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AN ULTRASTRUCTURAL STUDY OF
DEVELOPING AORTAS FROM
ATHEROSCLEROSIS SUSCEPTIBLE WHITE
CARNEAU AND ATHEROSCLEROSIS
RESISTANT SHOW RACER PIGEONS

PETER HAYMAN COOKE

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AORTAS FROM ATHEROSCLEROSIS-SUSCEPTIBLE
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AN ULTRASTRUCTURAL STUDY OF DEVELOPING
AORTAS FROM ATHEROSCLEROSIS-SUSCEPTIBLE WHITE CARNEAU
AND ATHEROSCLEROSIS-RESISTANT SHOW RACER PIGEONS

BY

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B. S., Springfield College, 1964

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SECTION I

INTRODUCTION

Marchand (1904) first used the term "atherosclerosis" to designate fatty and fibrotic changes in arteries. Atherosclerosis differs from the other arteriosclerotic changes in that the primary or initial change takes place in the tunica intima. This distinction is expressed in the World Health Organization's definition of atherosclerosis as:

a variable combination of changes of the intima of arteries consisting of a focal accumulation of lipids, complex carbohydrates, blood and blood products, fibrous tissue, and calcium deposits associated with medial changes.

However, recent evidence indicates that medial lesions can also influence the development of intimal atheromata (Schenk, et al., 1966).

There are many differences of opinion concerning the initial change in atherosclerosis. Balo (1963) has reviewed the work on general theories of atherogenesis from (1) lipid deposition, (2) edema, and (3) elastic fiber destruction. According to Constantinides (1965) there are two major theories regarding the pathogenesis of human atherosclerosis. The lipid infiltration theory states that lipid along with plasma-borne materials infuse into the arterial wall and initiate a local phagocytic reaction to the

lipid by cells of the vascular wall. The similarity of plaque lipids to blood lipids supports this theory, but arguments have been raised on the grounds that induced hyperlipemia in experimental animals does not produce atheromata identical to human atheromata and that the hyperlipemia required is excessive. The thrombogenic theory of atherogenesis postulates that lesions are mural thrombi which have undergone endothelialization. Evidence for this theory came from observations of mural thrombi in continuity with fibrous plaques (Crawford, 1963; Constantinides, 1965). The absence of many transitional forms and the inability to produce this type of lesion experimentally detract from this theory. Other theories (i.e. the lipid synthesis theory, lipophage migration theory, and capillary hemorrhage theory) are more speculative, and lack substantial proof and observations (Constantinides, 1965).

Microanatomy and fine structure have played an important role in atherosclerosis research by characterizing cell types and delineating cytological changes involved in atherosclerosis. The discovery that smooth muscle cells were intimately involved in the atherosclerotic process came from the use of the electron microscope in studies on spontaneous and experimental atherosclerosis (Geer, et al., 1961; McGill and Geer, 1963; Geer, 1965a; Geer, 1965b; Parker and Odland, 1966; Imai, et al., 1966; and Balis, et al., 1964).

Information on the morphology of normal and atherosclerotic aortas has come primarily from mammals, but mammals are not the only animals that are susceptible to atherosclerosis. Many birds, both wild and domesticated, have been shown to have extensive atherosclerotic lesions in their elastic arteries (Ratcliffe, et al., 1960).

The White Carneau pigeon is highly susceptible to spontaneous atherosclerosis, while the Show Racer pigeon is highly resistant (Clarkson, et al., 1959; Prichard, et al., 1964). In order to describe the pathogenesis of atherosclerotic lesions in White Carneau pigeons, the development of the aortic elements in both breeds was studied. Since the vascular smooth muscle cell is suspected to play an important role in atherosclerosis special emphasis was placed on changes in this cell.

In primary muscle diseases, all types of muscle (i.e. skeletal, cardiac, and smooth) are involved in the pathological process (Pruzanski and Huvos, 1967). This suggests greater similarities between muscle types than previously realized. Aspects of smooth muscle cell development were compared with skeletal myogenesis to demonstrate basic similarities in their differentiation since the way in which cells differentiate is a central problem in biology. This study describes stages in the development of vascular endothelium, smooth muscle cells,

fibroblasts, and intercellular substances of aortas from
White Carneau and Show Racer pigeons.

SECTION II
REVIEW OF THE LITERATURE

The Model System. The White Carneau is a class of Carneau pigeon reported to be descended from a French variety (Dietz, 1929). However, according to Levi (1957), the White Carneau did not originate in France, but was an American creation produced from splashed French and Belgian Red Carneau with an abundance of white plumage. This product was outcrossed to larger white birds whose breed identity is unknown. White Carneau pigeons used in atherosclerosis studies have been acquired from the Palmetto Pigeon Plant which started a strain in 1915 (Clarkson, et al., 1959).

The White Carneau has been shown to spontaneously form atherosclerotic lesions (Clarkson, et al., 1959; Clarkson, et al., 1964). In White Carneau pigeons older than three years, 100% of the birds examined had grossly visible lesions in the distal portions of the thoracic aorta. No sex difference was observed in either the frequency, locale, or extent of the condition (Prichard, et al., 1962). Microscopic lesions were found in 30% of one week old White Carneau pigeons and were characterized by intracellular and extracellular lipid often accompanied by proliferation of round cells and smooth muscle cells in the intima. At two weeks of age the plaques consisted of large cells with dense

round nuclei, suspected to be macrophages. Smooth muscle cells were believed to constitute a part of these plaques. Stainable lipid appeared to be chiefly extracellular and presented a granular appearance in the plaque. Intracellular droplets distorted some plaque-involved cells making verification of cell type difficult (Prichard, et al., 1962). Microscopic features of the lesions in birds older than three years were nearly identical with human lesions (Clarkson, et al., 1959; Prichard, et al., 1964). The severity of atherosclerotic involvement was evaluated in terms of an atherosclerotic index which is equal to the total area of the plaques divided by the total area of the aorta. In a group of White Carneau birds from four to eight years old, an atherosclerotic index of ten per cent was obtained (Prichard, et al., 1962).

The Homer pigeon was bred in large numbers for exhibition and flying, and today the various kinds of Homers can be treated as separate breeds (Dietz, 1929). Prichard, et al., (1962) indicated that the Racing Homer has been bred in this country for 150 years. The Show Racer is identical to the Racing Homer except that it has been bred for exhibition purposes.

Show Racer pigeons have been shown to be highly resistant to spontaneous atherosclerosis (Prichard, et al., 1962). This resistance could only be overcome with 6000 Roentgen whole body x-irradiation combined

with feeding a high cholesterol diet (Lofland, et al., 1965). At one week of age, ten per cent of this resistant breed showed microscopic accumulations of lipid in the aorta (Clarkson, et al., 1964), and at six months of age thirty per cent of this breed showed small lesions. Presumably the lesions regressed during aging, since by three years, only rarely was a lesion encountered. Early lesions were correlated with the nutritional history of squabs (Lofland and Clarkson, 1963). Formula fed squabs of both breeds showed fewer microscopic lesions than did squabs receiving "pigeon milk" from their parents.

Total blood cholesterol, esterified cholesterol, phospholipids, and the cholesterol: phospholipid ratio did not differ significantly between White Carneau and Show Racer pigeons. Beta-lipoprotein levels did not differ between males of the two breeds, but variation in females was high due to phospholipemia associated with egg-laying (Prichard, et al., 1962). Both breeds showed similar uptake of C^{14} -acetate into digitonin precipitable material by minced aortic tissue (Lofland, et al., 1960), but further studies on perfused aortas (Lofland, et al., 1965) indicated little incorporation of C^{14} -acetate into digitonin precipitable material, the isotope being incorporated into fatty acids. Cholesterol administered to perfused aortas caused an enhancement of fatty acid synthesis in the plaque. No

differences of acetate incorporation into fatty acids by the aortic wall existed between the two breeds.

Breeding experiments by Goodman and Herndon (1963) and Herndon, et al., (1962) demonstrated that genetic factors, through a polygenic mechanism, played a major role in pigeon atherosclerosis. Factors responsible for the initiation of the disease were largely independent of those responsible for progression of the disease.

The Aorta. Hughes (1943) described the histogenesis and morphology of the developing chick aorta from a light microscope study. The first recognizable feature was an endothelial cell layer. By the fourth day of development, mesenchymal cells began to form additional layers around the endothelium. Between the fifth and seventh days of development reticular fibers appeared between successive cellular layers. At hatching the aorta consisted of an endothelium within a tunica intima of up to ten layers of non-muscular cells between which were found longitudinally oriented elastic fibers. The intimal cells had elongated nuclei. The tunica media consisted of alternating muscle cell layers and elastic layers. The muscle cells formed layers which coursed helically along the circumference of the vessel and whose orientation varied in successive layers. The elastic layers consisted of elastic fibers and non-muscular cells with rounded nuclei between the muscle

layers. The inner portions of the aorta had elastic layers three or four cells thick. In the outer half of the aorta the elastic layers were thinner consisting of a single cell layer.

In an electron microscopic study, Pease (1955) reported that the tunica media of the kitten's thoracic aorta contained flattened smooth muscle cells forming continuous sheets alternating with elastic "membranes". The smooth muscle cells were circularly oriented and frequently attached to the elastic "membranes". The endoplasmic reticulum and myofilaments were prominent in smooth muscle cells. Collagen was present between elastic lamellae and cells.

Karrer (1960) studied the ultrastructure of the developing chick aorta. The embryonic aorta consisted mainly of an endothelium and a tunica media which gradually merged into the tunica adventitia. The endothelium was non-porous, and the cell number per unit area was greater in a younger aorta than in an older aorta. The media was composed of cell layers alternating with intercellular elements. Medial cells were globular in young aortas, but later appeared flattened. The long axes of the medial cells appeared to be oriented perpendicularly to the long axis of the vessel, but the orientation was not studied in detail. The outer portion of the aortic wall was gradually being organized into additional cell layers contributing to the media.

In contrast to the observations of Hughes (1943), no evidence of alternating muscular and non-muscular layers was found in these embryonic aortas. In younger embryos all cells of the media looked morphologically alike, and the cells appeared to be fibroblasts rather than myoblasts. In older embryos medial cells showed indistinct traces of filaments. These cells were suggested to have "arisen" from fibroblasts or secondarily immigrated into the media. Continuous elastic laminae did not exist up through eighteen days of development.

The thoracic aorta of the rat (Pease and Paule, 1960) had an intima composed of endothelial cells, and a media composed solely of smooth muscle cells which alternated in successive layers with elastic lamellae in a three dimensional network. The smooth muscle cells were arranged obliquely in a longitudinal, tangential section of the aorta indicating a helical orientation. The smooth muscle cells were without innervation, and they lacked the well developed set of pinocytotic vesicles and myofilament attachment devices seen in smooth muscle cells of muscular arteries (Pease and Molinari, 1960).

From a study of the ultrastructure of the ascending rat aorta, Keech (1960) defined the aorta as a myoelastic tube whose diameter is controlled by a thick medial coat composed of alternating concentric layers of elastin and smooth muscle cells. The smooth

muscle cells were oriented obliquely to the radius of the vessel, and the lumen was lined with endothelial cells which were separated from the internal elastic lamina by a subendothelial space. The endothelium of normal rat aorta was described as a single layer of lobular cells with pinocytotic vesicles along their luminal surfaces. The media consisted of seven to eleven interlamina spaces alternating with smooth muscle cell layers. Smooth muscle cells were characterized by the presence of myofilaments, scattered mitochondria, and a sparse endoplasmic reticulum. The cells were enclosed with a narrow zone of collagen fibers, and many fine pinocytotic vesicles lined the plasma membrane of the cells. The only areas on smooth muscle cells devoid of small pinocytotic vesicles were sites of contact with elastic laminae. The smooth muscle cells formed continuous spirals of compact, obliquely-directed, fusiform cells. Desmosomes were reported at sites of smooth muscle cell-elastic lamina appositions.

Karrer (1961) also investigated the fine structure of the thoracic aorta in young and aging mice. The intima consisted of an endothelium, and the media was composed of five elastic lamellae alternating with layers of smooth muscle cells. The elastica showed a filamentous component, but little fine structure of this element was clear. Since the smooth muscle cells were presumed to be active in the synthesis of elastin and collagen, it was

pointed out that the smooth muscle cell was not typical of most connective tissue cells.

Paule (1963) reported the ultrastructure of the newborn and adult rat aorta. The smooth muscle cell was the only cell-type present in the tunica media of newborn and adults. Layers of muscle one or two cells thick alternated with elastic membranes. In the media, the smooth muscle cells had their long axes parallel, but in adjacent layers the direction of the long axis changed. The myofilaments in young smooth muscle cells were less obvious than in adults, but young cells contained more organelles. Basement membranes were infrequently observed. The elastin was vesicular in newborn rat aortas when stained with phosphotungstic acid. The adventitia contained elastin and fibroblasts.

In a review of work on elastic arteries by Pease (1963), a regular alternating pattern of elastic lamellae and cellular layers was described throughout the media. Smooth muscle layers which were oriented in different directions in successive layers spanned the distance between the two adjacent elastic lamellae. The smooth muscle cells lacked well defined basement membrane envelopes and plasma membrane dense bodies, and had multiple extensions of the cell surface. Nerves were present in the adventitia, but absent in the media.

The Endothelium. Several recent reviews deal with the vascular endothelium. Rhodin (1962) and Majno

(1965) discussed the vascular endothelium and emphasized the structural invariability of endothelium in vessels with a tunica media. Variability in endothelial structure appeared in capillaries where functional adaptation had occurred (Rhodin, 1962).

The vascular endothelium of large vessels is described as a continuous layer of cells separating subendothelial zones of the vessel from the lumen. Lobular and attenuated cell shapes were observed (Karrer, 1960 and 1961; Rhodin, 1962). Normal vascular endothelium cytoplasm contained only a few common organelles. The evolution of the current structural concept of the basement membrane, intercellular junctions, pseudopodia, and endothelial flaps is reviewed by Majno (1965).

The endothelium is known to undergo age related changes and specific changes related to injury (Zweifach, 1959). In experimentally induced atherosclerosis, the endothelium underwent vacuolization (Still, 1963; Geer, 1965a) and membraneous organelles became more abundant.

Luft (1963) and French (1963) supported the structural concept of this tissue as described by Rhodin (1962).

The Media. The conclusion has been drawn that the smooth muscle cell is the only cell component of the media of mammals. This cell type was the subject of an extensive review (Eichna, 1962) in which Rhodin

described the fine structure of the mammalian vascular wall with special reference to the smooth muscle component. The vascular smooth muscle cell was described as an elongate cell which coursed helically on the long axis of the vessel. The sarcolemma consisted of a basement membrane and a plasma membrane with micropinocytotic vesicles. The nucleus was an elongate ellipsoid, and the granular endoplasmic reticulum and mitochondria were found in the juxta- and perinuclear regions. The Golgi complex was rudimentary and centrosomes were rarely found. The contractile elements of aortic smooth muscle consisted of myofilaments approximately sixty to eight μ in diameter, (Shoenberg, et al., 1966). Using flow birefringence and viscosity measurements, Obinata, et al., (1966) presented strong evidence that the thin filament of skeletal muscle represented actin. In contrast to striated muscles and some invertebrate smooth or plain muscles, only a single filament type was observed. This filament was identified as actin (Shoenberg, et al., 1966). Myosin has not yet been structurally identified, although it was demonstrated biochemically (Shoenberg, et al., 1966; Laszt and Hamoir, 1961).

Rhodin (1962) reported filament diameters between 30 and 200 μ for various types of smooth muscle cells. Dense bodies were located at the plasma membrane and free in the cytoplasm. Myofilaments appeared to

enter or penetrate these dense bodies. The dense bodies consisted of protein but not contractile protein (Prosser, et al., 1930). In elastic arteries, the smooth muscle cell component has been described as lacking a clearly defined basement membrane and having few micro-pinocytotic vesicles along the plasma membrane. These cells also possessed a comparatively small number of fusiform densities when compared with smooth muscle cells from muscular arteries (Pease and Paule, 1960). Elastica and collagen constituted the structurally identifiable extracellular materials. Elastin occupies most of the interlamellar spaces in the media. It is generally described as consisting of a filamentous and amorphous component (Greenlee, et al., 1966; Fahrenbach, et al., 1966), although some authors have observed only the amorphous component (Pease and Paule, 1960; Cox and Little, 1961).

The Adventitia. This region of the vessel consists of irregularly arranged connective tissue, which merges with the loose connective tissue compartment investing the vessel (Rhodin, 1962). Capillaries and nerves are usually apparent. As an aid to penetration of fixative in electron microscopic studies, the adventitia is commonly stripped from the vessel prior to immersion in fixative.

Aortic Atherosclerotic Lesions. Lesions have been characterized by extreme disorganization of the

normal vascular architecture due to cell migration (Poole and Florey, 1958; Marshall and O'Neal, 1966; Parker and Odland, 1966), and alterations in extracellular materials (Bertelsen, 1963). Foam cells appear in experimental and spontaneous lesions, but their derivation has been controversial. Marshall and O'Neal (1966) described the role of the blood monocyte-derived lipophage in foam cell production in experimental lesions, and Geer (1965a) indicated that round or ovoid cells, which come from the blood, evolved into foam cells in human lesions. Haust and More (1963), Balis, et al., (1964), and Geer, et al., (1961) observed that smooth muscle cells could degenerate into foam cells while Parker and Odland (1966) also demonstrated a myogenic foam cell in experimental rabbit lesions. Moderately lipid-laden smooth muscle cells are seen in a variety of lesions (Marshall and O'Neal, 1966; Geer, et al., 1961), and a mesenchymal cell type, considered to be a dedifferentiated smooth muscle cell or endothelial cell (Haust and More, 1958), has often been implicated in the origin of foam cells.

SECTION III

MATERIALS AND METHODS

White Carneau and Show Racer pigeons were originally obtained from the Palmetto Pigeon Plant (Sumter, South Carolina). Seventy-eight pigeons (40 White Carneau and 38 Show Racers) were studied. Birds were sacrificed at 6, 12, and 18 days of incubation, as day-old squabs, at two and four weeks, at four and eight months, and at four years of age. Weaned immature and adult birds were reared on a diet of Purina Pigeon Checkers supplemented with Palmetto Pigeon Health Grit. Embryos and day-old squabs were maintained in a humidified and temperature controlled incubator at 99.5°C.

Excised aortas from sacrificed birds were fixed for light microscopy in calcium-formol (Barka and Anderson, 1963) or frozen at -20°C. in an International cryostat microtome.

Fixation for electron microscopy involved double fixation in 0.15M sodium phosphate buffered four to six per cent glutaraldehyde and 0.4M s-collidine buffered one per cent osmic acid, or, simply, in 0.15M sodium phosphate buffered two per cent osmic acid. Transected pieces of the aorta were washed in 0.3M sucrose, dehydrated in ethanol solutions and propylene oxide and embedded in epon (Luft, 1961). Thin sections

were cut with glass or diamond knives on Porter-Blum MT-1 or MT-2 ultramicrotomes and stained with two per cent uranyl acetate in seventy per cent ethanol and Reynolds' lead citrate solution (Reynolds, 1963). Six to ten blocks of each aorta were prepared in epon and sectioned at 0.2 to 1 micron, according to the methods of Richardson, et al. (1960) for light microscopy. On the average, eight to ten thin sections (0.05-0.1 micron) were prepared from each block for electron microscopy. Approximately 1,500 micrographs ranging in magnification from 3,000X to 80,000X were taken of a total sample of approximately 2,000 thin sections. Observations were made on an Akashi Tronscope 80, a JEM-7, and a Philips EM 200 electron microscope. All the micrographs in the Appendix were taken on the Philips electron microscope.

