Electron Microscope Study of the Normal Rat Aorta*

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ABSTRACT

The fine structure of the normal rat aorta is described. The presence of a subendothelial layer, the oblique orientation of the smooth muscle cells with respect to the aortic axis, and the occurrence of desmosomes between these cells and adjacent elastic laminae, are emphasized. Lead-stained collagen presented a characteristic signet-ring appearance on cross-section. The rats examined were the pair-fed controls for the lathyritic series described in a separate communication.

Electron microscope studies on the normal aorta and large arteries of mammals are scant. Buck (1) described the endothelium of the aorta, femoral and splenic arteries of rats and rabbits, and Parker (2) reported the normal architecture of rabbit coronary arteries. The only papers that could be found on the whole aortic wall were by Berrian (3) on the rat, and a short abstract on the kitten aorta by Pease (4). Although the present study is in general agreement with these, it differs with Berrian on several points.

It was considered of interest, therefore, to investigate the fine structure of the normal rat aorta using the newer embedding and staining techniques. In addition, this investigation serves as a basis for the lathyritic and enzyme studies reported in separate communications.

Materials and Methods

Five albino rats of Sprague-Dawley strain were weaned at 21 days and then fed Purina fox chow. Each was pair-fed with a litter mate given a sweet pea diet, and thus constituted the controls for the lathyritic animals reported in a separate paper (5). The intake averaged 9 to 11 gm. daily and all animals remained apparently healthy though hungry. They were sacrificed when 37, 38, 41, 54, and 60 days old.

After anaesthetizing the animal with ether, the thoracic cage was opened and both ascending and descending aortae were removed as quickly as possible. (The actual arch was carefully avoided owing to the multidirectional elastic laminae at the origin of the great vessels (6)). Unwanted fat and connective tissue were removed and 2 mm. lengths of the ascending aorta were placed in ice-cold 2 per cent O_sO_4 buffered to pH 7.3 with veronal-acetate and containing sucrose as described by Caulfield (7). Short lengths of the descending thoracic aorta were put in 10 per cent formalin for light microscopy. After fixation for 1 to 1½ hours at 0–5°C. and dehydration at room temperature, duplicate specimens for electron microscope examination were embedded in butyl methacrylate and araldite. The best results with methacrylate were obtained by polymerizing at 60°C. for 2 days. Difficulty was experienced in obtaining undistorted sections of tissue embedded in araldite, and this was partly due to the nature of the aortic wall which consists of alternating layers of elastin and muscle cells, each layer presenting a different cutting property. The commonest artifacts were washboarding and macroscopic disintegration of the specimen in the microtome collecting trough. Since methacrylate did not appear to produce such an accurate anatomical picture as araldite (owing to differences in polymerization and alterations occurring in the tissue on exposure to the electron beam), various procedures were tried in order to obtain a satisfactory method using araldite. The final technique employed three changes of 100 per cent araldite containing catalyst (in the proportion of 0.1 ml./ml. to 1 ml.) spread over 24 hours at room temperature, followed by 5 or more days' polymeriza-

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tion at 60°C. After several days' storage at room temperature the blocks were cut and sections collected on distilled water containing a detergent (25 drops of a 1 per cent solution of photo-flo[1] in 60 ml. water).

Transverse sections of the aortic wall were cut with a glass knife in a Porter-Blum microtome, picked up on carbon-coated grids, stained with saturated aqueous lead hydroxide for 30 minutes (8), and examined in an RCA EMU-2A electron microscope. The methacrylate sections were sandwiched (9) with formvar after staining. Some duplicate specimens were stained either with 10 per cent aqueous phosphotungstic acid (PTA) for 5 minutes or with lead in order to compare the effect on collagen.

Light Microscopy.—Sections of the descending thoracic aorta were stained with haematoxylin and eosin, McManus' periodic acid–Schiff (PAS) procedure, Verhoeff's elastic stain, and Heidenhain's azan.

RESULTS

None of the aortae presented any abnormality visible to the naked eye or with the light microscope.

The aorta is a myoelastic tube whose diameter is controlled by a thick medial coat composed of alternating concentric layers of elastin and smooth muscle cells, the latter being oriented obliquely to the radius of the blood vessel (see below). It is surrounded by an outer adventitial coat consisting of bundles of collagen interspersed with small blood vessels and nerves, and the lumen is lined by endothelial cells which are separated from the internal elastic lamina by a subendothelial layer. The outer aspect of the internal elastic lamina and both inner and outer surfaces of all the medial laminae are invested by collagen sheaths.

