Virchows Arch [Pathol Anat] (1981) 392:199–216

Virchows Archiv A

Ultrastructure of the Normal Human Aortic Media

Koert P. Dingemans, Norien Jansen, and Anton E. Becker
Department of Pathology, University of Amsterdam, Wilhelmina Gasthuis, Eerste Helmersstraat 104, 1054 EG Amsterdam, The Netherlands

Summary. The ultrastructural organization of the adult human aortic media was studied utilizing *aortic biopsies* from 14 patients, ranging in age from 28 to 67, who underwent cardiac surgery. Apart from solid elastic elements the tissue spaces contained a vast amount of ill-defined thin streaks of elasin, an observation much facilitated by utilizing a selective elastin staining technique. In favorable sections, these streaks were found to be continuous with the solid elastic laminae. Furthermore, most medial smooth muscle cells were in close contact with the thin streaks, but almost none directly with the elastic laminae. The smooth muscle cells had also virtually no connection with collagen fibers. These observations are in contrast with the organization of elastin and with cell-to-stroma connections in the more extensively studied rodent and porcine aortas; they bring into question the role of the smooth muscle cells in the regulation of the viscoelastic properties of the human aortic wall. Other findings were: large number of nexuses connecting the smooth muscle cells, a very small degree of smooth muscle cell degeneration, and the presence of flocculent, fine-granular material investing all formed elements, but especially associated with the thin streaks of elastin.

Key words: Aortic media – Human – Ultrastructure – Elastin – Smooth muscle cell

Introduction

Systematic ultrastructural studies of the human aortic media have not, as yet, been reported. Most of our knowledge is therefore based on animal studies (Karrer and Cox 1961; Seifert 1962, 1963; Bierring and Kobayasi 1963; Paule 1963; Cliff 1967; Stein et al. 1969; Cliff 1970; Gerrity and Cliff 1975; Clark and Glagov 1979). However, it is at present well appreciated that major differ-

Offprint requests to: Dr. K.P. Dingemans

0340-1227/81/0392/0199/$03.60

ences occur between tissues of animal species and those of man (Race 1971). We have therefore studied the normal adult human aortic media in order to obtain a plane of reference necessary to study pathology.

Material and Methods

Material from the ascending aorta of 14 patients, ranging in age from 28 to 67 years was studied. Relevant clinical data are shown in Table 1.

After testing several fixation procedures, the following method appeared to be simple and reliable in preserving both cells and extracellular elements throughout the thickness of the aortic wall. In the operating room, the specimens (usually a few mm^3) were immersed in a formaldehyde-glutaraldehyde mixture (Karnovsky 1965) at room temperature and they were left in this fixative until further processing. Fixation of less than 24 h stabilized the tissue insufficiently, resulting in mechanical damage when the specimens were cut into smaller pieces. Storage of the specimens in fixative for periods of up to two years, on the other hand, did not cause any deleterious effects. Before post-fixation in 1% OsO_4 and block staining with 0.25% uranyl acetate, 0.1–0.2 mm thick slices of tissue encompassing the entire wall were cut in an Oxford vibratome. Due to the irregular shape and the small size of most specimens, orientation in respect to the long axis of the aorta was impossible, but the slices were always embedded in a way which permitted sectioning in a plane perpendicular to the intimal surface. Thus the sections were longitudinal, transverse, or oriented at angles in between, but never tangiential. Two micra-thick sections for survey by light microscopy were cut with an LKB Ultrotome and stained with basic toluidine blue. In at least three of such sections per aorta studied, an area for ultrathin sectioning was selected that was located at about one-third wall thickness from the intimal surface and which contained intact tissue with regular "lamellar units" (Wolinsky and Glagov 1967). Thus, the focal lesions that are present in the human aortic media and that increase with aging (Schlatmann and Becker 1977) were avoided. "Mesas" exactly covering the selected areas were trimmed out with a modified LKB Pyramitome (Dingemans and Lettinga 1976). Ultrathin sections were cut with a diamond knife and stained with uranyl acetate and lead citrate.

In part of the preparations, tannic acid was used to enhance the contrast of extracellular elements, especially elastin which is normally highly electron-lucent. Initially we treated the blocks with tannic acid before dehydration (Simionescu and Simionescu 1976). At later stages tannic acid was added to the uranyl acetate used for the staining of the ultrathin sections (Kajikawa et al. 1975), and this proved to be a simpler method giving more consistent results.

In a control experiment, pieces of aortic tissue from young pigs were prepared following exactly the same procedures as used for the human material. In addition, the possible influence

Table 1. Relevant clinical data

Patient	Sex	Age (in years)	Type of operation
1	F	28	aortic valve replacement
2	M	35	aortico-coronary bypass
3	M	35	aortico-coronary bypass
4	M	37	aortico-coronary bypass
5	M	39	LV aneurysm resection, aortico-coronary bypass
6	M	53	aortic valve replacement
7	M	54	mitral valve replacement, aortico-coronary bypass
8	M	55	aortico-coronary bypass
9	M	56	aortico-coronary bypass
10	M	60	aortico-coronary bypass
11	F	65	aortic and mitral valve replacement
12	M	66	aortico-coronary bypass
13	M	67	aortico-coronary bypass
14	M	67	aortico-coronary bypass

of a delay that may occur during the collection of the material at operation was tested by fixing some pieces of porcine aorta at 15, 60, 120 or 240 min after the isolation of the aorta from the body.

Results

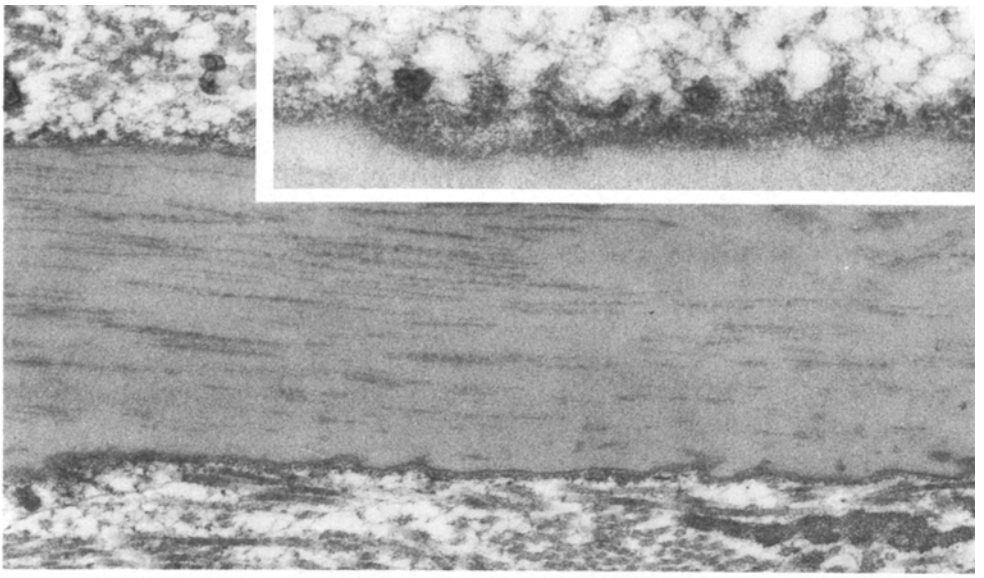
Since in our material the structure of the media was very similar at the different ages studied, the following is a general description of the various components of the adult human aortic media and their interrelations.