Paraffin-embedded sections were cut on a Spencer rotary microtome and stained with the PA-S reaction, acetylation and bromination procedures, and aldehyde fuchsin according to Barka and Anderson (1963). Frozen sections were cut on an International cryostat microtome and stained with Oil Red O and hematoxylin (Barka and Anderson, 1963).

SECTION IV

OBSERVATIONS

Gross Description of the Aorta. The thickness of the wall of the thoracic aorta is highly dependent on age and distance from the aortic arch. In adults, the wall is approximately 500 microns at the proximal end of the aorta, and 275 microns at the distal end. In embryos and squabs, the wall is 200-375 microns thick at the proximal end of the aorta and 60-150 microns at the distal end. The aorta, from the arch to the coeliac branch, is 7-10 millimeters long in embryos and 20-35 millimeters long in immature and mature birds.

Developmental Morphology of the Endothelium. The endothelium of the six day-old embryo forms a continuous, sometimes folded, internal lining of the wall of the aorta. The cells are lobular or trapezoidal in profile and approximately three microns in cross-sectional diameter. An extraneous, discontinuous band of intercellular material consisting of a filamentous component (100A diameter), and/or an amorphous component exists at the base of the endothelial cell layer, (Fig. 1). A plasma membrane, showing unit structure in many preparations, forms a continuous limiting membrane around the endothelial cell cytoplasm. This membrane is structurally involved in the fine structure of several

cellular attachment devices: (1) fine villous processes of the luminal cell surface which interdigitate to form a barrier between the lumen and the 200A close junctions between cells, (2) zonulae adherentes consisting of regions of increased plasma membrane density and decreased width (100-150A) of the intercellular space, and (3) hemidesmosomes consisting of dense areas of the basal plasma membrane where close apposition of the membrane to the basal extraneous coat exists. Hemidesmosomes are not related to cytoplasmic filaments, (Fig. 1). Caveolae intracellulares are present on the plasma membrane on the luminal and basal cell surfaces, (Fig. 1). No stable differentiations of the cell surface exist, and no motile cell processes are present. In the nucleus, the chromatin is coarsely aggregated. The nucleolus is approximately 2000A in diameter and consists of an inner pars amorpha, a fine granular area, and an outer region of coarse (200A) granules. The nuclear envelope has a granular outer membrane. Centrioles have not been observed in the cytoplasm. Mitochondria are spherical and elongate in profile. The cristae mitochondriales are plate-like, and the mitochondrial matrix is more dense than the background cytoplasm. The mitochondrial matrix contains dense (150A) granules. The Golgi complex consists of three to five stacked, flattened smooth surfaced cisternae with dilated cul-de-sacs. Two or three stacks of cisternae are located together in the

cell forming a Golgi area which is circular in profile. Granular endoplasmic reticulum is widely distributed and free ribosomes are numerous. Filaments (ca. 100A diameter) are present in loosely organized bundles which are 500-2000A in diameter, (Fig. 1). Microtubules (250-300A diameter) are generally observed in cross-section indicating that they are elongate on the long axis of the aorta. They are seen at the luminal and basal borders of the endothelial cells, (Fig. 1). No glycogen particles or cytoplasmic inclusions are observed, although small vacuoles (ca. 600 to 800A diameter) are occasionally observed, (Fig. 1).

In the twelve day-old embryo, the general organization of the endothelium is unchanged, (Fig. 3). At the basal surface of the endothelium an extraneous discontinuous layer of 800-1600A particles of amorphous substance bordered with 100A diameter hollow filaments is present, (Fig. 2). In many sulci of the basal cellular surface the filamentous component can be seen alone, (Fig. 2). Below these sulci, the combined amorphous units of elastin and filaments are found, (Fig. 2). The plasma membrane and surface differentiations have not changed, but zonulae occludentes are sometimes present at the lateral plasma membrane interface, (Fig. 3). The chromatin of the nucleus is coarsely aggregated, and two or three large granular, marginal chromatin aggregates are found in many nuclei. The nuclear pores have a

dense disc separating the nuclear and cytoplasmic compartments. In the cytoplasm, very few profiles of the granular endoplasmic reticulum are present in nuclear regions of the cell, but short cisternae are widely scattered in the peripheral portions of the cytoplasm. Free ribosomes have decreased in abundance. The ribosomes are in groups of four to six, but large aggregates are not found. Bundles of 100A diameter filaments are no longer found in the cytoplasm. Dense inclusions (2000-4000A diameter) are occasionally observed in the luminal and basal cytoplasm. These inclusions probably represent lipid droplets, (Fig. 3).

In the eighteen day-old embryo, there are no marked changes in the general organization of the endothelium, but extensive foldings of the endothelial layer are encountered in the proximal aorta, (Fig. 44). The cells are lobular in the region of the nucleus, but flattened in peripheral areas, (Fig. 4). The extraneous coat at the basal surface of the endothelium is a nearly continuous basement lamina consisting of a flocculent-appearing band of material subtended by amorphous units of elastin (ca. 1000A in diameter) each surrounded by 100A diameter hollow filaments. Some evidence of filaments within the amorphous core also exists. Interdigitating villous processes and flaps, close junctions, zonulae adherentes, zonulae occludentes, and hemidesmosomes constitute the attachment devices at this age.

There are no changes in the nucleus. Mitochondria are still not abundant, and the Golgi complex has not changed. The granular endoplasmic reticulum is limited to short cisternae widely distributed in the cytoplasm. Free ribosomes have decreased slightly in abundance. Cytoplasmic filaments are not observed. Dense inclusions (ca. 3000A diameter) are widely scattered in the cytoplasm.

In day-old squabs, the endothelium consists of flattened cells, or cells which are lobular in the region of the nucleus but flattened in the peripheral regions, (Fig. 5). Endothelial folds are still present. The basement lamina is complete. Its lamina rara is approximately 300A thick, and the lamina densa is 500A thick. Large fragments of elastin (greater than 1000A in diameter) are irregularly distributed below the endothelium, and occasionally interrupt the basement lamina in regions of contact with cells. Special attachment devices and the nucleus have not changed. Multivesicular bodies are found widely distributed in the cytoplasm and free ribosomes are less abundant.

At two and four weeks of age, the folding of the endothelial lining is no longer observed. The cells are flattened and rest directly upon a fenestrated elastic lamina: The extraneous basal lamina is incomplete in regions of contact with the subtending elastic lamina, (Fig. 6). In the cytoplasm, free ribosomes and

granular endoplasmic reticulum are much reduced. Loosely organized cytoplasmic filaments (ca. 100Å in diameter) which lack dense bodies are present in the cytoplasm, but there are no other detectable changes in the cytoplasm. No cytoplasmic inclusions are observed.

In the four and eight month old pigeons, the endothelium is similar in general architecture to earlier ages. The extraneous basal lamina is almost entirely obliterated due to the presence of the internal elastic lamina, (Fig. 7). Attachment devices have not changed. A dense marginal band of chromatin is present in the nucleus. The cytoplasm is nearly devoid of granular endoplasmic reticulum and free ribosomes. Loosely organized cytoplasmic filaments identical to the previous stage are widely distributed in the cytoplasm, but they are not structurally related to the plasma membrane, and they do not have fusiform dense bodies, (Fig. 9). There are a few dense cytoplasmic inclusions.

The four year old normal endothelium is similar to the endothelium of the four and eight month old pigeons. Observations on abnormal endothelium from four year old birds are presented in "Fine Structure of Lesions" (page 36).

Developmental Morphology of the Subendothelial Region. This region consists of three to four incomplete and irregular layers of cells in the proximal

half of the aorta, but only one discontinuous layer in the distal half, (cf. Fig. 45).

In the six day-old embryo, no extraneous enveloping cell coats are present surrounding the fibroblast-like cells, (Fig. 10), but small particles (ca. 700A diameter) of elastin and small bundles of collagen occasionally are found in contact with the surface of the cell. A reticular microfibril (50A diameter) is found widely distributed in the extracellular spaces. No hemidesmosomes or other special attachment devices are present in areas of intercellular contact, or at sites of approximation between cells and intercellular substances. Lobopodia of the fibroblast-like cells approximate each other forming a close junction in many cases. The nucleus is oblong or lobular in profile, and the chromatin is coarsely aggregated. The nucleolus is large and shows a pars amorpha, nucleolonema, and 200A peripheral granules. One or two marginal aggregates of chromatin are generally present. A nuclear envelope (with few pores) is present around the nucleus. The outer membrane of this envelope is studded with ribosomes. In the cytoplasm, centrioles are found as a diplosome. Mitochondria are circular or elongate in profile with plate-like cristae mitochondriales. The Golgi complex consists of two or three flattened cisternae surrounded by smooth vesicles and in certain sections these stacks of cisternae form an area which is circular

in profile. Elongate and ramified cisternae of the granular endoplasmic reticulum consist of a moderately dense lumen with ribosome studded parallel membranes. Cytoplasmic filaments are not present, but microtubules are widely distributed. A single rudimentary cilium can be found in these cells extending from the diplosome to a distance approximately one micron beyond the cell surface. The cilium consists of eight peripheral bundles of two tubules each. Axial filaments are absent in the cilia.

In the twelve day-old embryo, the organization of this zone, the elements of the cell surface, and the nucleus of the fibroblast-like cell have not changed. Centrioles associated into a diplosome or basal body give rise to a rudimentary cilium in many fibroblast-like cells. The granular endoplasmic reticulum is more widely distributed and more abundant than in the previous stage. The mitochondria and Golgi complex have not changed. Multivesicular bodies, usually two or three per cell section, are present. The contained vesicles are occasionally dispersed around the major vesicle. Acanthosomes closely resemble the dispersed small vesicles of the multivesicular bodies.

In the eighteen day-old embryo, units of elastin approximately 1000A in diameter are frequently in contact with the plasma membranes of the fibroblast-like cells. The number and size of units of elastin is

increased over previous stages. Collagen and reticular microfibrils are present. There are no changes in the nucleus and cytoplasm, (Figs.11 and 12).

In the day-old squab, the units of elastin are larger (greater than 1000A) and more numerous than before. Bundles of collagen have increased in size through an increase in the number of fibrils per bundle. Reticular microfibrils remain the same. Some fibroblast-like cells show small fragments of a 300A thick basement membrane envelope extending for a length of 600-800A. The Golgi complex has increased in the number of small vesicles and acanthosomes are present. No other cytoplasmic changes have occurred, (Fig. 13).

In two and four week old birds, elastic laminae are still incomplete having large fenestrations. Collagen and reticular microfibrils are abundant. A basement membrane envelope is not found around the fibroblast-like cells. The only "motile" cell process is in the form of a rudimentary cilium extending from a diplosome. There are no nuclear changes. The granular endoplasmic reticulum is limited to short cisternae. Cytoplasmic filaments (100A diameter) are loosely organized and widely distributed in the cytoplasm. No fusiform or plasma membrane dense bodies are present, (Fig. 14).

In four and eight month old birds, the fibroblast-like cells have had a marked reduction in amounts of granular endoplasmic reticulum and an increase in

100A diameter filaments. At this age, the cells are designated filament-laden mesenchymal cells because of their modified structure. All cellular interstices are filled with mature elastic laminae, collagen, or reticular microfibrils. The nucleus has a dense marginal band of chromatin, and it is occasionally folded. Centrioles are occasionally observed. Mitochondria are few and distributed widely. The cytoplasm is packed with loosely organized 100A diameter filaments which lack fusiform and marginal dense bodies. The Golgi area is still prominent in the juxtannuclear regions of the cytoplasm. Large dense inclusions are commonly present in the cytoplasm, (Fig. 9).

The subendothelial region in the four year old bird compares with that region in the eight month old bird.

Developmental Morphology of the Media. This region of the aorta consists of alternating layers of presumptive smooth muscle cells and non-muscular interlaminar cells.

In the six day-old embryo, the media consists of fusiform smooth myoblasts and non-muscular interlaminar cells in alternate layers, (Fig. 15). The smooth myoblasts are approximately five microns in diameter and approximately fifteen microns long. Their long axes are perpendicular to the vessel's long axis, and they

are circumferentially directed around the lumen. Small units of elastin and small groups of collagen fibrils are found randomly distributed in the cellular interstices. The interlamellar cell is fibroblast-like, resembling the subendothelial fibroblast-like cell, and isodiametric or slightly elongate and oriented circumferentially.

A basement membrane envelope is present around the smooth myoblast as an irregular 400A amorphous coat along short regions of the cell surface. Occasionally small units of elastin and collagen fibrils contact the surface of the smooth myoblast and, where contact exists, the basement membrane envelope is absent. Marginal dense bodies are present along the plasma membrane and associated with myofibrils of 60-90A diameter cytoplasmic filaments. Hemidesmosomes are present where contact between the plasma membrane and extracellular units of elastin exists. At terminally adjoining smooth myoblasts, villous extensions of the cells interdigitate, (Fig. 22). Intercellular junctions are close and tortuous, but the smooth myoblasts are not syncytial, since most cells are seen to be separated by close junctions of approximately 200A width. Fungiform differentiations of the cell occur along the lateral surface of some smooth myoblasts, (Figs. 18 and 19). The differentiations contain free ribosomes and a flocculent-appearing filamentous substance. A basement membrane envelope is not found

on the surface of these differentiations. The nucleus is elongate on the cell's long axis, and the chromatin is coarsely aggregated. A nucleolus is present in addition to one or two marginal aggregates of chromatin. A nuclear envelope is present, and the outer membrane is granular. In the cytoplasm, the centrioles are observed as a diplosome. The mitochondria are elongate or spherical in profile with a dense granular matrix. The Golgi complex consists of one to three stacks of flat cisternae with dilated cul-de-sacs. Many satellite Golgi vesicles are present in the immediate vicinity of the Golgi complex. The granular endoplasmic reticulum is composed of long cisternae oriented primarily on the long axis of the cell, and is often dilated to contain a flocculent-appearing, moderately electron-dense substance. Cytoplasmic filaments (60-90A diameter) are found along the plasma membrane and as longitudinal bundles deep in the cytoplasm. Other areas of the cytoplasm have randomly-dispersed 60-90A diameter filaments. These areas are devoid of cell particulates except free, single ribosomes. Fusiform dense bodies within groups of organized 60-90A diameter filaments and dense bodies on the plasma membrane with and without attachment to filaments are present. Some groups of filaments terminate in the plasma membrane dense bodies, but the filaments pass through the amorphous fusiform densities. Microtubules are oriented principally along the cell's long axis, (Figs. 17-20; 22-24).

In the six day-old embryo, the fibroblast-like interlaminar cells are located between layers of smooth myoblasts, but they are not intimately connected with each other or to the adjacent smooth myoblasts. They have no basement membrane envelope, but particles of elastin and small groups of collagen fibrils frequently contact the cell surface, (Fig. 17). No special attachment devices are present. The nucleus is elongate and the chromatin is coarsely aggregated. A nucleolus and nuclear membrane envelope are present. Centrioles are present as a diplosome, and one centriole commonly acts as a basal body for a rudimentary cilium. Mitochondria are widely distributed and elongate in profile. The Golgi complex consists of three to four stacks of three to five flattened cisternae. A granular endoplasmic reticulum is randomly distributed in the cytoplasm. Many free ribosome clusters are present. There are no cytoplasmic filaments, and microtubules are infrequently observed.

In the twelve and eighteen day-old embryo and day-old squab, the smooth myoblasts assume a helical orientation, and they are held together laterally by 200A close junctions into laminae of four to eight cells. Collagen, reticular microfibrils, and units of elastin are very abundant. The general plan of alternating layers of smooth myoblasts, interlaminar cells, and intercellular substances exists for these ages. A

complete 400-500A thick basement membrane envelope covers the entire surface of the laminae of smooth myoblasts, except for the surfaces of the round-shaped surface differentiations which are devoid of an extraneous coat. No micropinocytotic vesicles are present on the plasma membrane. The nuclei have not changed. The cytoplasm often has a diplosome and a rudimentary cilium. Elongate mitochondria are widely distributed in the cytoplasm. The granular endoplasmic reticulum consists of only a few short dilated cisternae filled with a flocculent-appearing filamentous material. Free ribosomes are greatly diminished in number. The Golgi complex is juxtannuclear and consists of stacks of cisternae. Golgi vesicles and acanthosomes are numerous. The cytoplasmic filaments (60-90A diameter) with fusiform and plasma membrane dense bodies are abundant along the cell border and in large areas of the cytoplasm. The development of these filaments is protracted since cells with varying amounts of filaments can be seen at any one age. The organized filaments are compactly grouped, being 200-250A from each other and running in parallel for lengths up to one micron. Bundles of organized filaments can be seen to terminate in plasma membrane dense bodies while others run parallel to the plasma membrane on the long axis of the cell or slightly oblique to it, (Fig. 25). These groups of filaments may or may not have fusiform densities. Areas of randomly-dispersed

60-90A diameter filaments are still observed, often in apposition to organized filament bundles. Multivesicular bodies are widely distributed in the cytoplasm. Groups of glycogen particles (ca. 350A diameter) are present in various areas of the cytoplasm and commonly in the surface differentiations. There are numerous membrane bound cytoplasmic inclusions with light electron density, (Figs. 21, 27-30).

The general organization of the interlaminar cells has not changed for the twelve and eighteen day-old embryo and day-old squab. There is an increase in size and number of elastin units and collagen bundles. A basement membrane envelope is not present around the interlaminar cell or the surface differentiations of smooth myoblasts which impinge on the surfaces of the interlaminar cells, (Fig. 26). The space at the interface of the interlaminar cell and smooth myoblast surface differentiations constitutes a uniform 200A close junction. The interlaminar cells have no other special attachment devices. The nucleus has undergone no changes. In the cytoplasm, a diplosome gives rise to a rudimentary cilium. There is no change in the mitochondria, but in the Golgi complex, small Golgi vesicles are very abundant. The granular endoplasmic reticulum and the free ribosomes have no diminished in number. No cytoplasmic filaments are present.

In the two and four week old pigeons, the base-

ment membrane envelope covering laminae of smooth myoblasts is obliterated by contact with elastic lamellae in many areas. Surface differentiations of the smooth myoblasts, which in many cases now appear to be mature smooth muscle cells, often extend through fenestrations in the elastica to impinge upon the adjacent interlaminar cells. The periphery of the nuclear chromatin is condensed to form a discontinuous dense marginal band along the inner nuclear membrane. Diplosomes are often observed, but no longer in relation to rudimentary cilia. Mitochondria are juxtannuclear, and much reduced in number. The Golgi complex is juxtannuclear and extensive in some cells. Granular endoplasmic reticulum and free ribosomes are very much reduced, and widely scattered in the cytoplasm. Cytoplasmic filaments with fusiform and plasma membrane dense bodies now occupy the majority of the cytoplasmic compartment, (Fig. 31).

In the interlaminar cells, the granular endoplasmic reticulum and free ribosomes are decreased. Cytoplasmic filaments (100A diameter) without dense bodies of either type are loosely organized and widely distributed in the cytoplasm. Many surface differentiations of the smooth muscle cells impinge the surfaces of interlaminar cells or are present in the immediate interstices. A few large osmiophilic droplets are found in most of the interlaminar cells, (Fig. 32).

In four and eight month old birds, the architec-

ture of the media is the same as previous stages, but the smooth muscle cells are no longer bound laterally by means of continuous close junctions. Short regions of close cell to cell approximation exist as special processes of the cell surface, (Fig. 33). Terminally, smooth muscle cells interdigitate through fine ramifications, but no close junctions are present in these regions. Basement membrane envelopes remain discontinuous, and elastic laminae are complete except for a few fenestrations which allow numerous smooth muscle cell surface differentiations to pass into adjacent layers of the media and impinge interlaminar cells. The surface differentiations are most numerous in the outer portion of the media. They contain fine (30-50A diameter) filaments, small (ca. 100A diameter) granules, and occasionally mitochondria and profiles of vesicles. The membraneous organelles are juxtannuclear. The myofilaments are not uniformly packed in the cytoplasm, so that areas of irregular orientation often exist, (Fig. 33).