Electron Microscope Findings:

Endothelium (Figs. 1 and 6).—The cytoplasm contains mitochondria and endoplasmic reticulum (10), some of the nuclei are sectioned at the level of the nucleolus, dense particles and small vesicles are concentrated along the luminal surface of the plasma membrane and the intercellular surfaces of the plasma membrane are clearly distinguished in areas where they are not oblique to the section. These observations agree with those reported by Buck (1) for rat aorta and Parker (2) for rabbit coronaries, including evidence of pinocytosis and frequent overlapping of adjacent cell margins (1). However, no filiform endothelial processes connecting the media through gaps in the internal elastic lamina as described for the rabbit coronary artery (2) are seen.

[1] Eastman Kodak Co.

A hitherto undescribed subendothelial layer is differentiated by the staining method used (Figs. 1 and 6). This extends from the outer surface of the endothelial plasma membrane to the luminal aspect of the internal elastic lamina and varies in width from area to area along the aortic wall, being wider between nuclei. It is definitely thicker in the 54-day old rat than in the animal aged 38 days. It is of low electron density in the lead-stained sections, presents an appearance suggestive of edema, and contains: (*a*) small whorls of fine collagen fibrils (about 200 A in diameter) situated close to the lamina with occasional branches extending towards the lumen (Figs. 1 and 6), (*b*) small scattered fibrous fragments that produce a reticulated effect and, in many pictures, appear to be attenuated units of the groups of the fine collagen fibrils just described (Fig. 1), and (*c*) blunt processes extending inwards from the edge of the internal elastic lamina. Sometimes the plane of section is such that these processes are separated from their parent lamina (Fig. 6).

Internal Elastic Lamina.—A collagen sheath is present as a continuous anatomical structure only on the *medial* aspect of the internal elastic lamina in contrast to all the laminae in the media which are sandwiched between well marked sheaths. The subendothelial aspect of the internal elastic membrane exhibits only scanty, very fine collagenous fibrils in addition to the blunt processes extending towards the lumen.

Media (Figs. 2 to 4).—This comprises 7 to 11 elastic laminae separated by interlaminar spaces containing smooth muscle cells and collagen. No blood vessels are seen.

Elastic Laminae.—Each is surrounded by a well differentiated collagen sheath, the outermost lamina being only one-half to one-third the width of the rest. The elastin appeared homogeneous but was easily distorted by sectioning. If cut in a direction parallel to the lamina, dense wrinkles (or the "garters" of histologists) were produced. This could be avoided by orienting the laminae at right angles to the direction of the knife, but where curving of the laminae occurred (as frequently visualized as "accordion folds" histologically), the artefact consequent on the change of direction could easily be seen (see Fig. 6, reference 5). If the knife were slightly blunt, either marked washboarding occurred or the elastin was heaped up in layers as the knife advanced through the tissue, as the alternating layers of elastin and interlaminar

cells presented markedly different cutting properties. As illustrated by Parker (2), the laminae contained dense linear streaks embedded in the homogeneous matrix, and, in the transverse sections examined in the present study, these followed the curve of the arterial wall (Fig. 4).

Smooth Muscle Cells.—The cytoplasm contains myofilaments and scattered mitochondria and ribonucleoprotein particles (11, 12), but the endoplasmic reticulum (10) is relatively scanty. These observations are consistent with the description of smooth muscle cells from mouse urinary bladder reported by Caesar *et al.* (13). However, unlike the bladder, all the aortic medial cells are surrounded by a narrow zone of collagen, no example of closely apposed adjacent cell membranes being seen. Numerous vesicles are arranged in a linear fashion immediately beneath the lateral borders of the cells, but, unlike the bladder, these continue along the entire membrane instead of alternating with thickenings of the cytolemma as illustrated by Caesar *et al.* in their Figs. 1 and 6. In bladder these local thickenings in the plasma membrane are found at apposing regions of adjacent cell borders, but no such intercellular attachments are seen in the aorta. However, parts of the plasma membrane bordering the collagen sheaths that invest the laminae are of increased electron density and lined by vesicles and appear to be specialized areas possibly representing local points of physiological activity (Fig. 3) rather than physical attachments. The only regions of the muscle cell membrane devoid of vesicles are the specialized zones of increased electron density occurring where the cell is closely apposed to the edge of the elastic lamina. These attachments are usually set at an oblique angle and overlap the lamina in a stepwise fashion to give what would appear to be a very firm anchorage (Figs. 2 and 3). Occasionally the plane of section includes one of the interconnecting elastic fibrils branching from the laminae, and this may also act as an attachment site (Fig. 2 *A*). The regions of higher density just described are found on the cytoplasmic side of the cell membrane, do not appear to extend into the elastin, and are not associated with vesicles. They resemble structures variously described as adhesion plates, desmosomes, intercalated discs, intercellular bridges, and "bobbins" (14–20) except that, instead of occurring between adjacent cells, they exist between a cell and an extracellular material. In this respect they resemble those described by Cannan-Selby (16) as discussed below. The morphology is the same whether embedded in methacrylate or araldite, but there is no evidence of the laminated structure recently described for epidermal desmosomes (21).