General Tissue Architecture and Extracellular Components

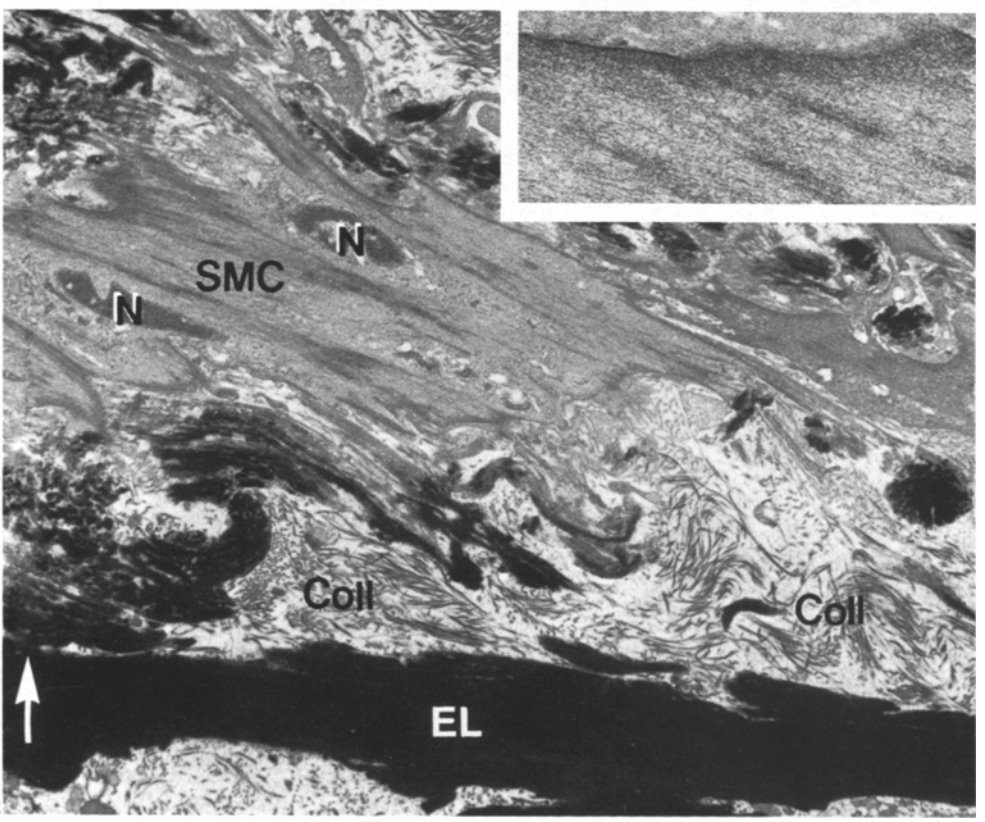
Elastic Elements. In the electron microscope most of the aortic medial tissue displayed the laminar units that were already evident light microscopically. Such units consisted of thick, homogeneous, parallel elastic laminae that were very electron-dense when treated with tannic acid and that alternated with layers containing smooth muscle cells and a variety of extracellular components. The interlaminar spaces were about 13–25 μ in width (average 17–18 μ), which corresponds with the values given by others (Wolinsky and Glagov 1967; Knieriem and Hueber 1970). The thickness of the individual elastic laminae was 1.1–1.8 μ (average about 1.5 μ). In material not treated with tannic acid, vague electron-dense lines or patches could be seen within the laminae (Fig. 1). An unexpectedly large amount of elastin was found in the interlaminar spaces. This was especially evident in tannic acid-treated material. Some of the interlaminar elastin was organized in solid elastic fibers that could be distinguished from the laminae only by their smaller diameter and by their haphazard orientation. However, most of it was present in the form of ill-defined streaks oriented parallel to the main axis of the smooth muscle cells and often closely associated with the surface of the smooth muscle cells (Fig. 2). In sections cut perpendicularly to the main axis the smooth muscle cells, on the other hand, such streaks appeared as irregular patches (Fig. 3). In favorable sections (Figs. 2 and 3) the ill-defined deposits of elastin were often found to be continuous with the elastic laminae. In material not treated with tannic acid they were sometimes difficult to recognize at low magnification. Studying the tissue at higher magnifications, however, demonstrated that the shape and localization of the electron-dense deposits in tannic acid-treated material corresponded precisely to the electron-lucent elastic elements in conventionally treated material.

As mentioned above, the tissue areas selected were all very regular light microscopically, but the electron microscope also revealed many irregular areas. In such areas all tissue elements were usually disorganized; notably the elastic laminae were branched, fragmented, fissured, etc., so that laminar units were not longer clearly definable.

Flocculent Extracellular Material. All elastic elements were invested by a flocculent, extracellular material of moderate electron density. Only a thin layer of this material was associated with the solid elastic laminae and fibers (Fig. 1), but it formed larger accumulations around the above mentioned thin streaks of elastin (Figs. 3 and 4). In such accumulations it was frequently impossible



1



2

to determine the exact places where elastin was present and where it was absent. When the streaks were sectioned longitudinally, the associated desposits of flocculent material were often oriented in the same direction, but a truly fibrillar organization was either absent or obscured by the granularity of the surrounding material (Fig. 4, inset). Near the medial smooth muscle cells the flocculent material gradually merged with the basal laminae surrounding the cells (vide infra). Treatment with tannic acid slightly increased the electron density of this material.

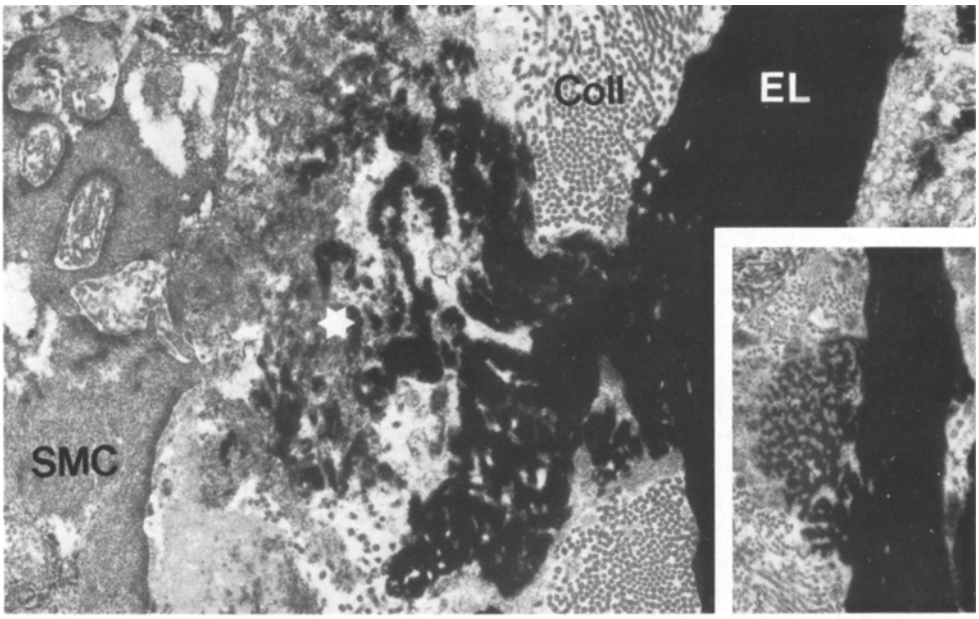
Collagen. The intercellular space contained large amounts of collagen, both as coherent, wavy bundles of fibers and as criss-crossing fibers without apparent orientation (Figs. 2 and 3). Collagen was also somewhat more electron-dense when treated with tannic acid.

Cell Debris and Matrix Vesicles. Variable amounts of cell debris were present; small vesicles similar to the "matrix vesicles" or "granulovesicular bodies" described by others (Trillo and Haust 1975; Kim 1976; Riede and Staubesand 1977) were present in addition. In aortas containing many of these structures they were concentrated especially near the elastic laminae and the largest elastic fibers (Fig. 5). In most aortas many small matrix vesicles contained an amorphous, electron-dense material (Fig. 5, inset A); needle-shaped crystals, probably representing hydroxylapatite (Kim 1976), were found in the larger ones (Fig. 5, insets B and C). With increase of crystal deposition and size of the bodies, their limiting membranes gradually became irrecognizable (Fig. 5, insets A–C). Calcification of matrix vesicles was never associated with an accumulation of residual bodies within smooth muscle cells as reported by Kim (1976). Neither intracellular calcification (Massmann and Weidenbach 1975), nor calcium deposition onto elastic elements (e.g., Massmann and Weidenbach 1975; Theman et al. 1979) was observed (for a discussion of this point, see Morgan 1980).

