A study of intracranial aneurysms with ultrasoft x-rays

Ove Hassler, M.D
Institute of Pathology, University of Umeå, Umeå, Sweden

Ten saccular aneurysms obtained postmortem from large cerebral arteries were examined with ultrasoft x-rays. At the border toward the aneurysms the elastic lamella generally showed duplication, thickening, and fragmentation, with irregular variations in the absorption of ultrasoft x-rays. Calcification could only be discovered in the intimal collagenous tissue several millimeters from the aneurysm mouth. Eosinophilic substance was found in one case to be markedly radiopaque. The observations support the hypothesis that at the site of a media defect there is a compensatory hypertrophy of the elastic lamella, resulting in degeneration and decay. Fluorescent microscopy of the elastic lamella gave similar results.

Key Words • intracranial aneurysm • elastic tissue • ultrasoft x-rays

The microscopic appearance of cerebral arteries with saccular aneurysms has been studied by a great number of methods. These studies have chiefly utilized autopsy material, because aneurysms occur extremely rarely in animals, and because in living aneurysm cases only the aneurysm sac and not the parent artery with the aneurysm mouth is available for study. The parent artery is particularly interesting in investigation of the underlying pathogenesis.9

The development of saccular aneurysms is probably to a great extent dependent on a degenerative process in the internal elastic lamella; this is the main wall component at the site of the congenital media defects that are so common.6,9 The elastic component of the arteries in general is known to show a considerably higher degree of absorption of ultrasoft x-rays than most other soft tissues.5 Consequently, it seems reasonable to use these x-rays in the study of the aneurysms to gain further information about the underlying mechanisms of elastin degeneration.

Material and Methods
Ten postmortem specimens of saccular aneurysms from 10 different subjects were studied (Table 1).1 All had ruptured, except one which occurred in a patient with polycystic kidneys (Case 7). All the aneurysms were situated on large cerebral arteries supplied by the carotid arteries. In all cases, 1.5 cm of the main arterial trunk carrying the aneurysm and 1 cm of the branch were studied. The aneurysms were taken less than 24 hours postmortem. The material was fixed in formalin, buffered to pH 7.2 with phosphate, and embedded in filtered paraffin (Merck). In one case (Case 8) elastase treatment was carried out before the fixation. From each aneurysm at least three representative paraffin sections, 5 μ thick, from different levels were examined with ultrasoft x-rays.

In addition, some special examinations were made. To exclude the possibility that changes secondary to the fixation and embedding procedures might have occurred, fresh material from one aneurysm (Case 4)
Ultrasoft x-ray study of aneurysms

TABLE 1

Clinical data regarding ten aneurysm cases

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age, Sex</th>
<th>Main Disease</th>
<th>B.P. over 145/95*</th>
<th>Heart Size†</th>
<th>Atherosclerosis of the Large Cerebral Arteries‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>weight (gm)</td>
<td>enlarged left side</td>
</tr>
<tr>
<td>1</td>
<td>36 M</td>
<td>subarachnoid hemorrhage</td>
<td>no</td>
<td>390</td>
<td>no</td>
</tr>
<tr>
<td>2</td>
<td>38 M</td>
<td>subarachnoid hemorrhage</td>
<td>yes</td>
<td>410</td>
<td>yes</td>
</tr>
<tr>
<td>3</td>
<td>42 F</td>
<td>subarachnoid hemorrhage</td>
<td>no</td>
<td>490</td>
<td>yes</td>
</tr>
<tr>
<td>4</td>
<td>49 M</td>
<td>subarachnoid hemorrhage</td>
<td>no</td>
<td>380</td>
<td>no</td>
</tr>
<tr>
<td>5</td>
<td>51 M</td>
<td>subarachnoid hemorrhage</td>
<td>yes</td>
<td>480</td>
<td>yes</td>
</tr>
<tr>
<td>6</td>
<td>52 F</td>
<td>subarachnoid hemorrhage</td>
<td>no</td>
<td>300</td>
<td>no</td>
</tr>
<tr>
<td>7</td>
<td>54 M</td>
<td>polycystic kidneys</td>
<td>yes</td>
<td>470</td>
<td>yes</td>
</tr>
<tr>
<td>8</td>
<td>55 F</td>
<td>subarachnoid hemorrhage</td>
<td>yes</td>
<td>290</td>
<td>no</td>
</tr>
<tr>
<td>9</td>
<td>62 F</td>
<td>subarachnoid hemorrhage</td>
<td>no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>65 F</td>
<td>subarachnoid hemorrhage</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Recorded over 145/95 on more than one occasion.
† The weight and enlargement of the heart was obtained from postmortem reports.
‡ Graded from Group I (no or slight changes) to Group IV (very marked changes) according to Baker, et al. (1960), see ref. 1.

was also sectioned and dried in a cryostat and examined with ultrasoft x-rays. To study the picture after elastin dissolution, fresh material from one aneurysm (Case 8) with adjoining arteries was treated with elastase (2X Crystalline from Sigma, St. Louis, Missouri) and then fixed, embedded in paraffin, sectioned, and examined with ultrasoft x-rays. Furthermore, paraffin sections from another two aneurysms with adjoining arteries (Cases 1 and 9) were deparaffinized, treated with elastase, and examined with ultrasoft x-rays.

