STRUCTURE AND ADRENERGIC INNERVATION OF THE AORTA IN THE GOAT *(Capra hircus)*

by

Julius Alexander Ogeng'o

A thesis submitted in fulfillment of the requirements for the Degree of Doctor of Philosophy in the University of Nairobi

Declaration

This thesis is my original work, and has not been presented for a degree in any other university.

Sign _____ Date 27/6/08

Julius A. Ogeng'o, BSc, MBChB
Department of Human Anatomy
University of Nairobi

This thesis has been submitted with our approval as university supervisors

Sign _____ Date 27-6-2008

Prof. Adel A. K. Malek, MBChB, MSc, MMed, PhD
Department of Human Anatomy
University of Nairobi

Sign _____ Date 2/7/08

Dr. Stephen G. Kiama, BVM, MSc, PhD
Department of Veterinary Anatomy and Physiology
University of Nairobi

Dedication

This thesis is dedicated to the Late Prof. James Kirumbi Kimani, Professor of Human Anatomy, University of Nairobi, for teaching me the value of organization and endurance; and to my wife Dorinah, children Mercy and Silas, for always standing with me and persevering many hours of mental absence while working on this thesis.

Acknowledgements

I would like to acknowledge the late Prof. James Kirumbi Kimani, Professor of Human Anatomy, University of Nairobi for exemplary mentorship, interesting me in the subject of vascular anatomy, continuous academic moulding, encouragement, constant interest, regular supervision and follow up of the work till his sudden death on September 15, 2006.

My deep gratitude and very special appreciation goes to Prof. Adel Kamel Abdel Malek, Professor of Human Anatomy, University of Nairobi. He was especially instrumental in focusing the study topic, developing the research objectives, text organization and moderating the discussion. His firm criticism, correction, insistence on high quality photographs, and clarity of presentation were invaluable.

I would also like to most sincerely thank Dr. Stephen G. Kiama, Chairman, Department of Veterinary Anatomy and Physiology, University of Nairobi for his consistent guidance in reviewing the various sections. This was critical in data presentation, image selection and refining of the discussion. Special gratitude goes to him for kindly allowing me to use his electron microscope and electron micrographic material. It would not have been possible to complete this work without the unlimited access he allowed me to this very indispensable equipment and valuable material.

I also wish to thank Messrs Leonard K. Wainaina, James Macharia, Jackson Gachoka, Moses Kibiru, Christopher Kamwaro for their invaluable technical assistance. I would like to remember all the BSc. Anatomy students over the years 2004-2007, especially Beatrice Wamuti, Beda Otieno, Peter Kitunguu, Paul Bundi and Philip Maseghe for their assistance in information technology. Special gratitude goes to Ms. Catherine Chinga for her patience, understanding and the meticulous manner in which she typed the thesis. I would also like to thank all the academic and supporting members of staff in the Department of Human Anatomy for their cordial support and continuous encouragement, and all other colleagues and friends who contributed in one way or another to the completion of the study. I sincerely valued their contribution.

Finally, I am deeply thankful to the University of Nairobi for waiving statutory fees, and for providing financial support in purchasing the equipment and chemicals necessary for the study. The Vice Chancellor, Professor George Magoha, Deputy Vice Chancellor (Administration and Finance), Professor Peter Mbithi, principals, Colleges of Biological and Physical Sciences, and Health Sciences, Professor Lucy Irungu and Joseph Kitonyi respectively, and Deans School of Medicine, Professors Dominic Makawiti and Ziporah Ngumi were especially helpful.

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Abbreviations Used

AP-I:	Activating Protein-I
CVCs:	Calcifying Vascular Cells
DIT:	Diffuse Intimal Thickening
ECM:	Extracellular Matrix
FAK:	Focal Adhesion Kinase
FCS:	Fetal Calf Serum
IEL:	Internal Elastic Lamina
MEF:	Musculo Elastic Fascicle
MLU:	Medial Lamellar Unit
MMP:	Matrix Metalloproteinases
MVECs:	Multinucleated Variant Endothelial Cells
NO:	Nitric Oxide
PAI-1-:	Plasminogen Activator Inhibitor - 1
PKC:	Protein Kinase C
SM:	Smooth Muscle
SMC:	Smooth Muscle Cells
SMEF:	Smooth Muscle Derived Extracellular Matrix Factor
SPG:	Sucrose Potassium phosphate Glyoxilic acid
VASP:	VasoActivator Stimulated Phosphoprotein
VSMC:	Vascular Smooth Muscle Cells
WSS:	Wall Shear Stress

Summary

The aorta, the principal efferent from the left ventricle, is an elastic artery which conducts blood to all parts of the body. Its four parts namely ascending, arch, thoracic and abdominal aortae vary in their susceptibility to diseases such as atherosclerosis and aneurysms. The histomorphology and innervation of its wall, and their regional variations may be important in understanding physicomechanical properties, pattern of aging and regional distribution of these diseases. The goat is a suitable model for vascular studies but the structure and innervation of its aorta are only scarcely reported. This study therefore investigated the structure, regional variations, adrenergic innervation and aging changes of the goat aorta.

Twenty four (24) healthy domestic goats (*capra hircus*) in three age groups 6-12; 18-24 and 60 – 120 months were euthanized with sodium pentobarbitone and specimens from various aortic segments processed for light microscopy, transmission electron microscopy and histochemistry. Morphometry was done on random samples from the various segments.

Observations revealed that the goat aorta is an elastic artery consisting of tunica intima, a prominent tunica media and a tunica adventitia. The tunica intima comprises a heterogenous endothelium with round and flat cells supported on a single, and in some cases, lamellated basement membrane. The round cells are

more frequent in the proximal than in the distal segments. Deep to the basement membrane, is the subendothelial zone which frequently widens to form diffuse intimal thickenings, comprising a heterogeneous population of cells, including monocytic and fibroblastic types, collagen and elastic fibres. The collagen and elastic fibres are physically interlinked. Unlike in typical elastic arteries, the internal elastic lamina is prominent in the distal segments, and gives anchorage to cells and fibres on its luminal and abluminal aspects.

The tunica media, like in other elastic arteries, comprises concentric elastic lamellae between which are cells, collagen and elastic fibres. The number of lamellae displays a craniocaudal decline while smooth muscle increases. The tunica media of the goat aorta, however, deviates from the typical medial lamellar unit (MLU) structure in two ways. Firstly, in the proximal segments, the lamellae of the adventitial zone are interrupted by aggregations of interlocked contractile smooth muscle cells which form muscle islands. These islands, are linked to collagen and elastic lamellae, and are conspicuously absent in the distal segments. The cells in the interlamellar spaces consist of various phenotypes of smooth muscles oriented in different directions, fibroblast-like cells and macrophages. Secondly, some of the elastic lamellae run longitudinally. Collagen is, quite prominent, oriented in various directions, consistently physically linked with elastic fibres, and frequently anchored onto the smooth muscle cells. The tunica media also contains vasa vasora, which penetrate into its inner half in the proximal segments. The density of the vasa vasora declines

craniocaudally but, they are still present even in aortic segments with less than 29 elastic lamellae and 0.5 mm in diameter. Surrounding the tunica media is the tunica adventitia, which consists of collagen and elastic fibres, a variety of cells and vasa vasora. The tunica adventitia shows a craniocaudal increase in thickness and elastic fibre content such that it is thickest and most elastic in the abdominal aorta.

Adrenergic innervation of the goat aorta is quite dense in the proximal segments where nerve terminals penetrate into the tunica media and co-localise with muscle islands. Distal segments are, however, sparsely innervated with few terminals confined to the media-adventitial border. Definite aging changes occur in all the layers. In the tunica intima, there are discontinuities and dendritic cells in the endothelium. Medial elastic lamellae fragmentation is more marked in the luminal and proximal than in the adventitial zones and distal segments. The most noticeable change in the tunica adventitia is the occurrence of strips of smooth muscles surrounded by elastic fibres, in distal segments.

In conclusion, the results of this study suggest that structure and adrenergic innervation of the wall of the goat aorta is designed to confer unitary functional integrity, spatial stability, temporal adaptability, immunological homeostasis, and an auxillary blood pumping mechanism. The contribution of the different layers to these functions may be reflected in the zonal and regional variations which probably influence the pattern of aging change and disease distribution.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The aorta, the principal systemic efferent from the left ventricle of the heart, is the largest artery in the body. It is made up of four main anatomical parts namely the ascending aorta, arch of the aorta, descending thoracic aorta, and abdominal aorta (Gabella, 1999). Available data suggest that these aortic regions vary in their structure (Wolinsky and Glagov, 1969; Awal *et al.*, 1999; Sokolis *et al.*, 2002) and susceptibility to different diseases. For instance it has been reported that atherosclerosis and related aneurysms occur more commonly in the abdominal than in the thoracic aorta (Haimorlich and Maier, 1966; Glagov *et al.*, 1988; Hoffman, 2005) while dissecting aneurysms and penetrating atherosclerotic ulcers, seem to be more common in the ascending and descending thoracic than in the abdominal aorta (Braverman 1994; Chiche and Leseche, 2000; Cho *et al.*, 2004). Age changes, characterized by elastic fibre degradation, branching and disorganization (Dellasandri *et al.*, 1994, Zhu *et al.*, 2001, Connat *et al.*, 2001b), affect the thoracic more than abdominal segments (Zarins *et al.*, 2004).

The reason for these differences has not been fully elucidated. Understanding the structure of the aorta may provide some insights into the basis of the observed variation in the predisposition of the aortic regions to disease. The

normal aortic wall comprises three typical layers of the vessel wall, namely tunica intima, media and adventitia. The tunica intima is made up of the endothelium, subendothelial zone of various cell types, collagen and elastic fibres, and a fenestrated, folded internal elastic lamina (Gabella, 1999; Junqueira and Carneiro, 2003). The tunica media, usually the widest layer, is made of fenestrated elastic lamellae between which are smooth muscle cells, ground substance, collagen and elastic fibres. The tunica adventitia, the most external layer, consists of collagen and elastic fibres, a variety of cells, vasa vasora and nervi vascularis (Gabella, 1999; Orsi *et al.*, 2004). The vasa vasora and nervi vascularis may extend into the tunica media (Abrahams, 1969; Stefanadis *et al.*, 1995).

Details of the composition and arrangement of the cells and matrix fibres, vascularization and innervation, and the manner in which they differ between the various aortic layers, different anatomical parts and age changes are not fully known. Knowledge of the histomorphological organization of the aorta, and in particular the different aortic regions may enhance understanding of its function and serve as a baseline for studying alterations that occur in aging and disease processes such as atherosclerosis and aneurysms (Crissman and Pakulski, 1984). The goat may be a suitable model for studying vascular disease, and also for use in experimental cardiovascular surgery (Lemson *et al.*, 1999; Zheng *et al.*, 2000), since the structure of some of its vessels, and its physiological cardiovascular

parameters resemble those of humans (Manrique *et al.*, 1977, Garcia *et al.*, 1995; Prassinos *et al.*, 2005). Further, utilization of bovines as animal models has led to great progress in understanding atherosclerosis (Sugitani *et al.*, 2002). In addition, successful development of bovine aortic vessel substitutes (Optiz *et al.*, 2004a,b) and production of autologous tissue engineered blood vessels have been reported (Rashid *et al.*, 2004). The structure, ultrastructure, innervation and their regional variations as well as age changes of the aorta in the goat, however, remain only scarcely reported. The present study aims at elucidating these features of the goat aorta and how they change by region and age.

1.2 Literature review

The mammalian aorta is a heterogenous organ which shows wide variations. Further, the composition and organization of the vascular extracellular matrix is influenced by genetic factors (Behmoaras *et al.*, 2005). The physicomechanical properties, functions, age changes and other structural disruptions of the aorta, and their effects, depend on the manner in which its various components are organized. Available data on the structure, regional variations and innervation of the aorta, however, is disparate.

1.2.1 Structure of the aorta

Information on the structure of the aorta is inconsistent. For example, whereas in most mammals the tunica media comprises a uniform arrangement (Wolinsky and Glagov, 1967; Stergiopulos *et al.*, 2001; Orsi *et al.*, 2004), in some animals, qualitative transmedial variation has been reported (Knieriem, 1967; Fukuda *et al.*, 1984). In all cases, however the structure of the aorta is designed to confer mechanical strength and reversible distensibility (Shadwick and Gosline, 1981; Gibbons and Shadwick, 1989; Faury, 2001). These functional attributes of the aorta result from the composition and organization of its various layers.

1.2.1.1 The tunica intima

The tunica intima of the aorta varies in thickness and composition depending on blood flow dynamics, blood pressure, age and animal species (Gabella, 1999; Ross *et al.*, 2003). The innermost layer, the endothelium, is composed of a single layer of squamous endotheliocytes which are linked to each other laterally by tight and gap junctions (Fishman, 1982; Ye *et al.*, 2000). The cells are characterized by elongated highly organized electron dense storage vesicles which contain blood clotting factor VIII (Weibel and Palade, 1964; Van Mourik *et al.*, 2002). In healthy arteries, the endothelial cells are generally arranged parallel to the long axis of the vessel (Silver *et al.*, 1989; Gabella, 1999).

It was previously believed that endothelial cells were usually stable, with smooth luminal surfaces and scanty organelles with a flat nucleus (Crawford *et al.*, 1976; Zarins, 1980; Langille and Adamson, 1981). Recent ultrastructural studies have, however, shown that endothelial cell morphology may vary depending on blood flow dynamics (Sugiyama *et al.*, 1997; Zhuang *et al.*, 1998; Nanjo *et al.*, 2001; Xu *et al.*, 2001). Increased flow in arteries causes endothelial activation characterized by lumen protrusions and increase in cytoplasmic organelles, while inactivation by decreased flow is characterized by decrease of endothelial cell number with reduced organelles (Masuda *et al.*, 2003).

The endothelial cells of the aorta, have also been shown to differ markedly in the amount of surface proteins, suggesting that there are several types of aortic endothelial cells with distinct functional differences (Karenyi *et al.*, 1984). In normal adult human aortas, Tokunaga *et al.*, (1998) observed unusually large endothelial cells which had multiple nuclei. These, so called multinucleated variant endothelial cells (MVECs), existed individually or in groups surrounded by typical endothelial cells.

Deep to the endothelium, is the so called subendothelial zone which extends from the endothelial basal lamina to the luminal aspect of the internal elastic lamina, and varies in width from one area to another along the aortic wall (Ross *et al.*, 2003, Junqueira and Carneiro, 2003). It contains cells embedded in variable amounts of extracellular matrix. These cells, mostly smooth muscles, may be represented by four morphological types; stellate, elongated, elongated with side processes, and irregularly shaped (Orekhov *et al.*, 1984; Babaev *et al.*, 1988). This zone displays a variegated structure such that in some regions, it contains only a few scattered cells, or a continuous layer of single cells; while in others it may pile up to form intimal cell masses (Kim *et al.*, 1985).

Other cells described in the subendothelial zone include monocyte-like cells (Duff *et al.*, 1957; Geer, 1965; Gerrity *et al.*, 1979). It is believed that the existence of monocytes in the subendothelial space of large vessels is a normal continuous

physiological event (Joris *et al.*, 1979; Kim *et al.*, 1985; Navab *et al.*, 1988). The subendothelium may also contain vascular dendritic cells (Bobryshev and Lord, 1995), pericyte-like cells (Andreeva *et al.*, 1998) and fibroblast-like cells (Seidel, 1997; Sartore *et al.*, 1997).

The extracellular matrix of the subendothelial zone has been demonstrated immunohistochemically to comprise various connective tissue components including elastic fibres (Sato *et al.*, 1994; Gabella, 1999), collagen type I, III, IV, V VI, and VIII, laminin, fibronectin, heparan sulphate and proteoglycan, (Palotie *et al.*, 1983; Leushner and Haust, 1984; Irue-Arispe and Sage, 1991).

The internal elastic lamina of the aorta is usually fenestrated (Song and Roach, 1984, 1985; Roach and Song, 1988), although in some regions may appear as a solid sheet formed by elastic fibrils of 0.1 - 0.2 microns thick (Ushiki and Murakumo, 1991). In other cases, it may consist of superficial longitudinally arranged bundles of elastin fibrils and underlying solid sheet containing few fenestrations (Sato *et al.*, 1994). Morphometric studies have demonstrated that the internal elastic lamina usually exists in a folded state creating intimal folds (Tindall and Svendsen, 1982; Song and Roach, 1985; Orsi *et al.*, 2004).

Aortic endothelial cells have been observed to be connected to the internal elastic lamina by long cellular protrusions which pass through a layer of subendothelial

granular and fibrillar material, (Laver-Rudich *et al.*, 1978). The cells also attach to the extracellular matrix at their basal surface (Kramer, 1985). Endothelial cells are connected to the subjacent internal elastic lamina by microfibrillar filaments related to elastin associated microfibrils (Davies, 1994).