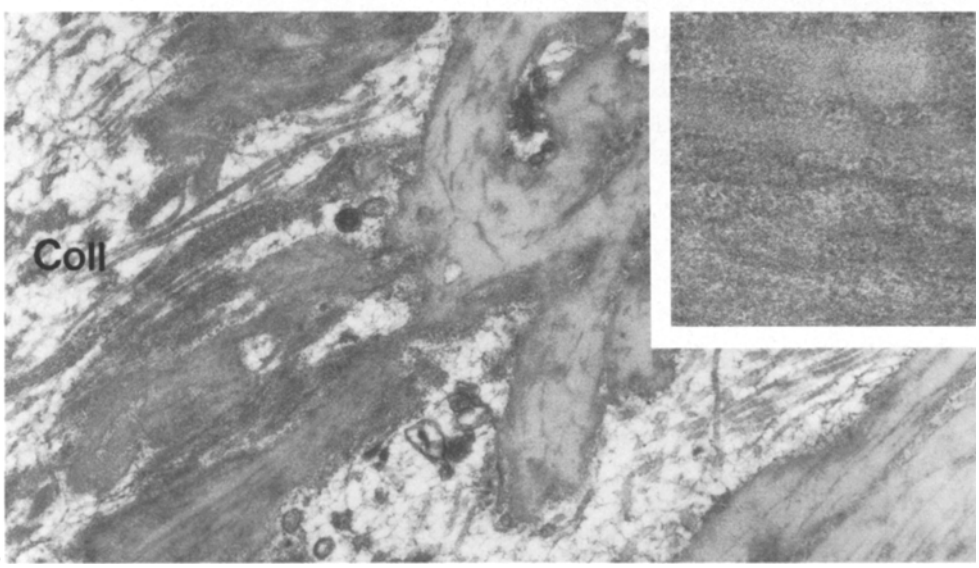
Ground Substance. The intercellular ground substance appeared almost empty in some specimens, but in others it was found to contain a meshwork investing all formed elements (e.g., Figs. 1, 4 and 5). In favorable specimens (Fig. 6) this meshwork appeared to be identical to the ruthenium-red positive proteogly-

Fig. 1. Elastic lamina with vague electron-dense lines and patches internally and with a thin layer of fine granular material covering the surface. Note the presence of collagen fibers, and the meshwork structure of the ground substance (inset). 39-year-old subject. ×51,000. inset ×69,000

Fig. 2. Section cut parallel to the main axis of a smooth muscle cell (*SMC*), showing the presence of thin electron-dense streaks of elastin near the cell surface. Note: the continuity between the streaks and the elastic lamina (*EL*) at the arrow; the absence of a direct contact between the elastic lamina and the smooth muscle cell; the distance between the cell surface and the collagen fibers (*Coll*); and the multilayered basal laminae associated with the cell surface (right side of the micrograph), not to be confused with the more electron-dense collagen fibers. The smooth muscle cell has serrated outlines and contains oblique myofibrillar tracts alternating with organelle-rich regions. The nuclear profiles (*N*) probably represent different parts of a single nucleus. The inset illustrates the apparent termination of myofibrils at the electron-dense patches on the cell surface. 39-year-old subject ×6,400. inset, ×33,000



3



4

Fig. 3. Section cut perpendicular to the longitudinal axis of a smooth muscle cell (*SMC*) demonstrating that the thin streaks of elastin appear as irregular patches when cut transversally. Note: the continuity between the patches of elastin and the elastic lamina (*EL*); the complexes of small elastin deposits and flocculent extracellular material near the surface of the smooth muscle cell (asterisk); the distance between the smooth muscle cell surface and both the elastic lamina and the collagen fibers (*Coll*), and the deep recessions of the smooth muscle cell surface containing branched, multilayered basal laminae (upper left). The inset shows the close association between the elastic lamina and the electron-dense patches that was frequently found. 28-year-old subject. Tannic acid; ×17,300. inset, 60-year-old subject ×15,600

Fig. 4. Transitions from solid elastic laminae (lower right) and fibers (center) to ill-defined streaks of elastin (left part of the figure). Flocculent material forms only a thin layer around the elastic lamina, a thicker layer around the elastic fibers, and large accumulations around the streaks of elastin. Although the deposits of flocculent material are oriented parallel to the collagen fibers (*Coll*) and to the streaks of elastin, a higher magnification (inset) reveals no truly fibrillar but rather a granular organization. The inset also demonstrates that in specimens not treated with tannic acid elastin frequently appears only as small, vague areas of reduced electron density, so that the exact places where elastin is present are difficult to determine. Note the faintly visible meshwork structure of the ground substance. 39-year-old subject ×23,000. inset, ×65,000

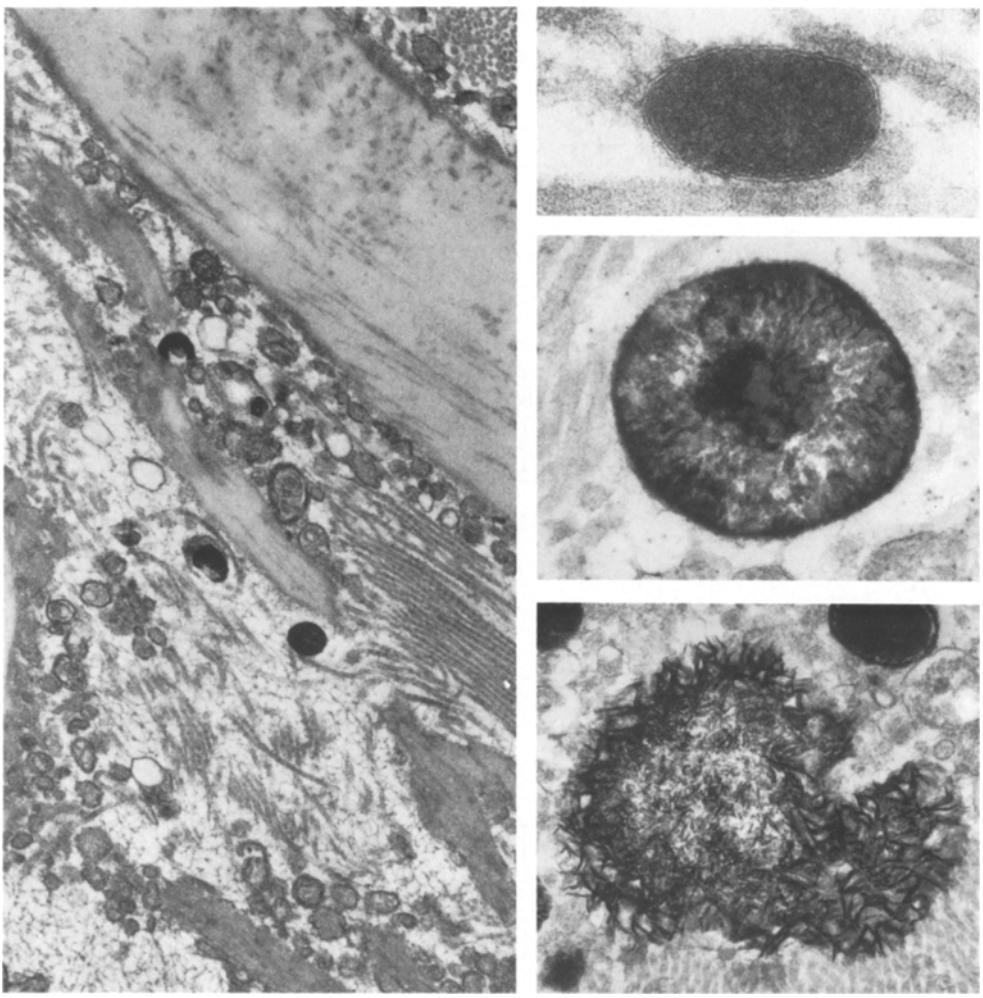
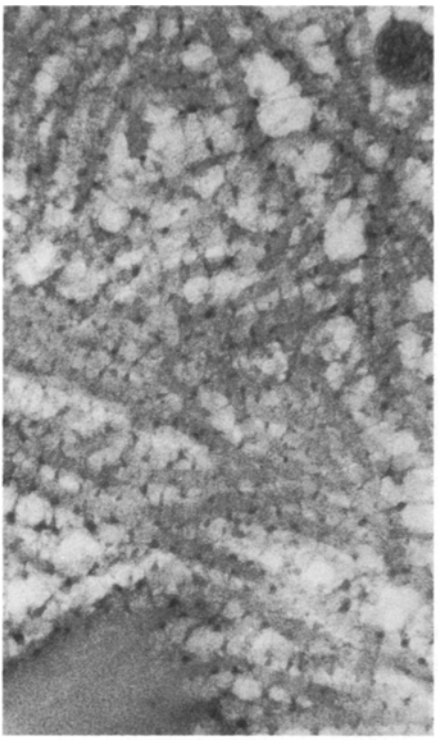
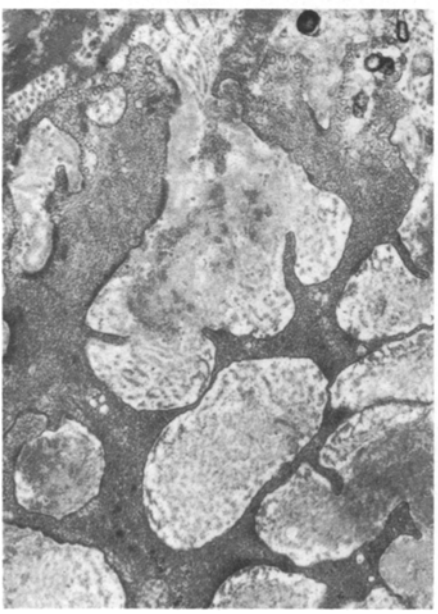