A noticeable feature of the medial smooth muscle cells is their oblique orientation between the elastic laminae (Figs. 4 and 5, reference 5). This was seen repeatedly in all the normal aortic specimens studied and contrasts with the radial orientation associated with the increased interlaminar space found in lathyrism (5). Care was taken to orient the blocks so that true transverse sections were obtained, the angle of cut being verified by thick sections stained with methylene blue and examined under the light microscope. The muscle cells were frequently seen with each end attached to adjacent elastic laminae by the desmosomes[2] described above (Fig. 4).

Interlaminar connective tissue completely surrounds the muscle cells except at their terminal attachments, albeit the space is quite narrow between some adjacent cells. It consists of a loose network of collagen fibrils of moderate size (about 500 A in diameter) which in cross-section and stained with lead appear as dense rings with less dense centers (Fig. 4). Where the cell boundaries were not well defined, these cross-section images of collagen could be confused with cytoplasmic vesicles. They could, however, usually be correctly identified either by the presence of adjacent fibrils cut at a different angle or by the characteristic adherent lead particle described below.

Adventitia.—This consists of bundles of collagen with fibrils of larger size (about 700 A) than those found in the media, the bundles running both longitudinally or encircling the aorta. Blood vessels and occasional nerves, elastic fibers, and muscle cells are interspersed between the collagen.

Duplicate specimens treated with lead or with PTA differed in the way the collagen was stained (Figs. 5 and 7). Both stains clearly defined the cross-banding in fibrils viewed longitudinally, but in cross-sections PTA stained diffusely whereas lead stained the periphery of the fibrils more densely than the center. Scattered larger particles of (presumed) lead appeared to be deposited at the *outer* aspect of the fibrils to give a characteristic signet-ring appearance. No such particles were visible between the fibrils although some

[2] The term *desmosome* will be used throughout this investigation.

could be seen randomly distributed on nearby longitudinal fibrils (Fig. 5). It is noticeable that the particles producing this signet-ring appearance in cross-section tend to be at the same position on all the fibrils in a given specimen and oriented in the direction of knife marks when these are present. This suggests that as the knife cuts the tough collagen bundles the periphery of each fibril furthest from the knife is slightly torn away at one point to give a tiny space. It is suggested that during the subsequent staining a particle of lead is deposited in this space and thus is protected from removal by washing. It should be stated that this ring appearance was found for fibrils of all sizes, from the very fine subendothelial collagen to the large examples of the adventitia, but only in the thinnest sections. Transversely cut fibrils in thicker sections stained diffusely with lead.

DISCUSSION

Unlike Berrian (3) who could not find any divisions between the endothelial cells, the present study is in agreement with more recent reports (1, 2), intercellular boundaries being clearly seen. In addition the lead stain used in the present study differentiated a subendothelial layer which varied in width along the aortic wall, being wider between nuclei, and, in the 5 rats examined, this layer increased in width with age. It contained blunt processes extending from the endothelial aspect of the internal elastic lamina and localized whorls of fine collagen with what appeared to be attenuated fibril units nearer the lumen, giving a reticulated effect. The low density produced an appearance suggesting edema, and it is possible that this is essentially a collagenous ground substance layer from which material has been extracted during the fixation and embedding procedures. Such swelling might be prevented in the media by the limits imposed by the elastic laminae. The subendothelial layer may be of importance in anchoring the endothelium to the internal elastic lamina, yet allowing free play of the endothelial sheet during the rhythmic contraction and dilatation of the vessel during life. If pathologically affected it could present a barrier to the passage of nutriment to the media which lacks a blood supply of its own. Buck (1) states "the endothelium lies directly on the internal elastic lamina or on a fine network of unit fibrils of collagen," but did not emphasize the possible significance of this layer. In lathyrism this layer not only increases in width but contains large inclusions not present in the controls (5).

Again the present findings regarding the elastic laminae disagree with Berrian (3), but agree with those of Parker (2) for rabbit coronary, in showing linear streaks of fibrous appearance embedded in the matrix. The nature of these non-striated filaments is under investigation at the moment.