1.2.1.2 The tunica media

The thick tunica media is composed of medial lamellar units (MLU), each comprising an elastic lamella and the adjacent smooth muscle cells and collagen (Wolinsky and Glagov 1967a; Awal *et al.*, 1999; Orsi et al 2004). The medial lamellar units could be resolved into composites of overlapping musculo-elastic fascicles (MEF) each made up of a group of commonly oriented, elongated smooth muscle cells and an encompassing array of branching similarly oriented elastic fibres (Clark and Glagov, 1985). Successive layers of cells and their surrounding elastic fibres are separated from one another by a narrower, intervening acellular zone containing thick wavy collagen fibre bundles. These musculoelastic fascicles are thought to constitute the structural and functional units of the aortic tunica media (Clark and Glagov 1979; 1985; Zarins *et al.*, 2004).

The smooth muscle cells are usually placed obliquely between the elastic lamellae (Bierring and Kobayashi, 1963; Cliff, 1970; Osborn-Pellegrin, 1978; Clark and Glagov, 1979; Dingemans *et al.*, 1981). Occasionally, however,

longitudinally oriented (Schmid *et al.*, 1982; Wasano and Yamamoto, 1983; Clark and Glagov, 1985), and circumferentially arranged (Humphrey, 1995) smooth muscle cells have been described. Other studies have demonstrated fairly complex shapes and orientations of aortic smooth muscle cells (Clark and Glagov, 1979; Fujiwara and Uehara, 1992).

The smooth muscle cells in the tunica media are of two phenotypes, synthetic and contractile (Sprinkle and Subian, 1987; Minorov *et al.*, 1995) with occasional intermediate forms (Kanda *et al.*, 1995). The synthetic phenotype is characterised by the presence of well developed rough endoplasmic reticulum and Golgi apparatus (Gerrity and Cliff, 1975; Manderson *et al.*, 1989). The contractile phenotype, on the other hand, is characterised by large amounts of myofilaments (Cliff, 1970; Gerrity and Cliff, 1975; Clark and Glagov, 1979), and the abundance of nexuses (Dingemans *et al.*, 1981). Mature vascular smooth muscle cells in the adult aorta may however, change from one phenotype to another (Sanz-Gonzalez *et al.*, 2000).

Aortic smooth muscle cells also show heterogeneity in the type of protein expressed (Bochaton-Pillat *et al.*, 1993; Lemire *et al.*, 1994), cytoskeletal protein content (Travo *et al.*, 1982; Orlandi *et al.*, 1994; Villaschi *et al.*, 1994; Miyazaki *et al.*, 2003) and growth potential (Wohrley *et al.*, 1995; Bochaton-Pillat *et al.*, 1996; Benzakour *et al.*, 1996). These different varieties show regional (Giachelli *et al.*,

1993; Frid *et al.*, 1994) as well as zonal variation (Walker *et al.*, 1986; Orlandi *et al.*, 1994).

The smooth muscle cells are connected by occluding (Stein *et al.*, 1969), and gap junctions (Dingemans *et al.*, 1981; Berry and Sosa-Melgarejo, 1989; Sosa Melgarejo and Berry, 1991). Further, there are elaborate junctions linking two cells end-to-end, or side-to-side or end to side. In each case, the contact is characterized by an increase of the surface of both cells by means of folds, invaginations and projections (Henderson, 1975; Gabella, 1977; Litwin, 1980).

The tunica media also contains cells which do not bear ultrastructural features resembling smooth muscle cells, that is packed arrays of myofilaments, peripherally situated dense bodies, a basal lamina and clusters of peripheral vesicles (Moss and Bendit, 1970; Toda *et al.*, 1984; Arciniegas *et al.*, 1989). These cells are characterized by the presence of long delicate finger-like extensions of cytoplasm that insinuate between and around elastic fibres. They are designated "interlamellar connective tissue cells" (Moss and Bendit, 1970). In addition to these, other cells demonstrated in the tunica media include fat laden macrophages and foam cells (Hineck and Rosnowski, 1975; Hineck and Konsinski, 1975) and vascular dendritic cells (Krupa *et al.*, 2002; 2004; Weyand *et al.*, 2005). Cells resembling fibroblasts, mesenchymal cells (Minorov *et al.*, 1995;

Abedin *et al.*, 2004), pericyte-like cells (Andreeva *et al.*, 1998) and calcifying vascular cells (CVC) (Tintut *et al.*, 2003), have also been described.

Elastic tissue in the aortic tunica media is organized in fenestrated concentric elastic membranes called lamellae, interconnected by interlamellar elastic fibres (Berry *et al.*, 1974; Song and Roach, 1985; Roach and Song, 1988) to form a single structural complex (Mukhalev *et al.*, 1978). The elastic lamellae are closely associated with thick collagen fibres (Dingemans *et al.*, 2000), between which are circumferentially oriented streaks of elastin protruding from the lamellae. In some instances, the elastic fibres appear as irregular, undulated laminae of variable size and shape (Raspanti *et al.*, 2006).

Most smooth muscle cells are in direct contact with elastic lamellae (Bierring and Kobayashi 1963; Cliff 1970; Berry *et al.*, 1974; Clark and Glagov 1979; Dingemans *et al.*, 1981), although some attach onto the interlamellar fibres running between the cells (Dingemans *et al.*, 2000). Lateral branches of elastic lamellae, namely elastic fibres connect to the surface of smooth muscle cells (Fornieri *et al.*, 1992; Davies, 1993; Quaglino *et al.*, 1993). From these attachment points, filaments span the cell, and connect the opposite portions of the cell membrane. This complex of elastin and smooth muscle cells forms "contractile elastic units" which confer to the whole media, a continuous functional organization (Davies, 1993).

Collagen fibres surround the smooth muscle cells and their predominant orientation appears to be parallel to the long axis of the artery although they are observed in all directions (Keech, 1960). The collagen fibrils are most numerous close to the elastic lamellae, where they form organized fibres (Dingemans *et al.*, 2000). These fibres are oriented roughly circumferentially, parallel to the main axis of the smooth muscle cells as well as to the streaks of elastin protruding from the elastic lamellae. In the interlamellar spaces, there are thin haphazardly oriented collagen fibres or isolated fibrils.

The main collagen types in the tunica media include type I, III, IV and V (Mayne *et al.*, 1980; Bartholomew and Anderson, 1983). Type I and type III collagen are more prominent with type I collagen being located between elastic fibres, while type III collagen appears to envelope the elastic fibres. Type IV and V collagen usually surround smooth muscle cells. Other collagen types described in the aortic wall include type VIII (Irue-Arispe and Sage, 1991) type XI (Lawrence *et al.*, 1994), and type VI (Dingemans *et al.*, 2000). These collagens out of the over 29 collagen types based on the molecular composition and morphological characteristics of tropocollagen (Ross *et al.*, 2003), confer mechanical strength and prevent overdistension of the aorta due to excessive pressure.

Interactions between matrix molecules and smooth muscle cells occur primarily via the structurally related cell surface receptors of the integrin superfamily

(Humphries, 1990; Humphries *et al.*, 1991). Dense plaques of smooth muscle, composed of cytoskeletal proteins linked to matrix proteins via membrane integrin receptors are the major site of anchorage between the muscle cells and extracellular matrix (Bezie *et al.*, 1998; 1999; Integan and Shiffrin, 2000). Some areas of the cell membrane are in contact with a basal lamina, and immediately beyond, with microfibrils and collagen fibrils (Bezie *et al.*, 1998).

The structure of the aortic tunica media may display transmural variation in the disposition of extracellular matrix fibres, cellular orientation and ultrastructure (Knieriem, 1967). The outer media contains more elastin than collagen, while the inner media contains more collagen than elastin (Feldman and Glagov, 1971). The elastin on the luminal side exists in the form of fenestrated sheets while that on the adventitial side is a more fibrous network (Roach, 1983). The smooth muscle cells in the outer lamellae differ from those in the inner interlamellar spaces in shape, orientation and size. The outer ones are more irregular, larger and interconnect, overlap or cross each other forming lattice-like structures, as opposed to the inner ones which are regular, smaller and generally transverse (Fujiwara and Uehara, 1992). The thickness of elastic lamellae and collagen increases from the luminal to the adventitial side (Sans and Moragas, 1993; Dingemans *et al.*, 2000).

1 2.1.3 The tunica adventitia

Aortic tunica adventitia contains collagen which consists of closely packed bundles oriented in all directions (Keech, 1960; Osborne-Pellegrin, 1978). The main collagen types in the tunica adventitia are type I and III (Farquhason and Robins 1989; Howard and Macarak, 1989). The diameter of the collagen fibrils in the adventitia is higher than of those in the tunica media (Buck 1987, Merrilees *et al.*, 1987; Dingemans *et al.*, 2000). The elastic fibres of the tunica adventitia on the other hand, comprise fibrous forms containing tubular elastin surrounding vasa vasora (Roach and Song, 1988), cord like shapes entangled among the fibroblasts (Ushiki and Murakumo, 1991) and lamellar forms with transverse, oblique and longitudinal arrangements forming a mosaic (Hass *et al.*, 1990; Orsi *et al.*, 2004).

The cells in the tunica adventitia include fibroblasts, mast cells, macrophages and smooth muscle cells (Keech 1960; Humphrey, 1995; Gabella, 1999). In addition, other cells include myofibroblasts (Wilcox and Scott, 1996), "pericyte-like" cells (Canfield *et al.*, 2000), and mesenchymal cells (Howson *et al.*, 2005; Torsney *et al.*, 2005)). Vascular dendritic cells displaying distinct unique ultrastructural features, as well as B and T lymphocytes have also been identified (Bobryshev and Lord 1995; Weyand *et al.*, 2005; Galkina *et al.*, 2006).

1.2.1.4 Vasa vasora in the aortic wall

Vasa vasora are small vascular channels found within, and provide nutrition to the walls of larger vessels. They play an important role in aortic wall structure and function (Heistad and Marcus, 1979; Stefanadis *et al.*, 1995; Angouras *et al.*, 2000). Those which arise from the lumen of the vessel they supply are called vasa vasora interna, while those that arise from adjacent vessels are called vasa vasora externa (Gossl *et al.*, 2003; Lametschwandtner *et al.*, 2004). Based on their location, there are three groups of vasa vasora namely those in the tunica media, those between the tunica media and tunica adventitia, and those in the tunica adventitia (Chuncher and Somana, 2005).

In the aortic wall, the type and density of vasa vasora are influenced by the size of blood vessel, thickness of the vascular wall (Geiringer, 1951; Wolinsky and Glagov, 1967b; Kachlick *et al.*, 2002); oxygen content of luminal blood (Heistad *et al.*, 1981) and the activeness of the vascular wall in regulating the amount of blood flow to organs (Chuncher and Somana, 2005). A commonly accepted view is that the first 0.5 mm thickness or 29 lamellae after the internal elastic lamina is avascular and obtains nutrition by diffusion from the luminal blood (Heistad *et al.*, 1978; Stefanadis *et al.*, 1995; Angouras *et al.*, 2000). In portions of the aorta where the media exceeds the critical thickness of 0.5 mm and 29 lamellae, nutrition of the wall is supplemented by vasa vasora penetrating into the tunica media (Heistad and Marcus, 1979; Marcus *et al.*, 1985; Stefanadis *et al.*, 1995).

1.2.2 Regional variations in the structure of aorta

The most widely documented regional variation of the aortic wall is the quantity of elastin relative to collagen in the tunica media. Biochemical studies demonstrated that, followed craniocaudally, elastin decreased while collagen increased (Harkness *et al.*, 1957; Grant, 1965; Stefanovitch, 1970). Accordingly, elastin is the major component of the wall of the proximal aortic segments while collagen constitutes the major component of the abdominal aorta (McCloskey and Cleary, 1974). For example, elastin makes upto 67% of the thoracic aorta, and 28% of the abdominal aorta (Song and Roach, 1984). Further, morphometric studies have revealed that the number of elastic lamellae in the aortic media decreases craniocaudally from the aortic arch to the iliac bifurcation (Kartzberg 1966; Wolinsky and Glagov, 1967a, 1969; Roach, 1983). The reduction in the number of lamellar units corresponds to the reduction in the diameter of the aorta (Awal *et al.*, 1995; Sokolis *et al.*, 2002; Mello *et al.*, 2004; Orsi *et al.*, 2004).

The type of collagen has also been shown to exhibit regional variation (Howard and Macarak, 1989). In the ascending aorta, type I, III and IV collagen co-localize in the intimal and medial layers, while in the descending thoracic and abdominal aorta, type III collagen is only present in the tunica adventitia. The proportion of smooth muscle cells increases craniocaudally (McCloskey and Clearly, 1974; Yamanouchi *et al.*, 1995). These workers further reported that irregularity of medial smooth muscle cell contours and an amount of smooth muscle cells

associated with collagen fibres were more prominent in the thoracic than in abdominal aorta.

The thickness of the tunica adventitia shows regional variation such that it is thickest in the ascending and thoracic aorta, and thin in the abdominal aorta (Hass *et al.*, 1990; Orsi *et al.*, 2004). The frequency and density of vasa vasora progressively decreases down the aorta, with a sharp drop in the abdominal aorta (Wolinsky and Glagov, 1967b, 1969; Glagov, 1972). In the thoracic aorta, numerous vasa vasora form a perivascular plexus and penetrate into the tunica media (Wolinsky and Glagov, 1969; Heistad and Marcus, 1979; Marcus *et al.*, 1985; Stefanadis *et al.*, 1995). In the abdominal aorta, on the other hand, it has been reported that vasa vasora are absent (Gerringer, 1951; Wolinsky and Glagov, 1969 Heistad *et al.*, 1978).

Apart from the biochemical and anatomical differences between the thoracic and abdominal aortae, studies of aortic embryogenesis have revealed that neural crest cells contribute to the formation of smooth muscle cells in the aortic trunk, and proximal arch, while distal thoracic and abdominal aortic smooth muscle cells derive primarily from mesoderm (Gadson *et al.*, 1997). Furthermore, there are striking differences in gene expression patterns between the thoracic and abdominal aortae (Absi *et al.*, 2003).

1.2.3 Adrenergic Innervation of the aorta

The aorta is innervated by adrenergic nerves which penetrate into the tunica media to form plexuses whose end fibres extend between the muscle cells (Kirby and Burnstock, 1969; Abraham, 1969; Dolezel, 1972; White *et al.*, 1973; Nilson *et al.*, 1988). In many cases each nerve ending contacts specific muscle fibres in the tunica media, while in others, nerve fibres permeate between the muscle cells of the tunica media and form neuro-muscular junctions (Abrahams, 1969).

In general, however, adrenergic innervation of the aorta is believed to be less compared to that of muscular arteries (Bevan *et al.*, 1972; Burnstock and Costa, 1975; Cowen and Burnstock, 1980; Luff and McLachlan, 1989), and there is no direct innervation of the tunica media (Leung *et al.*, 1976). Further, the catecholamine containing neurons are confined to the media-adventitia border, (Burnstock, 1975; Burnstock and Costa, 1975). Notably, even in the cases where there is medial penetration of nerves, the few adrenergic nerve terminals do not penetrate deeper than the outer half of the aortic wall (Gerova *et al.*, 1973). The localization of nerves predominantly at the media-adventitial border has also been demonstrated for the non adrenergic modes of innervation such as cholinergic and VIPergic (Amenta *et al.*, 1980; Uddman *et al.*, 1981; Connat *et al.*, 2001b).

1.2.4 Function and physicomechanical properties of the aorta

The aorta serves as a low resistance conduit to transfer blood from the heart to the peripheral vessels. Besides, it acts as an elastic buffering chamber behind the heart, which stretches and stores about 50% of the left ventricular stroke volume during systole. In diastole, the recoil of the elastic fibres of the aortic wall forwards this blood to the peripheral circulation, thus creating a nearly continuous peripheral blood flow. This systolic-diastolic interplay is referred to as the "**windkessel mechanism**" (Belz, 1995; Roberts *et al.*, 1995). The efficiency of this mechanism is essential to ensure left ventricular performance, myocardial perfusion and the normal function of the entire cardiovascular system (Boudoulas and Wooley, 1996). The stretch-recoil mechanism further enables the aorta to dampen out the pulsatile flow and blood pressure delivered by the heart, thus limiting distal shear stress and allowing regular irrigation of the peripheral organs (Faury, 2001).

The most important mechanical property of the aorta is non-linear elasticity, characterized by bending away from the axis of elongation with increased pressure (Burton, 1954; Roach and Burton, 1957; Cameroon, 1999; Levy, 1999; McVeigh *et al.*, 2002). According to these workers, the non linear elasticity with J-shaped stress-strain curve may be a product of a parallel arrangement of elastin and collagen. In this model, elastin serves to bear stress at low pressure and distributes it evenly throughout the wall and onto collagen fibres, which in turn

bears the stress at, and above physiological pressure (Roach and Burton 1957; Glagov and Wolinsky, 1963; Wolinsky and Glagov, 1964). Recent studies support this postulation and suggest that, high strain behaviour is consistent with in-series arrangement of collagen fibres and smooth muscle cells (Silver *et al.*, 1989, 2003a).