Fig. 5. Matrix vesicles and cellular debris, accumulated around elastic elements. The variable electron density probably reflects a variable deposition of hydroxylapatite. Note the faintly visible meshwork structure of the ground substance. The insets illustrate the supposed sequence of development of the deposits. Inset A, higher magnification of a matrix vesicle with an electron-dense, homogenous content; the limiting membrane is clearly discernible. Inset B, a matrix vesicle in which a particulate electron-dense material has been deposited so as to obscure the limiting membrane that still may be present. Inset C, accumulation of hydroxylapatite crystals, no longer recognizable as being derived from a matrix vesicle. Main figure, 39-year-old subject ×20,500. Inset A, 39-year-old subject ×97,000. Inset B, 66-year-old subject ×70,500. Inset C, 66-year-old subject ×30,000

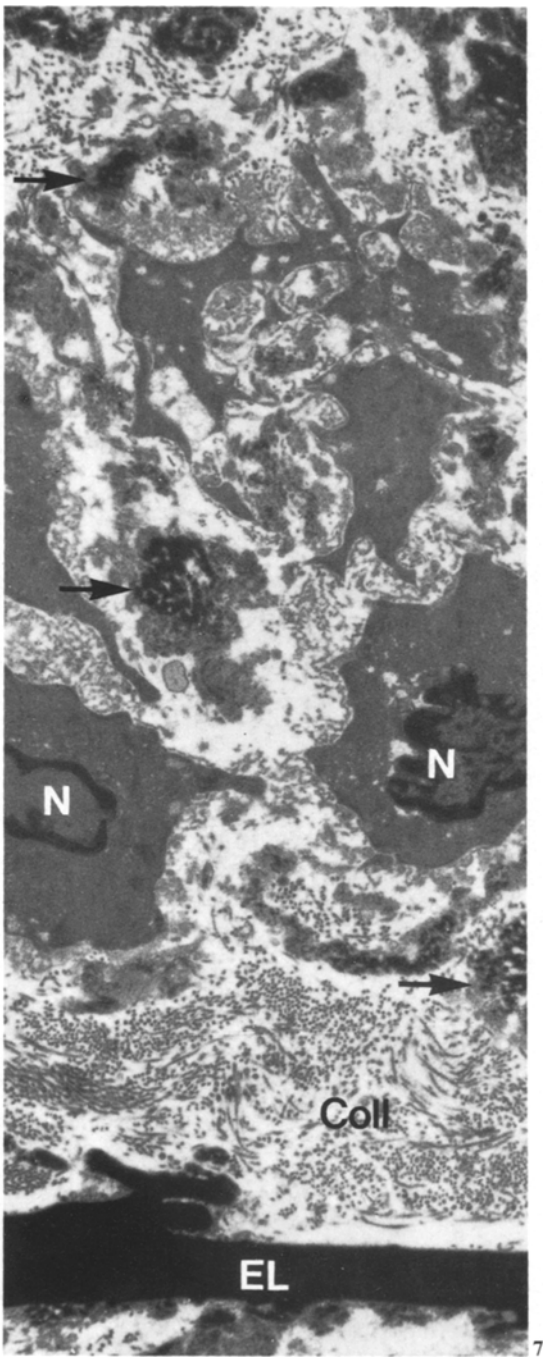
can meshwork described by others (Kádár et al. 1972; Kádár 1974; Wight and Ross 1975), which is assumed to hold together the various components of the intercellular matrix (Wight and Ross 1975). In our material it also contained angular granules, roughly 20–50 mm in size, connected by 3–6 nm filaments. Many of the granules were deposited along the surfaces of elastic elements and at regular 50–55 nm intervals along collagen fibers (Fig. 6).



6



8



7

Smooth Muscle Cells

General Cell Architecture. The smooth muscle cells displayed highly variable profiles in sectioned material, which was partially dependent on the angle of sectioning (Seifert 1962, 1963; Cliff 1970; Clark and Glagov 1979). Longitudinal and transverse profiles could usually be clearly distinguished on the basis of both their overall shape and the orientation of the myofibrils.

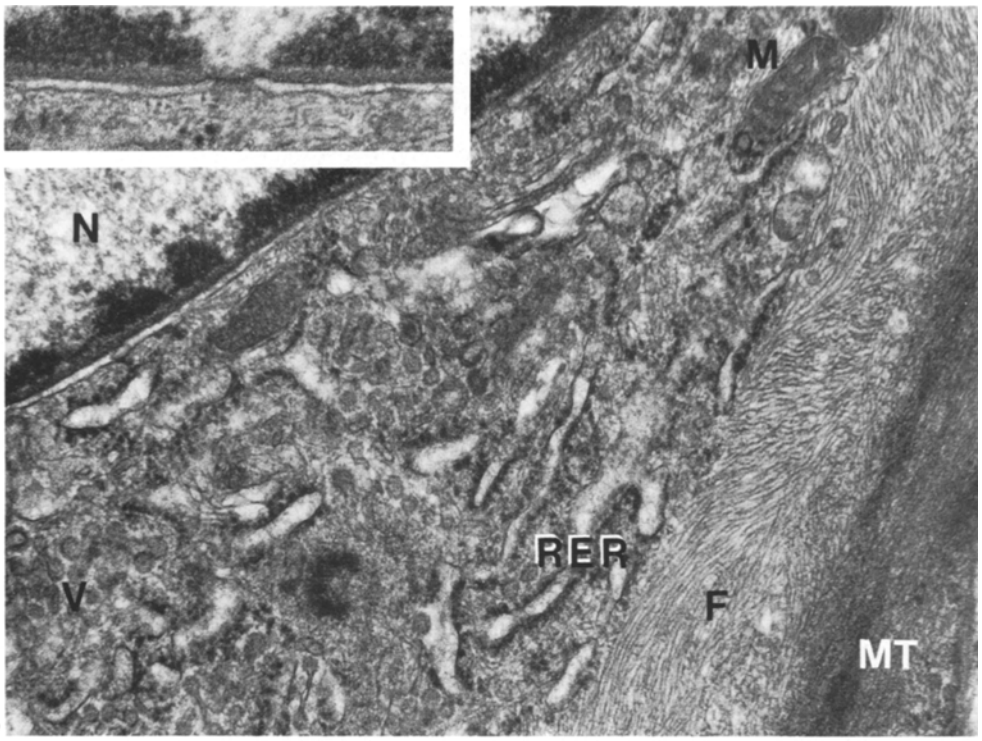
Longitudinal cell profiles had serrated outlines with conspicuous oblique myofibrillar tracts, closely resembling the descriptions of Cliff (1967, 1970) and Gerrity and Cliff (1975). These tracts ran across the cells and terminated at electron-dense patches on the cell surface (Fig. 2). According to Clark and Glagov (1979) the impression of myofibrils terminating at the cell surface is erroneous and only due to configurational changes of the cells occurring when undistended aortic tissue is fixed. Transverse cell profiles were highly variable in shape, in part depending on the level of sectioning. At the level of the nucleus they were roughly isodiametric (Fig. 7) and often had many surface folds imparting a lacy appearance upon the cellular borders (Fig. 3). More towards the cell ends, however, the profiles mainly consisted of multiform laminar projections (Fig. 7; cf. Gabella 1977). Extremely irregular cell profiles such as illustrated in Fig. 8 were also common. Such profiles probably reflected highly irregular overall cell shapes rather than representing sections through the laminar projections near the ends of otherwise normally formed cells.