A major point of interest in this study is the direction and attachment of the smooth muscle cells of the media to the elastic laminae and their relationship to normal function and the changes found in lathyrism. Strong (22) demonstrated by microdissection of macerated distributing arteries in animals and humans that the media was composed of a continuous spiral of compact, obliquely directed fusiform muscle cells. These muscular arteries, like the coronary, do not contain the elastic laminae typical of aorta but only an internal elastic membrane. Parker (2) illustrated for rabbit coronary artery an oblique orientation of the smooth muscle cells relative to this membrane in his Fig. 4 but no comment was made. A feature of all the specimens of normal aorta examined in the present study was the oblique orientation of the smooth muscle cells in relation to the elastic laminae. Cells were frequently seen with both ends attached to adjacent laminae. The possible significance of this observation was only realized when the orientation was found to be radial in lathyrism and associated with an increased interlaminar space. Perhaps the obliquity provides a more controlled regulation of the circumference of the vessel, each concentric ring of smooth muscle acting on adjacent elastic laminae with each pulse wave. On the other hand, radially oriented cells might not be so effective in controlling the diameter of the aorta, their more passive role rendering the multilayered wall less able to withstand the constant impact of blood with resultant dilatation. This and additional features are amplified in the paper on lathyrism (5).

Pease (4) published a short abstract on the electron microscopic appearance of kitten aorta and noted that the smooth muscle cells had long processes definitely attached at many points to adjacent elastic membranes, and it was presumed that their contractile force was transmitted directly to these. The term "desmosome" is now applied to localized attachments between adjacent cells in a wide variety of tissues and species (14–20). Cannan-Selby's work (16) on the attachments of

the basal cells of the epidermis to the dermo-epidermal junction presents many similarities with those described in the present paper. She actually outlines (but does not define it as such) *two* types of desmosome for the basal epidermal cells: first, the intercellular bridges between adjacent cells. These conform to the usual pattern of duplicated structures at adjacent sites in each cell, the tonofibrillae being anchored to elongate granules associated with localized, dense areas at the cell membrane. The second type (illustrated in her Figs. 15, 17, 19, and 22; reference 16) attach the basal aspect of the cell to the structure termed the "dermal membrane" to differentiate it from the thicker "basement membrane" of histologists. Again, the tonofilaments run towards granules and localized bands of density, but this is *not* mirrored in the dermal membrane itself, which is a horizontal structure running at right angles to the epidermis and does not contain any visible filaments or localized thickenings.

In the aorta, the well marked zones of increased electron density found at the edge of the elastic lamina or its processes and unassociated with vesicles were not mirrored in the lamina or its sheath. Cannan-Selby (17) believes that the function of the desmosomes in the epidermis is to maintain cohesion, and that the morphological changes observed in the superficial skin layers were related to the loss of cohesion and desquamation. Caesar *et al.* (13) suggest that the localized thickenings at apposing regions in adjacent smooth muscle cells may play a role in intercellular transmission of excitation. Certainly there is a localized concentration of tiny vesicles in these and other sites believed to be concerned with electrophysiologic events, such as neural synapses (18, 23). It may well be that in aorta the attachments directly to the elastic laminae are related to anchorage and the movement of the aortic wall with each pulse wave, whereas the narrower lines of increased density associated with vesicles and bordering the collagen sheaths may be concerned with physiological activities. In addition each muscle cell was surrounded laterally by a narrow zone of connective tissue and numerous vesicles were lined up beneath the lateral cell membranes. These may be concerned with the activation of contraction as suggested by Peachey and Porter (24). Again, it was noted that the quantity of intercellular collagen increased with the growth of the rat.

The characteristic lead staining of thin sections of transversely cut collagen proved of value in identifying the smaller fibrils of the media and subendothelial layers, especially in oblique sections or when the cell boundaries are not well defined and confusion could arise with cytoplasmic vesicles. PTA was without value in this respect. In this connection, it is of interest to note that a tubular appearance of collagen has been reported previously in unstained tissue "cut on the bias" (25, 26). In the present study there was a preferential deposition of the lead stained round the periphery of the fibrils as compared with their centers, to give a false appearance of "hollow collagen."

