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Annulo-aortic ectasia

Light and electron microscopic changes in aortic media

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Summary. The aortic wall of the human ascending aorta from 44 patients operated on for annulo-aortic ectasia (AAE) was studied. Light microscopy revealed significantly greater cystic change, elastic fragmentation, fibrosis and disappearance of smooth muscle cells in aortic media in AAE than in control specimens taken at autopsy. Occasional aortae, however, were morphologically almost normal. Eight of the patients had Marfan's syndrome. No significant differences were observed between them and the other 36 patients, except for a tendency to have less pronounced fibrosis. There were 9 patients who, in addition to the changes mentioned, had advanced atherosclerosis, and their aortae showed more extensive fibrosis and medial necrosis. Pooling of proteoglycan matrix, degeneration of elastic lamellae, increased amount of collagen and necrosis of smooth muscle cells characterized the electron microscopic findings of 13 patients. The collagen fibers seemed to be of normal shape. In conclusion, changes in annulo-aortic ectasia are characterized by severe cystic medial necrosis. The changes are basically similar in Marfan and non-Marfan patients.

Key words: Aneurysm – Aorta – Collagen – Cystic medial necrosis – Elastin

Introduction

Annulo-aortic ectasia (AAE) has been defined as an entity comprising dilation of the ascending aorta, dilation of the aortic annulus and progressive insufficiency of the aortic valve (Ellis et al. 1961; Inberg et al. 1985). Patients can be classified as those with the classic Marfan's syndrome and those without it. Diagnosis of Marfan's syndrome is based on the presence of certain abnormalities of the skeletal, ocular and cardiovascular systems inherited as an autosomal dominant (McKusick 1972). Annulo-aortic ectasia is the most important cardiovascular sign of Marfan's syndrome and cystic medial necrosis (Gsell 1928; Erdheim 1929, 1930) the histopathological term used to describe the abnormalities found in the aortic wall. Schlatmann

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and Becker (1977) have shown a critical attitude towards cystic medial necrosis and offered a more profound classification of the alterations which occur in dissecting aortic aneurysms. They have also demonstrated that there are only quantitative differences between the normal "ageing" aorta and the overtly abnormal aorta. In order to clarify the concepts associated with aneurysms of the ascending aorta in cases of annulo-aortic ectasia, we studied the frequency of histological abnormalities by both light and electron microscopy in samples taken from surgically resected aneurysms.

Material and methods

The material consisted of resected ascending aortae from 44 patients (37 male, 7 female, aged 10–66 years, mean 40.3) with annulo-aortic ectasia. There was no serological evidence of syphilis in any of the patients. Eight of the patients (6 male, 2 female, aged 10–35 years, mean 20.6) had the classic Marfan's syndrome. In seven patients the aneurysm of the ascending aorta was accompanied by an acute or chronic dissection of the aorta. For control material, ascending aortae were obtained from 43 consecutive autopsies (30 male, 13 female, aged 15–70 years, mean 55.6) carried out in the Departments of Pathology and Forensic Medicine in the University of Turku. Autopsies performed on individuals of less than 10 years and more than 70 years of age were excluded.

Samples for histological investigation, covering 2–4 cm of the circumference at 1 (controls) to 4 different levels above the aortic ostium were taken and fixed in 10% buffered formalin, embedded in paraffin, sectioned and then stained with haematoxylin and eosin, van Gieson, Verhoeff's elastic tissue stain, Alcian blue at pH 2.5, or with toluidine blue. The samples were graded according to the severity of the light microscopic changes in the aortic media, as described by Schlatmann and Becker (1977). Four different types of degeneration were distinguished: 1) cystic change, defined as pooling of mucoid material, 2) elastic fragmentation, characterized by disruption of elastic lamellae, 3) fibrosis, defined as an increase in collagen, and 4) medial necrosis, defined as areas with loss of smooth muscle cell nuclei. Slight alterations were classified as Grade 1, moderate as Grade 2 and severe as Grade 3. For cases of unchanged morphology, Grade 0 was used. For statistical analysis the χ^2-test was employed. Because of the small number of patients in a particular grade, 0 and 1, or 2 and 3, were sometimes amalgamated for the purpose of the analysis.