1.2.5 Age related changes of the aorta

Studies on age related chemical changes in the aorta of several species showed that vascular aging follows the same general pattern in mammals (Berry *et al.*, 1972; Looker and Berry, 1972; Cox, 1977). Endothelial cells of old rat aorta have irregular shapes and sizes, long protrusions, develop solitary cilia and buldge into the lumen (Laver-Rudich *et al.*, 1978; Haudenschild *et al.*, 1981). They also show more pinocytotic vesicles on their luminal surface (Laver-Rudich *et al.*, 1978; Briffeuil *et al.*, 1994). The cells assume bizarre shapes with irregular nuclei (Haudenschild *et al.*, 1981). An increase in the proportion of binucleate cells, and those with pycnotic nuclei was reported by Gansburgskii and Pavlov, (1994). Furthermore, the number of leucocytes adhering to the endothelium also increased with age (Aliev *et al.*, 1995). In addition, apoptosis, reduced cell density and increased frequency of aneuploidy of aortic endothelial cells occurs with age (Asai *et al.*, 2000; Aviv *et al.*, 2001).

In the subendothelium, the age related structural changes include increase in number and heterogeinity of cells including mononuclear cells, macrophages, lymphocytes and smooth muscle cells (Danon *et al.*, 1980; Haudenschild *et al.*, 1981; Aliev *et al.*, 1995). The subendothelium may also increase in thickness with basement membrane-like granular material accounting for the bulk of this thickening (Guyton *et al.*, 1983; Bonert *et al.*, 2003).

Age-related changes in the tunica media of the aorta are characterized by degenerative changes in smooth muscle cells (Toda *et al.*, 1980; Kojimahara, 1985; Ooyama and Sakamato 1995a, b). Numerous smooth muscle cells become more secretory and form collagen and glycosaminoglycans, while others completely de-differentiate to the proliferative phenotype with advancing age (Bouissou *et al.*, 1987; Levy, 1992; Moon *et al.*, 2003). Tetraploidy also increases exponentially with age, and may serve as an indicator of age (Jones and Ravid, 2003).

The most consistent extracellular change in the tunica media is elastic fibre degradation, with branching, breakage and disorganization of elastic lamellae (Dalessandri *et al.*, 1994; Ooyama and Sakamato, 1995a, b; Zhu *et al.*, 2001; Connat *et al.*, 2001b). There is also an increase in thin collagen fibrils between medial smooth muscle cells (Nakamura and Ohtsubo, 1992; Zhu *et al.*, 2001).

Calcification of the different layers of the various segments of the aged aorta have been reported (Nagpal *et al.*, 1976a,b; Tohno *et al.*, 2001; Moriwake *et al.*, 2001; Ohnishi *et al.*, 2003). This age-related calcification occurs at two sites of the vessel wall namely in the tunica media where it is known as Monckenberg's sclerosis, and in the intima where it is commonly associated with atherosclerosis (Shanahan *et al.*, 1998).

1.2.6 Common disruptions of the aortic wall

Most of the diseases of the aorta involve disruption of one component or the other in the wall. In most cases the diseases are associated with each other and their effects are synergistic. The most common of these are hypertension, atherosclerosis and aneurysm formation. Hypertension is associated with increased wall thickness (Tobian, 1969; Jiang *et al.*, 2000; Bacarini-contri *et al.*, 2003; Palivoda *et al.*, 2004). This increased wall thickness results from changes in the tunica intima (Hadjiisky and Peyri, 1982) tunica media (Ferrante *et al.*, 1994; Kacem *et al.*, 1995) and tunica adventitia (Howard and Macarak, 1989; Xu *et al.*, 2000). In the tunica intima, the main changes are increased endothelial cell volume and subendothelial thickness (Gabbiani *et al.*, 1979; Lesauskaite *et al.*, 1999).

Most of the changes due to hypertension, occur in the tunica media, where there is increased accumulation of all major extracellular matrix components including both elastin and collagen (Wolinsky, 1970, 1971; Cleary and Moont, 1976; Keeley and Alatawi, 1991; Baccarani-Contri *et al.*, 2003). The additional elastin and collagen accumulated during hypertension are deposited in the interlamellar spaces (Wolinsky, 1971; Tudorovich-Hunter *et al.*, 1988; Bashey *et al.*, 1998), resulting in decreased distensibility (Cox, 1981; Tozzi, *et al.*, 1994).

Other structural changes which occur in the aortic wall during hypertension include smooth muscle hyperplasia (Daly and Gurpide, 1959; Tobian *et al.*, 1969; Bodin *et al.*, 1987) and hypertrophy (Weiner *et al.*, 1977; Owens *et al.*, 1981; Owens and Schwartz, 1982); altered orientation with jumbling up (Yoneda *et al.*, 1981); increase in nexus junctions (Berry and Sosa-melgarejo, 1989; Sosa-Melgarejo *et al.*, 1991), and increased cell-matrix anchoring (Bezie *et al.*, 1998; 1999).

Atherosclerosis is a vaso-occlusive disease characterized by formation of fatty streaks and fibromuscular plaques, usually in the subendothelial zone (Ross and Agius, 1992; Rosenfeld, 1996). The main histopathogenic events may be summarized as follows: (i) adhesion of monocytes and lymphocytes to the endothelial surface, (ii) migration of monocytes by chemotaxis into the subendothelial zone and differentiation into macrophages, (iii) ingestion of low density lipoproteins, and modified or oxidized low density lipoproteins by macrophages, (iv) accumulation of cholesterol esters and formation of "foam cells". These foam cells together with T lymphocytes form the fatty streak.

Vascular smooth muscle cells migrate from the tunica media into the intima, proliferate, and synthesize connective tissue leading to the formation of atherosclerotic plaques. Simultaneously, there is inflammatory (Ross, 1999; Libby, 2002) and immune activation of macrophages and dendritic cells (Johasson *et al.*, 1986; Glass and Witztum, 2001; Yilmaz, 2004) and lymphocytes

(Caligiuri *et al.*, 2002; Whitman, 2004; Tupin, 2004). The overall effect of this inflammatory response is extensive destruction of smooth muscle cells and elastic lamellae in the tunica media (Nakatake and Yamamoto 1987; Lavezzi *et al.*, 2005), focal necrosis (Zhdanov, 1993), and duplication of the internal elastic lamina (Lee, *et al.*, 2005; Jones *et al.*, 2005).

The commonest complication of atherosclerosis is stenosis and occlusion of the artery causing ischaemic injury of the dependent vascular bed (Ross 1999; Libby, 2002). Other potentially lethal complications of atherosclerosis are penetrating atherosclerotic ulcers (PAU) (Welch *et al.*, 1990; Braverman, 1994; Chiche and Leseche, 2000; Cho *et al.*, 2004) and aneurysms. The ulcers usually occur more frequently in the ascending aorta and thoracic aorta (Sato *et al.*, 1994), and may be complicated by transmural aortic rupture, embolization, or progressive aneurismal dilatation (Braverman, 1994).

Aortic aneurysm formation is characterized by medial weakness due to fragmentation and loss of elastic fibres, interstitial collections of collagenous tissue and basophilic ground substance (Hasham *et al.*, 2002; McFadden *et al.*, 2004; Sakalihasan *et al.*, 2005) and apoptosis of smooth muscle cells (Sinha *et al.*, 2005). Dissecting and inflammatory aneurysms occur more commonly in the proximal aorta, while atherosclerotic aortic aneurysms occur more commonly in the infrarenal segment of the abdominal aorta (Hoffman, 2005).

1.3 Objectives of the study

1.3.1 Broad Objective

To describe the structure and adrenergic innervation of the goat aorta.

1.3.2 Specific Objectives

a) To determine the structure of the various layers of the goat aortic wall.

b) To identify the regional differences in the wall of the goat aorta.

c) To demonstrate the pattern of adrenergic innervation of the goat aorta.

d) To find out the pattern of histomorphological changes that occur in the wall of the goat aorta during aging.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Experimental animals

The present study was done on 24 healthy male domestic goats *(Capra hircus)* aged between 6 and 120 months and weighing 10 - 60 kg (Figure 1). The animals were purchased from private livestock farmers in Nairobi. Only animals certified to be healthy by a veterinary doctor and whose age was known from farm records were used. The goats were divided into three age groups of eight animals each. These groups were, juvenile, 6 -12 months; young adults, 18 -24 months and old, 60 - 120 months. Four animals in each age group (total of 12) were used for light microscopic studies and eight (four young adults and four old ones) were used for electron microscopy. Histochemistry was done on four juveniles only.

The animals were weighed then euthanised with an overdose of sodium pentobarbitone 20 mg/ml injected intravenously. After attaining complete euthanasia, the ventral aspect of the animals was shaved. To expose the heart, and abdominal cavity, the thorax was opened by cutting through the costochondral junctions and lifting off the ribs and the sternum, and the abdomen opened through a ventral midline incision in the abdominal wall.

Figure 1 A-D: Photographs of Experimental animals

A: Photograph of a herd of domestic goats, showing various age groups. Note the herdsmen in the background.

B: Photograph of a 6 months old goat in a goat cave.

C: Photograph of a 24 months old goat beside a Maasai Manyatta (M). No a herd's boy holding the left forelimb, and other goats in the backyard.

D: Photograph of a 120 months old goat.

Figure 1

2.2 Fixation and sampling of the aorta

The animals were fixed by gravimetric perfusion. The pericardium was opened through a vertical incision to expose the entire heart. To clear blood from the animal, a canula was inserted into the left ventricle, 0.2 litre/Kg body weight of normal saline introduced from a container 150 cm above the heart, and the right auricle punctured to drain out the blood. After satisfactory clearing, in the animals for light microscopy, 10% formaldehyde solution 0.2 litre/Kg body weight was introduced over 30 minutes.

The aorta was then exposed by retracting away the lungs, slitting the thoracic diaphragm, and turning the abdominal viscera to the right side. Periaortic fat was gently dissected away and five millimeter long transverse specimens taken from each of the following areas: the ascending aorta; aortic arch, at each vertebral level down to T13 and abdominal aorta from pre-renal, renal and post renal segments (Figure 2). At adjacent sites of the same vertebral level, longitudinal sections were also taken. The specimens were further fixed by immersion in 10% formaldehyde solution for 72 hours.

In the animals for electron microscopy, similar procedure was followed except that 3% phosphate buffered glutaraldehyde solution 200 ml/Kg body weight was used to fix the aorta, and specimens were taken from the ascending aorta,

aortic arch, proximal thoracic aorta (T6); middle thoracic aorta (T9) and distal thoracic aorta (T12), and abdominal aorta. The specimens were immersed in 3% phosphate buffered glutaraldehyde solution for 72 hours for further fixation, and subsequently post fixed with 1% phosphate buffered osmium tetroxide solution for 48 hours.

Histochemistry was done to demonstrate adrenergic nerves. In the animals for this method of study, specimens were obtained as soon as possible after the animal was euthanized, without fixation, from the same regions and intervals as described for electron microscopy.

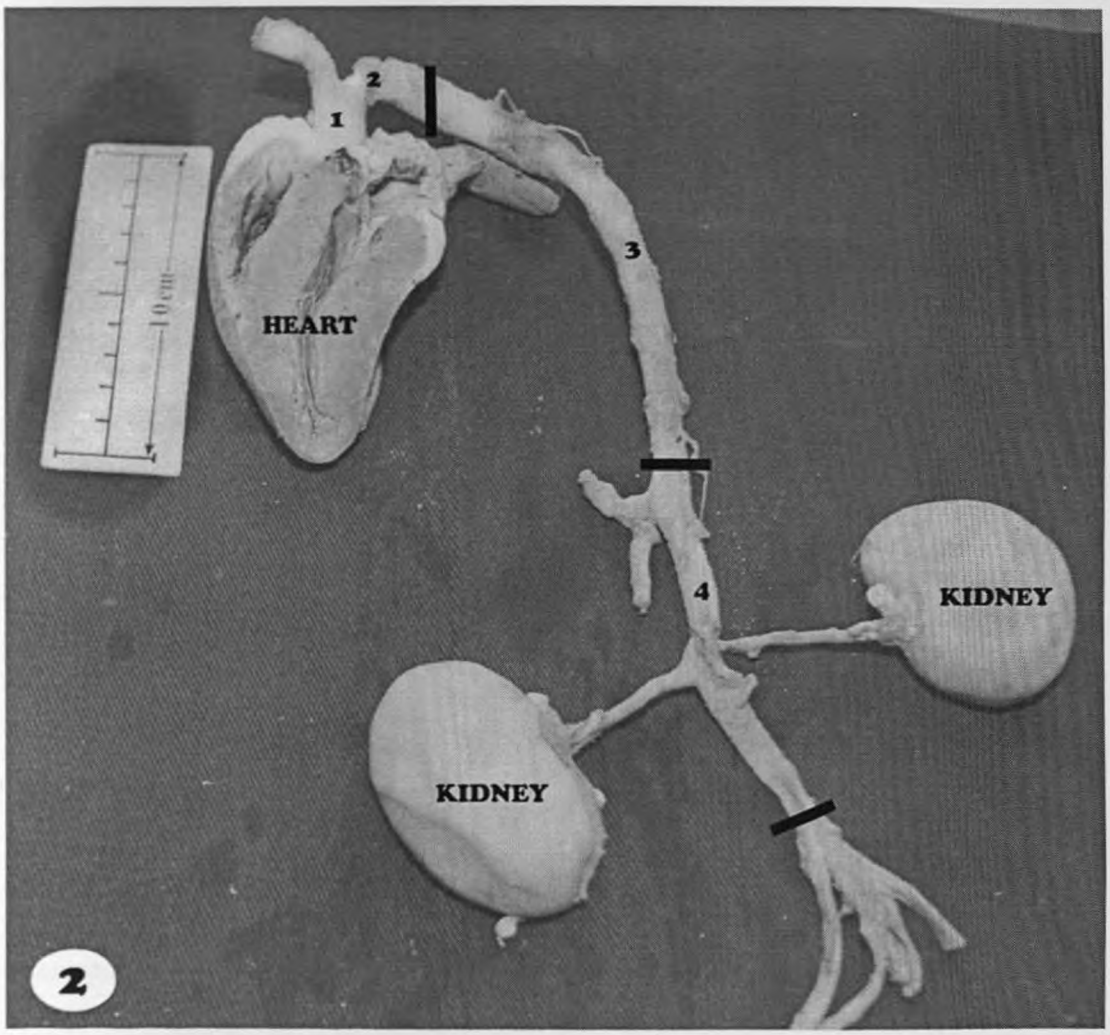


Figure 2: Photograph of the goat aorta showing the sites from where samples were taken. Ascending aorta (1), aortic arch (2), thoracic aorta (3) and abdominal aorta (4).

2.3 Processing of specimens

The specimens were processed using different protocols for light microscopy, electron microscopy and histochemistry.

2.3.1 Processing for light microscopy

The sections were trimmed and processed routinely for paraffin embedding by passing them through graded concentrations of Isopropyl alcohol, (50%; 60%; 70%; 80%; 90%; 95% and 100%) each for one hour and cleared with cedar wood oil for 12 hours. The sections were then infiltrated with fresh molten paraplast wax for 12 hours, and embedded in wax, in steel moulds. Seven micrometer sections were made using a ***leitz wetzlar***© sledge microtome, floated in water at 37^0c, then stuck onto glass slides using egg albumin, applied as a thin film with a sponge. The slides were dried in an oven at 37^0c for 24 hours, then stained with the Weigert resorcin - fuchsin stain and counterstained with Van Gieson stain for demonstration of elastic fibres; and with Mason's trichrome stain for demonstration of other mural components (Drury *et al.*, 1967). The slides were then examined at various magnifications, using a ***Leica BM*** © microscope.

2.3.2 Processing for transmission electron microscopy

The post-fixed specimens were rinsed in sodium phosphate buffer for 15 minutes then dehydrated by passing through increasing concentrations of ethanol (50%;

60%; 70%; 80%; 90%; 95% and 100%) for 30 minutes each, and twice for 1 hour each in absolute ethanol. The sections were then cleared in propylene oxide for 30 minutes. Subsequently, the sections were infiltrated in catalyst free durcupan mixture I as follows: propylene oxide: durcupan 3:1 - 30 minutes; propylene oxide: durcupan 1:1 - 30 minutes; propylene oxide: durcupan 1:3 - 30 minutes and absolute durcupan at 60°C in oven for one hour. The sections were then embedded in 100% durcupan with catalyst, and polymerized in an oven at 60°c, for 48 hours. The blocks were trimmed and 1µ thick (semithin) sections cut using ***Reichert ultramicrotome***©. The sections were then stained by applying a drop of tuolidine blue, mounted on DPX, examined with ***Leica BM***© light microscope at magnification of x 100, 250, 400 and photographed using ***orthomax-Zeiss***© camera on ***ortholux***© light microscope.

For electron microscopy, the blocks were trimmed and ultrathin sections made with ***Reichert ultramicrotome***©. These sections were collected on 200 mesh copper grids, stained with uranyl acetate, counterstained with lead citrate (Glauert, 1965), and examined by ***EM 201 Phillips***© electron microscope.