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References

1. Buck, R. C., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 187.
2. Parker, F., *Am. J. Anat.*, 1958, **103,** 247.
3. Berrian, J. H., United States Naval School of Aviation Medicine Research Reports NM 001 057.10.02, and NM 001 057.10.03, 1953.
4. Pease, D. C., *Anat. Rec.*, 1955, **121,** 350, abstract.
5. Keech, M. K., *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 539.
6. Bachhuber, T. E., and Lalich, J. L., *A.M.A. Arch. Path.*, 1955, **59,** 247.
7. Caulfield, J. B., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 827.
8. Watson, M. L., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 727.
9. Watson, M. L., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 1017.
10. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 85.
11. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 59.
12. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 171, 671.
13. Caesar, R., Edwards, G. A., and Ruska, H., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 867.
14. Palade, G. E., and Porter, K. R., *J. Exp. Med.*, 1954, **100,** 641.

15. Weiss, P., and Ferris, W., *Exp. Cell Research*, 1954, **6,** 546.
16. Cannan-Selby, C., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 429.
17. Cannan-Selby, C., *J. Inv. Dermat.*, 1957, **29,** 131.
18. Palay, S. L., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 193.
19. Moore, D. H., and Ruska, H., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 261.
20. Sjöstrand, F. S., Cadergren, E. A., and Dewey, M. M., *J. Ultrastruct. Research*, 1958, **1,** 271.
21. Hibbs, R. G., and Clark, W. H., *J. Biophysic. and Biochem. Cytol.*, 1959, **6,** 71.
22. Strong, K. C., *Anat. Rec.*, 1938, **72,** 151.
23. de Harven, E., and Coërs, C., *J. Biophysic. and Biochem. Cytol.*, 1959, **6,** 7.
24. Peachey, L. D., and Porter, K. R., *Science*, 1959, **129,** 721.
25. Wyckoff, R. W. G., *in 3rd Conf. Connective Tissues*, Josiah Macy, Jr. Foundation, New York, 1952, 38.
26. Kennedy, J. J., *Science*, 1955, **121,** 673.

Explanation of Plates

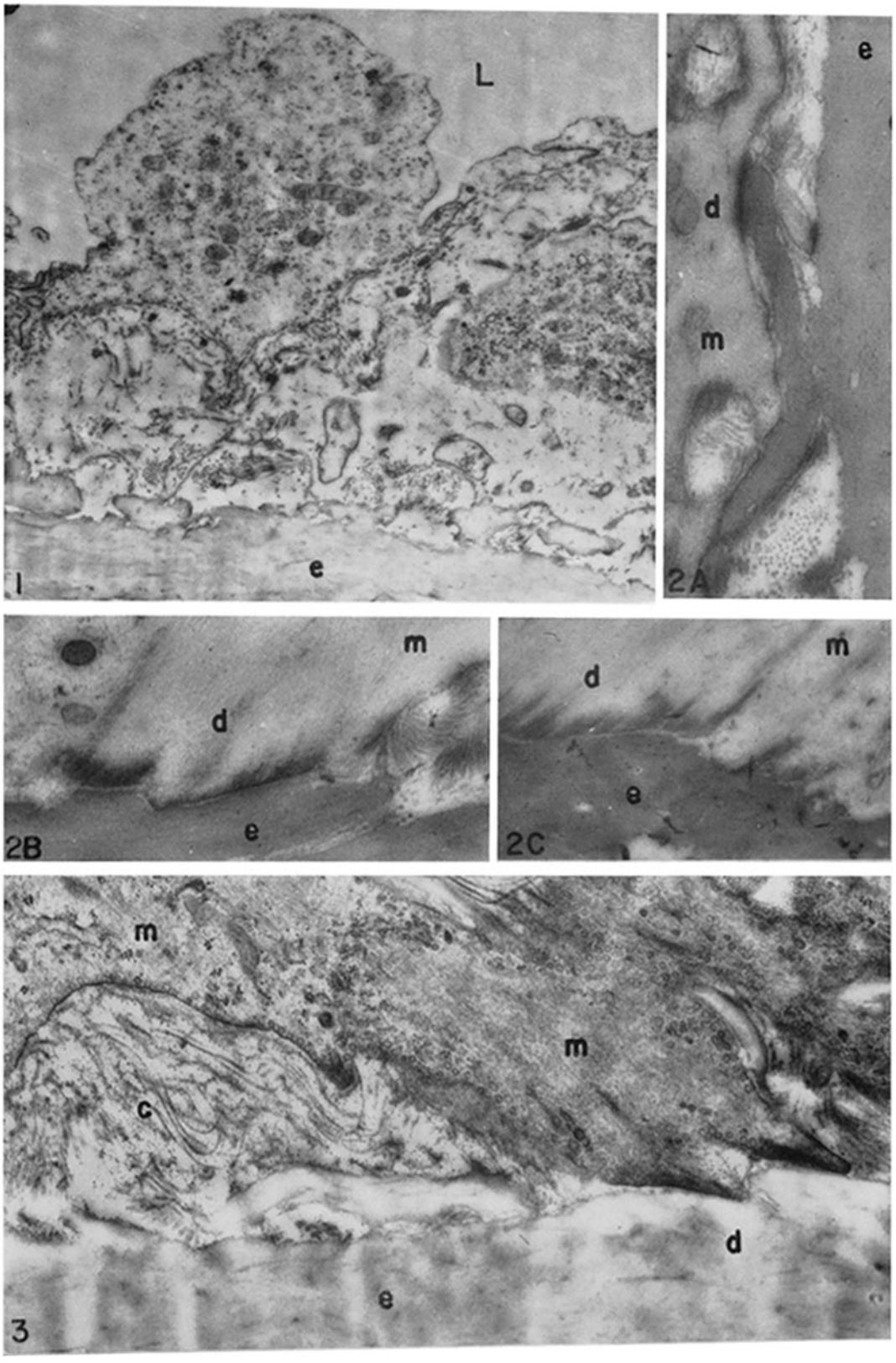
Unless otherwise stated, all the electron micrographs illustrate sections of normal rat aorta embedded in araldite and stained for 30 minutes with a saturated solution of lead hydroxide.