For electron microscopy, tissues were obtained from 13 patients with AAE. Samples from the ascending aortae of 6 additional patients were included as control material, in order to compare the normal fine structure of the human aorta. Four of the samples were taken from patients operated on for aortic valve disease (sex and age: f 30, f 40, f 54, f 61), and 2 were taken in autopsy from patients suffering from acute leukaemia (m 16) or cerebral lymphoma (f 57).

The samples were fixed in 3.0% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4, and postfixed in 1.0% osmium tetroxide. The pieces of aortic tissue were embedded in plastic (Epon 812) in such way that it was possible to examine the whole thickness of the aortic wall in semi-thin (1 μm) plastic sections stained with alkaline toluidine blue. Thin sections taken from different parts of the aortic media were mounted on copper grids with or without treatment for 10 min at room temperature in an elastic fibre stain composed of 0.3 g orcein, 1 ml 25% HCl, and 100 ml 70% ethanol (Nakamura et al. 1977). The sections were counterstained with uranyl acetate and lead citrate and studied with a Jeol JEM-100 C electron microscope.

Results

Morphology

Cystic change in the aortic media was characterized by pooling of basophilic material between the elastic lamellae. Within a single lamellar unit only

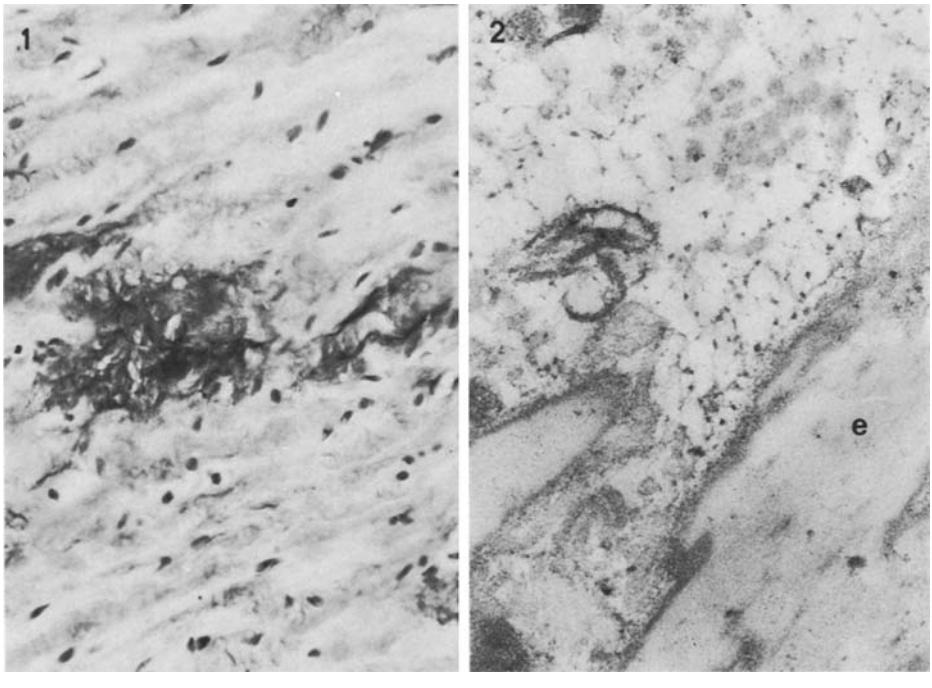

Fig. 1. Advanced cystic change extending over the area of three lamellar units. HE ×320

Fig. 2. Proteoglycan matrix, some cross-sectioned collagen fibres and elastic lamellae (*e*) ×51,000

minute cysts were seen in Grade 1 lesions. The cysts occupied up to the total width of one lamellar unit in Grade 2 and extended beyond the total width of the lamellar unit in Grade 3 (Fig. 1). In the Alcian blue stained sections, acid mucopolysaccharides were unevenly distributed and in toluidine blue a metachromatic staining pattern occurred in paraffin sections.

Electron microscopy revealed a delicate network of proteoglycan matrix, intermingled with a variable number of collagen fibres (Fig. 2).

Grade 1 was characterized by up to five foci of elastic fragmentation of two to four neighbouring lamellae in a single microscopic field comprising the total width of the aortic media. In Grade 2 there were more than five foci, and in Grade 3 there was also disarray of smooth muscle cells (Figs. 3 and 4).