2.3.3 Processing for histochemistry

Fresh 2 mm long transverse specimens were taken, wrapped in aluminium foil and stored in dry ice. Embedding of the material was done using OCT compound (Tissue - Tek II) in a cryostat chamber at -30°c. Sections of 16 µm

thickness were cut and picked by use of a clean but non-treated glass slides at room temperature. Cut sections were prepared for demonstration of tissue monoamines by the sucrose-potassium phosphate - glyoxylic acid (SPG) method as described by De La Torre and Surgeon (1976), and applied by Kimani *et al.* (1991).

The sections were then given 3 dips (1dip/sec) in the SPG solution. Excess solution was drained off and the slides dried at a temperature of 40^0c with a hair drier. The slides were then placed in an oven maintained at 100^0c under liquid paraffin for 5 minutes. They were removed and cover-slipped using fresh liquid paraffin. Examination of the slides was done under a ***Leitz ortholux***© fluorescent microscope, using a 250/4 ultra high pressure mercury lamp with a Leitz Bp 546/filter block.

2.4 Morphometric analysis of the aorta

Morphometry was done to determine luminal diameter, wall thickness and lamellar counts. To measure the luminal diameter, 1 mm thick transverse sections taken from the ascending aorta; aortic arch, each vertebral level of the thoracic aorta, prerenal, renal, and postrenal segments of the abdominal aorta were laid on a black horizontal surface. The internal diameter was measured using a Varneer© calipers to the nearest 0.1 mm in four different directions and

the average taken. Wall thickness was measured on photomicrographs taken on every fourth random slide from the various segments of the aorta at four different points at magnification of x35. The thickness was measured and the obtained values corrected for magnification, then the average taken.

Concentric lamellar unit counts were done on transverse sections. For each of the regions namely ascending aorta, aortic arch and at each vertebral level of the thoracic aorta; pre-renal, renal, and post-renal segments of the abdominal aorta, twenty good quality slides stained with Weigert Resorcin Fuchsin/Van Gieson stain were selected. For each of the regions, every fourth slide was used. On each slide, the section was photographed at magnification x100 in four systematically selected random fields. Complete lamellae were then counted, and the mean of the four fields recorded as the count for each region. The most luminal and adventitial lamellae were regarded as the internal and external elastic laminae respectively and were therefore not included. Disintegrated, interrupted or longitudinal lamellae were excluded from the count.

The mean values, and the standard deviations of these means were calculated for each parameter namely luminal diameter, wall thickness and elastic lamellae counts, at the different levels and presented in form of graphs.

CHAPTER THREE

RESULTS

3.1 General observations on the aorta

The goat aorta, consists of four main parts namely the ascending aorta, aortic arch, descending thoracic aorta and abdominal aorta (Figure 3A-D). The ascending aorta is a short segment extending from the left ventricle to about the 3rd thoracic vertebra, where it gives the brachiocephalic trunk and continues as the aortic arch, an almost imperceptible segment, at the level of the 4th thoracic vertebra, which continues caudally as the descending thoracic aorta (Figure 3A). This part gives esophageal branches and intercostal arteries and, extends from the 5th thoracic vertebra to the diaphragmatic hiatus at the level of the 13th thoracic vertebra (Figure 3B). It continues caudally as the abdominal aorta, which extends down to the 6th lumbar vertebra to give rise to the terminal internal iliac arteries (Figure 3C,D).

The diameter of the aorta decreases as it progresses caudally (Figure 4A,B). In addition, the various segments vary in wall thickness, with values showing a progressive craniocaudal decline. Both the mean diameter and wall thickness show a sharp drop at T8 (Fig 4C,D; N=16).

Figure 3A-D: Parts of the goat aorta

A: Macrograph of the goat aorta showing its parts, namely ascending (1), arch (2) and proximal part of the descending thoracic aorta (3). Note that the ascending aorta divides into the brachiocephalic trunk (bct) and aortic arch.

B: Macrograph of the goat thoracic aorta, showing intercostal arteries (IC).

C: Macrograph of goat abdominal aorta (4). Note that it extends from the thoracic diaphragm (TD) and gives among other branches, the coeliac artery (5), renal artery (6).

D: Macrograph of the goat abdominal aorta (4), showing the external (EI) and, the internal iliac arteries (stars) branches.

Figure 3

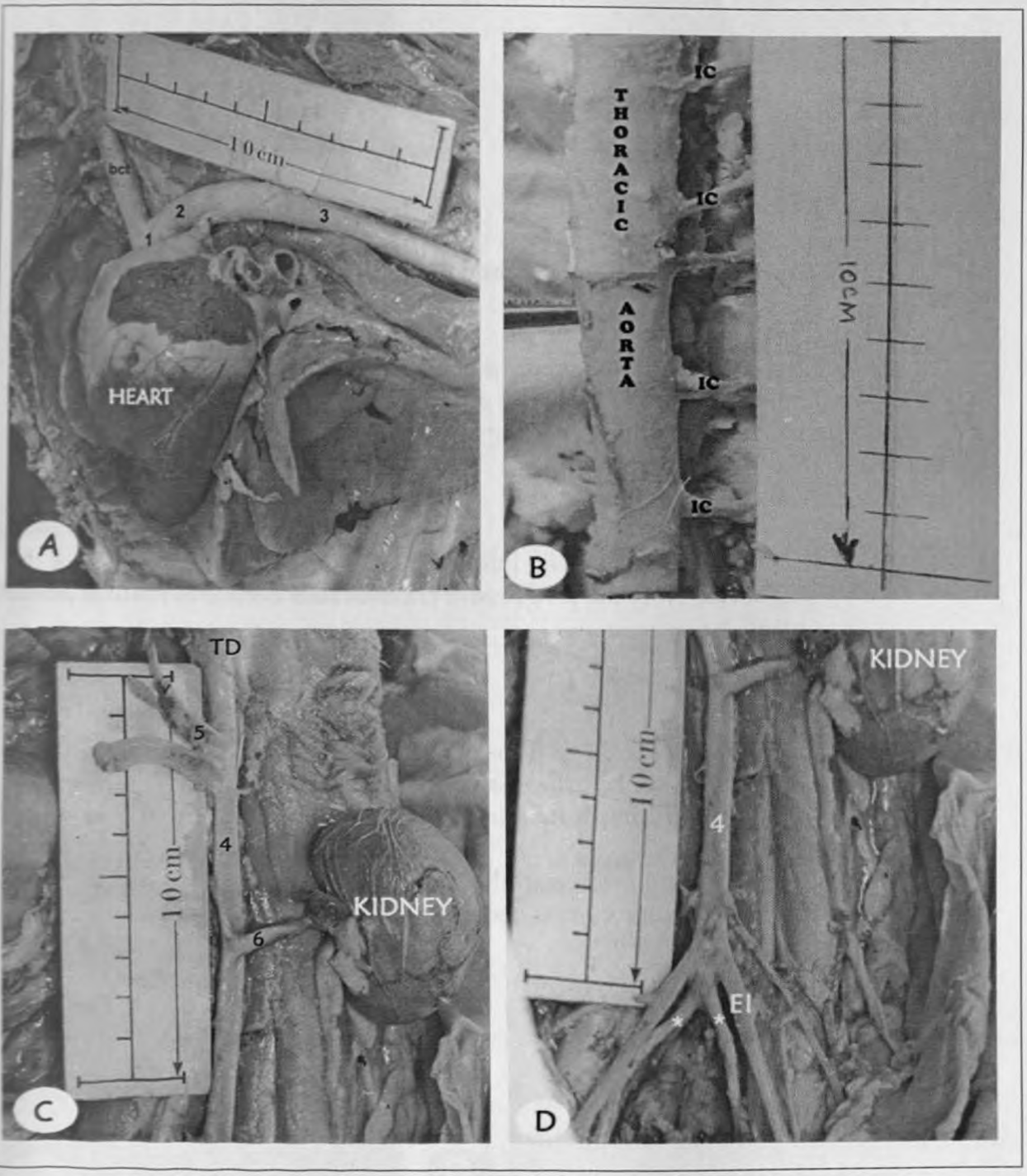

10 cm
bct
1
2
3
HEART
A
THORACIC
AORTA
IC
IC
IC
IC
10CM
B
TD
5
4
6
10 cm
KIDNEY
C
KIDNEY
10 cm
4
EI
D

Figure 4A-D: Luminal diameters and wall thickness of goat aorta

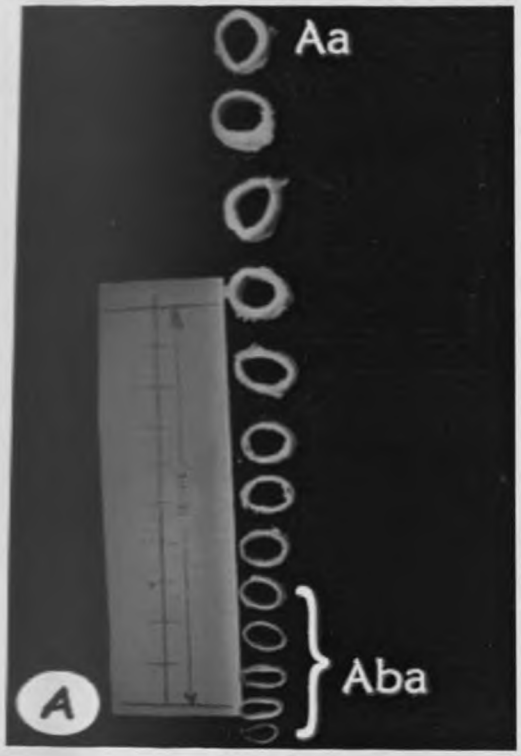
A: Macrograph of cross sections of goat aorta at various levels showing luminal diameter. Note the progressive craniocaudal decline in luminal diameter from the ascending aorta (Aa) down to the abdominal aorta (Aba).

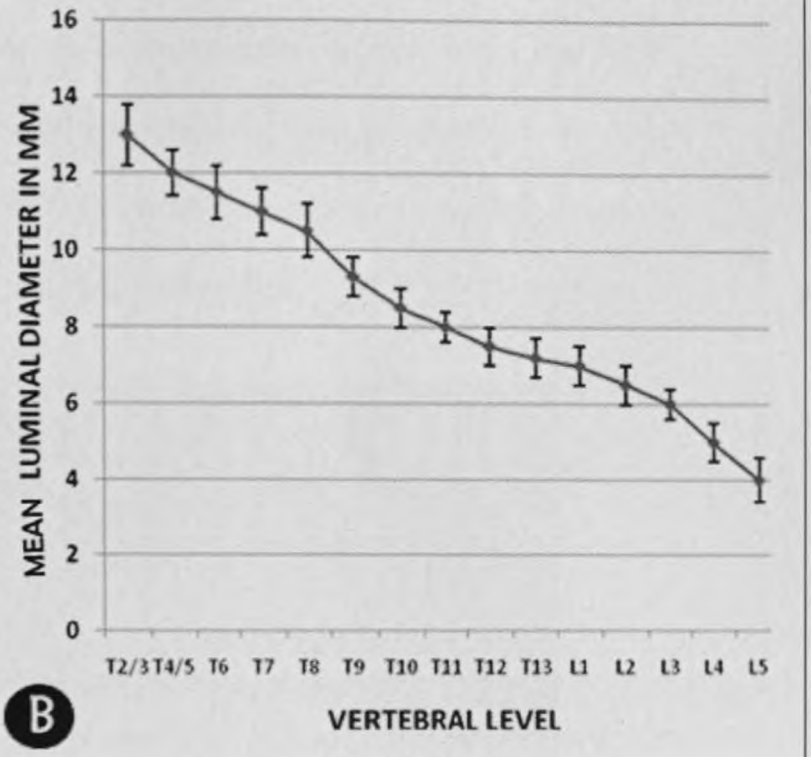
B: Graph showing the mean internal diameter of different segments of the goat aorta. Note the progressive craniocaudal decline in luminal diameter (n=16)

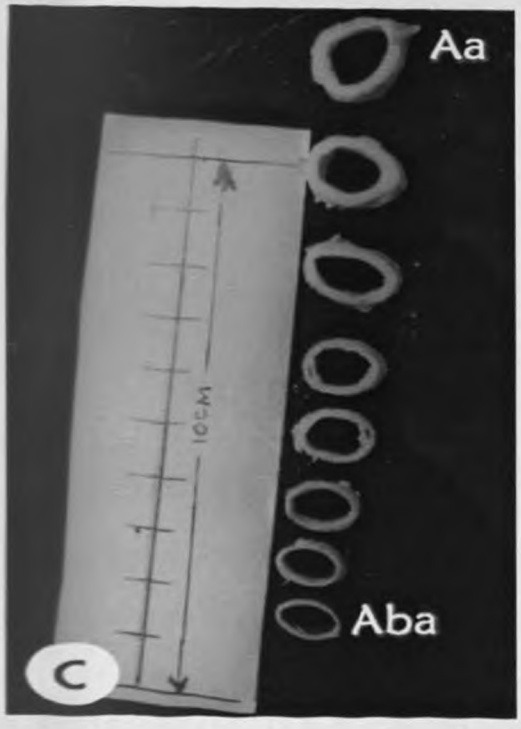
C: Macrograph of cross sections of goat aorta at various levels showing wall thickness. Note the progressive craniocaudal decline in wall thickness from the ascending aorta (Aa) to the abdominal aorta (Aba).

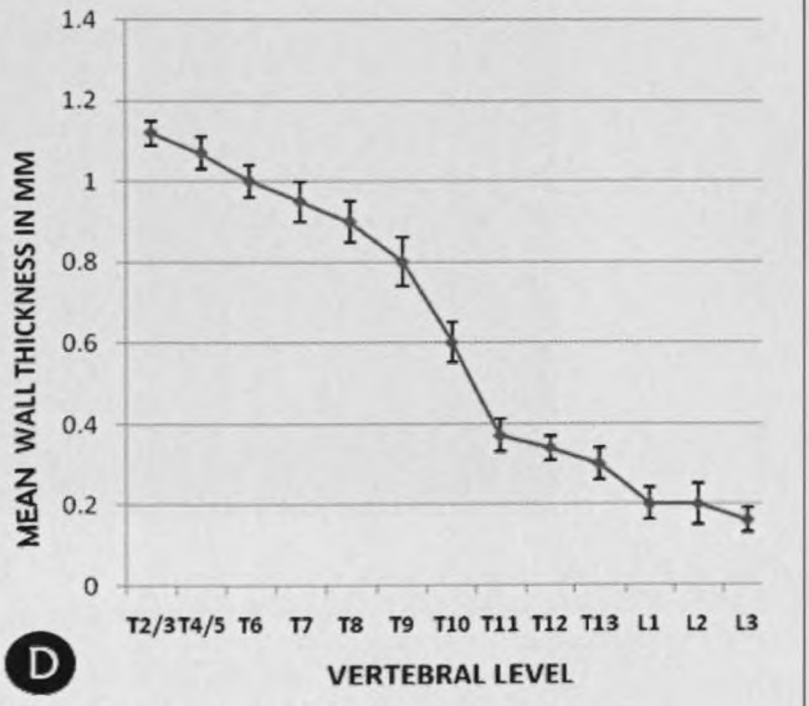
D: Graph showing mean wall thickness of the goat aorta. Note the progressive craniocaudal decline in the wall thickness.

Figure 4


Aa
Aba
A


MEAN LUMINAL DIAMETER IN MM
16
14
12
10
8
6
4
2
0
T2/3 T4/5 T6 T7 T8 T9 T10 T11 T12 T13 L1 L2 L3 L4 L5
VERTEBRAL LEVEL
B


Aa
Aba
C


MEAN WALL THICKNESS IN MM
1.4
1.2
1
0.8
0.6
0.4
0.2
0
T2/3 T4/5 T6 T7 T8 T9 T10 T11 T12 T13 L1 L2 L3
VERTEBRAL LEVEL
D

3.2 Structure of the aorta

The goat aortic wall comprises, a tunica intima, tunica media and tunica adventitia (figure 5). General structural features of juvenile and young adult goat aortae are similar, so results are reported together, from the tunica intima outwards to the tunica adventitia.