Plate 286

Fig. 1. Electron micrograph of aortic endothelium and part of the internal elastic lamina (*e*). The vesicle-lined endothelial cells are separated from the internal elastic lamina by a subendothelial layer which presents an appearance suggestive of edema and contains groups of fine collagen fibrils as well as blunt processes extending inwards from the endothelial aspect of the lamina. Attenuated fibrous units extend towards the lumen (*L*) to give a reticulated effect. × 8,400.

Figs. 2 *A* to *C*. Examples of desmosomes (*d*) in methacrylate-embedded tissue. These consist of areas of increased electron density along the plasma membrane of the smooth muscle cell (*m*) which border or overlap the edges of the elastic laminae (*e*), usually at an oblique angle (Figs. 2 *B*, *C*). The interconnecting elastic fibrils branching from the laminae can also serve as attachment sites (Fig. 2 *A*). The plasma membrane at the desmosome is devoid of vesicles, but no morphological change could be detected in the elastin. × 17,580.

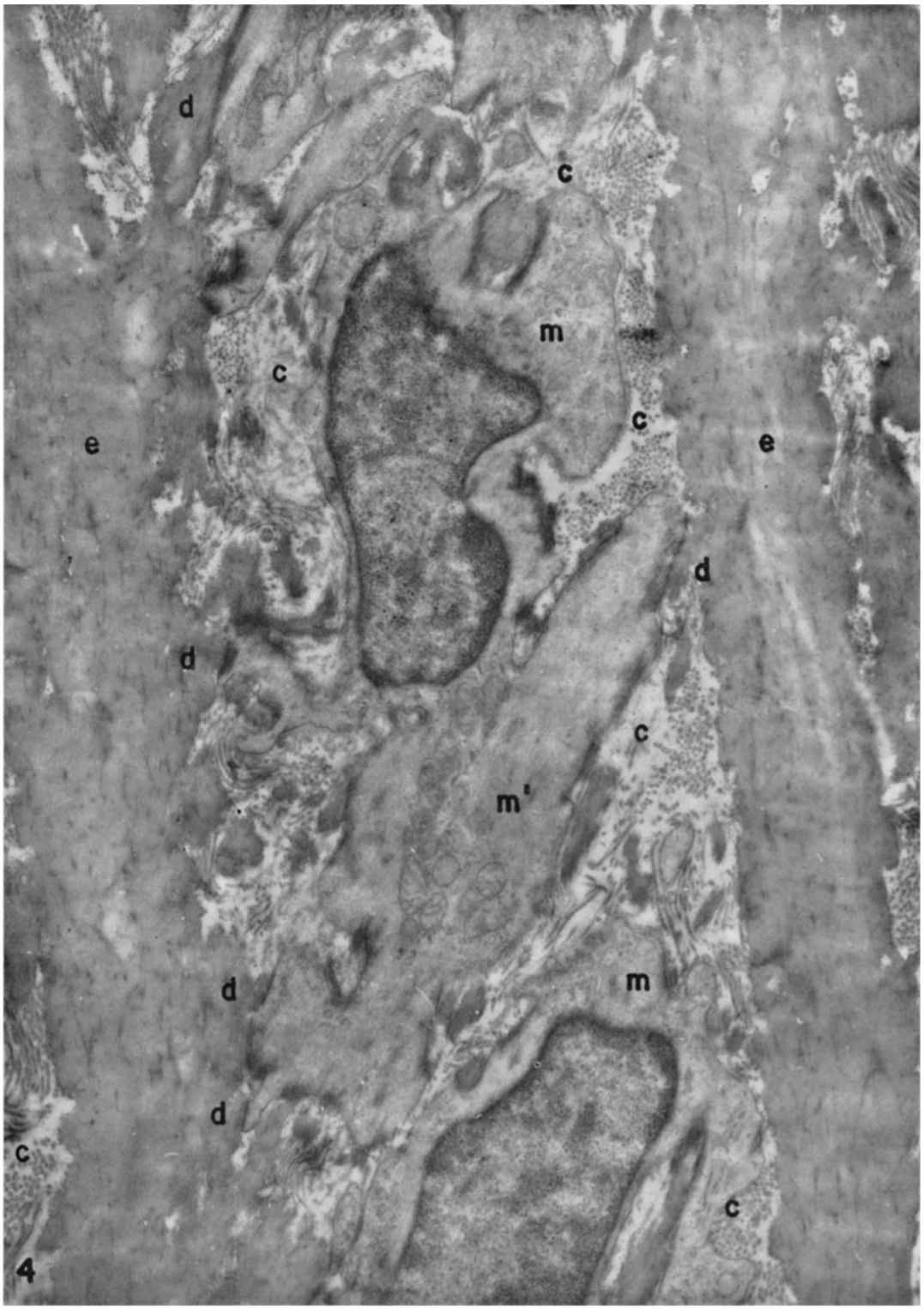
Fig. 3. Araldite-embedded aorta showing a typical desmosome (*d*) overlapping the elastic lamina (*e*) in a stepwise fashion. The muscle cell (*m*) plasma membrane bordering the collagen sheath (*c*) that invests the lamina exhibits areas of increased electron density lined by vesicles. × 17,580.