Qualitative microradiography with ultrasoft x-rays was performed using the techniques outlined by Greulich and Engström and Engström, et al. Paraffin sections were mounted on celloidin-coated Eastman Kodak High Resolution Plates (1 × 3 in.). After drying, the paraffin was removed with benzene. Exposures were made at 1.0 kV and 50 mA, using an improved version of an automatic instrument described by Friberg and Burke. The target-to-plate distance was 25 cm and the time of exposure, 55 min. One section from each case was exposed at 10 kV and 55 mA for 25 sec, to exclude calcification. After exposure the sections were floated on acetone. The plates were developed in Kodak D19B, dried, and mounted under coverslips. The microradiograms were examined under a light microscope.

Both the section examined with ultrasoft x-rays and that cut immediately preceding it were stained with Gomori's elastin stain combined with van Gieson's stain. The x-rayed section was, as a rule, partly destroyed, because it had to be handled too long in the dark room and in various liquids. The section cut immediately after the x-rayed section was stained with hematoxylin and eosin. In addition, portions from each slice were taken for fluorescence microscopy. One of these was mounted unstained. Others were mounted after treatment with Gomori's elastin stain or the Morel-Sisley reaction. Color photographs were made from representative areas of the elastic lamella in all sections examined with fluorescent microscopy using constant times of exposure and development.

As a control, cerebral arteries were obtained from the autopsies of 10 newborn infants and 24 subjects aged 1 to 79 years (three from each age decade below 80 years).

Results

Sample sections of the aneurysms studied are shown in Figs. 1, 2, and 3. The aneurysm wall had a moderate density to ultrasoft x-rays (Fig. 1 C). The external part, which was composed of adventitial connective tissue (Fig. 3A) had largely the same radiopacity as the internal part, which was composed of intimal connective tissue. Small
Fig. 1. Case 4.  A. Radiogram (ultrasoft x-rays) of a 4-μ-thick paraffin section through an anterior cerebral artery with a saccular aneurysm situated in the upper right corner of the picture. The internal elastic lamella of the artery (arrow) appears white, indicating a high x-ray absorption; it also shows thickening, duplication, and disintegration in the neighborhood of the aneurysm. The erythrocytes in the lumen and in the adventitia also have a high x-ray absorption, presumably because of their content of iron. ×75.  B. Detail enlargement of the changed elastic lamella. ×700.  C. Detail enlargement of the aneurysm wall, showing the same moderate radiopacity in the intimal (to the left) and the adventitial (to the right) connective-tissue layers. No elastic tissue or smooth muscle is present in the aneurysm wall. ×900.
Fig. 2. Ultrasoft radiograms. A. Case 9. Section through the mouth of an aneurysm demonstrating the end of the ruptured lamella (to the right) embedded in connective tissue. The elastic lamella was attenuated at its end. \( \times 135 \). B. Case 5. Another aneurysm mouth showing hyperplastic, moderately radiopaque intima with no calcifications. Near the rounded border of the tunica media there are some rather radiopaque clumps (arrows). \( \times 60 \). C. Case 8. Aneurysm treated with elastase before fixation and embedding. The enzyme has dissolved the elastin, so that the residue of the elastic lamella (arrows) appears less radiopaque and is split up into several thin lamellae. \( \times 300 \). D. Case 9. Aneurysm treated with elastase after fixation, embedding in paraffin, and sectioning. The picture resembles that in Case 8 (Fig. 2 C), but the structure is better preserved. \( \times 300 \).
FIG. 3. Histological sections through the same specimens from which the radiograms in Figs. 1 and 2 were prepared. 

A. Adjacent part of the aneurysm wall in Fig. 1 A. The wall is only built up of endothelium and collagenous connective tissue. Gomori’s elastin stain combined with van Gieson’s stain. × 600.

B. Adjoining portion of the elastic lamella in Fig. 1 C. The lamella shows duplication, thickening, disintegration, and irregular elastin-staining properties. Gomori’s elastin stain and van Gieson’s stain, × 600.