Intracellular Structures. The smooth muscle cell nuclei were highly lobulated, so that in longitudinal profiles the impression of several nuclei being present within one cell was sometimes created (Fig. 2). The nuclei had prominent heterochromatin, aggregated marginally, an inconspicuous nucleolus, and a prominent lamina densa; nuclear pores were present in small numbers (Fig. 9). Organelle-rich cytoplasmic regions (Fig. 9) alternating with the myofibrillar tracts, mainly contained cisternae of the rough endoplasmic reticulum, small electron-dense mitochondria, a small Golgi apparatus associated with many 70 nm vesicles some of which had an electron-dense content (cf. Joris and Majno 1974; Trillo

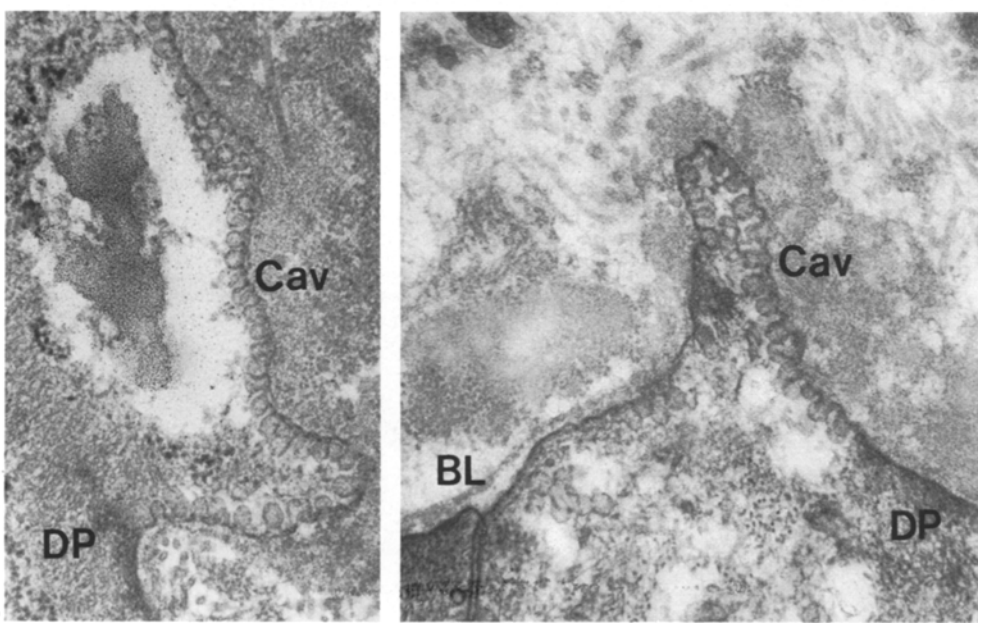
Fig. 6. Well recognizable proteoglycan meshwork, investing collagen fibers, elastic elements, and matrix vesicles. Note the regular spacing of the proteoglycan granules along the collagen fibers. 39-year-old subject × 57,000

Fig. 7. Transversally sectioned smooth muscle cells. At the level of the nucleus (*N*), the cell profiles are roughly isodiametric. When terminal portions are cut, the profiles mainly consist of irregular laminar projections. Note that the cells are associated with irregular patches of elastin that are embedded in flocculent material (some of them indicated by arrows) and with basal laminae that sometimes form multilayered or branched structures. On the other hand, the elastic lamina (*EL*) and the collagen fibers (*Coll*, distinguished from the multilayered basal laminae by their higher electron density), are at a considerable distance from the cells. 66-year-old subject. Tannic acid; × 8,300

Fig. 8. A highly irregular cell profile. The surface of the thinnest cell projections lacks the caveolae and the electron-dense patches that are just discernible in the more massive parts of the cell. The branched extracellular structures probably represent multilayered basal laminae. 66-year-old subject. × 14,200



9



10

and Haust 1975), and numerous intermediate filaments, easily distinguishable from the finer myofilaments. Part of the cells contained conspicuous lipofuscin bodies, but smaller lysosomal elements were found only rarely. Clear spaces without a limiting membrane containing remnants of a very fine-granular material, possibly extracted glycogen, were found especially at the cell periphery where they bulged into adjacent cells or into the extracellular space (Fig. 10). In many cells both the rough endoplasmic reticulum and the mitochondria were swollen, but unequivocal signs of irreversible damage (Cliff 1970; Imai et al. 1970) were not observed. Only the presence of extracellular debris and the occasional finding of "ghost bodies" (Veltman et al. 1975) suggested that some degeneration of smooth muscle cells did occur.

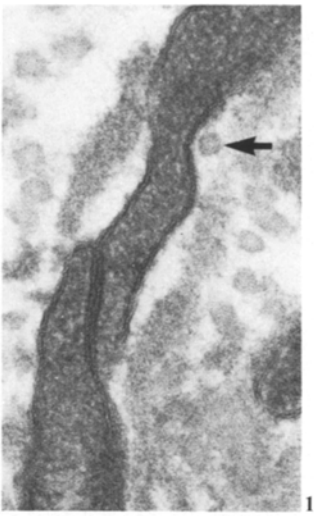
Cell Surface. Most of the smooth muscle cell surface was occupied, at the cytoplasmic side, by either electron-dense patches or caveolae. The electron-dense patches could often be followed over a considerable distance parallel to the main cell axis and might therefore better de designated "electron-dense bands" (cf. Gabella 1977). They were found predominantly in the surface recessions whereas the caveolae were especially abundant in the cell projections (Fig. 10 and 11; cf. Lane 1965; Gabella 1976). Only the thin, outermost projections were usually devoid of caveolae (Figs. 8, 13–15). At times, flat sacs of sarcoplasmic reticulum were associated with the caveolae (Gabella 1972). On the outside, the cell surface had short stretches of recognizable basal lamina predominantly associated with the electron-dense patches at the cytoplasmic side of the plasma membrane; they often bridged the interface between two closely apposed cells (Fig. 11). In addition, there were complicated branched structures, especially in deep cell surface recessions, that probably represented multilayered basal laminae (Figs. 7 and 8). Yet other parts of the cell surface were covered by the previously mentioned complexes of flocculent material and thin streaks of elastin (Fig. 11); occasionally, elastin even seemed to be a major component of these complexes (Fig. 12).