At the ultrastructural level most lamellae appeared to be normal (Fig. 5), varying in width from 1.2 to 1.5 μm. Lamellae with irregular outlines or with granular densities, cracks or holes in the amorphous centre were present in the areas showing the most pronounced degeneration in light microscopy (Fig. 6). Orcein stained the otherwise electron lucent lamellae intensely and revealed small deposits of elastin in the intercellular space of degenerated areas. When atherosclerotic plaques were plentiful there was also severe fragmentation in the elastic lamellae (Figs. 7 and 8).

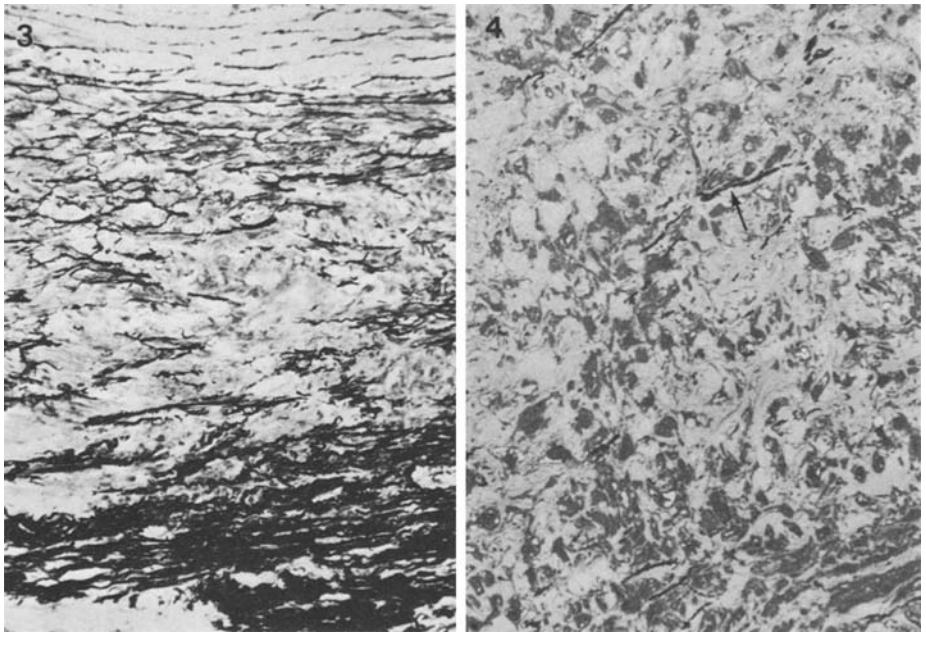

Fig. 3. Severe elastic fragmentation. Verhoeff × 100

Fig. 4. Disarray of smooth muscle cells. Elastin (*arrow*) has almost completely dissappeared. Plastic section, toluidine blue × 260

An increase in collagen content over an area less than one third of the medial thickness indicated Grade 1 fibrosis. In Grade 2, the fibrosis occupied between one and two thirds of the media, and in Grade 3 more than two thirds (Figs. 9 and 10).

In electron microscopy bundles of collagen fibres were observed between the lamellae and also in areas where elastin had disappeared (Fig. 11). No abnormalities were found in the structure of any of the fibres examined (Fig. 12).

Grade 1 medial necrosis was characterized by focal loss of smooth muscle cell nuclei over an area less than one third of the medial thickness. In Grade 2, the necrosis occupied between one and two thirds of the media and in Grade 3 lesions more than two thirds (Fig. 13).

Whereas light microscopy showed no cells between the lamellae, electron microscopy revealed degenerated smooth muscle cells or remnants of cytoplasmic organelles (Fig. 14).

There was no evidence of syphilitic mesaortitis.