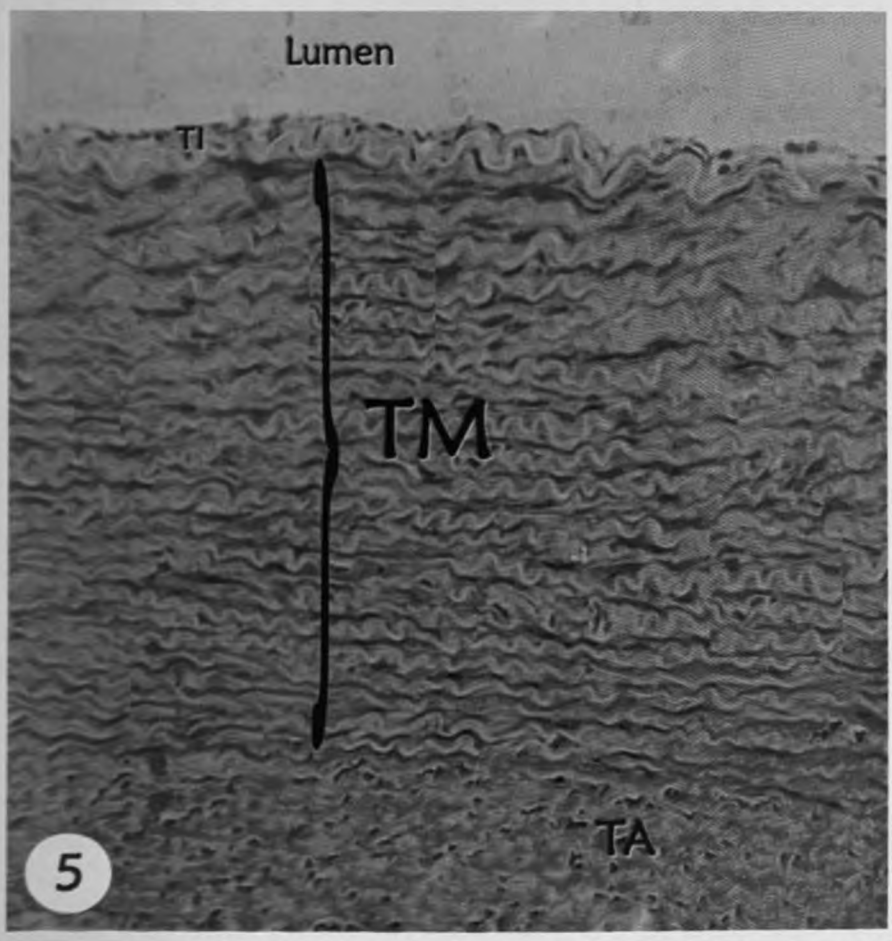


Figure 5: Photomicrograph of the goat abdominal aorta showing layers of the wall, namely tunica intima (TI), tunica media (TM) and tunica adventitia (TA). Mason's trichrome stain. Magnification x100.

3.2.1 The tunica intima

Tunica intima comprises three layers, namely endothelium and its basal lamina, a variable subendothelial zone and internal elastic lamina (Figure 6A). Endothelium consists of a single layer of flat and round cells (Figure 6B-D). In the flat cells, the cell membranes are pitted with caveolae (Figure 6C). The generally smooth basal surface is supported on a thin basal lamina, frequently connected to the subendothelial elastic fibres while the nucleus is dented, elongated and predominantly euchromatic (Figure 6C). Round endothelial cells, on the other hand, display an irregular luminal surface and a basal surface, bearing long branched processes which project into the subendothelial zone, (Figure 6D). Lateral processes of these round cells also show protrusions into the subendothelial zone. The large preponderantly euchromatic nucleus is generally irregular.

Intercellular junctions between the endothelial cells are of two types, namely a plane angular junction with a single extension anchoring into a cleft (Figure 6E) and an interdigitation between finger like lateral extensions of the cell membranes (Figure 6F). In both cases, adjacent cell membranes show high electron density. The basal surface of the endothelial cell is connected to the basal lamina by focal areas of high electron density (Figure 6G). Endothelial cells are attached to the subendothelial connective tissue, either through a thin simple basal lamina (Figure 6E,G); or a lamellated basement membrane (Figure 6F, H).

Figure 6A-H: Endothelial cells and their attachments in the goat aorta

A: Photomicrograph of the goat distal thoracic aorta showing components of the tunica intima, namely endothelium (arrow) subendothelial zone (SEZ) and internal elastic lamina (arrowhead). Mason's trichrome stain. Magnification x250.

B: Photomicrograph of transverse section of the goat ascending aorta, showing two morphological types of endothelial cells. Note the round (arrow), and flat (arrowhead) endothelial cells. Mason's trichrome stain. x400.

C: Electronmicrograph of the tunica intima of the goat proximal thoracic aorta, showing a flat endothelial cell. Note the shallow caveolae on the luminal surface (arrow) and a thin basal lamina (BL), connected to subendothelial elastic fibres (ef). Magnification x27,800.

D: Electronmicrograph of the tunica intima of the goat distal thoracic aorta showing a round endothelial cell. Note the basal cytoplasmic extensions (stars) projecting into the subendothelial zone (SEZ) x27,800.

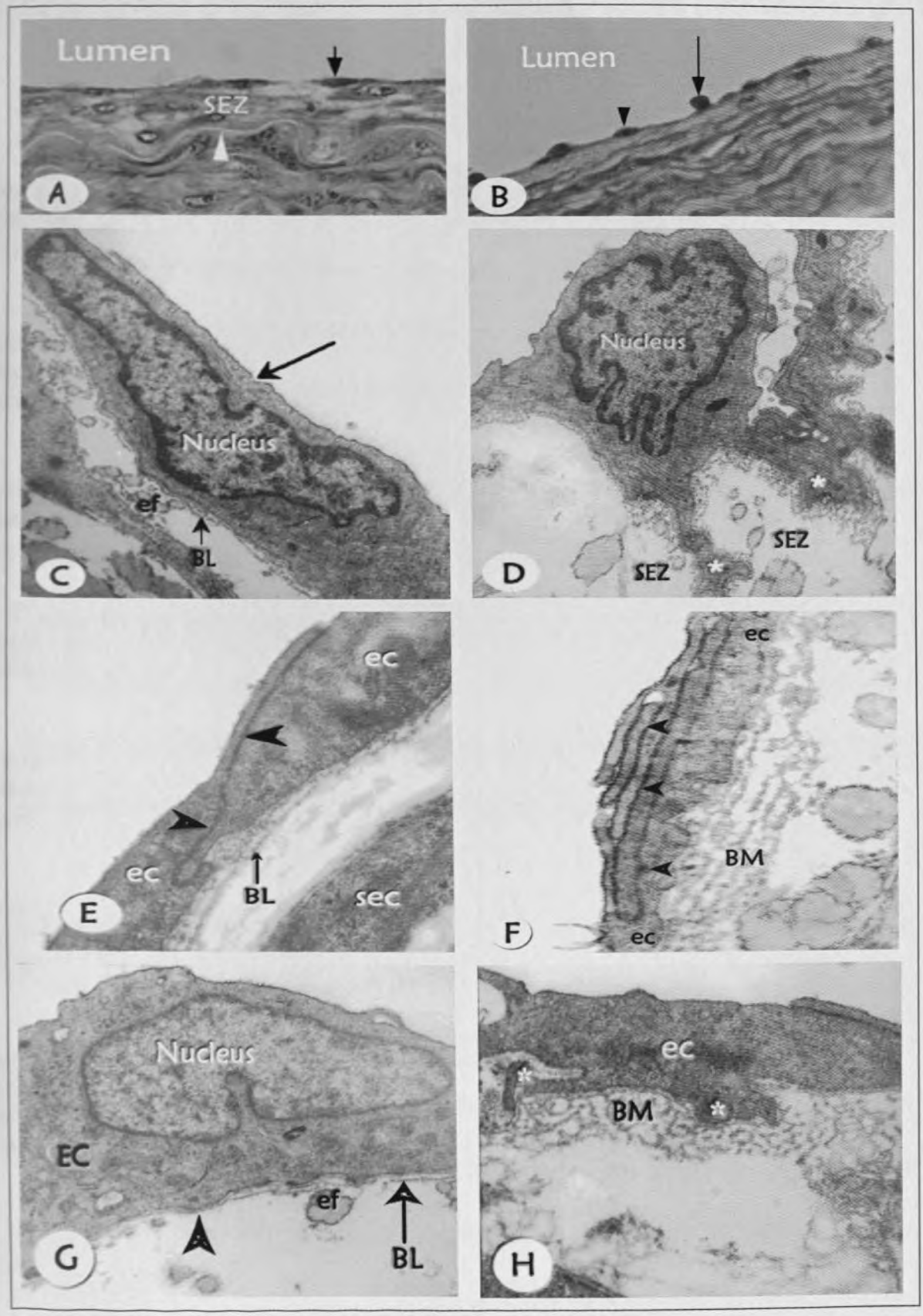
E: Electronmicrograph of the tunica intima of goat proximal thoracic aorta showing a plane intercellular junction (arrowheads) between two adjacent endothelial cells (ec). Note the thin basal lamina (BL) and the subendothelial cell (sec). Magnification x27,800.

F: Electronmicrograph of the tunica intima of the goat proximal thoracic aorta showing an interdigitating junction (arrowheads) between two adjacent endothelial cells (ec). Note lamellated basement membrane (BM). Magnification x8,760.

G: Electronmicrograph of the tunica intima of the goat abdominal aorta showing an endothelial cell (EC) resting on a simple basal lamina (BL). Note the electron dense areas of fusion between the endothelial cell and the basal lamina (arrowhead); and the close association between the basal lamina and subendothelial elastic fibres (ef), Magnification x63,400.

H: Electronmicrograph of the tunica intima of the goat abdominal aorta, showing a lamellated basement membrane (BM) beneath the endothelial cell (ec). Note the basal cell extensions (stars). Magnification x63,400.

Figure 6


Lumen
SEZ
A
Lumen
B
Nucleus
ef
BL
C
Nucleus
SEZ
SEZ
D
ec
ec
BL
sec
E
ec
BM
ec
F
Nucleus
EC
ef
BL
G
ec
BM
H

The subendothelial zone is of variable thickness such that it may be very thin (Figure 7A), or enlarged to form the so called diffuse intimal thickenings (DIT) (Figure 7B). These diffuse intimal thickenings appear mainly on the dorsal aspect, and are made up of a variety of cells, collagen and elastic fibres (Figure 7B,C). They enlarge gradually (Figure 7C). The subendothelial zone is also thickened at bifurcation points, where it is characterized by elastic fibres running at an angle to the elastic lamellae of the tunica media (Figure 7D).

The subendothelial zone contains a morphologically heterogeneous population of cells (Figure 8A-E), divisible into three main categories. The first category of the cells are characterized by presence of remnants of a basal lamina, rough endoplasmic reticulum and a large euchromatic nucleus (Figure 8A). These cells vary in morphology, some with regular cell surfaces, and others with highly irregular surfaces. Many of them are intimately associated with elastic fibres.

A second category of subendothelial cells are large with irregular euchromatic nuclei, have a thin rim of cytoplasm, lack remnants of basal lamina, and contain definite rough endoplasmic reticulum. These cells and their processes are also intimately associated with the elastic fibres (Figure 8B,C). The third category of subendothelial cells are relatively round, with several finger-like projections, a cytoplasm containing electron dense structures resembling primary lysosomes

and a large irregular nucleus. There are also vacuoles in the cytoplasm, some of which appear filled with dark structures (Figure 8D).

Connective tissue of the subendothelium comprises both collagen and elastic fibres generally oriented in all directions. The collagen and elastic fibres are frequently interlinked, insert onto the surface of the smooth muscle cells and connect the basement membrane onto the subendothelial cells (Figure 8E).

Figure 7A-D: Subendothelial zone of the goat aorta

A: Photomicrograph of the tunica intima and media of the goat thoracic aorta showing endothelial cell (ec) and the internal elastic lamina (iel). Note that the subendothelial zone is highly attenuated. Mason's trichrome stain. Magnification x400.

B: Photomicrograph of the tunica intima and media of the goat abdominal aorta, showing enlargement of the subendothelial zone. Note the endothelial cells (ec) separated from the internal elastic lamina (iel) by the subendothelial zone (sez). Mason's trichrome stain. Magnification x400.

C: Photomicrograph of the tunica intima and media of the goat thoracic aorta showing gradual enlargement of the subendothelial zone (sez) and its content of elastic fibres stained black. Note that the endothelial cells (ec) and the internal elastic lamina (iel) get further apart toward the right. Weigert elastic stain. Magnification x100.

D: Photomicrograph of the goat thoracic aorta at the level of 7th thoracic vertebra where an intercostal artery branches off. Note an enlarged subendothelial zone (sez) with longitudinal elastic fibres separated from the tunica media (TM) by a prominent internal elastic lamina (iel) Weigerts elastic stain. Magnification x250.

Figure 7

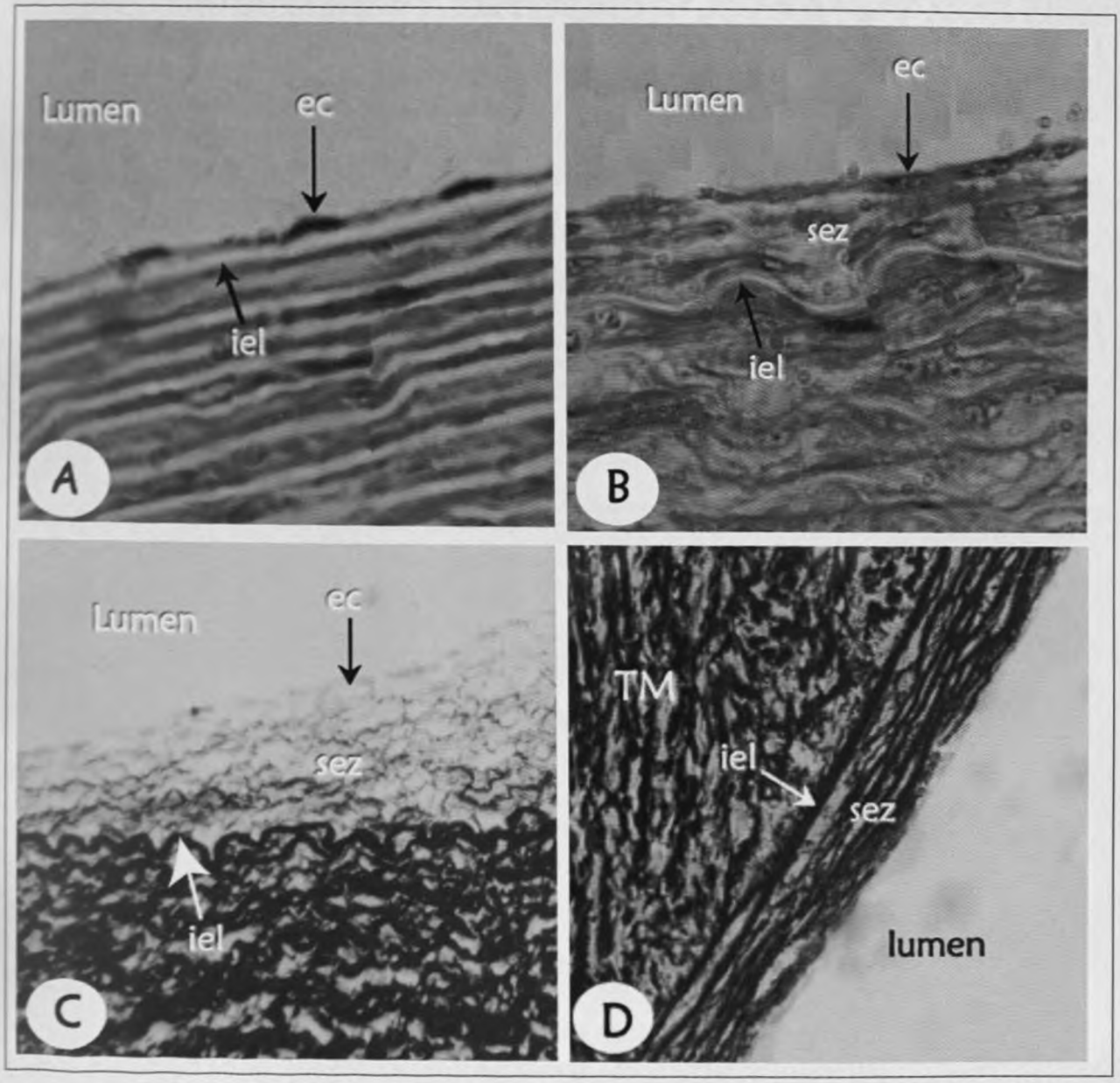

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Figure 8A-E: Subendothelial cells and connective tissue in the goat aorta

A: Electronmicrograph of the tunica intima of the goat proximal thoracic aorta showing an endothelial cell (ec) and subendothelial smooth muscle cell (sec). Note remnants of basal lamina (BL), euchromatic nucleus, rough endoplasmic reticulum (white star) and intimate association with elastic fibres (ef). Magnification x8,760.