Interlaminar cells from four and eight month old birds are commonly situated at fenestrations in the elastic lamellae. When this occurs, the interlaminar cell is impinged with surface differentiations from nearby smooth muscle cells. Close junctions obtain between impinging surface differentiations and interlaminar cells. The nucleus has a dense marginal band of chromatin and a nuclear membrane with few pores. The

cytoplasm has no centrioles. Granular endoplasmic reticulum is limited to short cisternae. The Golgi complex is prominent and widely distributed in the cytoplasm. Loosely organized filaments (100A diameter) are found widely distributed in the cytoplasm. These cells commonly have a large, densely osiophilic inclusion, (Figs. 34-37).

The media of normal portions of the aorta in four year old pigeons is similar to the media in eight month old birds, but in some sections, clusters of surface differentiations were larger than in younger birds.

Fine Structure of Lesions. Lesions first appear at the distal end of the thoracic aorta in four month old White Carneaux. They are characterized by a thickened vascular wall consisting primarily of modified smooth muscle cells. These cells are fusiform but smaller than normal smooth muscle cells, and they are not organized into the typical laminae characteristic of normal smooth muscle cells. Elastic laminae are not abundant in the plaques, but when present are not oriented in alternating layers with smooth muscle cells. Interlaminar cells are rarely encountered in these plaques, but they are abundant at the boundary between the plaque and normal areas of the aorta.

Microscopically, the grossly visible lesions in four year old White Carneau consisted of a modified

endothelium, a lamina of subendothelial mesenchymal cells, modified smooth muscle cells, and foam cells, (Fig. 38).

The endothelial cell is rounded with fine villous and flap-like processes on the luminal surface. The nucleus is round or elongate with a dense band of chromatin at the periphery. The chromatin is coarsely clumped. The cytoplasm has an abundance of granular endoplasmic reticulum and profiles of Golgi complex are frequently observed. Normal endothelium rests upon an elastic lamina, but foamy-lesion endothelium is separated from the subendothelial cell population by a thin layer of ground substance. The subendothelial mesenchymal cells are rectangular in profile, forming layers with adjacent cells, (Fig. 39). The nucleus is elongate on the cell's long axis and contains a discontinuous marginal band of chromatin, with two or three peripheral clumps. The cytoplasm contains many free ribosomes, little granular endoplasmic reticulum, and a scanty complement of small vesicles, smooth cisternae, and mitochondria. These cells underly and appear to subtend thin strands of endothelial cell cytoplasm which are in a state of dissolution. Many dense membrane-bound inclusions are found in the mesenchymal cell cytoplasm, (Fig. 38).

The modified smooth muscle cell is elongate and roughly fusiform in outline, (Figs. 40 and 41). The

nucleus is very dense with a wide discontinuous, dense marginal band and several large marginal clumps of chromatin. The cytoplasm contains myofilaments with fusiform dense bodies, plasma membrane dense bodies, granular endoplasmic reticulum, and a few mitochondria with pale matrices. These cells may contain large dense inclusions, and dilated, clear granular cisternae containing a flocculent-appearing material. The vacuoles of moderately vacuolated smooth muscle cells appear to be dilated cisternae of the granular endoplasmic reticulum. Myofilaments are prominent and ensheath the vacuolated areas. In severely vacuolated smooth muscle cells, circular vacuoles are observed to have limiting membranes in only a few preparations. Filaments in these cells are scanty and dense bodies are lacking.

The foam cell is round or elongate with a peripherally located nucleus which resembles the smooth muscle cell nucleus. The cytoplasm contains clear vacuoles, myelin figures, and cholesterol clefts. Mitochondria are circular in profile with pale matrices. Granular endoplasmic reticulum is lacking, but free ribosomes and small vesicles are abundant. Amorphous fragments of material are present at the surface of foam cells, possibly representing dissolution products of the cell. Peripheral areas of foam cell cytoplasm often contain densely packed myofilaments, (Fig. 42).

In regions around smooth muscle cells, the extra-

cellular space is occupied primarily by collagen. Many of the smooth muscle cells have thickened but discontinuous basement membrane envelopes. Thin bands of elastin frequently have irregular dense objects at their surfaces, possibly representing lipid. In areas around foam cells, the extracellular space is filled with vesiculated material with numerous dense objects resembling those on elastin. The surfaces of some foam cells are in continuity with the vesiculated material indicating that it may represent dissolution products of foam cells, (Fig. 43).

Although not true atherosclerotic lesions, intimal thickenings in embryos and day-old squabs of both breeds exist in the proximal half of the aorta, and consist of focal enlargements of the subendothelial region and an endothelial fold, (Fig. 44). These microscopic alterations are visible in sections of epon, paraffin, and frozen sections, and for this reason are believed not to be a fixation, embedding, or shrinkage artifact. Periodic acid-Shiff positive and aldehyde fuchsin positive material in embryonic aortas is limited to thin bands adjacent to all cell surfaces. The largest bands are alongside smooth myoblasts. Aldehyde fuchsin and PA-S stains failed to demonstrate any significant differences in amounts of intercellular material between these thickenings and normal areas.

SECTION V

DISCUSSION

Developmental Changes in the Endothelium. Buck (1963) suggested that in vivo perfusion fixation caused the flattening of otherwise round or cuboidal endothelial cells in arteries. In conditions of in situ low pressure perfusion fixation it was also flattened (Buck, 1963). In embryonic and adult pigeon aortas, a flattened endothelium was encountered with immersion fixation... usually in the distal thoracic aorta. Lobular and cuboidal endothelial cells were common in the proximal thoracic aorta of embryos, but few were encountered in adults. Karrer (1960; 1961) found a gradual flattening of endothelial cells with age. In general, however, the shape of pigeon endothelial cells was very variable, so much so that any generalization correlating their shape and age would not be justified.

Of the cytoplasmic elements, only tonofilaments and free ribosomes underwent detectable changes during development. Bundles of approximately 100A diameter filaments (tonofilaments) disappeared from the cytoplasm after six days of incubation. At the same time, hollow 100A diameter filaments appeared in abundance beneath the endothelium and made up the filamentous component which was embedded in and surrounded the amorphous component of elastin. Cytoplasmic tonofilaments in

endothelial cells reappeared at maturity and occupied a great proportion of the cytoplasmic compartment. Tonofilaments are generally considered to be a cytoskeletal structure as explained by Rhodin (1962). Because of the great variability of endothelial cell shape, a contractile or elastic function has also been suggested (Rhodin, 1962). In view of Buck's perfusion experiments, changes in cell shape appear to be passive in response to luminal pressure. This suggests that the observed filaments may fulfill a skeletal function to the cells but reasons for the loss of filaments during the latter half of the incubation period up to maturity are unknown.

Free ribosomes are generally considered a sign of an undifferentiated condition or of synthesis of protein not destined for transport to the extracellular spaces (Hay, 1963; Konigsberg, 1965). The gradual decrease in numbers of free ribosomes during development is probably an expression of endothelial cell differentiation. The slight decrease in extent of granular endoplasmic reticulum may express a depression of protein synthesis which would supply subunits for microfibrils or amorphous units which combine or transform to produce elastin beneath the endothelium, although this synthetic function has not been unequivocally established for the endothelium.

Special attachment devices of the endothelial

cell surface include: (1) interdigitations of villous and flap-like surface and lateral projections, (2) close junctions, (3) zonulae adherentes, (4) zonulae occludentes, and (5) hemidesmosomes. These structures were present in normal endothelium at every stage investigated. Recent interpretations of endothelial intercellular junctions were reviewed by Majno (1965), and the present observations agree with those presented. Hemidesmosomes in pigeon endothelium correspond to similar structures in frog vascular endothelium (Stehbens, 1966).

The endothelial basement lamina was fragmentary at six days of incubation, but by the end of incubation, it was complete and consisted of a lamina rara and densa. In the early stages it was largely filamentous with some surrounding amorphous substances, but the simultaneous appearance of elastin microfibrils makes the identification of a particular group of filaments as basement lamina or elastin unit difficult. After hatching, the internal elastic lamina obliterated the lamina rara and densa in areas of contact between elastica and endothelial cells. A similar condition existed in the chick embryo (Karrer, 1960) and newborn mouse (Karrer, 1961; Paule 1963). This obliteration will be considered in more detail in a later discussion (page 48).

In thoracic aortas of mature pigeons, where smooth muscle cells lie directly beneath the internal elastic lamina, some endothelial cells were impinged

with surface differentiations of smooth muscle cells which passed through fenestrations in the internal elastic lamina. This association between the smooth muscle cells and endothelial cells indicated that the smooth muscle cell-interlaminar cell relationship may not be a specific association. However, no smooth muscle cell-smooth muscle cell impingements were observed in any area.

Developmental Changes in the Subendothelial Region. The cells in the subendothelial region underwent a rapid transition from very simple fibroblast-like cells of embryos and squabs to filament-laden mesenchymal cells at two weeks of age. A drastic reduction of granular endoplasmic reticulum and free ribosomes, and the appearance and rapid accumulation of 100A diameter, loosely organized filaments (probably tonofilaments) characterized this transition.

The development of extracellular substances in the subendothelial region was gradual. At the earliest stage investigated, small units of elastin and small groups of collagen fibers could be demonstrated. Elastic laminae were complete by four weeks after hatching. Units of elastin enlarged by accretion and fusion with other units. No suggestions are advanced as to what initiates the formation of an elastin unit, but evidence herein indicates that their formation is begun as a group of hollow elastin microfibrils surrounding a 200-300A diameter amorphous core. Similar observations on

elastin development have been made recently by Greenlee, et al. (1966). Collagen bundles gradually increase in size until the extracellular spaces are filled at four weeks of age. Previous studies of the developing aorta have been concerned with the synthesis of collagen, and smooth myogenesis has been neglected (Karrer, 1960; 1961).

The cells of the subendothelial region in pigeons apparently have no counterpart in mammalian aortas, since smooth muscle cells are reported to subtend the endothelium and internal elastic lamina (Paule, 1963). The criteria for establishing the separate cell type are as follows: (1) absence of cytoplasmic filaments with dense bodies, (2) absence of a basement membrane envelope, (3) fibroblast-like characters up through the day-old squab, and (4) absence of cell to cell attachments and special attachment devices at the plasma membrane.

Developmental Changes in the Media. The smooth myoblasts of the media were approximately three to five microns in cross sectional diameter and 15 to 20 microns in length at all ages. The long axis of the smooth myoblasts changes from a circumferential orientation in six day-old embryos to obliquely longitudinal at all later ages. Smooth myoblasts are distinguished from the inter-laminar cells in the adjacent layers by the presence of (1) cytoplasmic filaments with dense bodies, (2) a base-

ment membrane envelope, (3) lateral close junctions forming laminae of cells, and (4) existence of large fungiform and spherical surface differentiations. The 60-90A diameter randomly-oriented filaments represent the earliest structure of the contractile apparatus to appear. The single 200A diameter particles associated with these filaments are interpreted as ribosomes serving as a template for filamentogenesis (Allen and Pepe, 1965; Obinata, et al., 1966). Organized groups of 60-90A diameter filaments with fusiform and plasma membrane dense bodies are interpreted as myofibrils because of their similarity to adult myofibrils. Randomly-oriented filaments become organized into myofibrils and contribute to the growth of myofibrils by apposition. The orienting influence is unknown.

The randomly-oriented filaments in smooth myoblasts closely resemble their counterparts in mononucleate myoblasts of presumptive striated muscle. They are 60-90A in diameter and associated with single or small groups of ribosomes. No other cellular organelles or inclusions are associated with the developing filaments. Similar conclusions concerning the formation of myofilaments in skeletal muscle were drawn by Allen and Pepe (1965) and Pryzbylski and Blumberg (1966).

The first phase of myogenesis is a mesenchymal cell-myoblast transformation. This event apparently occurs very early in the development of the aorta since

no observations on this transformation were made in the present study. The myoblasts were fibroblast-like, but there is little question of their muscular nature.

The second phase of myogenesis is the aggregation of smooth myoblasts into laminae. Smooth myoblasts form close junctions but remain non-syncytial.

Ribosomes are associated with the earliest appearance of myofilaments and possibly control the synthesis of these thin filaments. The literature on this point is critically reviewed by Fischman (1967). Substantial support for the hypothesis that myosin is synthesized on a template of large polyribosomes is lacking. Indeed, large polyribosomes never appear in smooth myoblasts, but myosin is known to be present (Shoenberg, et al., 1966).

The organization of myofilaments into myofibrils in smooth muscle is described on page 45. In skeletal muscle (Pryzbylski and Blumberg, 1966) randomly-oriented thin filaments became oriented into non-striated myofibrils before the appearance of thick filaments, suggesting that the cell must initially synthesize more actin than myosin (Fischman, 1967). As in smooth myoblasts, no cellular organelles are associated with aligning myofilaments, and the organization of the organellar elements is considered to be secondary to the organization of myofilaments (Fischman, 1967). Free myofilaments in skeletal muscle and randomly-

oriented myofilaments in smooth muscle appear to become oriented on the long axis of the myoblasts before becoming incorporated into a myofibril. Fischman (1967) advances the hypothesis that microtubules involved in cellular elongation cause cytoplasmic streaming and, in this way, longitudinally orient the asymmetrical myofilaments. Longitudinally oriented microtubules are quite common in smooth myoblasts in areas of randomly-oriented myofilaments and forming myofibrils. Such a mechanism of alignment appears to be possible for smooth muscle. Fischman (1967) advances three hypotheses for myofibril formation. In the first model, Z band material is necessary as a prerequisite for myofibril formation. The second model suggests that thick filament linkages are responsible for myofibril development, and the third model suggests that thick and thin filament linkages are responsible for myofibril development. Of these models, Fischman (1967) favored the third, since Z band material without thin filaments had not been detected, and I bands had not been observed without attached thick filaments. Since the homologous nature of the dense body in smooth muscle and the Z bands in skeletal muscle has not been demonstrated, it is not possible to directly compare the mechanisms of myofibril formation in the two muscle types. In regard to the first model, if plasma membrane dense bodies are homologous to Z bands, myofibrils are not always observed to contact plasma membrane dense

bodies. As for the latter two models, no thick filaments are ever present in smooth myoblasts, but myofibrils exist. None of the hypotheses advanced by Fischman (1967) appear to satisfy the situation in smooth muscle.

Myofibrils in smooth muscle consist of tightly organized myofilaments. Perhaps the fusiform dense bodies are responsible for organizing myofilaments into groups. However, in smooth myoblasts, myofibrils can be observed without fusiform densities. No structural feature of smooth muscle cells appears to satisfy the requirements of a "myofilament-organizer" of forming myofibrils, except perhaps the intrinsic properties of the myofilaments.

The basement membrane envelope is incomplete at six days of incubation but complete by hatching, consisting of a finely fibrillar substance. The envelope is not present within the close junctions of smooth myoblast laminae, but it is continuous over cellular junctions enveloping the entire lamina. The envelope is obliterated by one month of age in regions of contact between smooth muscle cells and elastica. Haust, et al., (1965) believe that the basement membrane envelope is involved in elastogenesis. In the present study, elastin shows a fibrillar component and an amorphous component which combine or transform to form vesicular units. On the other hand, the basement membrane envelope consists of a finely fibrillar or amorphous component which is more

electron dense than amorphous units of elastin. For this reason, they are interpreted as two separate structures without a developmental relationship.

Fungiform surface differentiations in six day-old embryos bear no physical relationship to interlaminar cells, but by hatching, some spherical surface differentiations of smooth myoblasts were embedded in adjacent interlaminar cells leaving only a 200Å wide close junction. This peculiar feature of smooth muscle cells in the aorta has not been previously reported, but it is a prominent feature of the pigeon aorta. Clusters of surface differentiations impinge interlaminar cells in adult birds, but their precise function is completely unknown, (cf. page 59).

The membraneous organelles of smooth myoblasts are widely distributed in the cytoplasm of embryos. After hatching, they become primarily juxtannuclear. This period of concentration of membraneous organelles corresponds to the period of rapid accumulation of filaments. The myofilaments in smooth muscle cells contact the plasma membrane at certain sites termed attachment devices (Pease and Paule, 1960). This attachment is essential to contraction in smooth muscle. (Rosenbluth, 1965). The membraneous organelles appear to occupy the juxtannuclear regions of the cell because of space restrictions due to the essential position of myofilaments. In synthetically active smooth muscle cells, the mem-

braneous organelles continue to occupy the juxtannuclear regions (Ross and Klebanoff, 1967) while the myofilaments continue to ensheath the cytoplasm. When myofilaments are lost from smooth muscle, the endoplasmic reticulum and mitochondria become widely distributed, (Parker and Odland, 1966).

The development of the interlaminar cells in the media parallels the development of the fibroblast-like cells of the subendothelial region, except for the presence of impinging smooth muscle surface differentiations. With the exception of one electron microscope study of the aorta (Saxl, 1961), ultrastructural studies of avian (Karrer, 1960), and mammalian (Pease, 1955; 1963; Pease and Paule, 1960, and Paule, 1963) aortas indicated that the smooth muscle cell was the only cell type present in the media. This investigation shows the presence of two cell types in the media. The interlaminar cell is a previously undescribed cell type. It is present in alternating layers of the media with elastic laminae intervening between smooth muscle cells and interlaminar cells. The interlaminar cells lack a basement membrane envelope, filaments with dense bodies, and they are not associated laterally into laminae. They are impinged with smooth muscle cell surface differentiations, and this association is a feature not previously described for vascular smooth muscle, although visceral smooth muscle is known to have projections of the plasma

membrane (Thaemert, 1963).

Greenlee, et al., (1966) identified young and mature elastic fibers from calf ligamentum nuchae and rat flexor digital tendon. In the earliest stages of development electron microscopic observation revealed collections of 100A diameter filaments, surrounding central non-staining areas. At higher magnifications, the 100A filaments were tubular in profile having a light central core around 40A in diameter. Longitudinal sections of elastic fibers revealed parallel aggregates of filaments with 100A diameters. The central non-staining area became more prominent in size with increasing age. Phosphotungstic acid staining did not show the filamentous portion, but it increased the density of the central core region of elastin units. The developing elastin of the embryonic pigeon aorta at six days consists of aggregates of fibrils identical to the filaments of Greenlee, et al., (1966). The central core region undergoes a relative increase in size with age, so at the time of hatching 0.2-0.5 micron units of elastin with a perimeter of 100A diameter fibrils were scattered throughout the vascular wall. These vesicular units grew by accretion and fusion after hatching so that between two weeks and one month, complete elastic laminae were formed. Elastin from ligaments and tendons appears to form in a manner similar to aortic elastin. Amorphous cores and aggregates of 100A diameter fibrils

combine or transform and increase in size by accretion and fusion.

Pathogenesis of the Lesion. Vascular smooth muscle cells were associated with the production of connective tissue components (Haust and More, 1963) and the intracellular accumulation of lipid to form foam cells in human atherosclerotic lesions (Geer, et al., 1961; Balis, et al., 1962; Haust, et al., 1962; McGill and Geer, 1963; Balis, et al., 1964). Macrophages (Balis, et al., 1964) and/or a blood-derived round or ovoid cell type (Geer, 1965a) were also involved in foam cell origin. Often foam cells of both origins were found in the same lesion (Balis, et al., 1964; Geer, 1965a).