Gradation

Control patients were divided into three age groups (15–55, 56–65 and 66–70) and the effect of age on aortic morphology was determined. Although

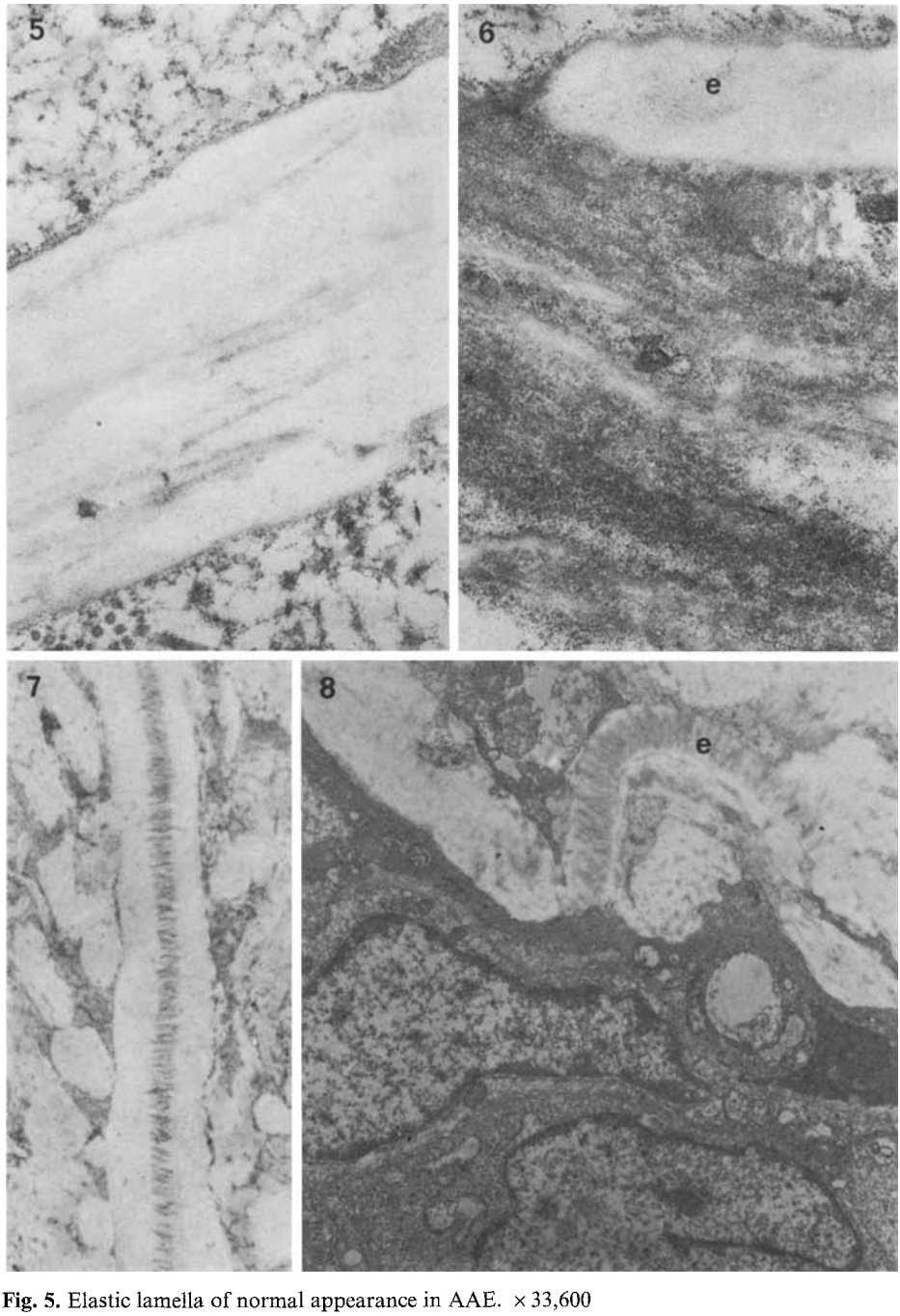

Fig. 5. Elastic lamella of normal appearance in AAE. × 33,600

Fig. 6. A thin elastic lamella (*e*) and irregular filamentous or granular elastin. × 43,300

Fig. 7. Degenerative elastic lamella. × 5,500

Fig. 8. Damaged elastic tissue (*e*) in aortic media. Some cells have discontinuous basement membrane and resemble fibroblasts. × 9,100