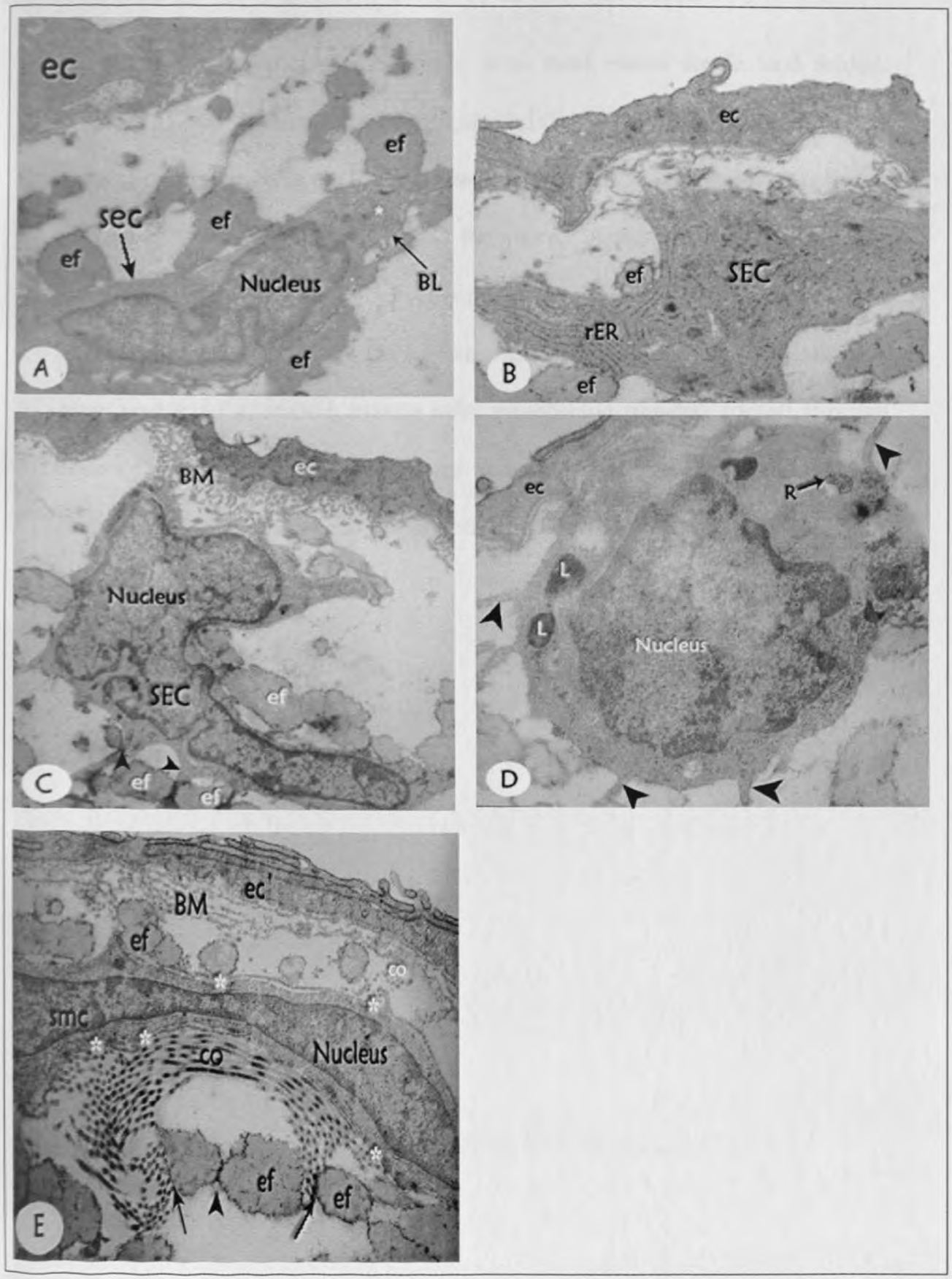
B: Electronmicrograph of the tunica intima of the goat proximal thoracic aorta showing an endothelial cell (el) and a process of subendothelial cell (SEC) with extensive rough endoplasmic reticulum (rER), and close association with elastic fibres (ef). Note absence of a basal lamina. Magnification x27,800.

C: Electronmicrograph of the tunica intima of the goat proximal thoracic aorta showing a subendothelial cell (SEC) with large euchromatic nucleus. The cell is connected to the endothelial cell (ec) by lamellated basement membrane (BM). Note the finger-like processes (arrowheads) extending between elastic fibres (ef), and also absence of basal lamina. Magnification x8,760.

D: Electronmicrograph of the tunica intima of the goat aorta showing a round subendothelial cell with a dented nucleus, "lysosome-like" (L) structures, vacuoles with residual bodies (R) and finger-like cytoplasmic extensions (arrowheads). Note its intimate association with the endothelial cell (ec). Magnification x27,800.

E: Electronmicrograph of the tunica intima of the goat aorta showing a subendothelial cell and connective tissue fibres. Note that both collagen (co) and elastic fibres (ef) are intimately associated with smooth muscle cell (smc), (stars). The elastic fibres are interconnected (arrowhead) and also connected to collagen fibres (arrow). The subendothelial components are separated from the endothelial cell (ec) by a lamellated basement membrane (BM). Magnification x27,800.

Figure 8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The prominent internal elastic lamina is in most places single, and folded, (Figure 9A), occasionally appearing duplicated (Figure 9B). Frequently, there are areas of discontinuity of the internal elastic lamina which are filled with collagen fibres (Figure 9C). The luminal side of the internal elastic lamina is connected to the subendothelial smooth muscle cells by elastic and collagen fibres oriented in all directions (Figure 9C,D). On its abluminal side, the internal elastic lamina is often connected to smooth muscle cells, establishing physical contact through areas of high electron density. In other areas, collagen fibres intervene between the internal elastic lamina and the smooth muscle cell (Figure 9E).

Figure 9A-E: Organization of the internal elastic Lamina of the goat aorta

A: Photomicrograph of tunica intima of the goat aorta showing the prominent folded internal elastic lamina (iel). Weigert's elastic stain. Magnification x400.

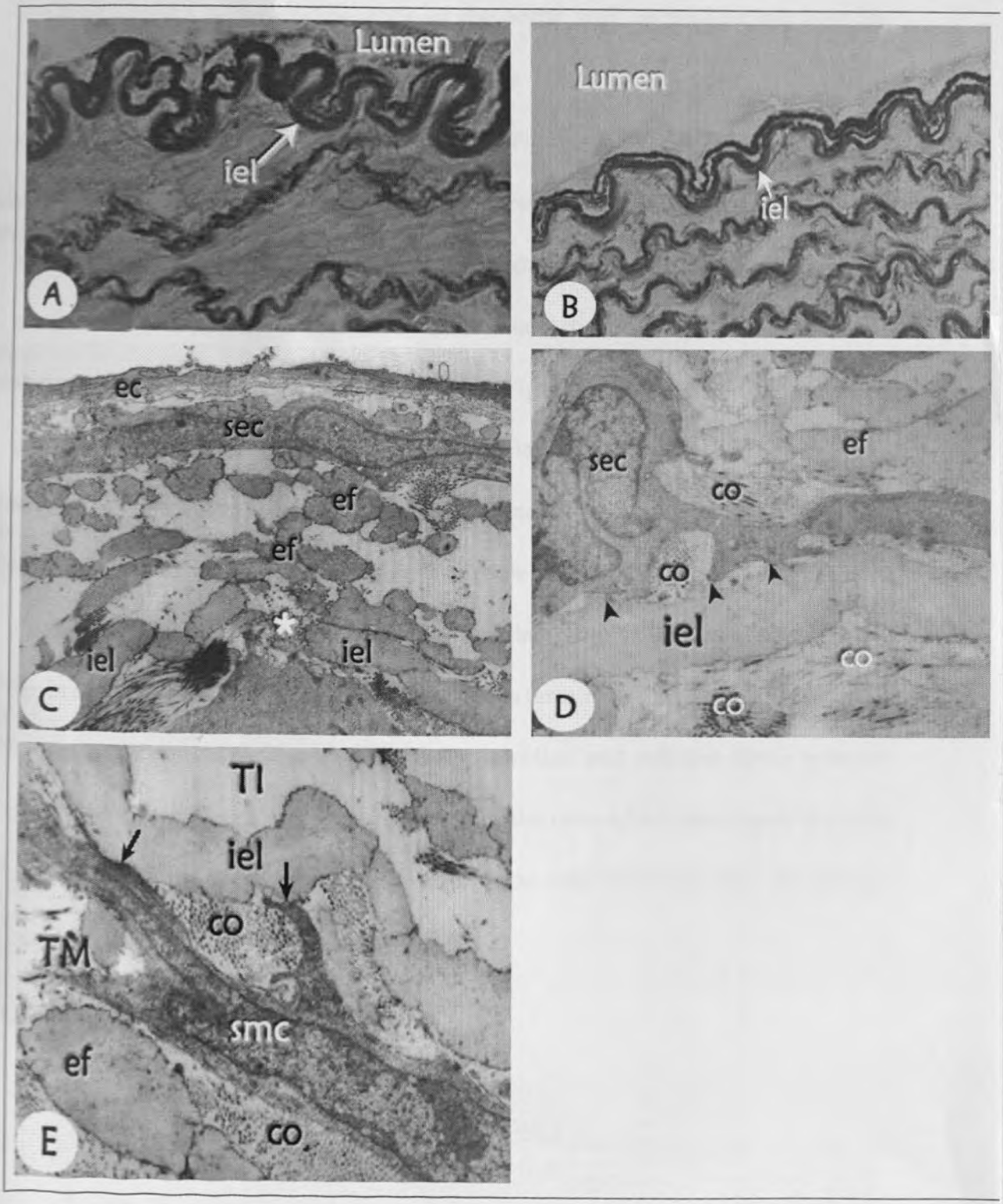
B: Photomicrograph of tunica intima of the goat aorta showing duplication of the internal elastic lamina (iel). Weigert's elastic stain. Magnification x250.

C: Electronmicrograph of tunica intima of goat aorta showing a discontinuity (star) in the internal elastic lamina (iel). Note how collagen fibrils fill the gap. Elastic fibres (ef) connect the iel to the subendothelial smooth muscle cell (sec) just beneath the endothelial cell (ec). Magnification x4,000.

D: Electronmicrograph of the tunica intima of the goat aorta, showing the association of the internal elastic lamina (iel) with subendothelial cell (sec). Note the collagen (co) on the luminal and abluminal side of the iel, and attachment of the smc onto the iel (arrowheads). Magnification x8,760.

E: Electronmicrograph of the goat aorta, showing junction between the tunica intima (TI) and the tunica media (TM). Note close association of the internal elastic lamina (iel) and a subintimal smooth muscle cell (smc) and also the physical linkage between the smooth muscle cell and the internal elastic lamina (arrows). Collagen (co) intervenes between the internal elastic lamella and the smooth muscle cell. Magnification x27,800.

Figure 9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3.2.2 The tunica media

The tunica media is the middle zone of the aortic wall, extending between the internal and external elastic laminae (Figure 10A). It is generally made of interconnected concentric elastic lamellae (Figure 10B), between which are smooth muscle cells, elastic and collagen fibres (Figure10C). In the ascending aorta, aortic arch and the proximal thoracic aorta down to T9, however, there is a transmedial zonation in which the tunica media is divisible into a luminal elastic and an adventitial musculoelastic zones (Figure 10D). The luminal zone comprises a uniform disposition of elastic lamellae (Figure 10E), while the adventitial one contains smooth muscle islands which interrupt some elastic lamellae (Figure 10F). In most of these muscle islands, the smooth muscle cells are disposed in series with thick elastic lamellae, with which they interdigitate. Some of the elastic lamellae and collagen fibres terminate into the muscle islands, while others transverse the island. Elastic lamellae and collagen fibres between the islands are compact and form fibrous bundles onto which the muscle bundles insert (Figure 10G). Often, the smooth muscle cells converge onto the fibrous bundle from various directions (Figure 10H).

Figure 10A-H: Organization of the tunica media of the goat aorta

A: Photomicrograph of the wall of the goat distal thoracic aorta, showing the tunica media (TM), extending between the internal and external elastic laminae (arrows). The tunica adventitia (TA) lies immediately external to the tunica media. Mason's trichrome stain. Magnification x100.

B: Photomicrograph of the luminal zone of the goat thoracic aorta, showing the tunica media. Note the oblique elastic fibres (arrow) linking the elastic lamellae (stars). Weigert's elastic stain. Magnification x250.

C: Photomicrograph of the goat abdominal aorta, showing the tunica media. Note the definite elastic lamellae (arrowheads), the interlamellar collagen (stars) and smooth muscle cells (arrows). Mason's trichrome stain Magnification x400.

D: Photomicrograph of the goat ascending aorta, showing tunica media. Note the transmedial zonation into luminal elastic (L) and adventitial musculoelastic (A) zones. Weigert's elastic stain. Magnification x35.

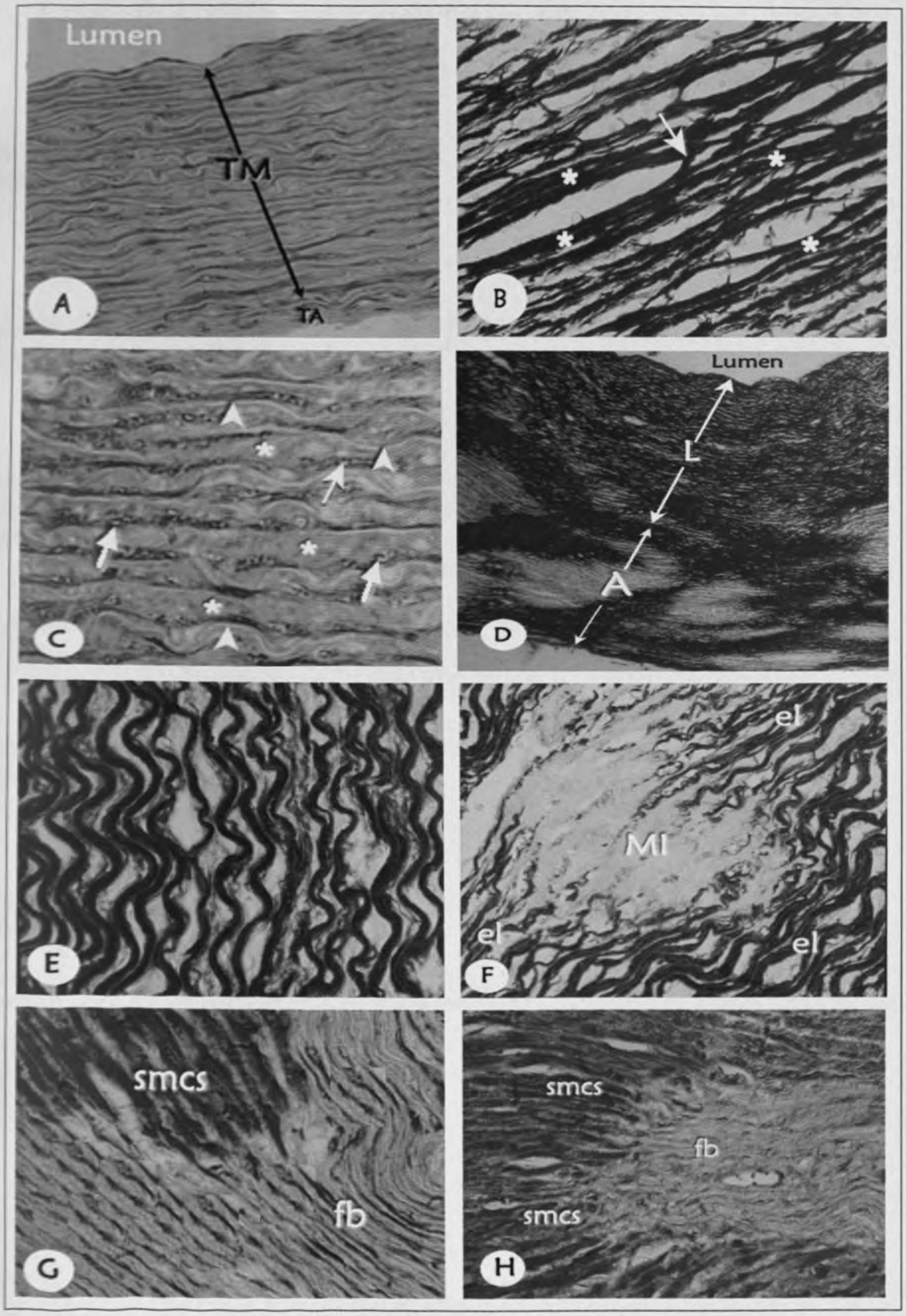
E: Photomicrograph of the luminal zone of the tunica media of goat ascending aorta showing concentric elastic lamellae. Weigert elastic stain. Magnification x250.

F: Photomicrograph of the adventitial zone of the tunica media of the goat ascending aorta showing a muscle island (MI) interrupting elastic lamellae (el). Weigert elastic stain. Magnification x250.

G: Photomicrograph of the adventitial zone of the tunica media of the goat ascending aorta showing smooth muscle cells (smcs)of the muscle islands terminating on a fibrous bundle (fb). Mason's Trichrome stain. Magnification x250.

H: Photomicrograph of the adventitial zone of the tunica media of the goat proximal thoracic aorta, showing a muscle island. Note how smooth muscle cells (smcs) from various directions converge on a fibrous bundle (fb). Mason's trichrome stain. Magnification x400.

Figure 10



In most of the islands, the smooth muscle cells run circumferentially but, frequently, some cells run longitudinally and obliquely (Figure 11A,B). Smooth muscle cells in these islands send projections which interdigitate with the matrix (Figure 11C,D). The parts of the cell adjacent to the matrix show areas of high electron density. The collagen and elastic fibres are haphazardly arranged (Figure 11C,D), and some of the elastic fibres terminate in series with the smooth muscle cell processes (Figure 11E).