In experimental atherosclerotic lesions, proliferating smooth muscle cells and modified smooth muscle cells (Parker and Odland, 1966; Imai, et al., 1966) were identified with myogenic foam cells. Marshall and O'Neal (1966) described a blood monocyte-derived lipophage in foam cell production in experimental rat lesions. It appears that foam cells are formed from transformed blood-derived cells and altered smooth muscle cells in spontaneous and experimental atherosclerotic lesions, but this dual origin makes a careful analysis of cellular modulations leading to foam cell production in lesions difficult. Since late stages of degenerating smooth muscle cells leading to foam cells

cannot always be distinguished from macrophage-derived foam cells, a natural model system which would allow the uncomplicated observation of smooth muscle cell degeneration without the presence of macrophage-derived foam cells would be of great interest in providing insight into the pathogenesis of atherosclerosis.

The reported close resemblance of advanced features of White Carneau lesions and human lesions (Clarkson, et al., 1959) makes a comparison of the ultrastructure of advanced lesions in humans and pigeons useful.

Aortic lesions in White Carneaux fail to show the filamentous type of intracellular lipid inclusion which is characteristic of human aortic lesions (McGill and Geer, 1963), but clear vacuoles and cleft-like profiles are abundant in the foam cells. No round or ovoid cells as frequently encountered in human lesions (Geer, et al., 1961; Geer, 1965a) were observed in pigeon lesions. In contrast to human lesion endothelium, pigeon lesion endothelium shows signs of dissolution and replacement by a lamina of subendothelial mesenchymal cells. This lamina appears to originate from subendothelial fibroblast-like cells of embryos and squabs and the filament-laden subendothelial mesenchymal cells of immature and mature birds. The latter two features of pigeons (i. e. lack of ovoid cells and presence of the subendothelial lamina) may be closely related. The round or ovoid cell

is generally believed to originate from the blood (Marshall and O'Neal, 1966), but the mode by which this cell enters the lesion is unknown. If this cell enters the vascular wall by migration through the endothelium as suggested by Poole and Florey (1963), then a compact subendothelial lamina may provide an effective barrier to cell immigration, and thus prevent the entry of round or ovoid cells into the substance of the lesion. This subendothelial lamina is an anatomic feature of pigeon lesions which apparently permits observation of smooth muscle cell degeneration into foam cells, uncomplicated by the presence of round or ovoid cell or macrophage-derived foam cells. It appears that modified smooth muscle cells beneath the subendothelial mesenchymal cell lamina become transformed to foam cells, since only smooth muscle cells are progressively vacuolated and appear to be abnormal in orientation.

Balis, et al., (1964) were able to distinguish between myogenic and macrophage-derived foam cells according to the presence or absence of membrane limited lipid vacuoles, remnants of a basement membrane envelope, and the compactness of the background cytoplasm. While it must be admitted that lipid droplets synthesized by cells only appear to be membrane bound at low magnification (Fawcett, 1966), Geer (1965a; 1965b) found that lipid droplets formed by fusion of agranular endoplasmic reticulum cisternae in smooth muscle cells and foam cells.

In the present study, foam cells having some properties of smooth muscle cells also had vacuoles with discernable discontinuous limiting membranes. In pigeons, the vacuoles appear to be derived from the dilated granular endoplasmic reticulum of modified smooth muscle cells, although the vacuole membrane in foam cells is agranular. Perhaps the inability to demonstrate a limiting membrane around most lipid droplets is due to an interface interaction, or in the case of vacuoles, extraction damage. The presence of a basement membrane envelope around myogenic foam cells is generally a good criterion for differentiation from blood-derived foam cells but fragments of the basement membrane envelope can be confused with degenerating units of elastin or other debris around foam cells. Finally, the background cytoplasmic density is probably highly variable with the health of the cells involved and the quality of fixation, and it would seem that judging cell origin from cytoplasmic compactness would, in general, be unsatisfactory for critical issues such as foam cell origin.

The present observations suggest that the macrophage or blood-derived cell type may fulfill an incidental role in the pathogenesis of spontaneous atherosclerosis, since pigeons form gross lesions from smooth muscle cells alone, and these lesions progress to resemble human lesions in terms of complications (Prichard, et al., 1964). A similar conclusion is

suggested in humans by Balis, et al. (1964) where it is expected that myogenic foam cells form and then macrophages phagocytize extracellular lipid deposited from myogenic foam cell dissolution.

Under some natural and experimental conditions vascular smooth muscle cells become altered to form foam cells in atherosclerotic lesions (Balis, et al., 1964; Geer, 1965a; Parker and Odland, 1966). The specific alterations vary with the system used, but always lead to the same result, myogenic foam cells. In pigeons, some of the alterations are regressive differentiation while others are not. The term "degeneration" is proposed to define those changes in vascular smooth muscle cells which are neither progressive or regressive differentiation.

In progressive differentiation, smooth myoblasts become normal smooth muscle cells by: (1) basement membrane envelope, collagen, and elastin synthesis, (2) cellular orientation, (3) surface associations with interlaminar cells, and (4) synthesis and organization of the contractile apparatus with a simultaneous loss of membranous organelles. Regressive differentiation includes the changes undergone by normal smooth muscle cells to become modified smooth muscle cells: (1) architectural disorganization, (2) dissociation from interlaminar cells, (3) increase in granular endoplasmic reticulum and partial loss of the contractile apparatus compartment.

The accumulation of lipid in droplets, myelin figures, and clefts is the first degenerative change in modified smooth muscle cells, then modified smooth muscle cells become foam cells by: (1) loss of cell shape, (2) loss of the granular endoplasmic reticulum and contractile apparatus, and (3) cellular dissolution. Progressive-regressive changes concern: (1) cellular organization, and (2) extent of the contractile apparatus and membranous organelles. Degeneration encompasses the accumulation of lipid and cell death leading to dissolution.

Normal smooth muscle cells never become totally dedifferentiated since they do not regain all the properties of myoblasts. Rather, they undergo some regressive changes then degenerate to become foam cells. Murray, et al. (1966) have shown that smooth muscle cells will dedifferentiate to fibroblast-like cells in healing arteries. Perhaps the regressive changes are a response to injury wherein the smooth muscle cells dedifferentiate to participate in the healing reaction, but become lipid-laden and die. Regressive and degenerative changes are overlapping, but they can be distinguished on the basis of a description of smooth muscle myogenesis.

In pigeon embryo aortic intimal thickenings the only cell type present is a fibroblast-like cell. Since no significant alterations of the wall were found in

either breed in subsequent ages up to four months, these intimal thickenings are not believed to represent a pathological or a pre-atherosclerotic condition. No focal accumulations of elastin or lipid accompanied these intimal thickenings, and these observations lend further credence to the idea that the pigeon embryonic and squab intimal thickening represents a temporary alteration of the vascular wall without further developmental consequences. Moreover, the pigeon embryo and squab intimal thickenings occur predominantly in the proximal portion of the thoracic aorta, a region in which gross lesions have not generally been observed (Kottke, et al., 1963). Hughes (1943) encountered similar alterations in a study of the chick aorta.

Human and pigeon lesions resemble one another in terms of the presence of thickened basement membrane envelopes on modified smooth muscle cells, loss of organization of elastic laminae, and abundance of collagen. Atherosclerotic lesions in pigeons and humans do not resemble one another in terms of general architecture, cell types present, and forms of lipid, but further observations on the biology of White Carneau smooth muscle cells may provide valuable insight into the atherosclerotic process. From the present observations, however, it is possible to advance a theory of atherogenesis in pigeons.

Surface differentiations occurred to the same

extent in both atherosclerosis-susceptible White Carneaux and Show Racers. However, differences in their distribution between proximal and distal portions of the thoracic aorta were found in both breeds. In the distal regions, aciniform surface differentiations were directly beneath or embedded in the endothelium elevating it into the lumen to cause a "microstenosis". In the proximal portion of the aorta, the smooth muscle cell surface differentiations were separated from the endothelium by several layers of non-muscular cells.

The distal portion of the thoracic aorta in White Carneau pigeons is highly susceptible to spontaneous atherosclerosis (Kottke, et al., 1965). Clusters of surface differentiations may focally influence insudation in this region since intimal lesions are reported to be induced by medial nodules or ligature (Schenk, et al., 1966; Buck, 1963), which, presumably, alter hemodynamics and infiltration of material from the plasma. The appearance of surface differentiations in both breeds indicates that they are not the only factor involved in atherogenesis. Reports have indicated that factors responsible for the initiation of atherosclerosis in pigeons are independent of those responsible for the progression of the disease (Herndon, et al., 1962). Aciniform surface differentiations may focally alter the vascular hemodynamics and increase insudation of material from the blood into the vessel wall. Local changes in

hemodynamics are reported to cause areas of hypoxia which can lead to cellular proliferation and increased cellular permeability to lipoprotein (Lazzarinni, 1965). Since these alterations would be present in both breeds, other factors inherent in smooth muscle cells of susceptible pigeons (e.g. mitochondrial defects; Smith, et al., 1966) may be responsible for the progression of the disease. Vascular smooth muscle cells alone are capable of producing advanced atherosclerotic lesions in pigeons, and their surface differentiations may also be the initial source of injury to the arterial wall.

SECTION VI

SUMMARY AND CONCLUSIONS

The vascular wall in developing aortas from White Carneau and Show Racer pigeons was observed to consist of three regions. A single layer of endothelial cells lined the lumen. A subendothelial region of variable thickness contained intercellular substances and non-muscular cells. An outer laminated media consisted of alternating layers of smooth muscle cells and non-muscular cells with intercellular substances.

In the normal aorta, endothelial cells underwent few developmental changes. Free ribosomes and granular endoplasmic reticulum gradually decreased through maturity, while tonofilaments gradually increased. Beneath the endothelium, non-muscular cells developed from fibroblast-like cells and were observed to accumulate cytoplasmic filaments while losing most of their membranous organelles. The media was observed to contain two types of cells. Interlaminar cells resembled the non-muscular cells of the subendothelial region. The smooth muscle cells formed fungiform surface differentiations which passed across elastic laminae and impinged upon non-muscular interlaminar cells. The surface differentiations of smooth muscle cells and the interlaminar cells are previously undescribed features of the

aorta. In differentiating smooth muscle cells, the early development of myofilaments closely resembles the early development of thin filaments in skeletal muscle.

Atherosclerotic lesions were only observed at the distal end of the thoracic aorta in White Carneau pigeons. Prior to the formation of lesions, clusters of surface differentiations from smooth muscle cells beneath the endothelium elevated the endothelial lining into the lumen thereby producing small stenoses. Since stenoses are known to produce local changes in luminal pressure, hypoxia, and infiltration of plasma-bourne materials into the vascular wall, a hypothesis was formulated to explain the susceptibility of the distal portion of the thoracic aorta to atherosclerosis based upon the subendothelial occurrence of clusters of surface differentiations from smooth muscle cells.

The first evidence of atherosclerotic alterations in the vascular wall was the presence of foci of abnormally oriented smooth muscle cells at the distal end of the thoracic aorta in White Carneau pigeons. Modified smooth muscle cells evolved from abnormally oriented smooth muscle by extension and dilatation of the granular endoplasmic reticulum. The muscle cells subsequently transformed to foam cells by accumulating lipid in droplets, myelin figures and clefts and by losing granular endoplasmic reticulum and myofibrils. The potential for these degenerative changes is expressed by smooth muscle cells

from White Carneaux but not Show Racers. No blood-derived cells were observed in these lesions. These observations indicate that the blood-derived cell type may fulfill an incidental role in the pathogenesis of spontaneous atherosclerosis, since pigeons form advanced lesions from smooth muscle cells alone.

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APPENDIX

Figure 1. Endothelial cells in a six day-old White Carnean embryo. Note the cytoplasmic filaments (dots) and microfibrils (arrow). Luminal surface is uppermost. 48,000X.

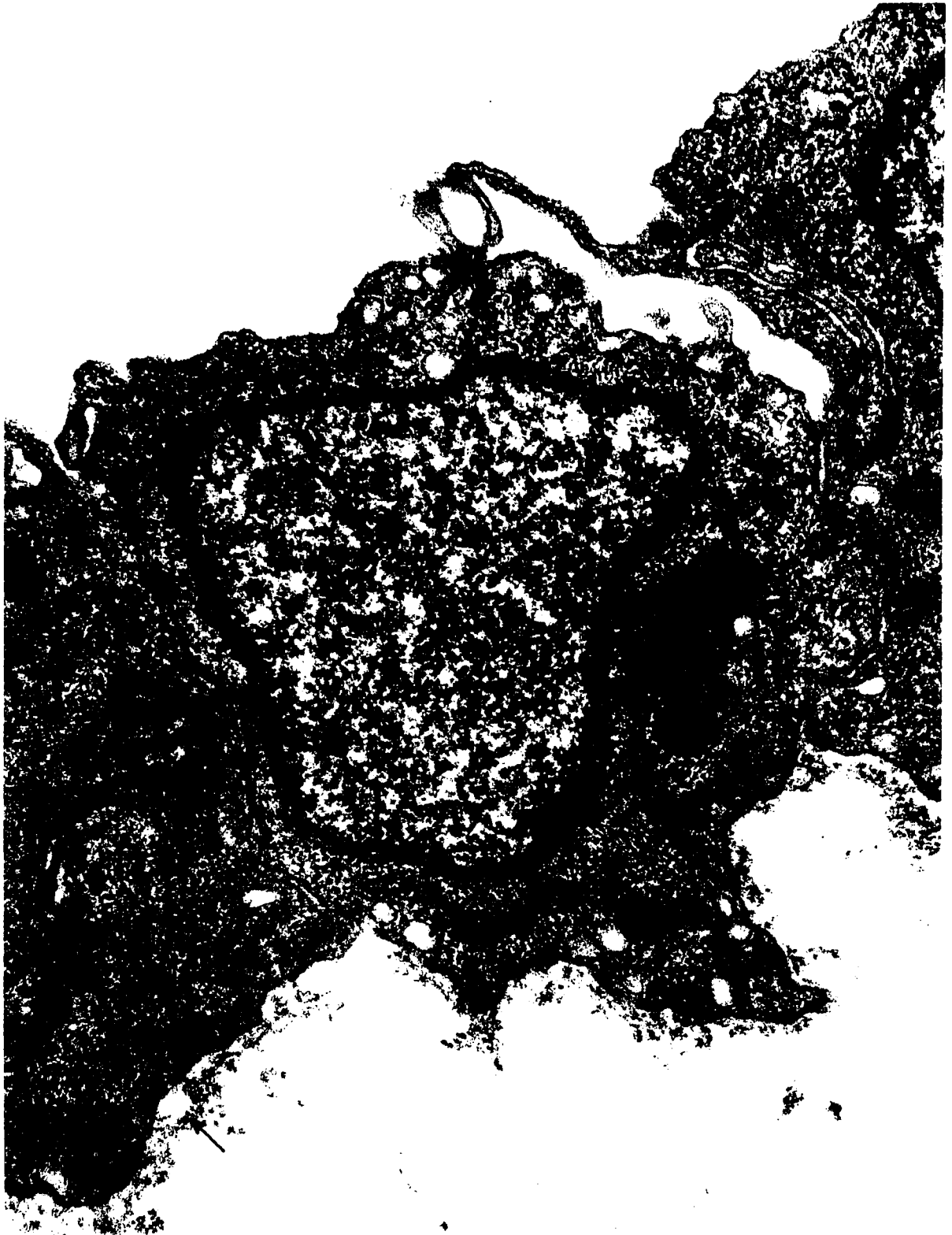


Figure 2. Basal portion of an endothelial cell with basement membrane lamina and elastin units from a twelve day-old Shew Racer. Elastin units consist of amorphous units (E) and microfibrils (arrow). 72,000X.



Figure 3. Epithelial cells in a twelve day-old Shaw Racer embryo. Luminal surface is uppermost. Close junctions (dart) and zonulae adherentes (arrow) are found at apposing plasma membranes. 21,900X.



Figure 4. Endothelial cell in an eighteen day-old White Carneau embryo. 48,000X.



Figure 5. Endothelial cells in a day-old Show Racer squab. Note interdigitations (d), collagen (c), and elastin units (e). 15,000X.

