudinal axes, as if they were made up of several thin fibers cemented together (fig. 3). The density of the network of external elastic fibers decreased gradually in the more distal part of the artery and only a few fibers could be found in the branches smaller than 0.5 mm (fig. 4). The external elastic layer is composed of fairly long fibers merging into each other at

![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** Operating microscopic view of the elastic sheets remaining in the stump of the middle cerebral artery of an adult human after removal of other tissue components by means of digestion by 90% formic acid at 45°C for 48 hours. a) elastic elements of the intima ("Begleitmembran"), b) internal elastic lamina, c) external elastic layer.
their endings, constituting a coherent network with relatively few branching points (fig. 5). “Free” fiber endings were observed in a negligible number.

In the media and adventitia of the non-digested arterial segments obtained from 32 of our 40 patients, the staining methods and silver techniques rendered visible only sporadic elastic fibers (fig. 6a). In the remaining 8 patient samples a weakly developed external elastic layer was stained, mainly in the vertebral, basilar and internal carotid arteries. In contrast, a dense elastic network was demonstrated in the media and adventitia in each of the partly-digested arterial segments of the circle of Willis and its main afferents and efferents (fig. 6b). The density of fibers gradually decreased with the diameter of the artery (fig. 6c, d). In general, the media contained fewer but thicker fibers than the adventitia, where they were located mainly in the vicinity of its inner boundary. There
FIGURE 3. High power scanning electronmicroscopic image of the preparation in Fig. 2. Note the furrows on the surface of the fibers running parallel to their longitudinal axes. ×30,000

were considerable individual variations in this pattern, e.g., 1) only the media contained elastic fibers; 2) the adventitial fibers were thicker and 3) were located in the outermost zone of the adventitia. The number of patients was too small for any conclusion to be drawn concerning the significance of the individual variations. No direct connection was found between the internal elastic lamina and the external elastic layer, but a small number of fibers connecting the medial and the adventitial elastic network was observed.

Each of the staining methods visualized the external elastic fibers in the partly digested as well as in the totally digested preparations. The silver technique demonstrating smooth muscle cells in non-digested materials3 proved to be the most suitable for this purpose. Even short treatment (partial digestion) by formic acid abolishes the capacity of the smooth muscle cells to react with silver ions under the circumstances required by the staining technique in question and enhances that of the elastic elements.10

The amino acid composition of the external elastic layer of the cerebral arteries (table) was similar to that of the ligamentum nuchae,14 young and old aorta, and
FIGURE 4. External elastic network in the a) internal carotid artery, b) middle cerebral artery, c) first main branch of the middle cerebral artery, d) in a thick cortical branch of the latter; isolated by formic acid and dried onto glass plates without the internal elastic lamina having been removed. The density of the external elastic layer decreases in a distal direction. ×250.

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>External elastic layer of human cerebral arteries</th>
<th>Elastin from bovine ligamentum nuchae</th>
<th>Elastin from young human aorta</th>
<th>Elastin from old human aorta</th>
<th>Elastin from human pulmonary artery</th>
<th>Cowhide collagen</th>
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<tr>
<td>Glycine</td>
<td>27.1</td>
<td>32.4</td>
<td>26.1</td>
<td>21.3</td>
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<td>Alanine</td>
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<td>23.2</td>
<td>21.6</td>
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<td>9.5</td>
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<tr>
<td>Valine</td>
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<td>13.5</td>
<td>13.0</td>
<td>11.5</td>
<td>12.9</td>
<td>3.4</td>
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<td>Leucine</td>
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<td>6.1</td>
<td>4.5</td>
<td>4.8</td>
<td>4.9</td>
<td>3.2</td>
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<tr>
<td>Isoleucine</td>
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<td>2.6</td>
<td>2.1</td>
<td>2.3</td>
<td>2.3</td>
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<tr>
<td>Phenylalanine</td>
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<td>3.1</td>
<td>1.7</td>
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<td>—</td>
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<tr>
<td>Methionine</td>
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<td>—</td>
<td>0.1</td>
<td>0.4</td>
<td>—</td>
<td>0.8</td>
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<tr>
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<td>3.0</td>
<td>1.8</td>
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<tr>
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<td>6.3</td>
</tr>
<tr>
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<td>15.1</td>
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<tr>
<td>Hydroxyproline</td>
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<td>1.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>14.0</td>
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<tr>
<td>Arginine</td>
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<td>4.3</td>
<td>—</td>
<td>8.8</td>
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<tr>
<td>Lysine</td>
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<tr>
<td>Tyrosine</td>
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<td>0.7</td>
<td>1.4</td>
<td>1.8</td>
<td>—</td>
<td>1.4</td>
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</tbody>
</table>
FIGURE 5. External elastic network in the anterior cerebral artery, isolated by formic acid, then dried on a glass slide in a moderately stretched state. The fibers are not uniform in thickness. Several branching points but no free fiber ending can be seen. ×10,000