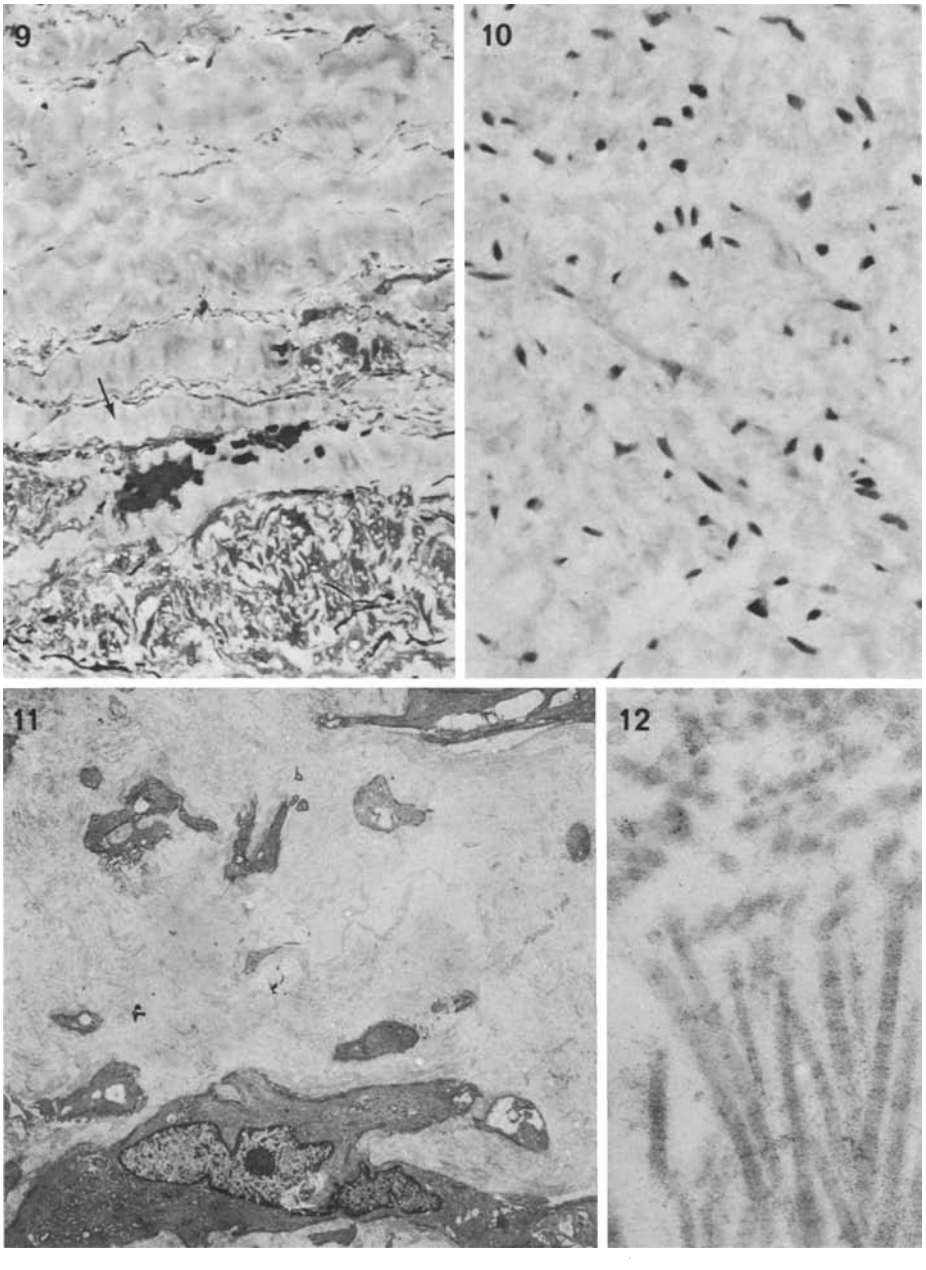

Fig. 9. Elastic lamellae and smooth muscle cells have almost completely disappeared from a fibrotic area. The arrow shows disappearance of smooth muscle cells between two lamellae (medial necrosis). Plastic section, toluidine blue × 340

Fig. 10. Collagen present around cells in fibrotic aortic media. Van Gieson × 350