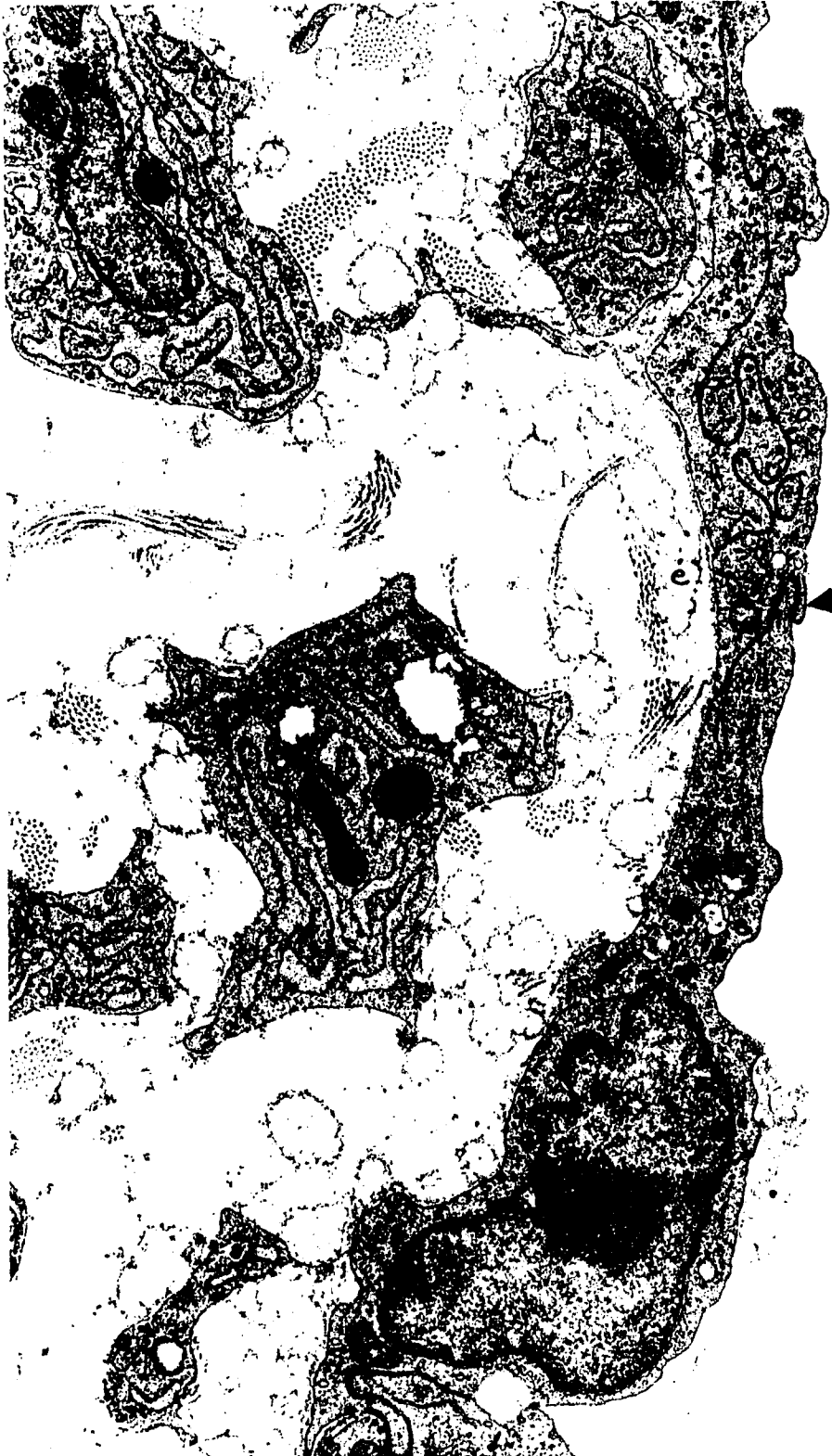


Figure 6. Endothelial cells in a two week old White
Carneau squab. 25,000X

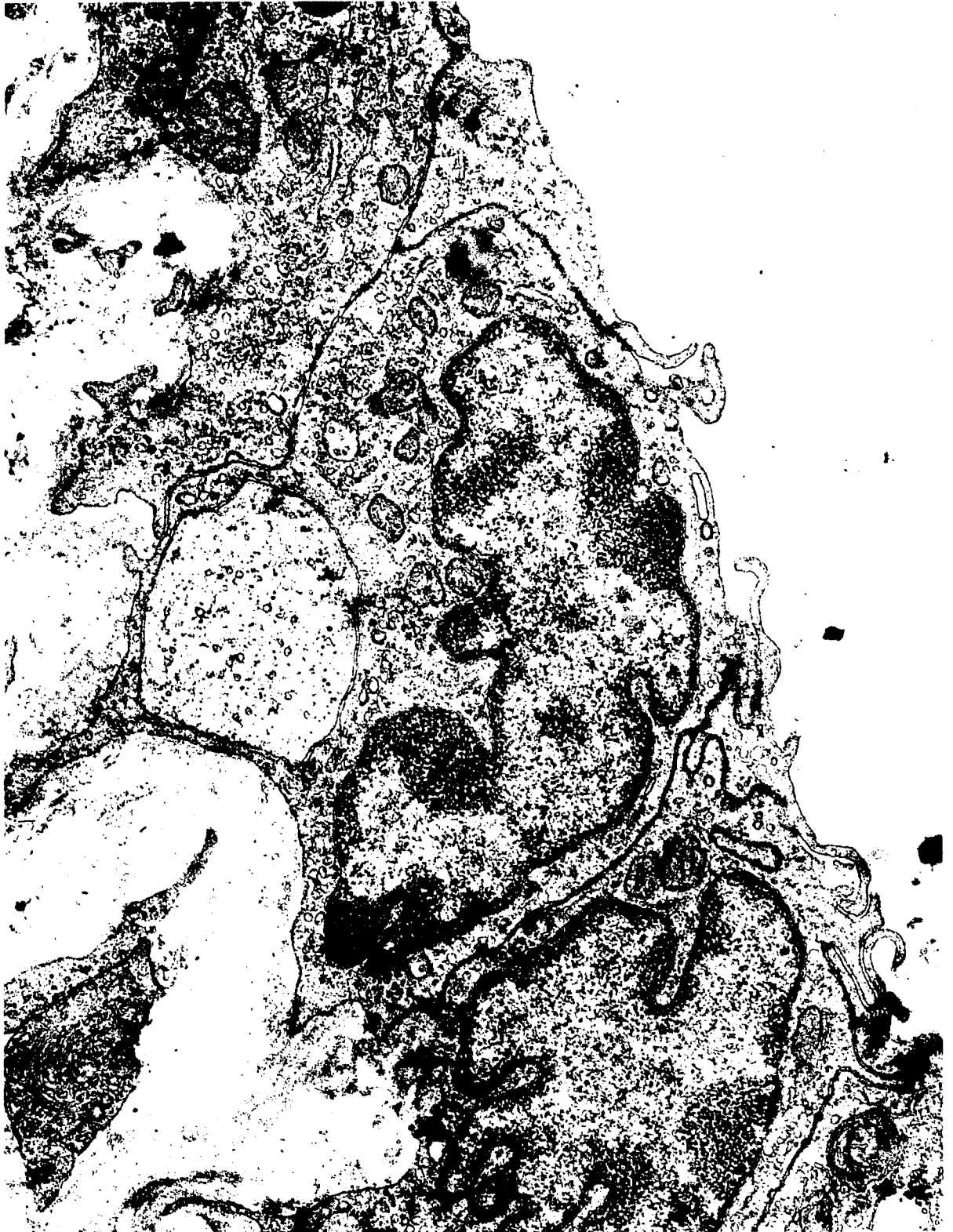


Figure 7. Endothelial cells in a four month old White Carneau. Elastic lamina (E). 21,900X.

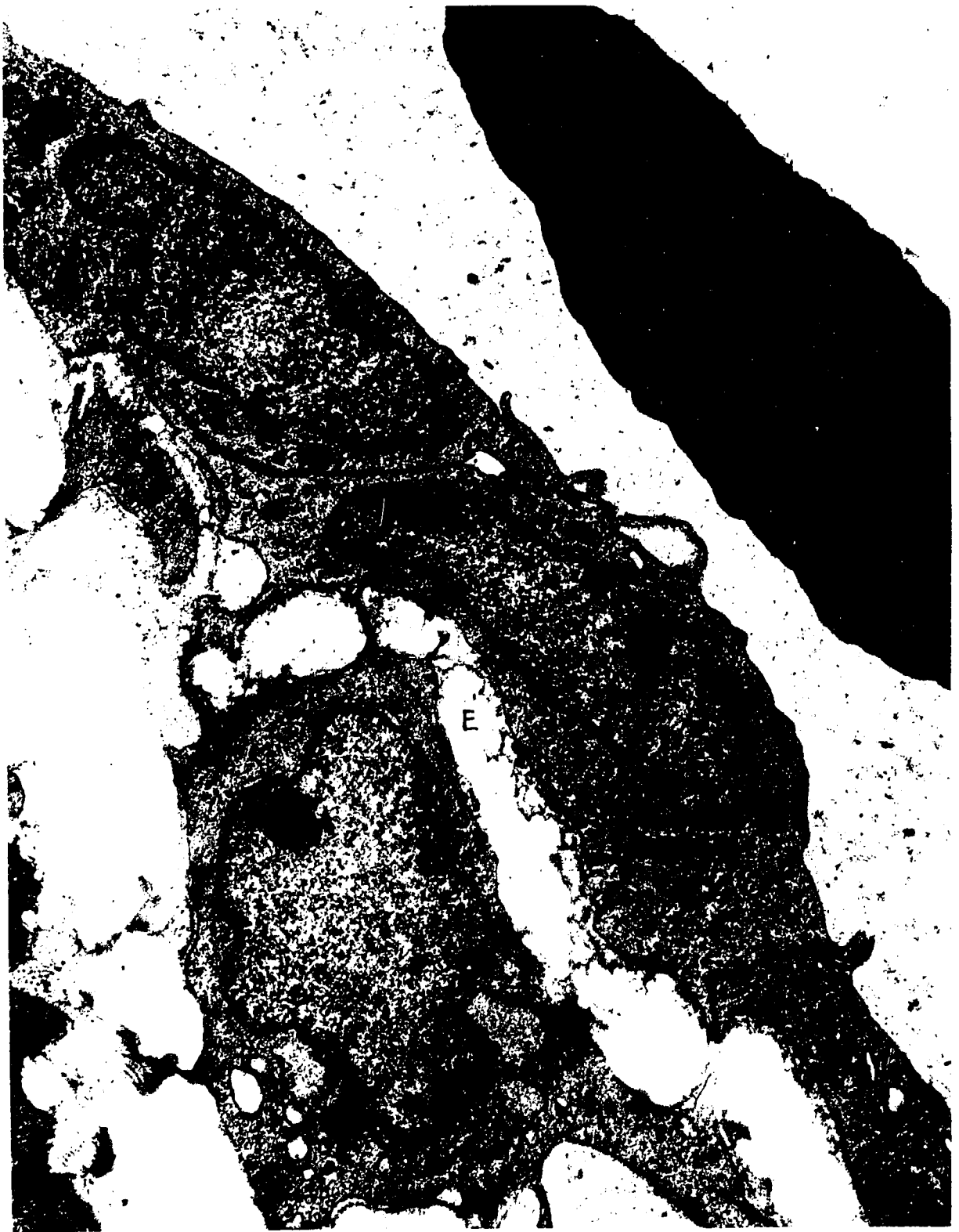


Figure 6. Endothelial cells in a four month old White
Carneau. Impinging surface differentiation (SD).
20,400X.



Figure 9. Endothelium and subendothelial region in an eight month old White Carneau. Many filaments (dart) occupy the cytoplasmic compartment. A subendothelial filament-laden coil (SE) appears embedded in collagen and elastica. 14,600X.

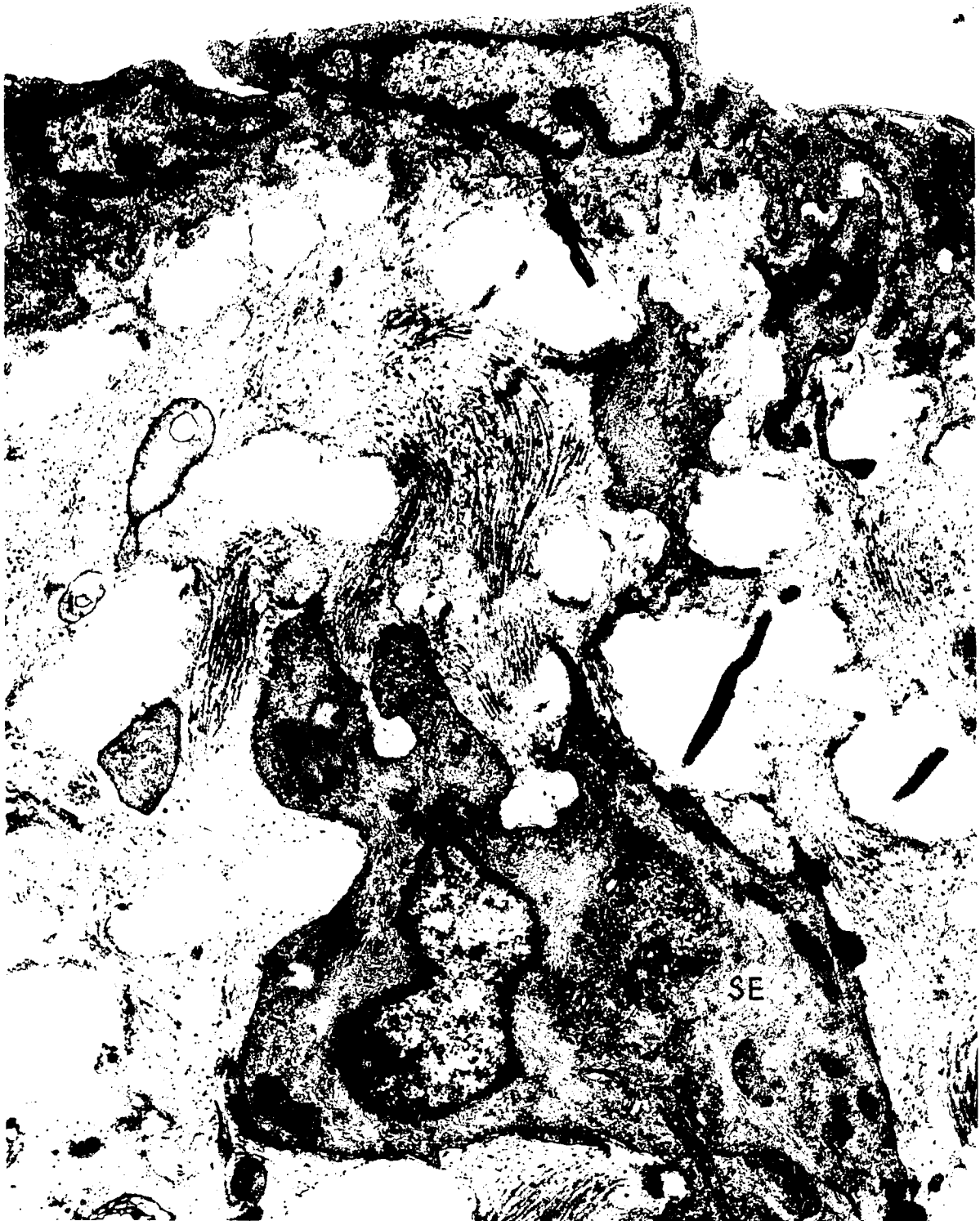


Figure 10. Subendothelial fibroblast-like cell of a six day-old Show Racer embryo. 16,200X.



Figure 11. Subendothelial fibroblast-like cell from an eighteen day-old White Carneau embryo. 21,900X.



Figure 12. Junction of subendothelial region and media of a day-old Show Racer squab showing fibroblast-like cells (FB) and smooth myoblasts (Sm). 8,500X

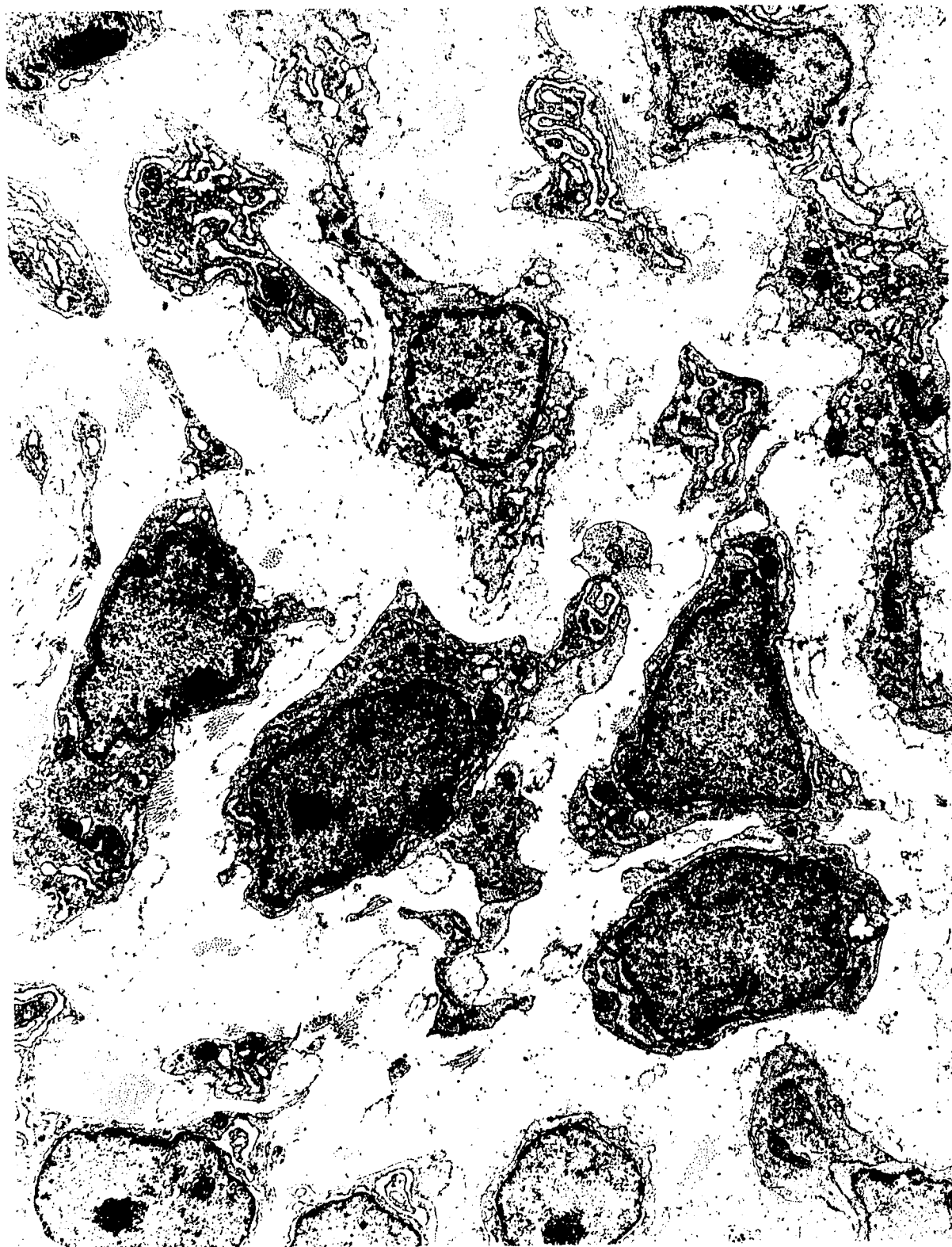


Figure 13. Subendothelial fibroblast-like cell of a day-old Snow Racer squab. Acanthosomes (darts) are present at the periphery of the Golgi complex. Elastin (E) and collagen (c) are present in the extracellular spaces. 51,200X.



Figure 14. Subendothelial fibroblast-like cell of a two week old White Carneau squab. Cytoplasm is filled with filaments (dart). 42,000X.



Figure 15. Laminae of smooth myoblasts (Sm) and inter-laminar cells (IL) in the media of twelve day-old Show Racer embryo. Many reticular microfibrils (rf) occupy the cellular interstices. 7,000X.

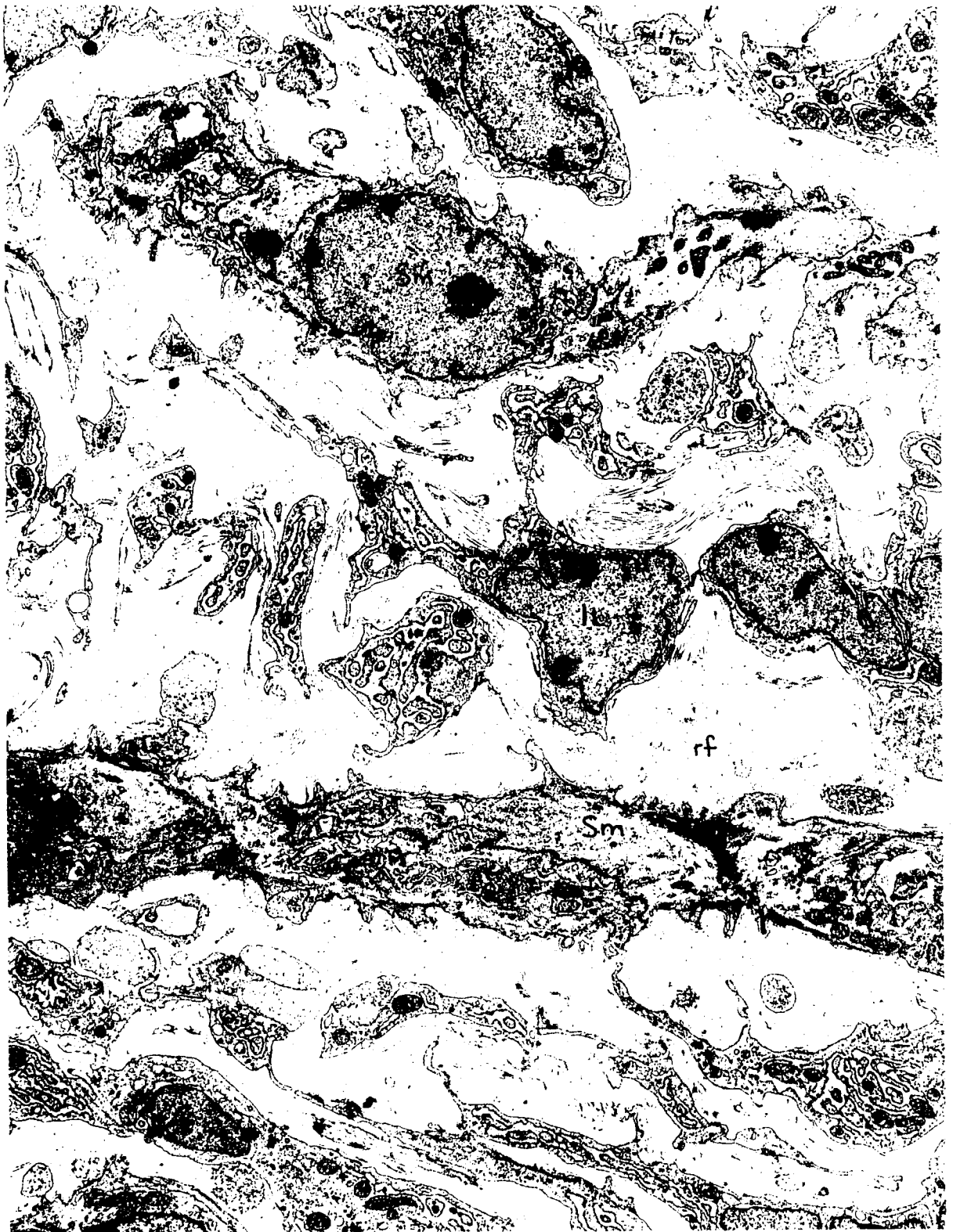


Figure 16. Portion of the media in an eighteen day-old Show Racer embryo showing smooth muscle cell laminae (arrow) and portions of two interlaminar cells (darts). 16,200X.