pulmonary artery in so far as it contained more than 80% non-polar amino acids and less than 2% hydroxyproline. In contrast, collagen can be characterized by a hydroxyproline content of about 14% and a relatively high proportion of other amino acids with charged side chains.16 The external elastic layer of the cerebral arteries was rich in lysine. A low content of lysine is regarded as characteristic of cross-linked/non-soluble/elastin.17

Discussion

Our findings obtained from arteries digested by various biochemical procedures for the isolation of elastic material suggest strongly that the arteries of the adult human brain are similar to other muscular arteries of comparable caliber in their content of elastic structures in the media and adventitia. Comparison of the non-digested preparations with the
partly digested ones indicates that the elastic fibers constituting these elastic structures are masked for the available staining methods, unlike the elastic fibers of the muscular arteries of other organs. The "masking" may consist of in vivo blocking of certain reactive groups in the molecular structure of the elastic tissue, which may play a role in the stainability of the latter. The treatment of non-fixed cerebral arteries with formic acid reverses this effect and restores the original staining character of the elastic fibers. Attempts to reproduce this effect of formic acid in paraffin sections of formol-fixed materials failed. The resistance of the external elastic layer of the cerebral arteries to the isolation procedures used, as well as their high-grade extensibility after the isolation, make it possible to conclude that the "masking" is not accompanied by a major transformation of the chemical composition and structure of the elastic material.

The chemical nature of a number of tissues, traditionally called "elastic" by histologists, such as the elastic membranes and fibers in intimal cushions and arteriosclerotic lesions, the internal elastic lamina of veins and small arteries, etc. has been questioned repeatedly by several workers,\textsuperscript{18-22} many of whom produced histochemical evidence indicating that these tissues were composed of a special kind of collagen — \textsuperscript{?} type III collagen. It was resistant to the procedures for isolating elastin and appeared as a homogenous mass under the transmission electron microscope. It could be demonstrated by both the so-called elastic staining methods and various staining and histochemical methods believed to be specific for collagen. Such tissues were termed by Wolff\textsuperscript{18} "pseudoelastica." To avoid ambiguity, Puchtler et al.\textsuperscript{22} introduced the following terminology: Elastin fibers or Membranes for structures composed of true elastin; Pseudo Elastica in the above sense; and Elastic Fibers and Membranes for structures composed of either true or pseudo elastin. This terminology is used throughout the present paper. The amino acid composition of the external elastic fibers of the cerebral arteries resembles that of elastin obtained from ligamentum nuchae or elastic arteries rather than that of collagen (see table), but this observation does not allow differentiation of its elastin or pseudo elastin nature.

The scanning electronmicroscopic findings on the structure and branching pattern of the fibers making up the external elastic layer of the cerebral arteries
is similar to findings obtained in ligamentum nuchae. This supports the somewhat older theory of Romhányi, formed from polarization microscopic observations that elastic fibers are composed of parallel subfibers cemented together.

Why are the external elastic fibers of the cerebral arteries invisible by the currently employed staining methods? Hassler and Larsson and Crompton assumed that the external elastic fibers of muscular arteries served to dampen the pulse. These fibers seem to appear when they are needed, such as after birth, but disappear gradually, becoming nonfunctional, i.e. the "lack" of elastic fibers over intimal cushions. The skull of the adult was regarded by those authors as a closed box because the total surface area of the skull. The skulls of animals cannot function as a rubber tube running through it. The outer surface area of the skull. Using this theory, it could be assumed that a special physiological process takes place in the external elastic fibers which have become dysfunctional, and this results in an alteration of their chemical composition. The latter might manifest itself in a change of the staining properties of elastin.

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References
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