(Keech: Normal rat aorta)

PLATE 287

FIG. 4. Part of aortic media showing two elastic laminae (*e*) with dense linear streaks embedded in the homogeneous matrix. Each is invested with a collagen sheath (*c*), the transversely cut fibrils presenting the ring appearance described in the text. The smooth muscle cells (*m*) are oriented obliquely with respect to the laminal axes and are attached to the elastin by desmosomes (*d*). Depending on the plane of section, cells are seen with each end attached to adjacent laminae (*m'*). Each cell is surrounded by a narrow zone of interlaminar connective tissue. × 13,185.



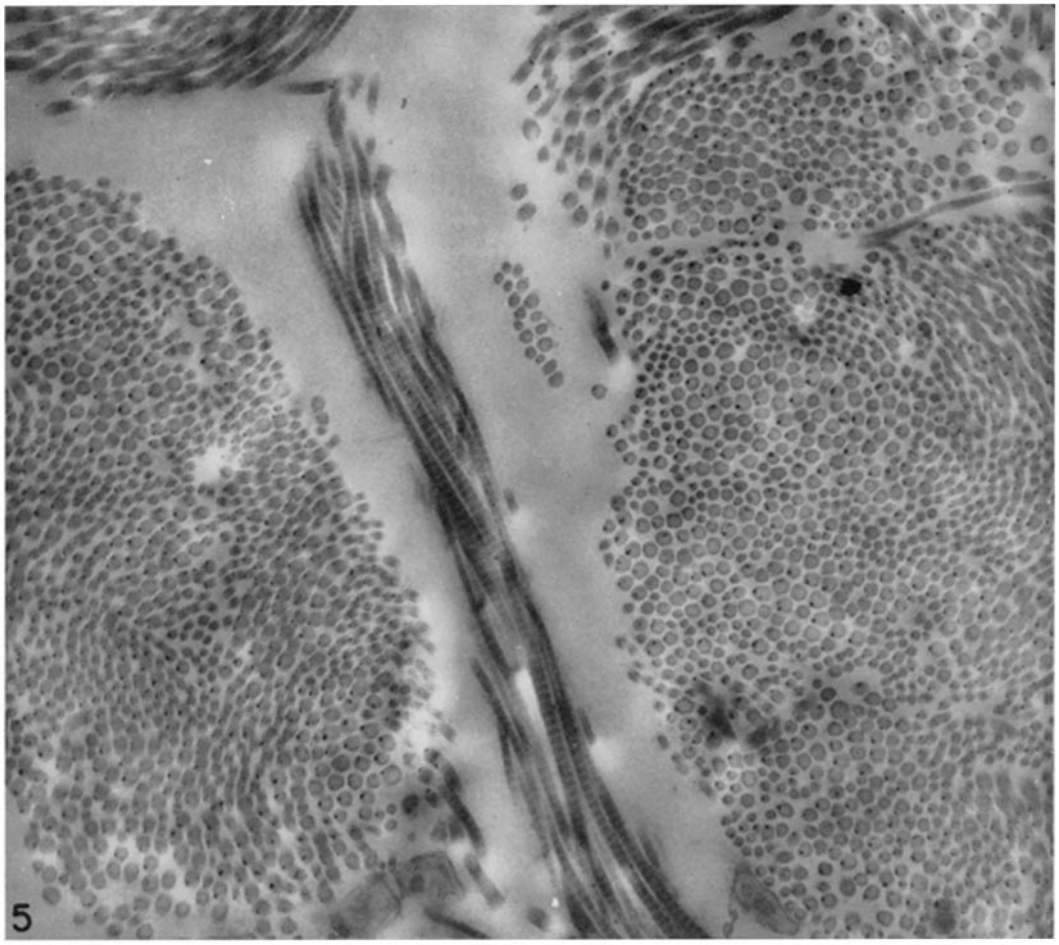
(Keech: Normal rat aorta)