Figure 17. Smooth myoblast of a six day-old White Carneau embryo with a discontinuous basement membrane envelope (dart) but no myofilaments. 21,900X.

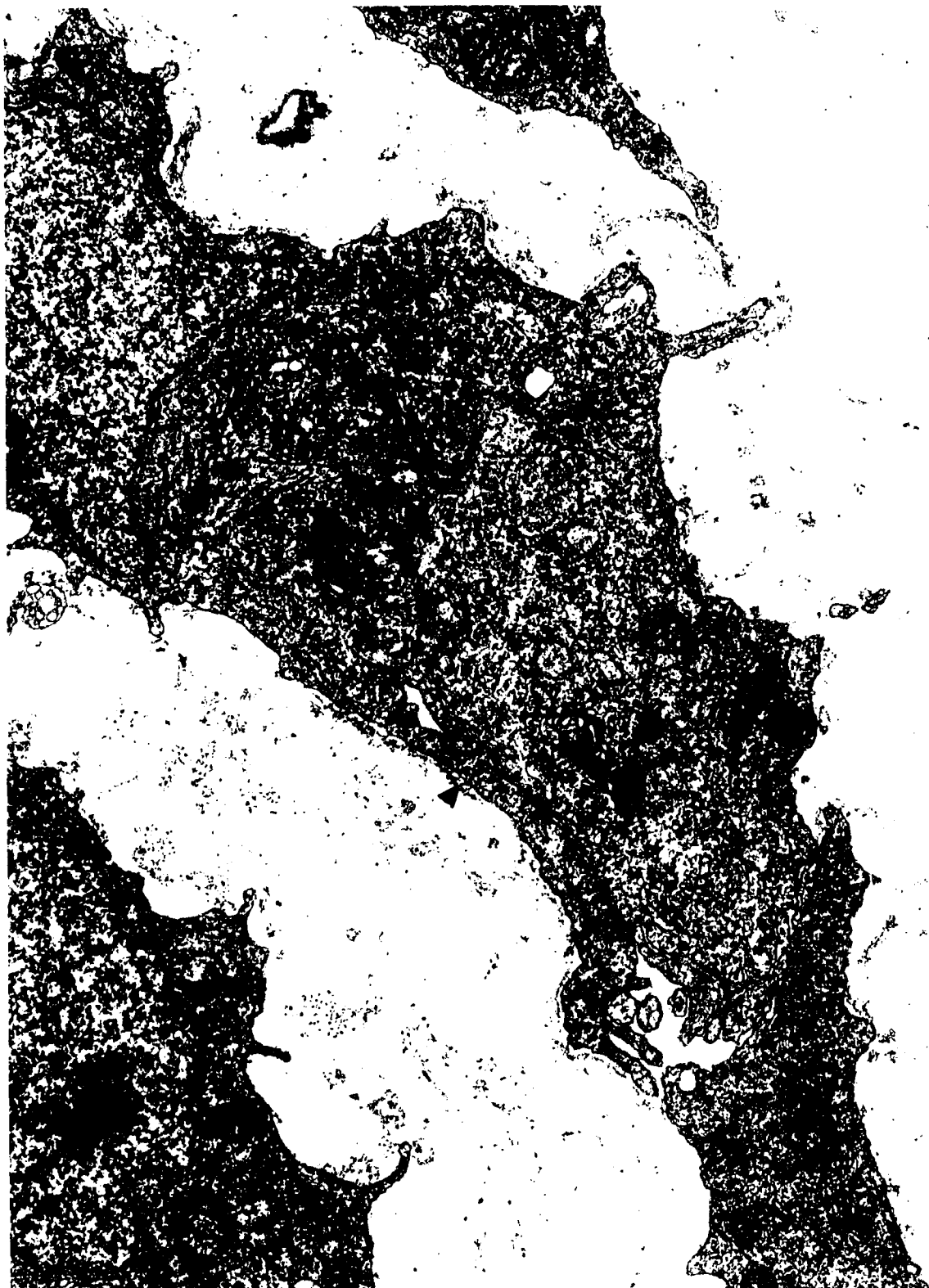


Figure 18. Smooth myoblast with a fungiform surface differentiation (arrow) with a myofibril (dart) and randomly-oriented filaments (mf) from a six day-old White Carneau embryo. 33,800X.



Figure 19. Smooth myoblast with fungiform surface differentiations (arrows) from a six day-old White Carneau embryo. 39,000X.



Figure 20. Randomly-oriented filaments (mf) in a smooth myoblast of a six day-old White Carneau embryo. Note single and small groups of ribosomes (darts). 57,000X.



Figure 21. Loosely organized filaments (mf) in a smooth myoblast of a twelve day-old White Carneau embryo. 45,000X.



Figure 22. Terminal portions of two smooth myoblasts showing randomly-oriented filaments (mf) from a six day-old White Carneau embryo. 52,000X.



Figure 23. Myofibril (M) of a smooth myoblast in a six day-old White Carneau embryo. 81,000X.



Figure 24. Fusiform dense body (F) in a smooth myoblast in a six day-old White Carneau embryo. 101,500X.

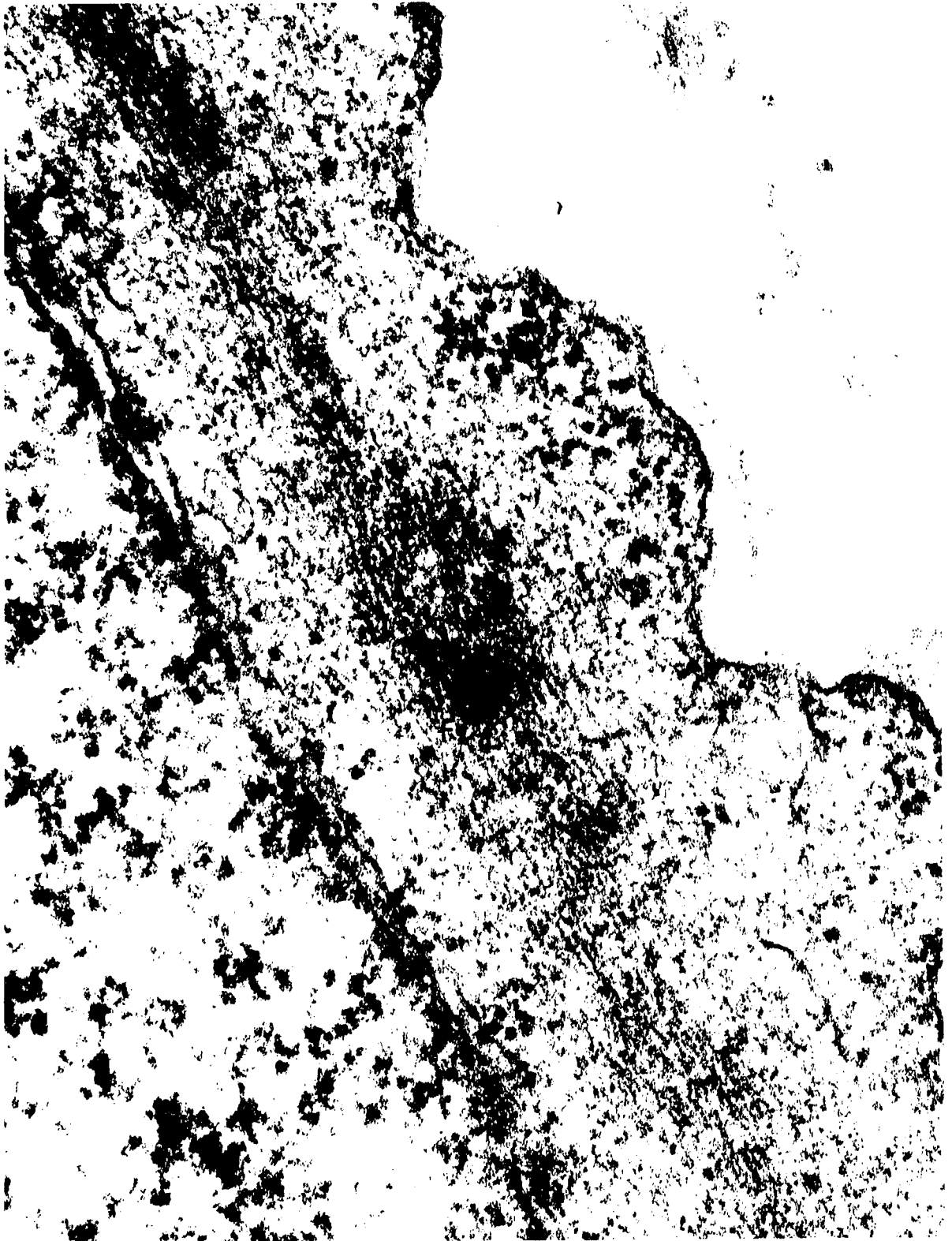


Figure 25. Myofibril with fusiform dense bodies (arrows) in a smooth myoblast of a day-old Show Racer squab. 88,000X.

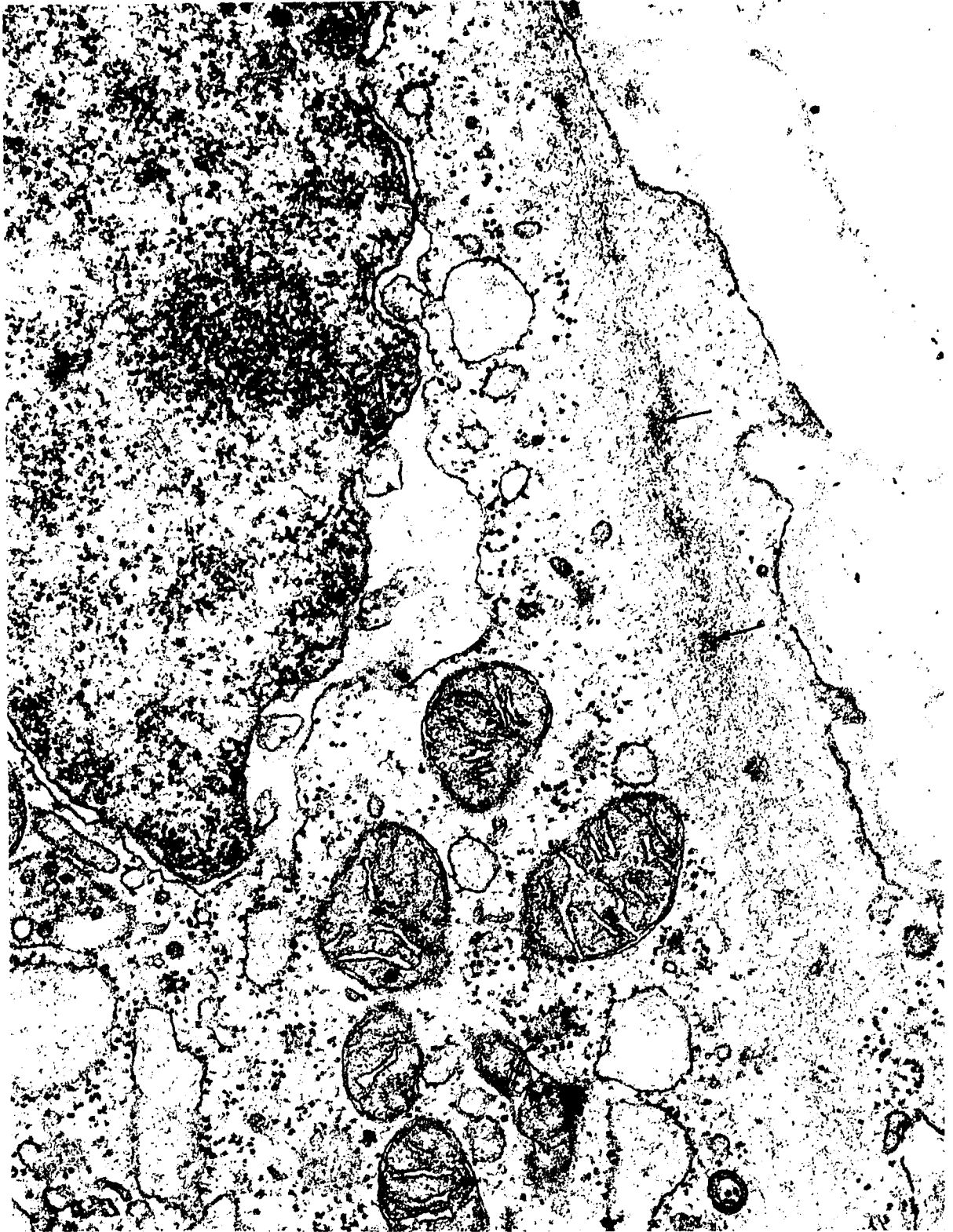


Figure 26. Interlaminar cell showing impinging surface differentiations (arrows) in a twelve day-old Show Racer embryo. 20,400X.



Figure 27. Lamina of smooth myoblasts showing ensheathing myofibrils (darts) and close junctions (arrow) from a day-old Show Racer squab. 17,500X.

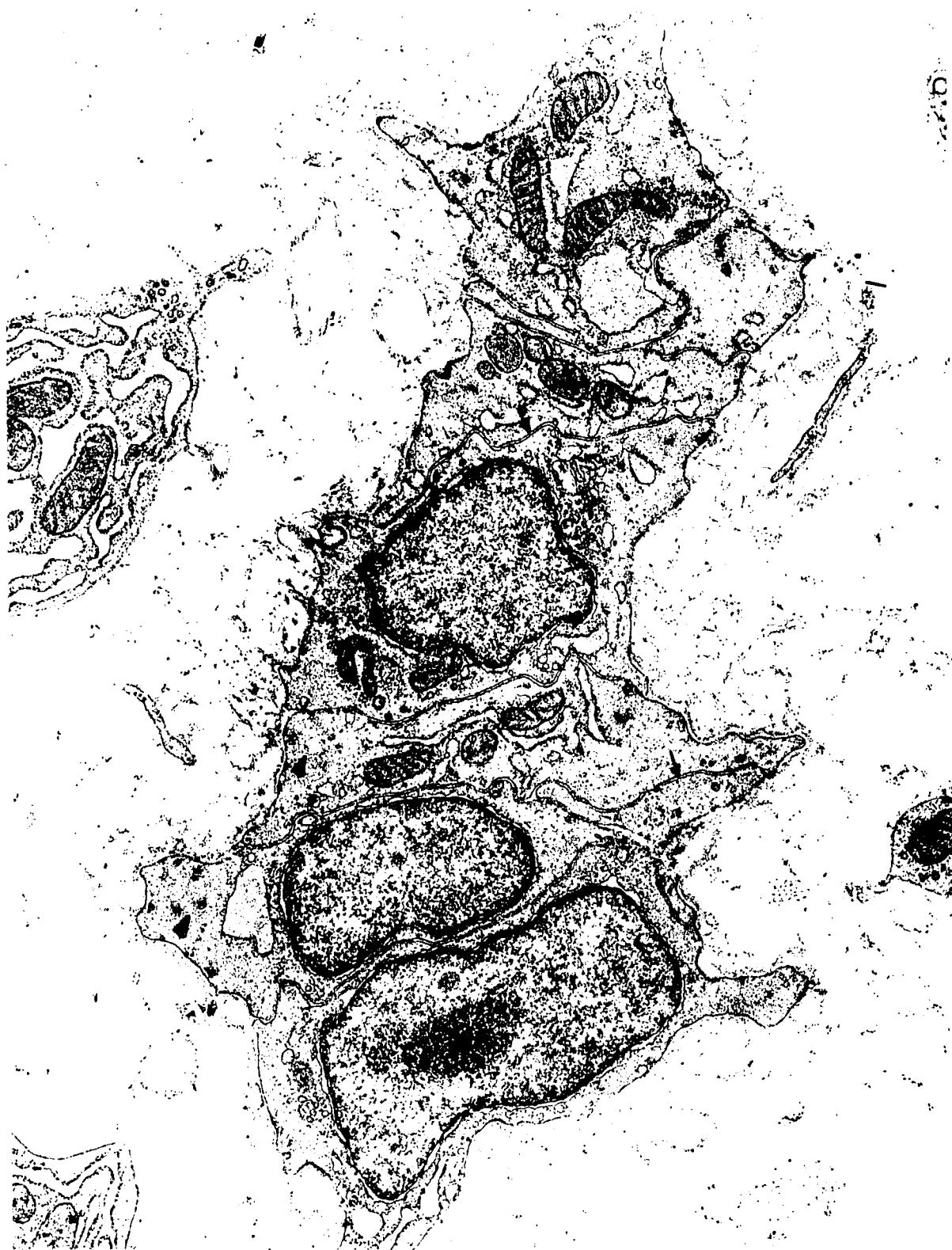


Figure 28. Smooth myoblast (Sm) and interlaminar cell (IL) with impinging surface differentiation (arrow) in a twelve day-old Show Racer embryo. Note basement membrane envelope (dart) and myofilaments (mf) in the smooth myoblast and absence of these features in the interlaminar cell. 28,000X



Figure 29. Smooth myoblast in a day-old Show Racer squab showing juxtannuclear concentration of membraneous organelles. 21,900X.



Figure 30. Smooth myoblasts in a day-old Show Racer squab showing myofilaments (mf), close junctions (dart) and diminishing numbers of membraneous organelles. 29,900X.