Fig. 11. Increased collagen bundles are seen around smooth muscle cells. × 2,100

Fig. 12. Collagen fibres show normal configuration. × 54,400

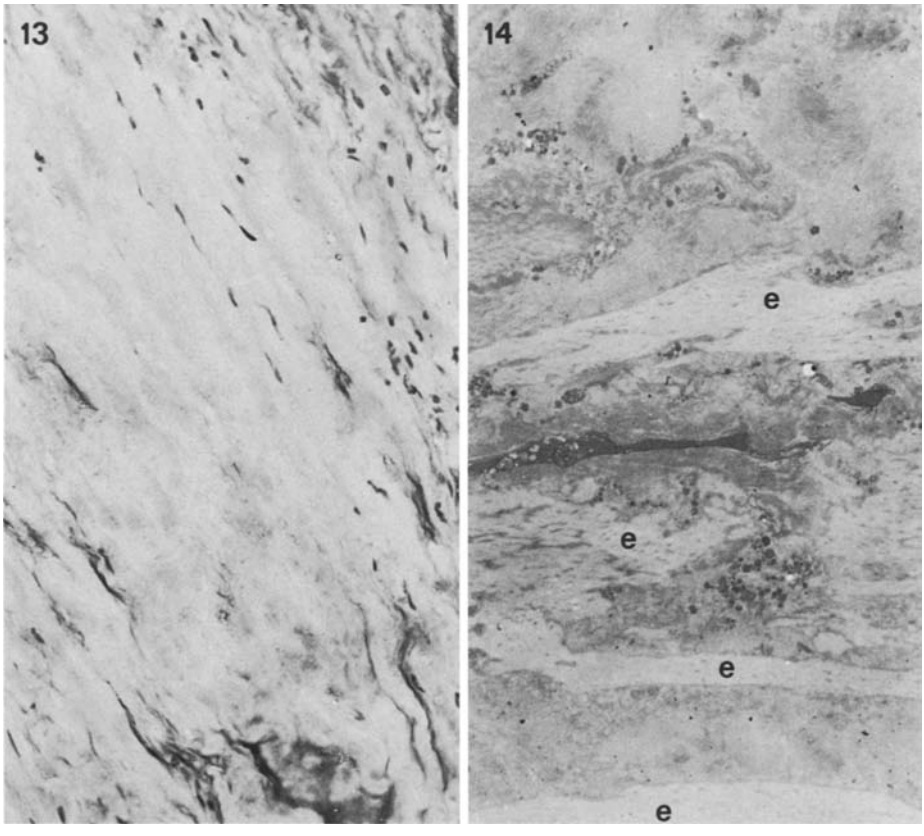

Fig. 13. No smooth muscle cell nuclei are visible in an area of medial necrosis. HE ×210

Fig. 14. Only cellular debris is present between elastic lamellae (*e*). ×5,700

there was a slight tendency towards more severe changes in the older aortae, no significant differences were found and therefore the control patients were compared with the others as a single group.

Changes in the aortic wall were graded and the results are shown in Table 1. There were significantly more severe cystic changes, elastic fragmentation, fibroses and medial necroses in AAE than in the control aortae ($p<0.001$). When Marfan and non-Marfan patients were compared separately with the controls, the differences were still significant ($p<0.001$). In AAE, the Marfan aortae seemed to be less fibrotic than the non-Marfan aortae ($p<0.05$). When advanced atherosclerosis was observed, cystic change was less ($p<0.01$), but fibrosis and medial necrosis more pronounced ($p<0.001$ and 0.01, respectively).

Discussion

Histological changes were significantly more pronounced in the ascending aortae of patients suffering from AAE than in normal control aortae. Idio-

Table 1. Gradation of changes in aortic media in AAE

Grades	0	1	2	3
Cystic change				
Controls (43)	8	20	14	1
AAE (44)	1	7	7	29
Marfan (8)	0	0	0	8
Non-Marfan (36)	1	7	7	21
Atherosclerosis (9)	1	4	0	4
No atherosclerosis (35)	0	3	7	25
Elastic fragmentation				
Controls	19	19	3	2
AAE	1	5	3	35
Marfan	0	0	0	8
Non-Marfan	1	5	3	27
Atherosclerosis	0	0	1	8
No atherosclerosis	1	5	2	27
Fibrosis				
Controls	18	21	4	0
AAE	2	11	19	12
Marfan	0	1	7	0
Non-Marfan	2	10	12	12
Atherosclerosis	0	1	1	7
No atherosclerosis	2	10	18	5
Medial necrosis				
Controls	25	16	2	0
AEE	0	12	20	12
Marfan	0	1	5	2
Non-Marfan	0	11	15	10
Atherosclerosis	0	0	3	6
No atherosclerosis	0	12	17	6