Smooth muscle cells also show lateral interdigitations where they come in contact with each other. At these sites, the cell processes interlock with each other (Figure 11F), and in some of them, the basal laminae of the cells fuse (Figure 10G). In others the processes establish cytoplasmic continuity in a manner akin to gap junctions (Figure 11H).

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Figure 11A-H: Smooth muscle cells in islands of the tunica media of the g aorta

A: Photomicrograph of longitudinal section of adventitial zone of t media of goat proximal thoracic aorta showing muscle islands. that some smooth muscle cells run longitudinally, (l-smcs), while o run circumferentially (c-smcs). Mason's trichrome stain. Magnific x100.

B: Photomicrograph of longitudinal section of adventitial zone of t media of goat proximal thoracic aorta showing oblique (o-smc) circumferential (c-smcs) smooth muscle cell in the island. Ma trichrome stain. Magnification x400.

C: Electronmicrograph of goat proximal thoracic aortic tunica m showing smooth muscle cells (smc) in the muscle islands. Not irregular cell surface of Smooth muscle cells (smc) connected to e fibres (ef), and also interdigitating with collagen fibres (co). Magnific x27,800.

D: Electronmicrograph of adventitial zone of tunica media of the proximal thoracic aorta, showing lateral surface of smooth muscl (smc) and the matrix. Note the irregular cell surface with the displaying high electron density (arrow), and interdigitating with el fibre (ef) Note also the "weaved" collagen (co). Magnification x63,400.

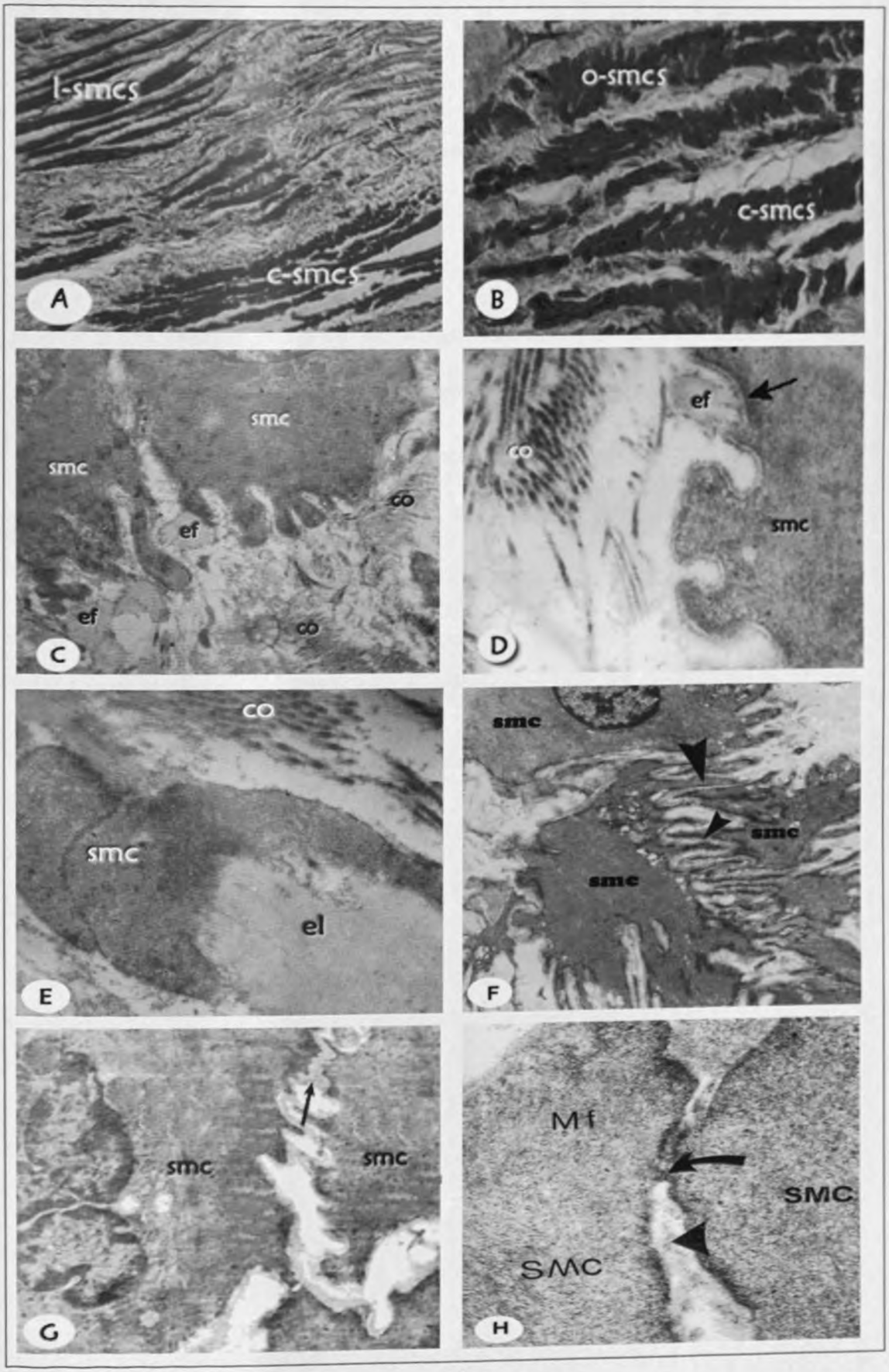
E: Electronmicrograph of the adventitial zone of the tunica media of ascending aorta showing an elastic lamella (el) connected to a sm muscle cell (smc) process. Note collagen fibres (co) in the neighbourh Magnification x63,400.

F: Electronmicrograph of an island in the adventitial zone of the tu media of goat ascending aorta, showing smooth muscle cells (smc). the extensive lateral muscle cell interdigitations, characterized by electron density (arrow heads). Magnification x27,800.

G: Electronmicrograph of adjacent cells in a muscle island in the adven zone of the tunica media of the goat aortic arch. Note the fusion of t lamina (arrow) of the smooth muscles (smc). Magnification x27,800.

H: Electronmicrograph of adjacent cells in a muscle island in the adven zone of the tunica media of the goat aortic arch. Note the gap (ar and tight (arrowhead) junction-like union between smooth muscle (smc) filled with myofilaments (mf). Magnification x63,400.

Figure 11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Smooth muscle cells of the luminal zone of the tunica media comprise a heterogenous population (Figure 12A-F). Many of them are either elongated, or spindle shaped, and lie between the elastic lamellae (Figure 12A). These cells hardly contain any synthetic organelles. Other cells are either characterised by irregularity, electron dense areas and close association with elastic lamellae and elastic fibres (Figure 12B), or are large with irregular nuclei, and prominent rough endoplasmic reticulum. These latter cells are also characterized by close association with elastic lamellae and, show areas of high electron density (Figure 12C). The smooth muscle cells run in various directions (Figure 12 D,E). Circumferentially oriented cells appear cut parallel to their long axis giving them an elongated appearance (Figure 12D), while those that run longitudinally, are cut transversely, and appear round. Occasionally, even in the same interlamellar space, both circumferential and longitudinal smooth muscle cells co-exist (Figure 12E).

Figure 12A-E: Heterogeneity of smooth muscle cells in the tunica me goat aorta.

A: Electronmicrograph of the luminal zone of the tunica media c aorta showing smooth muscle cells with spindle shaped (sm elongated cells (smc-e). Note the elastic lamellae (el), some i (star), and collagen (co) between the muscle cells. Ma x8,760.

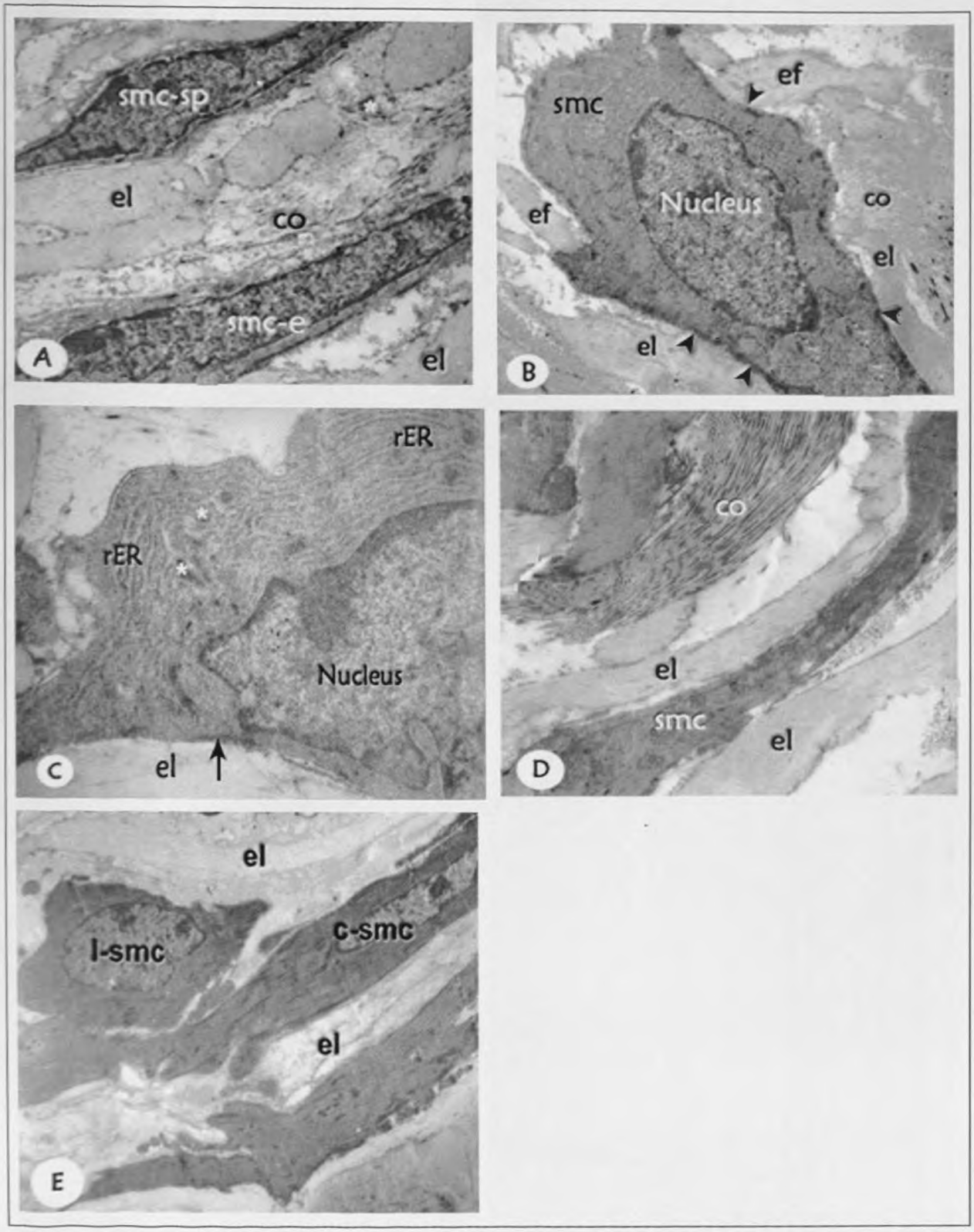
B: Electronmicrograph of the luminal zone of the tunica media c aorta showing an irregular cell with large euchromatic nucle attachment of elastic fibres (ef), elastic lamellae (el) and collagen onto the smooth muscle cell (smc) through areas of high electr (arrowheads). Magnification x27,800.

C: Electronmicrograph of the luminal zone of the tunica media c aorta showing an irregular cell with a large euchromatic nu abundant rough endoplasmic reticulum (rER). Notice intimate a of the cell with elastic lamella (el) and areas of high electr (arrow). Magnification x63,400.

D: Electronmicrograph of the tunica media of the goat aorta, circumferential smooth muscle cell (smc). Note the close associ elastic lamellae (el), and adjacent collagen (co). Magnification x2

E: Electronmicrograph of the tunica media of the goat aorta longitudinal (l-smc) and circumferential (c-smc) smooth m between two adjacent elastic lamellae (el). Magnification x8,760

Figure 12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In the muscle islands of the adventitial zone in the tunica media of the proximal aorta, the interfascicular space contains smaller cells with large euchromatic nuclei and prominent nucleoli (Figure 13A). These cells are characterized by extensive cytoplasmic processes which run between the matrix components. The cytoplasm in the perinuclear region, and within the processes shows prominent rough endoplasmic reticulum and mitochondria, but scarce microfilaments (Figure 13B). These cells also lack remnants of a basal lamina, and resemble fibroblasts. Similar fibroblast-like, but much more slender and elongated, cells are found between the elastic lamellae joining the muscle islands (Figure 13C). A second type of cells is observed in some folds of elastic lamellae. Such cells are characterized by a kidney shaped nucleus, irregular cell surface and numerous lysosome-like structures (Figure 13D).

Figure 13A –D: Non-muscle cells in the tunica media of the goat aorta

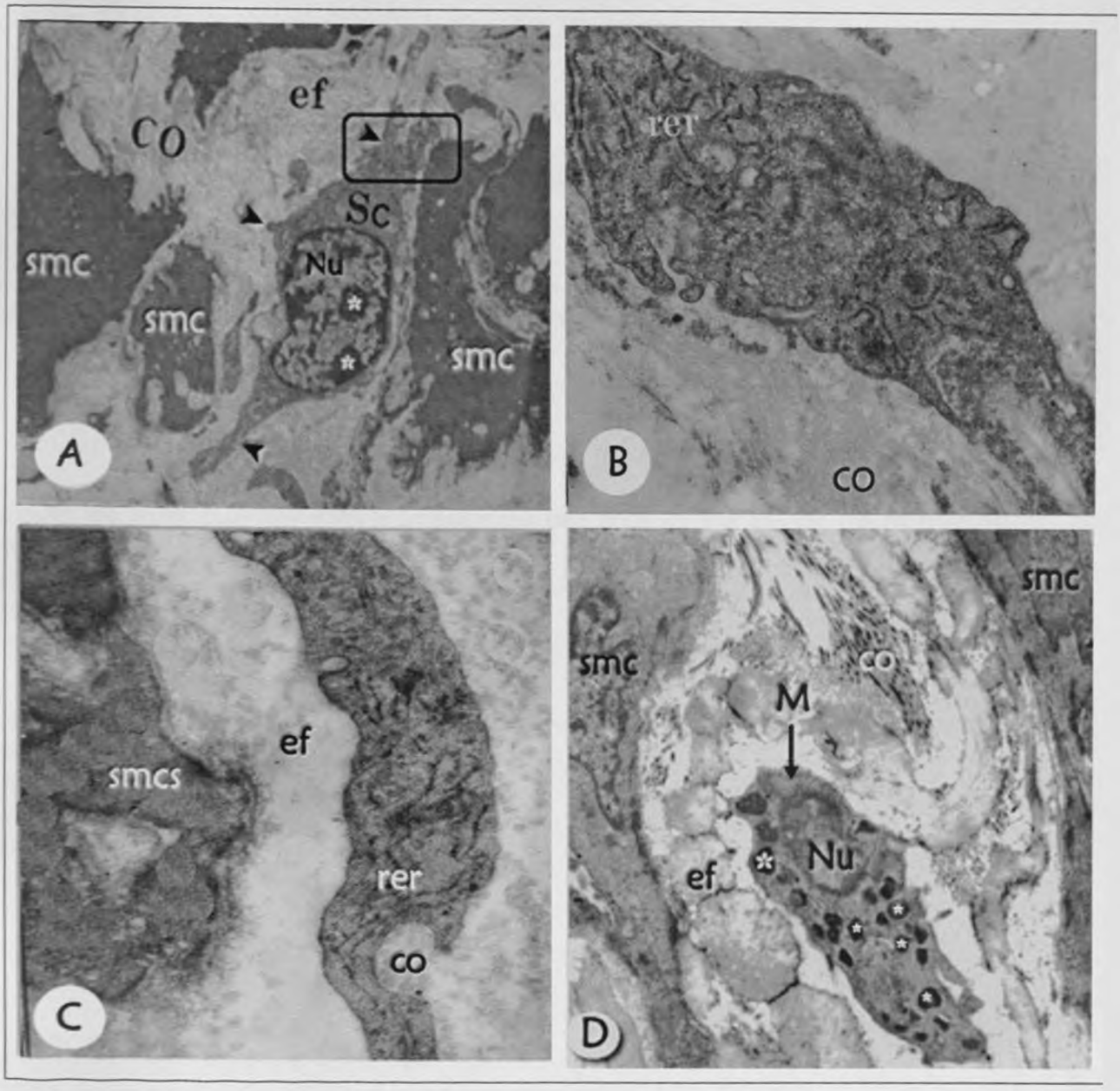
A: Electronmicrograph of muscle island in the adventitial zone of media in the goat proximal aorta, showing a synthetic cell (sc cytoplasmic extensions (arrowheads), and euchromatic nucleus two nucleoli (stars). Note the surrounding smooth muscle ce elastic (ef) and collagen (co) fibres. Magnification x8,760.