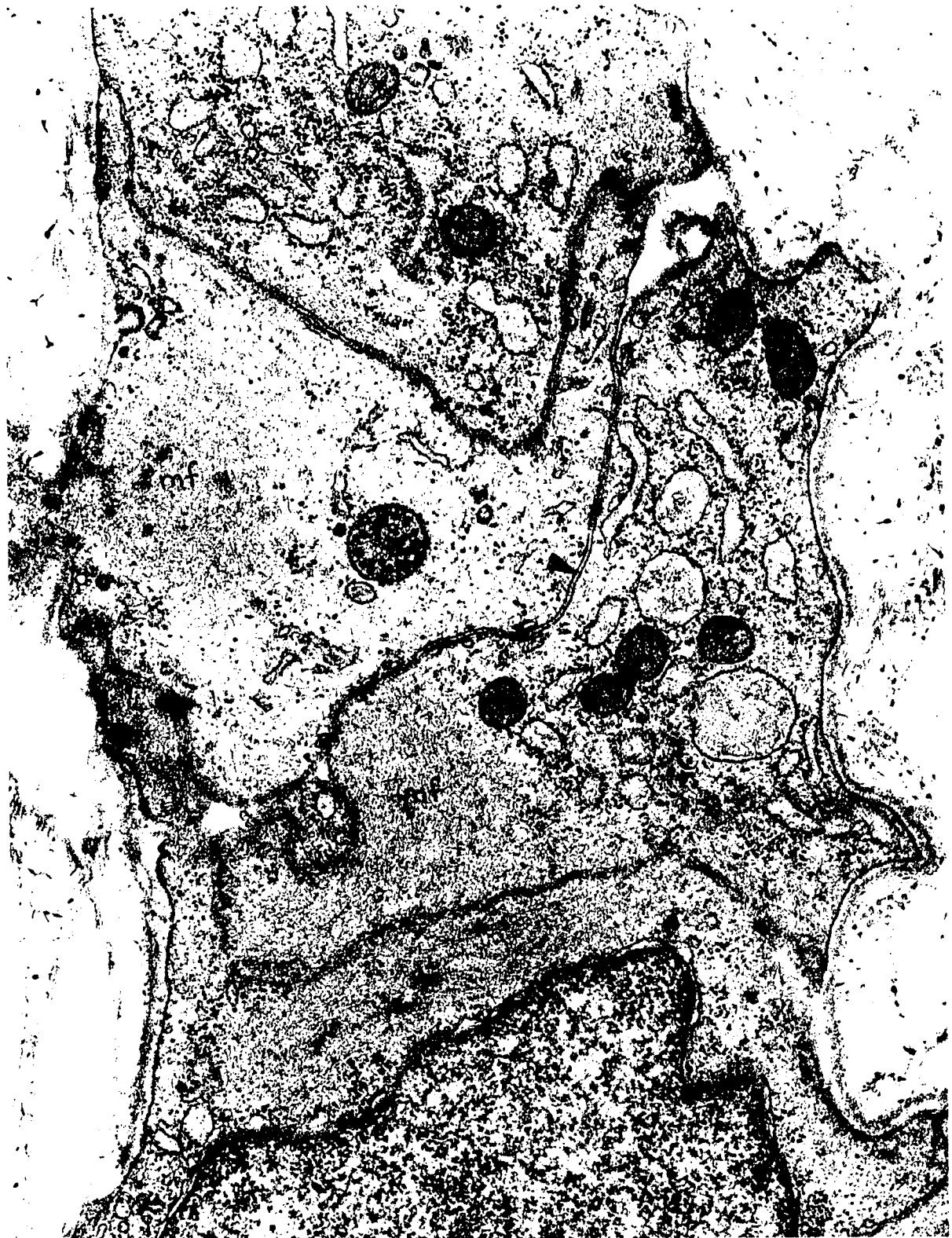


Figure 31. Smooth muscle cells in a two week old Show Racer squab showing special projections where close junctions obtain (arrow). Myofibrils (M) and plasma membrane dense body (dart) are present. 39,000X.

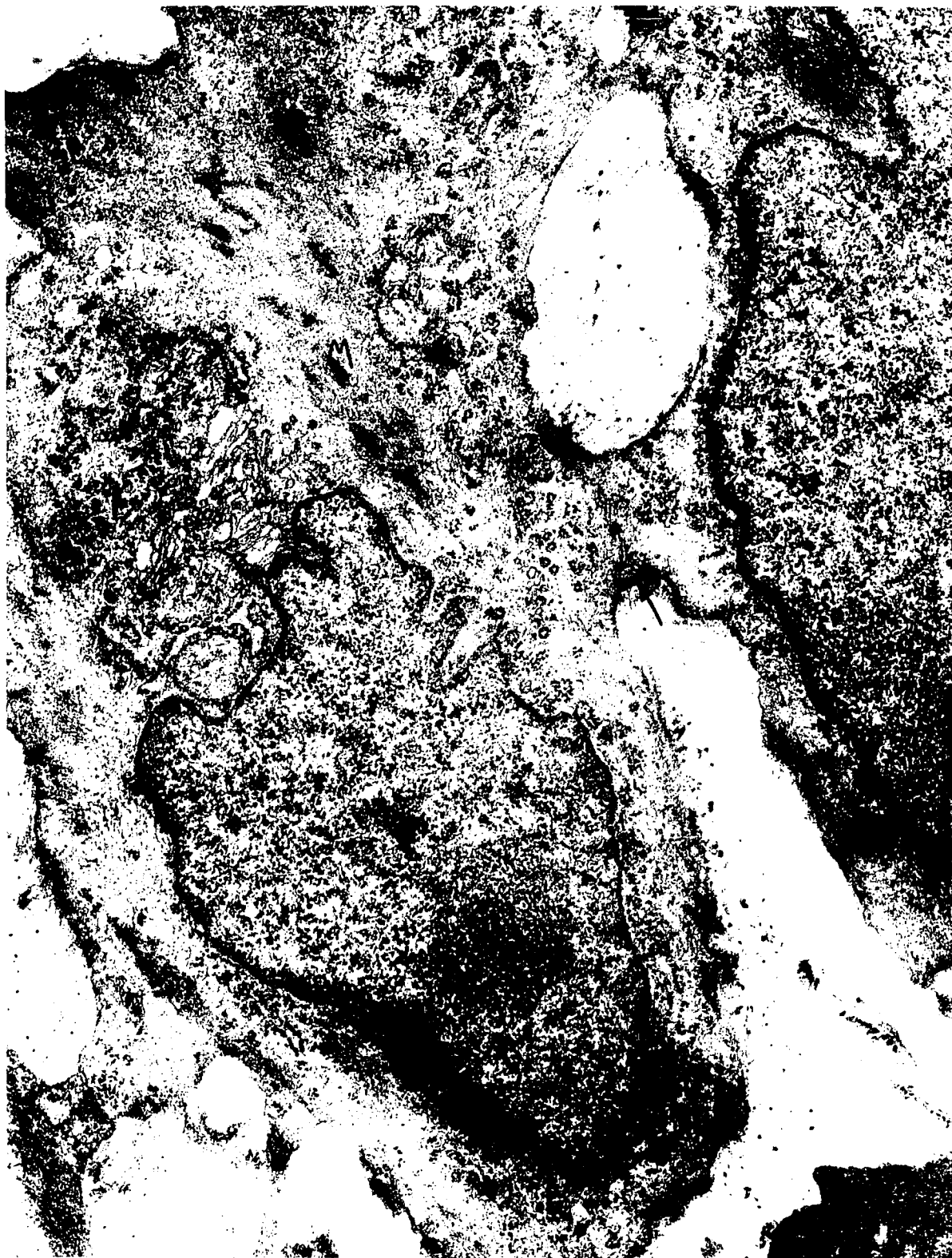


Figure 32. Interlaminar cell in a four week old White Carneau squab showing surface differentiations (SD) from smooth muscle cells and cytoplasmic filaments (dart). 20,500X.

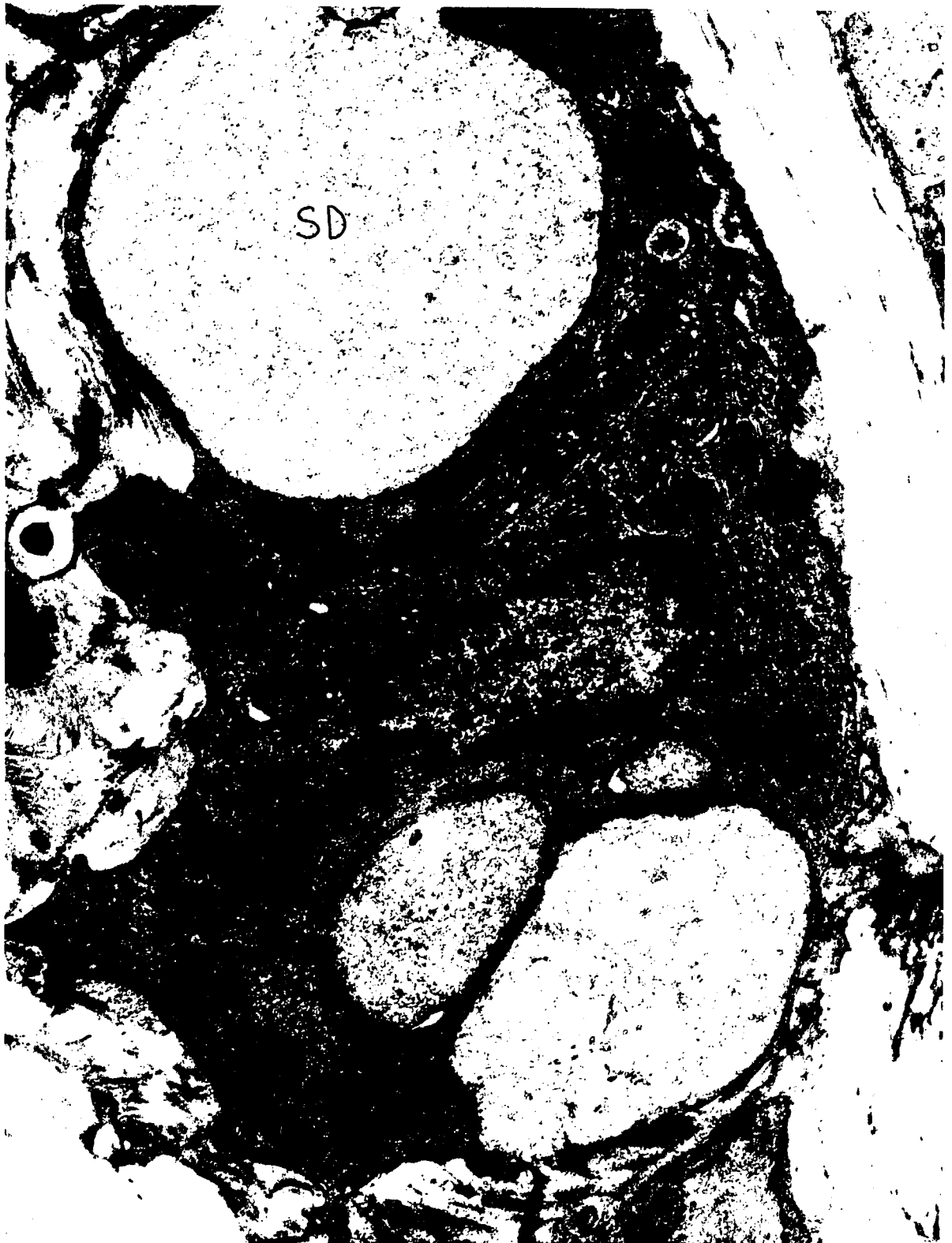


Figure 33. Smooth muscle cells in an eight month old White Carneau. Note that close junctions no longer exist between cells in a lamina, but short projections of the surface contact adjacent cells (dart). 31,000X.



Figure 34. Smooth muscle cells (Sm), elastica (E), and surface differentiations (SD) embedded in an interlamellar cell of an eight month old White Carneau. 21,000X.

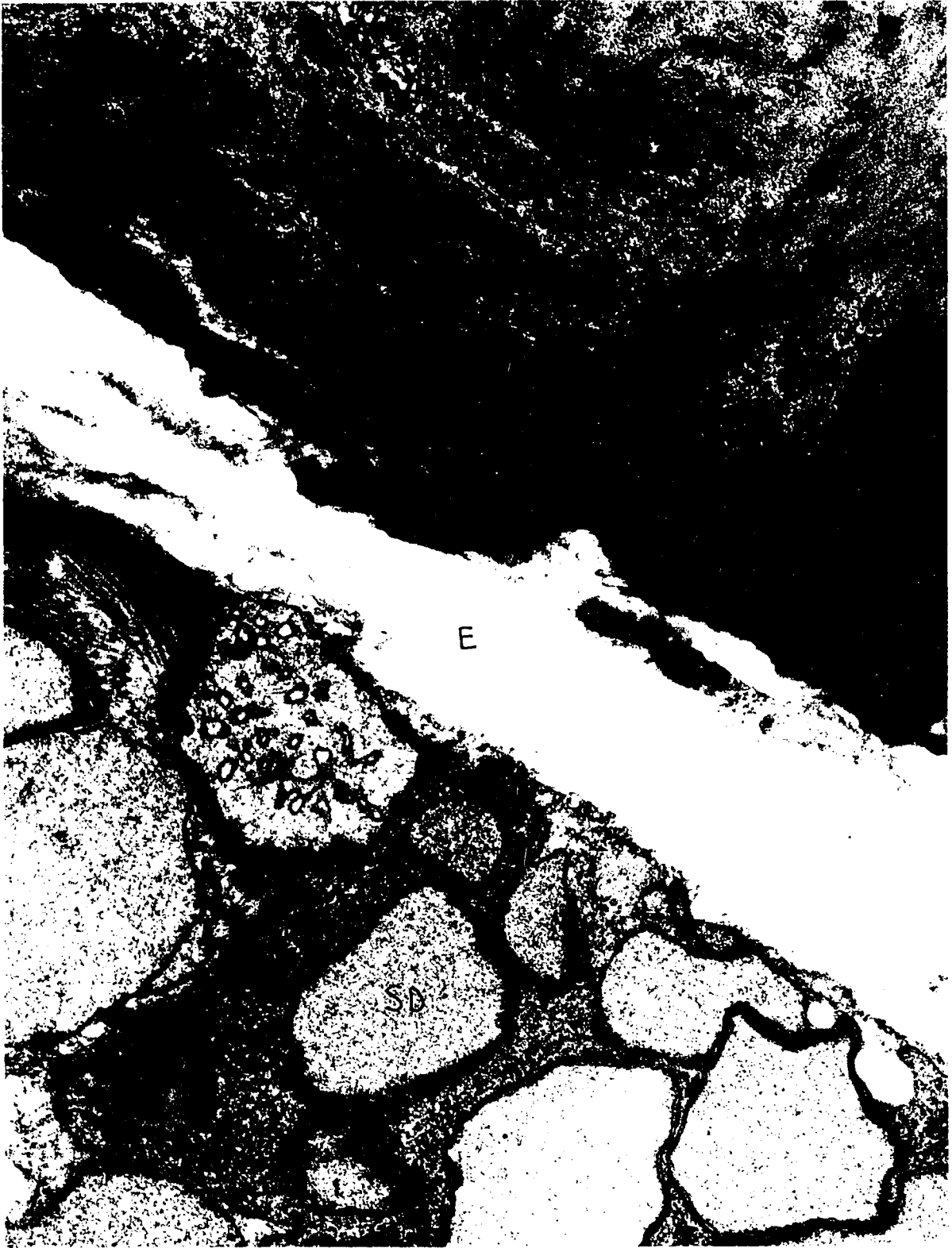


Figure 35. Fenestration in an elastic lamina (E) with surface differentiations (SD) from smooth muscle (Sm) impinging an interlamina cell (IL) in an eight month old White Carneau. 15,800X.

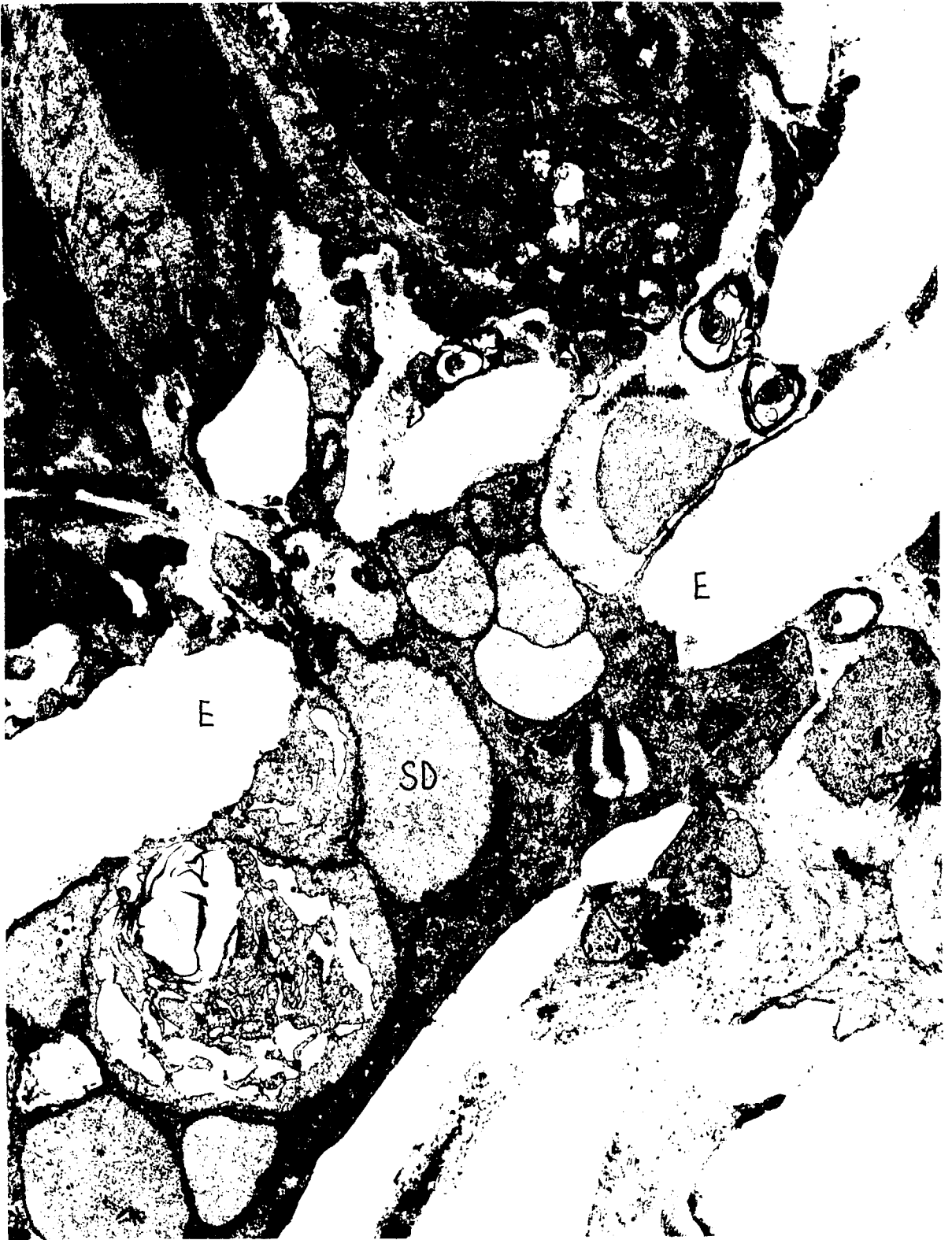


Figure 36. Interlaminar cell in an eight month old White Carneau showing surface differentiations (SD) elastica (E), and reticular microfibrils (arrow). 19,000X.