pathic cystic medial necrosis (Erdheim 1930) was diagnosed in most patients and apart from the fact that there appeared to be less fibrosis in patients with the Marfan syndrome there were no obvious differences between the Marfan and non-Marfan aorta.

Some non-Marfan aortae showed only slight degeneration, which was an almost universal finding in the controls. Schlatmann and Becker (1977) emphasized that degenerative changes, not related to hypertension, are present in approximately two thirds of normal ageing aortae and that severe (Grade 3) alterations are only seldom encountered. In the study by Klima et al. (1983) medial necrosis, fibrosis and atherosclerosis were found to correlate directly with age. This finding is in accord with our observation that fibrosis and medial necrosis were pronounced in aortae showing signs of atherosclerosis. Klima et al. (1983) also found that cystic change correlated inversely with age, and this is consistent with our findings that only slight cystic changes were found in atherosclerotic aorta.

According to the principal site of damage aortic wall degeneration can be divided into disseminated cystic (Erdheim-type) and microcystic (Gsell-

type) change (Doerr 1974). Pronounced degeneration in AAE, especially in the Marfan patients, corresponds to the disseminated cystic change, whereas slight degeneration of control and some AAE aortae can be regarded as microcystic lesions. The results support the idea that altered ground substance reflects genetic errors and that acquired factors can change the structure of aortic media during life. Indeed, our previous biochemical results point to altered elastin and collagen metabolism in the aortic wall of AAE patients (Halme et al. 1985).

Because of the small number of patients we did not analyse those cases where dissection was observed. In a series of 339 ascending aortic aneurysms, Klima et al. (1983) found that there was a tendency for dissection to occur more often in connection with medial abnormalities than with atherosclerosis. Hasleton and Leonard (1979) found no constant histological differences between 83 aortic dissections and 20 controls.

Our study indicated that electron microscopic alterations can be seen in the areas of light microscopic degeneration. Earlier examinations have revealed nonspecific changes, or the material has been too limited for major conclusions to be drawn (Saruk and Eisenstein 1977; Scheck et al. 1979; Theman et al. 1979). Smooth muscle cells grouped together and a normal appearance of connective tissue, including the 64 nm periodicity of collagen, have been demonstrated (Saruk and Eisenstein 1977). Scheck et al. (1979) reported morphologically abnormal collagen fibres in the aortic wall in one case of Marfan's syndrome. These investigators speculated that this was caused by a decrease of tensile strength in collagen Type 1 because of a defect in the development of the chains and cross links of the collagen precursors. In the present study, no ultrastructural changes in collagen fibres were observed.

Normal elastin is composed of fibres consisting of slender filaments about 3–4 nm diameter, running roughly parallel to the fibre. A regular periodicity of about 4 nm along the filaments can be resolved (Albert 1973; Gotte et al. 1974). Examination of the substructure calls for purification procedures which were not undertaken in our study. The filamentous structure cannot be seen in the lamellae (Thyberg et al. 1979) but the normal aorta contains thin streaks of elastin connected to the elastic lamellae (Dingemans et al. 1981).

Fragmentation of elastic fibres, irregular surfaces, increased granularity, cracks in the amorphous centres, and an increase of granulofilamentous substance around the fibre are considered to be indications of elastic degeneration. Bundles of non-banded microfibrils have been thought to indicate new elastin formation (Scheck et al. 1979). Degeneration of elastic tissue has, however, also been encountered in normal aortae (Scheck et al. 1979). It is often difficult, or even impossible, to decide whether the changes are signs of real degeneration or only a reflection of the normal regenerative organization of the elastic tissue.

In conclusion, the human ascending aorta showed in AAE degeneration of varying degrees, from almost normal morphology to overt cystic medial necrosis. Severe changes were, however, present in most cases.

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