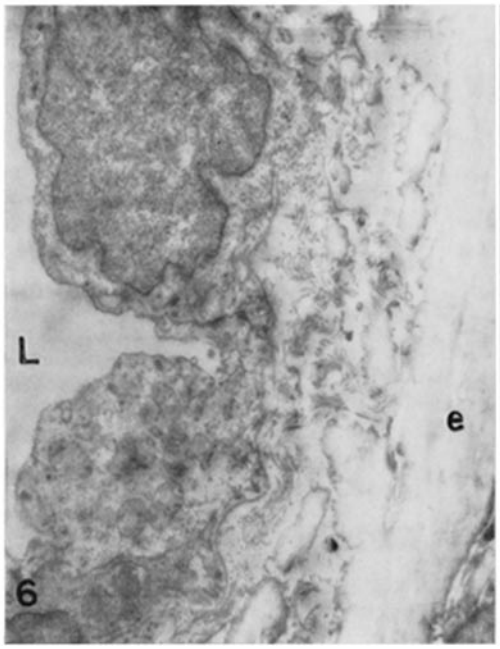
PLATE 288

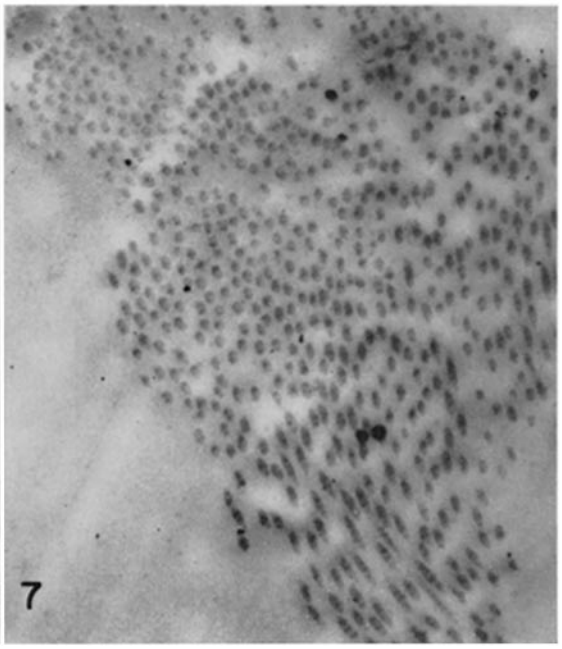
FIG. 5. Lead-stained adventitia showing clearly defined banding on the longitudinal fibrils, and occasional particles of lead distributed randomly on the surface, but not in between the fibrils. On cross-section the fibrils are less dense in the center than at the periphery and present a ring appearance. Frequently a single particle of lead is found at one edge, the position being the same for any given section, *i.e.*, in the direction of the knife marks. The probable cause of this signet-ring effect is discussed in the text. × 20,400.

FIG. 6. Endothelium showing collagenous subendothelial layer and blunt processes extending from the internal elastic lamina (*e*) towards the lumen (*L*). The plane of section is such that some of these processes are separated from their parent lamina. × 8,400.

FIG. 7. Adventitia after staining 5 minutes with 10 per cent phosphotungstic acid. On cross-section the collagen stains more diffusely than with saturated lead hydroxide (*cf.* Fig. 5). × 20,400.







(Keech: Normal rat aorta)