Fig. 9. An organelle-rich cytoplasmic region, situated between the nucleus (*N*) and a myofibrillar tract (*MT*). Note the rough endoplasmic reticulum (*RER*), 70-nm vesicles (*V*), mitochondria (*M*), and intermediate filaments (*F*). The inset shows a nuclear pore and the lamina densa. 39-year-old subject ×36,500. inset, ×53,500

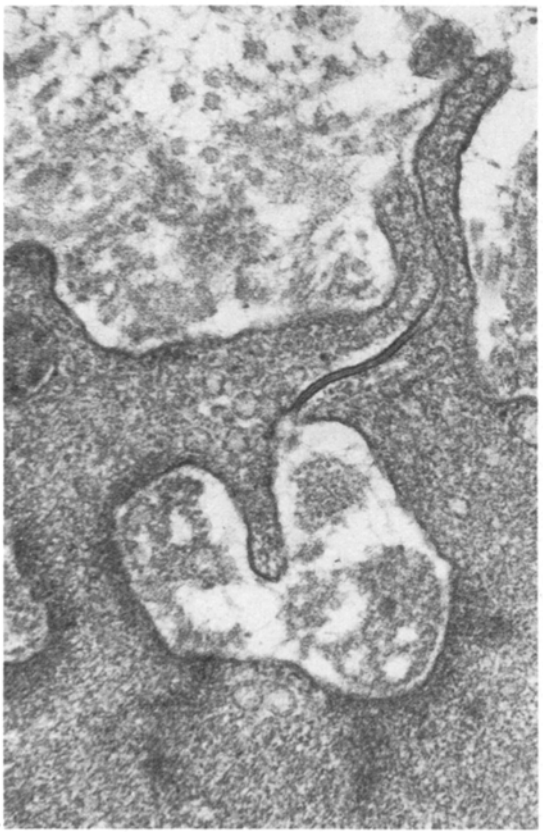
Fig. 10. A clear cytoplasmic space located near the cell surface and containing very fine granulated remnants of a material of unknown nature. Caveolae (*Cav*) line most of the surface of the cell projections whereas electron-dense patches (*DP*) are found more in the surface recessions. 66-year-old subject ×38,000

Fig. 11. Part of the surface of 2 adjacent smooth muscle cells, showing caveolae (*Cav*) mainly in the projections and electron-dense patches (*DP*) mainly in the surface recessions. At the left, a short stretch of basal lamina (*BL*) bridges the interface between the cells. At the right, the basal lamina seems to merge with complexes of flocculent material and ill-defined patches of elastin that appear as vague areas of reduced electron density in material not treated with tannic acid. 35-year-old subject ×46,000

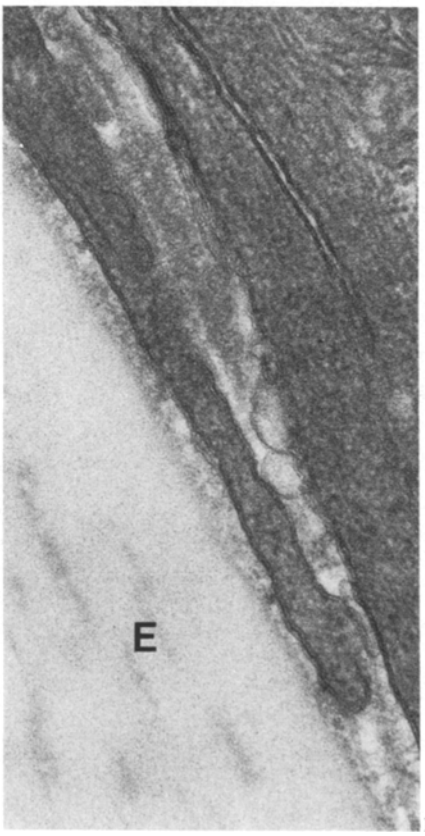
12



13



14



15

Smooth Muscle Cell Connections

Cell-to-Cell Connections. In most tissue areas studied, many smooth muscle cells were connected by well-developed nexuses or gap junctions, especially when cell extensions touched each other (Fig. 13). When, on the other hand, the central portions of the cell adjoined each other, nexuses were found relatively infrequently. Cell extensions forming a nexus were not necessarily parts of different cells: nexuses between extensions deriving from one cell ("reflexive gap junctions", Gabella 1975; Herr 1976) were as readily found as those between extensions of different cells (Fig. 14). For geometric reasons (Henderson et al. 1971), nexuses were generally much more conspicuous on transverse than on longitudinal cell profiles. Other types of cell junctions, e.g., clearcut intermediate junctions (Henderson et al. 1971), were not observed.

Cell-to-Stroma Connections. As shown above, there was often an intimate contact between the smooth muscle cell surface and complexes consisting of flocculent material and thin streaks of elastin (Fig. 3, 11 and 12) and a continuity could frequently be demonstrated between smooth muscle cells and the elastic laminae via such streaks (Figs. 2 and 3). However, *direct* contact between smooth muscle cells and elastic laminae was only occasionally observed. In fact there was almost invariably a considerable distance between the smooth muscle cell surface and the nearest elastic lamina (Figs. 2, 3 and 7). Even the infrequent examples of cell extensions abutting directly on elastic laminae or elastic fibers seemed to be largely chance appositions and the cell surface did not show any specializations at the site of contact (Fig. 15).

Collagen fibers were also generally located at a distance from the smooth muscle cell surface (Figs. 2, 3, 7 and 11); only occasionally were a few collagen fibers observed near the plasma membrane (e.g. Fig. 13).

Porcine Aorta

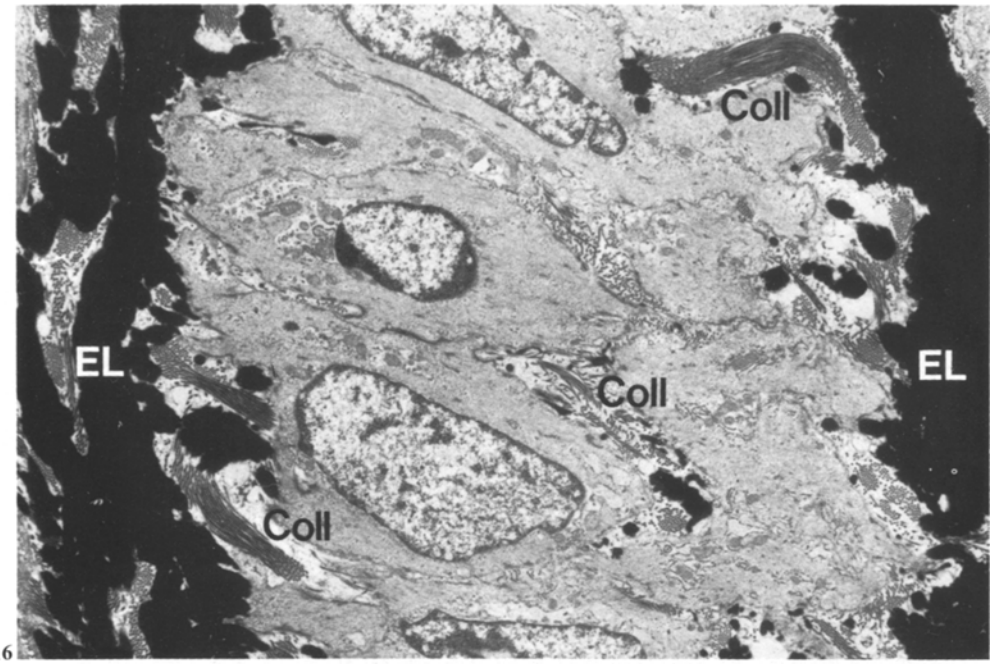
Most of the elastin in the porcine aorta was present in well-formed laminae and fibers; even the small aggregations of elastin that were found in addition to these consisted of sharply defined structures, strikingly different from the indistinct streaks found in the human material (Figs. 16 and 17). In correspondence with the observations of Seifert (1963), many elastic laminae had a conspic-