C. Adjacent part of the elastic lamella in Fig. 2 A and D as seen in fluorescence microscope. The fluorescence of the lamella (arrows) is moderately increased and corresponds well to the moderately increased radiopacity demonstrated in Fig. 2 A. No staining, × 600.

D. Area of intima thickening situated immediately to the left of the area shown in Fig. 2 B. The intima consists of split-up elastic lamellae, collagenous connective tissue, and some smooth muscle cells. Gomori’s elastin stain combined with van Gieson’s stain, × 250.
Ultrasoft x-ray study of aneurysms

masses of thrombotic material at some sites on the internal surface had an increased contrast, apparently because of the content of erythrocytes. Erythrocytes in the lumen and in the periadventitial space always had a high degree of x-ray absorption.

At the mouth of the aneurysm there was, as a rule, a diffuse proliferation of the intimal connective tissue (Fig. 2 B and 3 D). The density of this tissue was low to moderate. In Cases 7 and 10, the intima contained some small amorphous masses, which had the radiological and staining characteristics of calcifications. These masses were situated several millimeters from the mouth of the aneurysm.

The appearance of the elastic lamella showed great variations (Figs. 1 B and 2 A). It was thin and had a high density at places, with the normal appearance far away from the aneurysm. In the neighborhood (0.5 cm or less) of the aneurysm the elastic lamella showed thickening, duplication, and fragmentation, with irregular variations in density (Figs. 1 B, 3 B and 3 C). The density was sometimes slightly increased but generally decreased. Sometimes the lamella was attenuated (Fig. 2 A). The variations in x-ray density corresponded well to the variations in elastin-staining properties, so that the weakly stained lamella had a decreased density. No calcifications were encountered in the lamella near the rupture. After elastase digestion of fresh arteries with aneurysms, the microradiological picture of the elastic lamella had a changed appearance (Fig. 2 C). The lamella was less radiopaque and was split up into several thin lamellae. After elastase digestion of deparaffinized paraffin sections, the lamella was also less radiopaque (Fig. 2 D). The muscle layer and the adventitia showed a moderate density that was not noticeably changed in the neighborhood of the aneurysm. Near the edge of the muscle coat at the mouth of one aneurysm, one x-rayed section showed some clumps that had an increased radiopacity. These also occurred to a lesser extent in the adjacent routine-stained sections; they were amorphous and eosinophilic and were not stained by the elastin stain.

In the control material, the elastic lamella of the newborn showed low density to ultrasoft x-rays, with a gradual increase in its density to the age of 20 years. After that time the density of the lamella was rather constant. The adventitia and tunica media of this material always showed low density.

The autofluorescence of the elastic lamella was always well correlated to its density to ultrasoft x-rays. Thus, it was very weak in the newborn, increased to the age of 20, and was then constant. The variations in the intensity of the fluorescence at the border of the aneurysms was well correlated to the variations in density to x-rays. No qualitative variations in the fluorescence were noted. No eosinophilic material was available for fluorescent microscopy.

Discussion

It is already known from other works that elastic tissue has a comparatively high ultrasoft-x-ray absorption (high dry weight per volume). The changes in the elastic lamella at the border of saccular aneurysms may confirm the hypothesis that there has been an increased load on the lamella with compensatory duplication and hypertrophy, finally resulting in degeneration and decay. The results of the ultrasoft-x-ray examination of the elastic lamella are in good agreement with the light-microscopic findings in elastin-stained sections. The changes of the lamella are different from those seen in atherosclerosis where there is thinning but no hypertrophy; also, in atherosclerosis there often are calcifications while in our studies there were none in the elastic lamella, and only two cases in which atherosclerosis was found in the intima several millimeters from the aneurysm. Calcifications are demonstrated with exceptional sensitivity by the ultrasoft-x-ray method.

Elastase digestion caused a dissolution of the elastin but revealed no additional changes.

Clumps of eosinophilic material may be accidentally encountered at the mouths of aneurysms. In the present study a few small clumps of this material were found to be more radiopaque than most of the surrounding tissues. The high radiopacity of the clumps of eosinophilic material was unexpected. It may therefore be questioned whether it consists of fragments from the destroyed elastic lamella, although it did not take elastin stain.

J. Neurosurg. / Volume 34 / March, 1971 385
References


Received for publication April 17, 1970.
Supported by a research grant from the Swedish Medical Research Council (No. B70–12X–561–06).
Address reprint requests to: Ove Hassler, M.D., Pathology Department II, Institute of Pathology, University of Umeå, S-901 87 Umeå 6, Sweden.