E

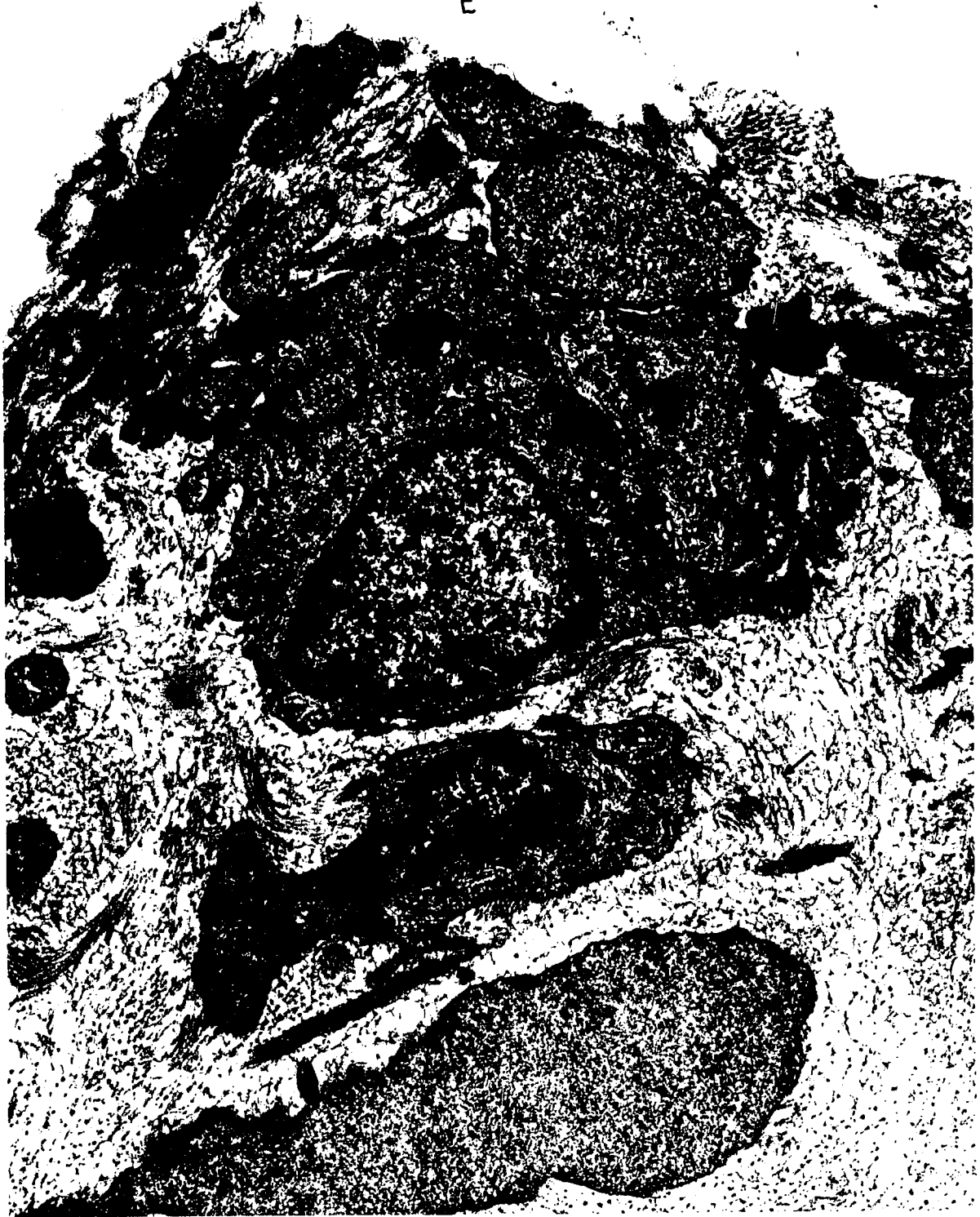


Figure 37. Interlaminar cell in an eight month old White Carneau showing surface differentiations (SD) and cytoplasmic filaments (dart). 28,000X



Figure 38. Endothelium (arrow) and subendothelial lamina in an advanced atherosclerotic lesion from a four year old White Carneau. 12,000X.

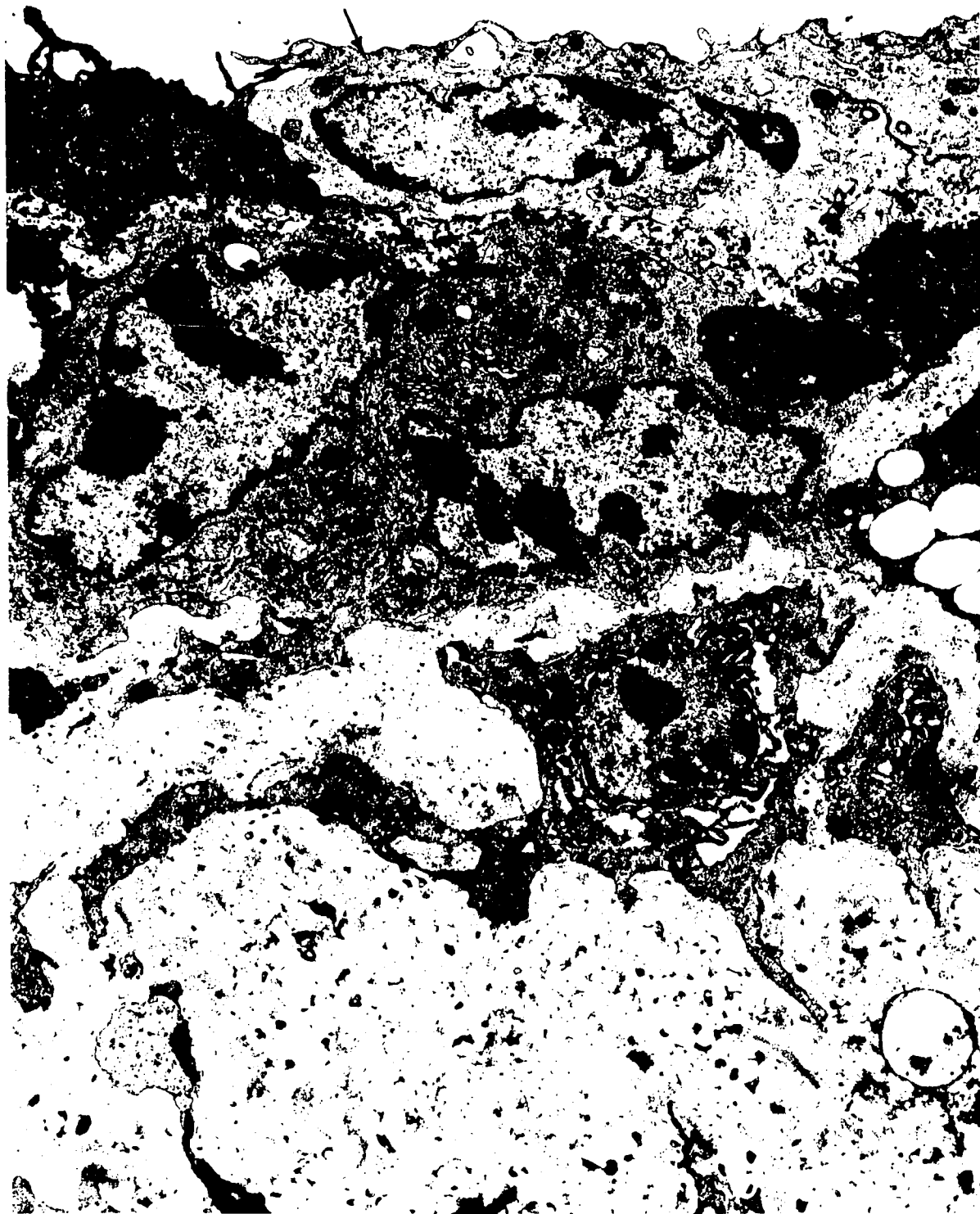


Figure 39. Subendothelial lamina (arrow) of an atherosclerotic lesion in a four year old White Carneau. Note the absence of an internal elastic lamina and the attenuated endothelial cytoplasm (dart). 40,200X.



Figure 40. Modified smooth muscle cell from a lesion. Note the extensive, dilated granular endoplasmic reticulum (dart) and diminished myofibril compartment from a four year old White Carneau. 14,500X.

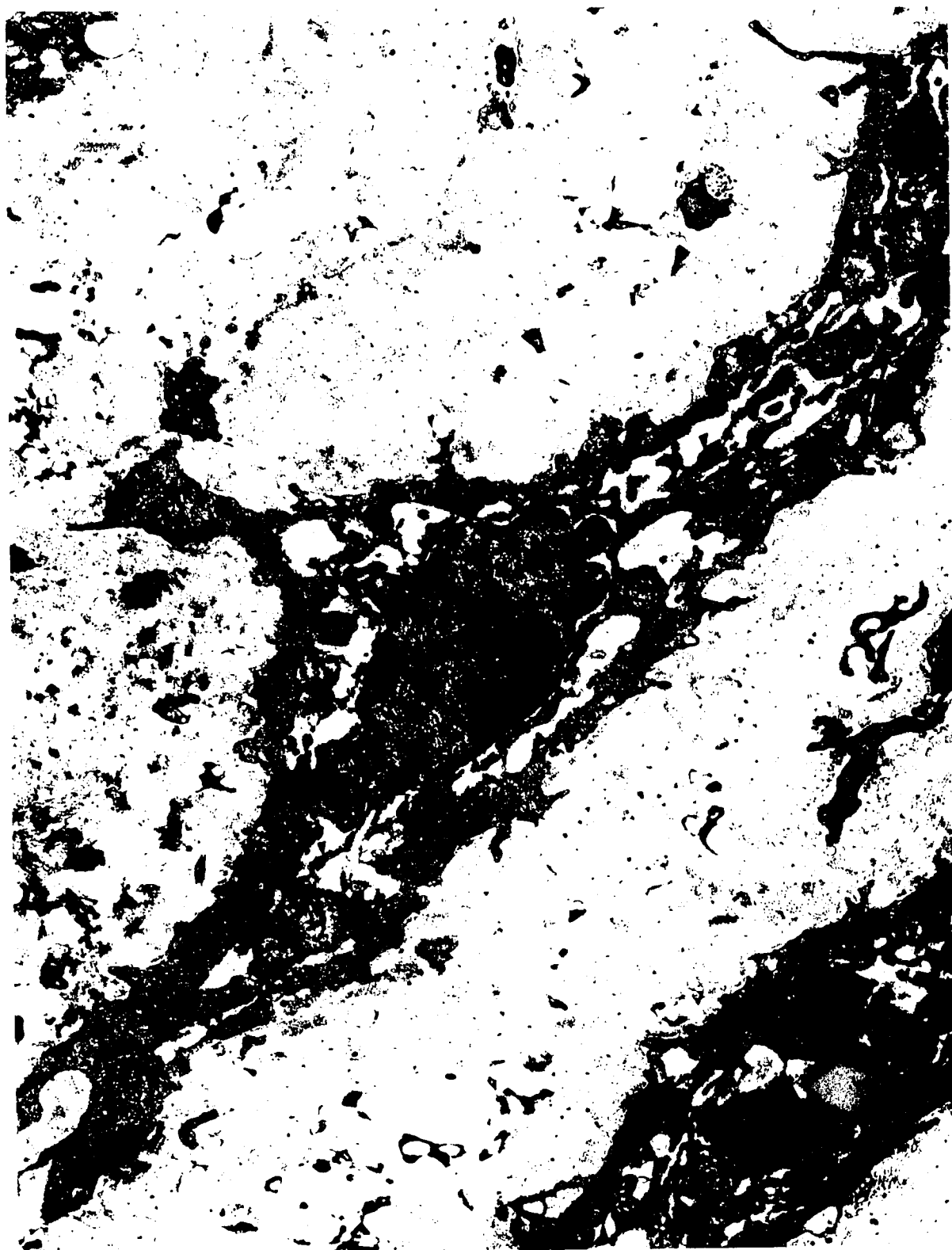


Figure 41. Modified smooth muscle cells from an advanced atherosclerotic lesion showing dilated granular endoplasmic reticulum (darts) and disrupted mitochondria (m) from a four year old White Carneau. 22,600X.



Figure 42. Foam cell showing vacuoles (V) and some cytoplasmic filaments (F). Note apparent dissolution of the cytoplasm (arrow) from a four year old White Carneau. 26,000X.

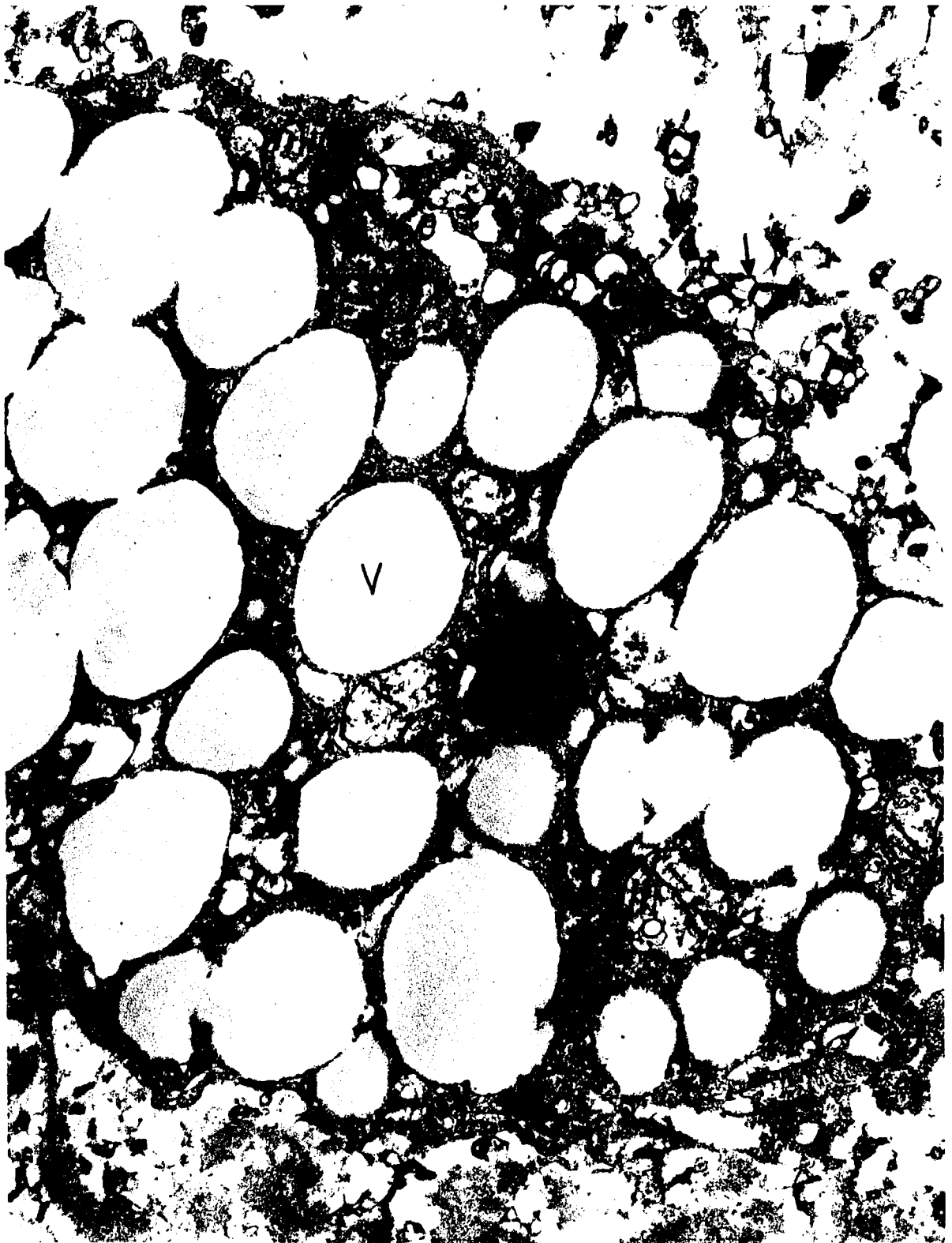


Figure 43. Profile of an atherosclerotic lesion showing endothelium at the top with attenuated cytoplasm (arrow), subendothelial lamina, and smooth muscle cells and foam cell embedded in collagen (C) from a four year old White Carneau. 21,600X.

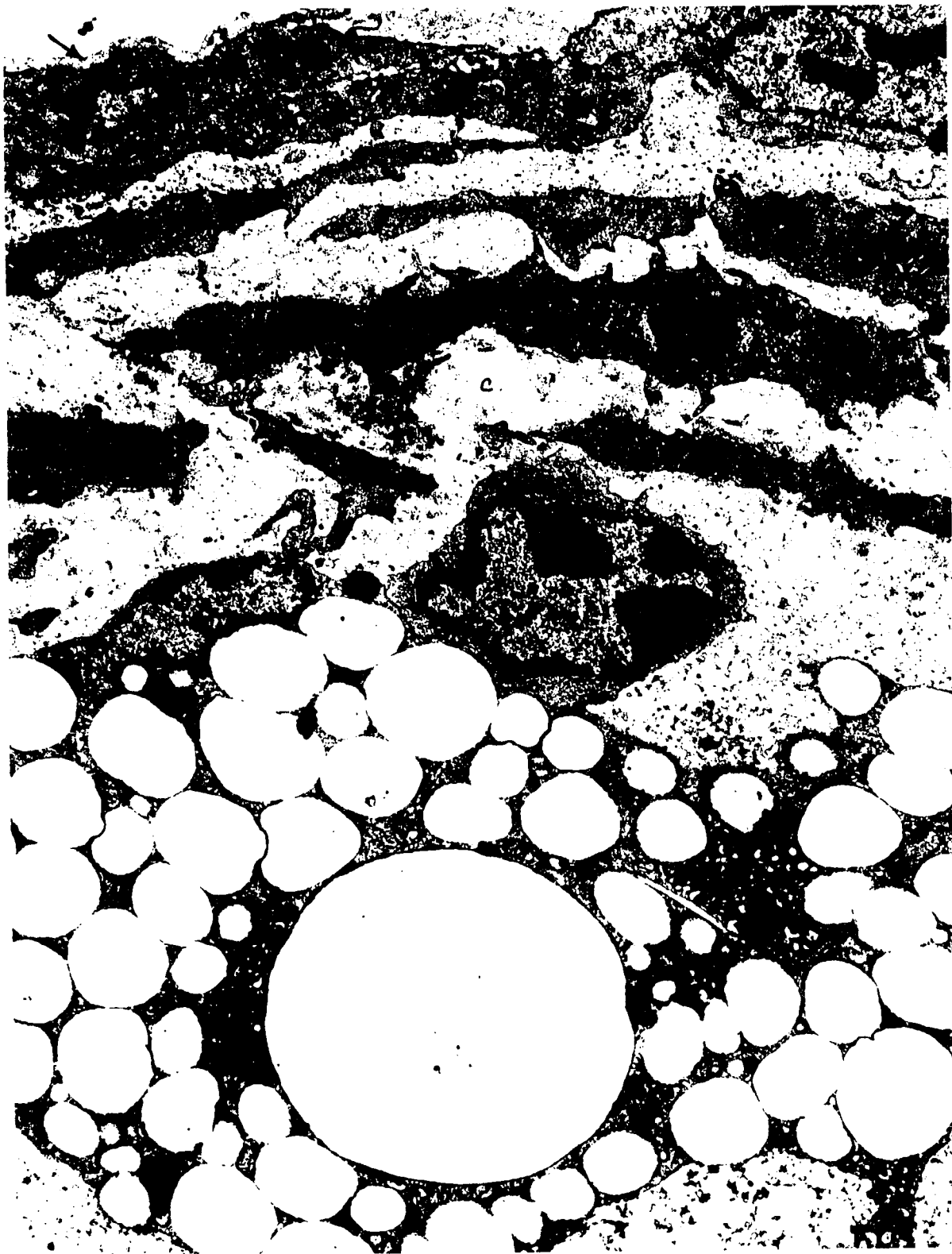
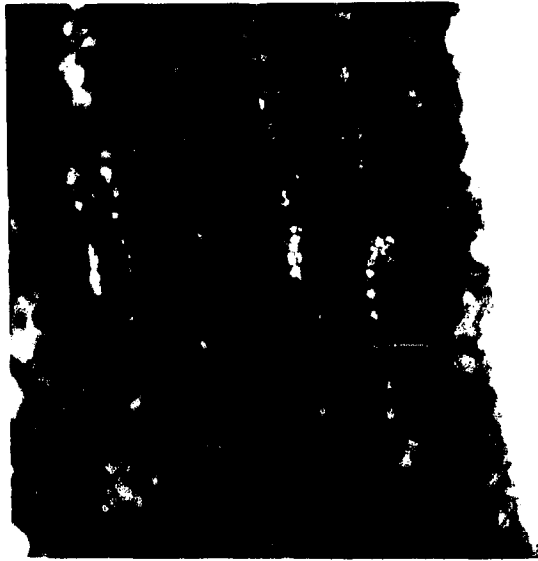


Figure 44. "Intimal thickening" in a day-old Show Racer squab. 7,300X.



Figure 45. (A) Clusters (arrows) of surface differentiations in the proximal aorta showing the intervening area of subendothelial non-muscular cells separating the clusters from the endothelium, and (B) cluster (arrow) of surface differentiations directly beneath the endothelium in the distal aorta, from an eight months old White Carneau. 500X.



A



B