Fig. 12. A complex of flocculent material and thin streaks of elastin, associated with the cell surface. In this instance, elastin seems to be the major component of the complex. 39-year-old subject × 54,000

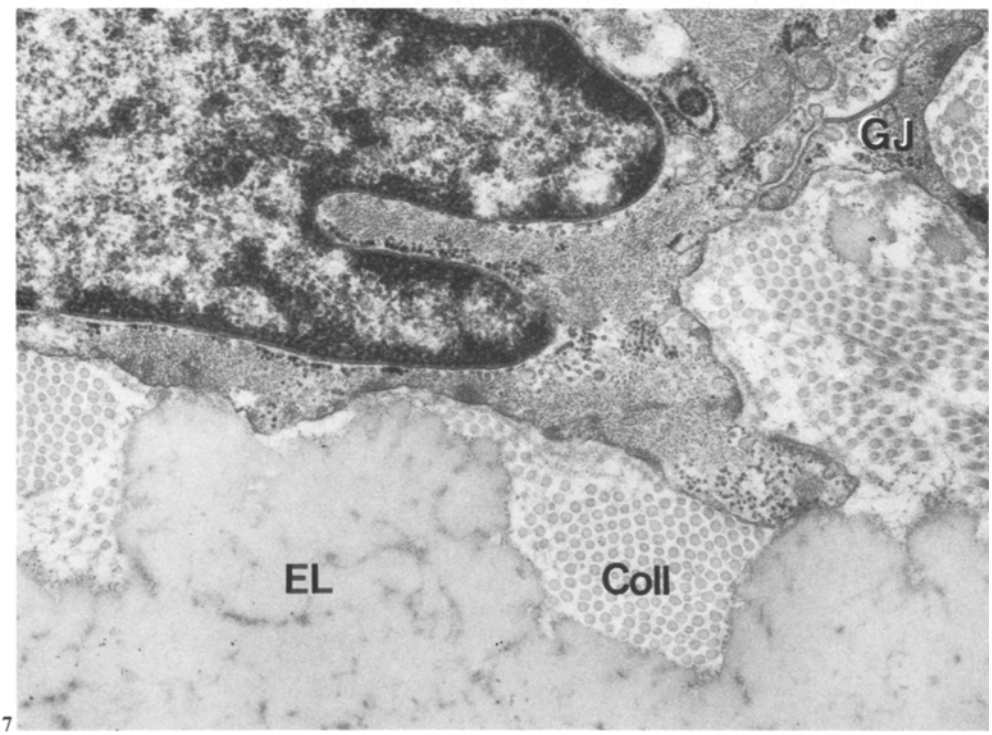
Fig. 13. Cell extensions connected by a nexus, characterized by the apparent fusion of the outer leaflets of the membranes. At the arrow, a collagen fiber is located near the plasma membrane. 39-year-old subject × 89,000

Fig. 14. A nexus connecting different processes of the same cell. 66-year-old subject × 60,500

Fig. 15. A cell extension abutting directly on a large elastic element (*E*). No surface specialization can be detected at the area of contact. 39-year-old subject × 85,500



16



17

Fig. 16. A lamellar unit in a porcine aorta. Smooth muscle cells fill most of the space between two elastic laminae (*EL*). Note: the scarcity of elastin the interlaminar space; the three-layered structure of the elastic lamina at the left, and the close association of the smooth muscle cells with both elastic laminae and collagen fibers (*Coll*). Tannic acid; ×4,600

Fig. 17. Detail of a porcine aorta, showing areas of close association of the smooth muscle cell surface with an elastic lamina (*EL*). Note: the compact structure of the elastic elements, including the small deposits of elastin at upper right, and the localization of bundles of collagen fibers (*Coll*) close to the cell surface, without an intervening basal lamina. *GJ*, gap junction. ×31,000

uous three-layered structure (Fig. 16). Most of the surface of all elastin elements was associated with microfibrils rather than with flocculent material. The cells had numerous contacts with the elastic laminae and fibers. The contact areas, usually located on cell extensions, were slightly concave and had a narrow electron-lucent gap between the elastin and the plasma membrane that frequently seemed to be obscured due to the angle of sectioning; they therefore corresponded to cell-to-elastin contacts as described by others (Seifert 1962, 1963; Bierring and Kobayasi 1963; Cliff 1967; Clark and Glagov 1979). Nexuses, however, were found only in small numbers. Furthermore, bundles of collagen fibers were frequently found in close association with the smooth muscle cells (Figs. 16 and 17). Finally, even a 4-h delay before fixation appeared to have only a very limited effect both on the general morphology of the cells and on the cell-to-stroma connections.

Discussion

This study demonstrates that the ultrastructural characteristics of the adult human aortic media differ markedly from descriptions of the aorta in various animal species (Karrer and Cox 1961; Seifert 1962, 1963; Bierring and Kobayasi 1963; Cliff 1967; Stein et al. 1969; Cliff 1970; Gerrity and Cliff 1975; Clark and Glagov 1979). Our own observations on porcine aorta fully endorse those of Seifert (1963) and Clark and Glagov (1979) and thus underline that the observed differences cannot be attributed to different fixation or staining procedures. It seems, therefore, more likely that species differences, age-related effects (virtually all animal studies were done on very young animals), or both, play a major role.

The question of whether the tissue samples in our patients represent "normality" can be raised. We feel confident that they do since none of these patients had an aortic disease clinically, in each instance the aorta was normal on gross inspection and the areas selected for electron microscopy appeared normal on light microscopic examination. Finally, the uniform morphology through all pieces suggests that at least these sites were not affected by disease.

In all aortas studied, an unexpectedly large amount of elastin was found in thin streaks between the more conspicuous solid elastic laminae and fibers. Although their detection was facilitated by the tannic acid staining, these streaks were clearly demonstrated after conventional specimen preparation as well, so that it seems unlikely that these streaks could have been overlooked in animal material. Several authors studying rodent aortas mentioned the occurrence of small shreds or fragments of elastin (e.g., Karrer and Cox 1961; Cliff 1970), but these always seemed to be scarce compared with those in our human material; moreover, they resembled the sharply defined "elastic units" found in developing elastic tissue (e.g., Haust et al. 1965) more than the indistinct streaks described in the present paper. It should be emphasized that the close correspondence between the shape and localization of elastin deposits in tannic acid-treated and in conventional specimens indicates that the staining procedure followed by us was indeed selective for elastin.

The localization of the flocculent, fine-granular material around elastic ele-

ments suggested a resemblance to "elastic fiber myofibrils" described by others (e.g., Haust 1965; Haust et al. 1965; Ross 1973). These descriptions were based almost exclusively on material from either young animals or young humans as opposed to the adult subjects in the present study. It seems therefore likely that the difference in age is responsible for the association of elastin with floccular rather than with fibrillar material. On the other hand, many authors have described a very similar flocculent material surrounding smooth muscle cells, which clearly represented thickened basal laminae (e.g. Ferrans and Roberts 1976). The precise nature of the flocculent material therefore remains to be determined.

Smooth muscle cells were often closely invested by the thin streaks of elastin, which probably are of little mechanical significance, whereas direct contact between smooth muscle cells and solid elastic elements was scarce. This observation is in sharp contrast to published reports on rodent and porcine aortas as well as our own observations on porcine aortas in which most smooth muscle cells are in direct contact with elastic laminae or elastic fibers (Karrer and Cox 1961; Seifert 1962, 1963; Bierring and Kobayasi 1963; Cliff 1967, 1970; Clark and Glagov 1979). Collagen fibers also had little contact with smooth muscle cells. This constitutes another difference between our human material and the rabbit and porcine material described by Clark and Glagov (1979), who observed prominent pericellular sleeves consisting of collagen fibers and basal laminae. The scarcity of cell-to-stroma connections in the adult human aorta brings into question the role of the smooth muscle cells in this tissue. In the literature it is almost generally accepted that the viscoelastic properties of the aortic wall are caused and modulated by smooth muscle cells and their tone. This conclusion seems to be based partly on the observed close association between smooth muscle cells, elastin, and collagen fibers (Wolinsky and Glagov 1967; Clark and Glagov 1979). Our own observations, on the other hand, shed doubt on the importance of the regulatory role of the smooth muscle cells in the adult human aorta.

The intracellular features do not offer a clue to the function of the smooth muscle cells since they are compatible with both tensile and secretory activities. On the one side, the large amounts of myofilaments found in our material, and also described by other authors (Karrer and Cox 1967; Stein et al. 1969; Cliff 1970; Gerrity and Cliff 1975; Clark and Glagov 1979), do suggest that some degree of contractile activity occurs. Also the presence of nexuses possibly indicates a contractile function. Their abundance in our human material is in contrast to our observations on porcine material as well as to most other studies in which nexuses were either not (Clark and Glagov 1979) or apparently infrequently (Cliff 1967, 1970) observed. A secretory function of the smooth muscle cells was suggested by the presence of well-developed rough endoplasmic reticulum and Golgi apparatus in most of our specimens. We did not, however, observe intracellular vesicles containing elastin and collagen as described in young rat aortas (Thyberg et al. 1979). The matter of the gradual change in composition of the aortic wall and the role played by the cellular constituents is at present under further investigation.

References

Bierring F, Kobayasi T (1963) Electron microscopy of the normal rabbit aorta. Acta Pathol Microbiol Scand 57:154–168

Clark JM, Glagov S (1979) Structural integration of the arterial wall. I. Relationships and attachments of medial smooth muscle cells in normally distended and hyperdistended aortas. Lab Invest 40:587–602

Cliff WJ (1967) The aortic tunica media in growing rats studied with the electron microscope. Lab Invest 17:599–615

Cliff WJ (1970) The aortic tunica media in aging rats. Exp Mol Pathol 13:172–189

Dingemans KP, Lettinga KP (1976) Modifications to an LKB Pyramitome. J Microsc (Oxford) 108:301–306

Ferrans VJ, Roberts WC (1976) The carcinoid endocardial plaque. An ultrastructural study. Hum Pathol 7:387–409

Gabella G (1972) The arrangement of sarcoplasmic reticulum in smooth muscle. Experientia 28:948–949

Gabella G (1975) Hypertrophy of intestinal smooth muscle. Cell Tissue Res 163:199–214

Gabella G (1976) Structural changes in smooth muscle cells during isotonic contraction. Cell Tissue Res 170:187–201

Gabella G (1977) Arrangement of smooth muscle cells and intramuscular septa in the taenia coli. Cell Tissue Res 184:195–212

Gerrity RG, Cliff WJ (1975) The aortic tunica media of the developing rat. I. Quantitative stereologic and biochemical analysis. Lab Invest 32:585–600

Haust MD (1965) Fine fibrils of extracellular space (microfibrils). Their structure and role in connective tissue organization. Am J Pathol 47:113–1137

Haust MD, More RK, Bencosme SA, Balis JU (1965) Elastogenesis in human aorta: an electron microscopic study. Exp Mol Pathol 4:508–524

Henderson RM, Duchon G, Daniel EE (1971) Cell contacts in duodenal smooth muscle layers. Am J Physiol 221:564–574

Herr JC (1976) Reflexive gap junctions. Gap junctions between processes arising from the same ovarian decidual cell. J Cell Biol 69:495–501

Imai H, Lee SK, Pastori SJ, Thomas WA (1970) Degeneration of arterial smooth muscle cells: ultrastructural study of smooth muscle cell death in control and cholesterol-fed animals. Virchows Arch [Pathol Anat] 350:163–204

Joris I, Majno G (1974) Cellular breakdown within the arterial wall. An ultrastructural study of the coronary artery in young and aging rats. Virchows Arch [Pathol Anat] 364:111–127

Kádár A (1974) The ultrastructure of elastic tissue. Pathol Europ 9:133–146

Kádár A, Gardner DL, Bush V (1972) Glycosaminoglycans in developing chick-embryo aorta revealed by ruthenium red: an electron microscope study. J Pathol 108:275–280

Kajikawa K, Yamaguchi T, Katsuda S, Miwa A (1975) An improved electron stain for elastic fibers using tannic acid. J Electron Microsc 24:287–289

Karnovsky MJ (1965) A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J Cell Biol 27:137A

Karrer H, Cox J (1961) An electron microscope study of the aorta in young and aging mice. J Ultrastruct Res 5:1–27

Kim KM (1976) Calcification of matrix vesicles in human aortic valve and aortic media. Fed Proc 35:156–162

Knieriem HJ, Hueber R (1970) Quantitative morphologische Untersuchungen an der Aorta des Menschen. Beitr Path Anat 140:280–297

Lane BP (1965) Alterations in the cytologic detail of intestinal smooth muscle cells in various stages of contraction. J Cell Biol 27:199–213

Massmann J, Weidenbach H (1975) Lichtmikroskopische und elektronenoptische Untersuchungen zur spontanen kalzifizierenden Mediasklerose des Kaninchens. Zentralbl Allg Pathol 119:179–188

Morgan AJ (1980) Mineralized deposits in the thoracic aorta of aged rats: ultrastructural and electron probe X-ray microanalysis. Exp Geront 15:563–573

Paule WJ (1963) Electron microscopy of the newborn rat aorta. J Ultrastruct Res 8:219–235

Race GJ (1971) In Atlas of human histology and ultrastructure, Matthews JL, Martin JH (eds). Lea & Febiger Philadelphia

Riede UN, Staubesand J (1977) A unifying concept for the role of matrix vesicles and lysosomes in the formal pathogenesis of diseases of connective tissues and blood vessels. Beitr Path 160:3–37

Ross R (1973) The leastic fiber. A review. J Histochem Cytochem 21:199–208

Schlatmann TJM, Becker AE (1977) Histologic changes in the normal aging aorta: implications for dissecting aortic aneurysm. Am J Cardiol 39:13–20

Seifert K (1962) Elektronenmikroskopische Untersuchungen der Aorta des Hausschweines. Z Zellforsch 58:331–368

Seifert K (1963) Elektronenmikroskopische Untersuchungen der Aorta des Kaninchens. Z Zellforsch 60:293–312

Simionescu N, Simionescu M (1976) Galloylglucoses of low molecular weights as mordant in electron microscopy. I. Procedure and evidence for mordanting effect. J Cell Biol 70:608–621

Stein I, Eisenberg S, Stein Y (1969) Aging of aortic smooth muscle cells in rats and rabbits: a morphologic and biochemical study. Lab Invest 21:386–397

Theman TE, Silver MD, Haust MD, McLoughlin MJ, Wigle ED, Williams WR (1979) Morphological findings in idiopathic calcification of the ascending aorta and aortic valve affecting a young woman. Histopathology 3:181–190

Thyberg J, Hinek A, Nilsson J, Friberg U (1979) Electron microscopic and cytochemical studies of rat aorta. Intracellular vesicles containing elastin- und collagen-like material. Histochem J 11:1–17

Trillo A, Haust MD (1975) The granulovesicular bodies of the arterial wall. Lab Invest 32:105–110

Veltman E, Backwinkel KP, Themann H, Hauss WH (1975) Elektronmikroskopische Untersuchungen zur Entstehung von "ghost bodies" in Aorten. Virchows Arch [Pathol Anat] 367:281–288

Wight TN, Ross R (1975) Proteoglycans in primate arteries. I. Ultrastructural localization and distribution in the intima. J Cell Biol 67:660–674

Wolinsky H, Glagov S (1967) A lamellar unit of aortic medial structure and function in mammals. Circ Res 20:99–111

Accepted March 25, 1981