B: Electronmicrograph of muscle island in the adventitial zone of media in the goat proximal thoracic aorta showing a part of the cell in figure 13A. Note the abundance of rough endoplasmic (rer), and also collagen (co) in the neighbourhood. Magnification

C: Electronmicrograph of the luminal zone of the tunica media o proximal thoracic aorta showing part of an interlamellar elon with extensive rough endoplasmic reticulum (rer), and abs lamina. Note elastic fibres (ef), collagen (co) fibres and th muscle cell (smc) in the neighbourhood. Magnification x63,400.

D: Electronmicrograph of luminal zone of goat thoracic aorta, s macrophage-like (M) cell with an irregular outline, kidney shape (Nu) and lysosome-like structures (stars). Note its proximity fibre (ef), collagen (co) and smooth muscle cells (smc). Magnifica x 8,760.

Figure 13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Collagen and elastic fibres anchor onto the surface of smooth muscle cells at various points (Figure 14A-D). Where collagen inserts, the basal lamina is prominent and together with the cell surface shows high electron density (Figure 14A), while where the elastic lamellae and fibres attach, the basal lamina is relatively less distinct (Figure 14B,C). Occasionally, smooth muscle cells, collagen and elastic fibres are all physically interlinked (Figure 14D).

In the interlamellar spaces, collagen and elastic fibres are intimately associated (Figure 15 A-D). Collagen fibres complexedly interweave then insert onto the end of the elastic fibre through its external microfibrillar component (Figure 15A), or form bundles which insert onto the ends of the elastic fibres (Figure 15B). In the other cases the collagen fibres insert onto the side of the elastic lamella (Figure 15C), or surround the elastic lamella or fibre, and insert along its entire circumference also through the peripheral microfibrillar component (Figure 15D).

Figure 14A–D: Cell-matrix linkages in the tunica media of the goat

A: Electronmicrograph of the luminal zone of the tunica media proximal thoracic aorta, showing a smooth muscle cell (smc) w (co) intimately linked to it through the basal lamina (arrow). N high electron density at those sites (arrowhead). Magnificatior

B: Electronmicrograph of the luminal zone of the tunica media proximal thoracic aorta, showing smooth muscle cell (smc) fibres (ef) linked to it. Magnification x8,760.

C: Electronmicrograph of tunica media of the goat distal thor showing smooth muscle cell (smc) with peripheral electron den (arrows) associated with attachment of elastic lamella (el), an (co). Magnification x27,800.

D: Electronmicrograph of tunica media of the goat distal thora showing linkage between collagen (co) and elastic fibre (ef) between elastic fibre (ef) and smooth muscle cell (smc) [ar Magnification x27,800.

Figure 14

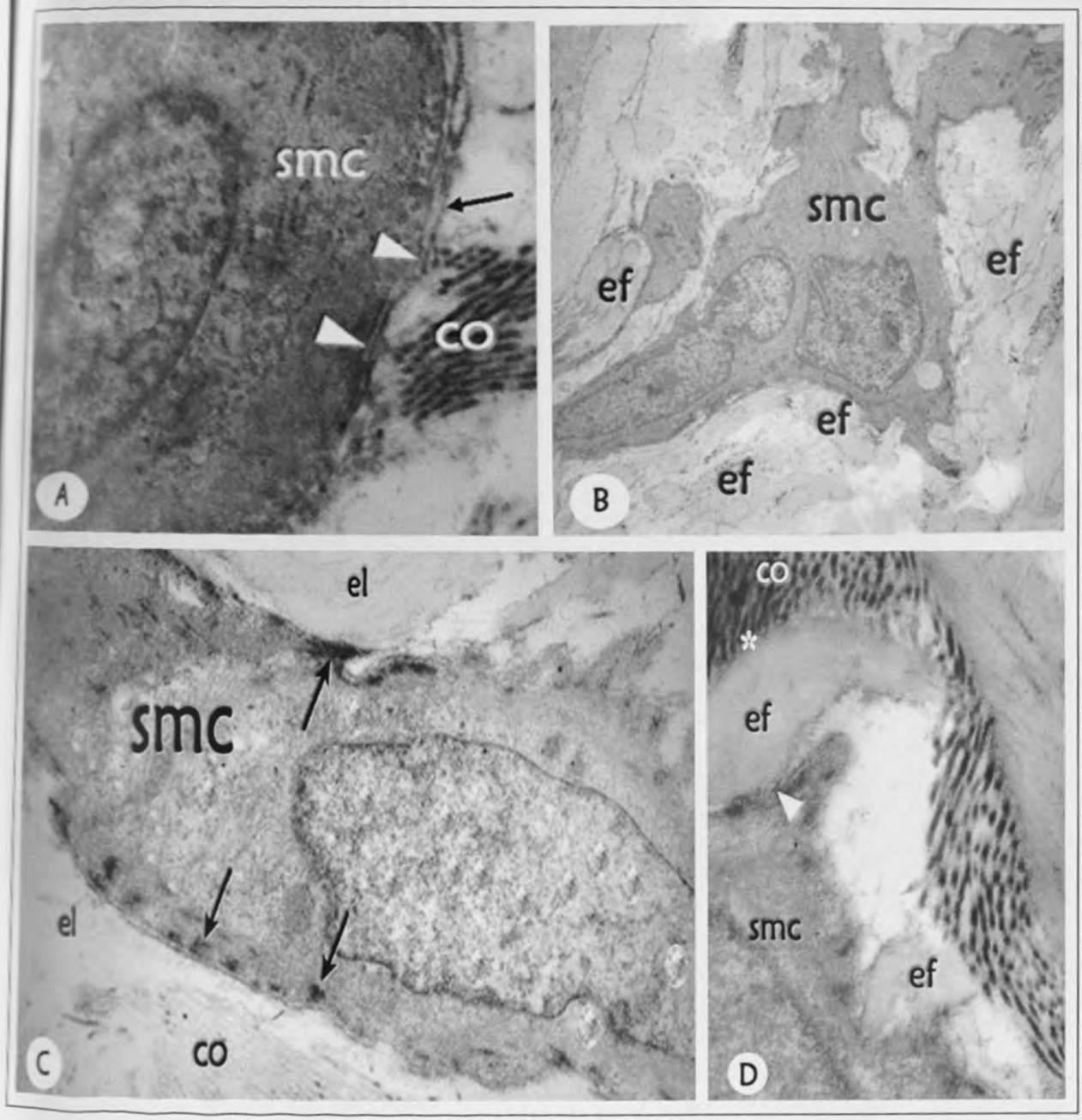


Figure 15A-D: Inter-linkages between collagen and elastin in the tunic

of the goat aorta

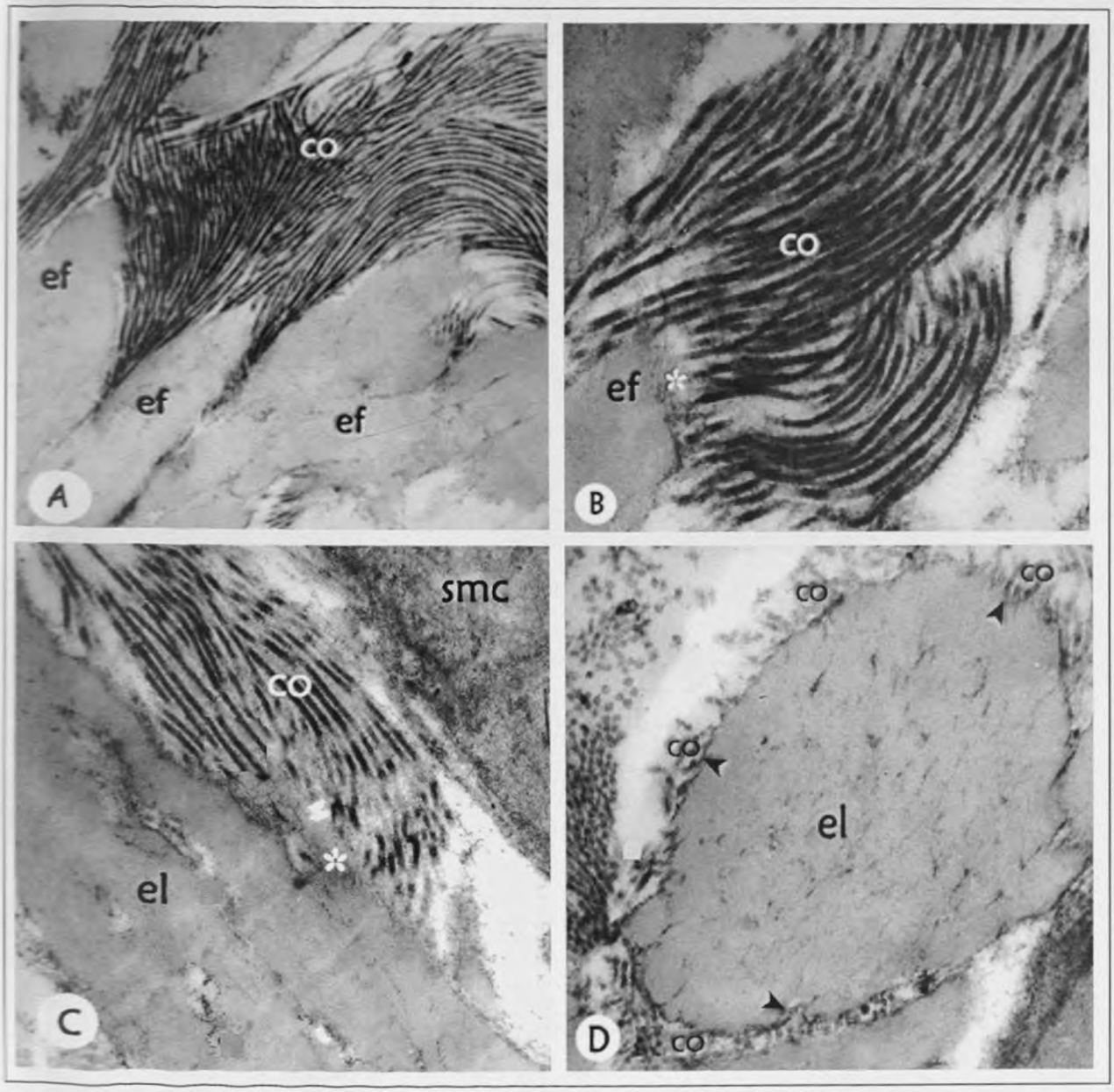
A: Electronmicrograph of luminal zone of tunica media of the goat ac showing a complex weave of collagen (co) inserting onto elastic fil (ef). Magnification x27,800.

B: Electronmicrograph of luminal zone of tunica media of the goat ac showing a collagen bundle (co) inserting onto the elastic fibre (ef) through its peripheral microfibrillar mantle (star). Magnification x63,400.

C: Electronmicrograph of tunica media of the goat distal descending showing a parallel collagen bundle (co) turning to insert onto the s an elastic lamella (el) through its peripheral microfibrillar mantle (Note smooth muscle cell (smc) in the neighbourhood. Magnificati X63,400.

D: Electronmicrograph of luminal zone of tunica media of the goat pr thoracic aorta, showing a transverse elastic lamella (el), surrounde collagen fibres (co) which attach onto its peripheral microfibrillar mantle (arrowheads). Magnification x63,400.

Figure 15



The tunica media in most places is made up of regular elastic lamellae between which are collagen and smooth muscle cells (Figure 16A) forming medial lamellar units (MLU). Some of the units can be resolved into musculoelastic fascicles (MEF) comprising composites of smooth muscle cells and elastic lamellae and adjacent collagen (Figure 16B). Elastic lamellae frequently branch (Figure 16B) while in others, they display discontinuities, which contain cell processes, or are bridged by collagen fibres (Figure 16C). In other instances, however, elastic lamellae run longitudinally (Figure 16D). Frequently, elastic lamellae run in parallel with collagen fibres (Figure 16E).

The most notable deviations from the MLU and MEF concepts is where either the cells are surrounded by large collagen bundles with elastic lamellae sandwiching both (Figure 17A), or where a cell is associated with collagen bundle on one side, and elastic lamella on the other (Figure 17B). Frequently, an elastic lamella and a collagen bundle run perpendicular to each other (Figure 17C), or alternate in parallel (Figure 17D).

Figure 16A-E: Organization of the tunica media of the goat aorta

A: Electronmicrograph of luminal zone of the tunica media of the showing smooth muscle cell (smc) and adjacent elastic la Observe paracellular collagen (co), and branching of elastic la Magnification x8,760

B: Electronmicrograph of luminal zone of the tunica media of the showing the three alternating components that form the mus fascicle (MEF), namely smooth muscle cell (smc), elastic lamel collagen bundle (co). Magnification x63,400.

C: Electronmicrograph of tunica media of goat aorta, showing di in an elastic lamella (el). Note a process of a smooth muscle c the gap (star) and collagen (co) bridging another discontinui elastic lamellae. Magnification x8,760.

D: Electronmicrograph of luminal zone of the tunica media of the g showing longitudinal elastic fibres (ef), and the elastic lamellar collagen fibres (co) physically linking onto the elastic fibres. Magnification x27,800.

E: Electronmicrograph of tunica media of the goat aorta showing lamella (el) surrounded by collagen fibres (co). Note that adjacent collagen fibres insert onto the elastic lamel Magnification x63,400.

Figure 16

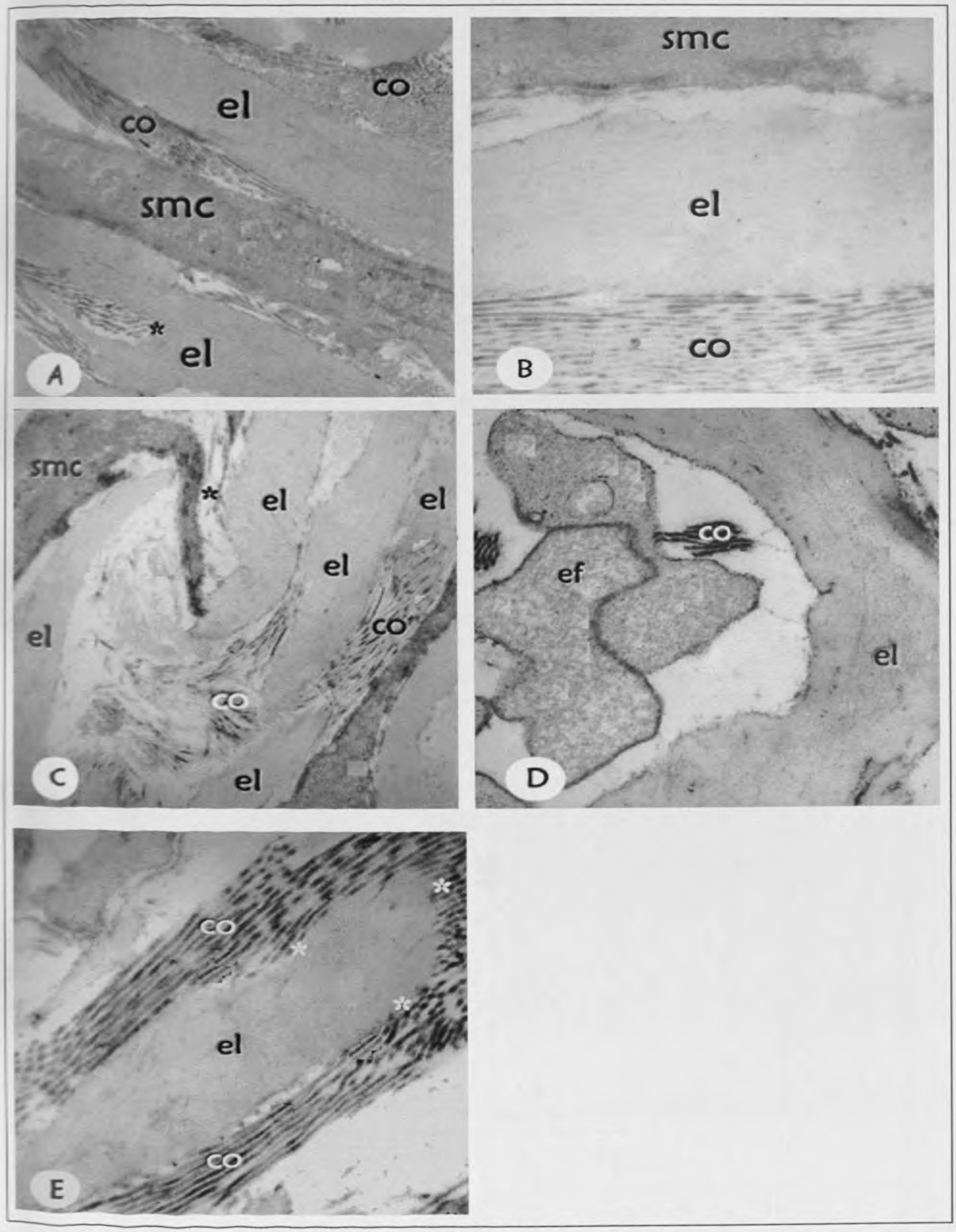

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Figure 17A-D: Deviation of goat aortic tunica media from the Muscul

Fascicle concept

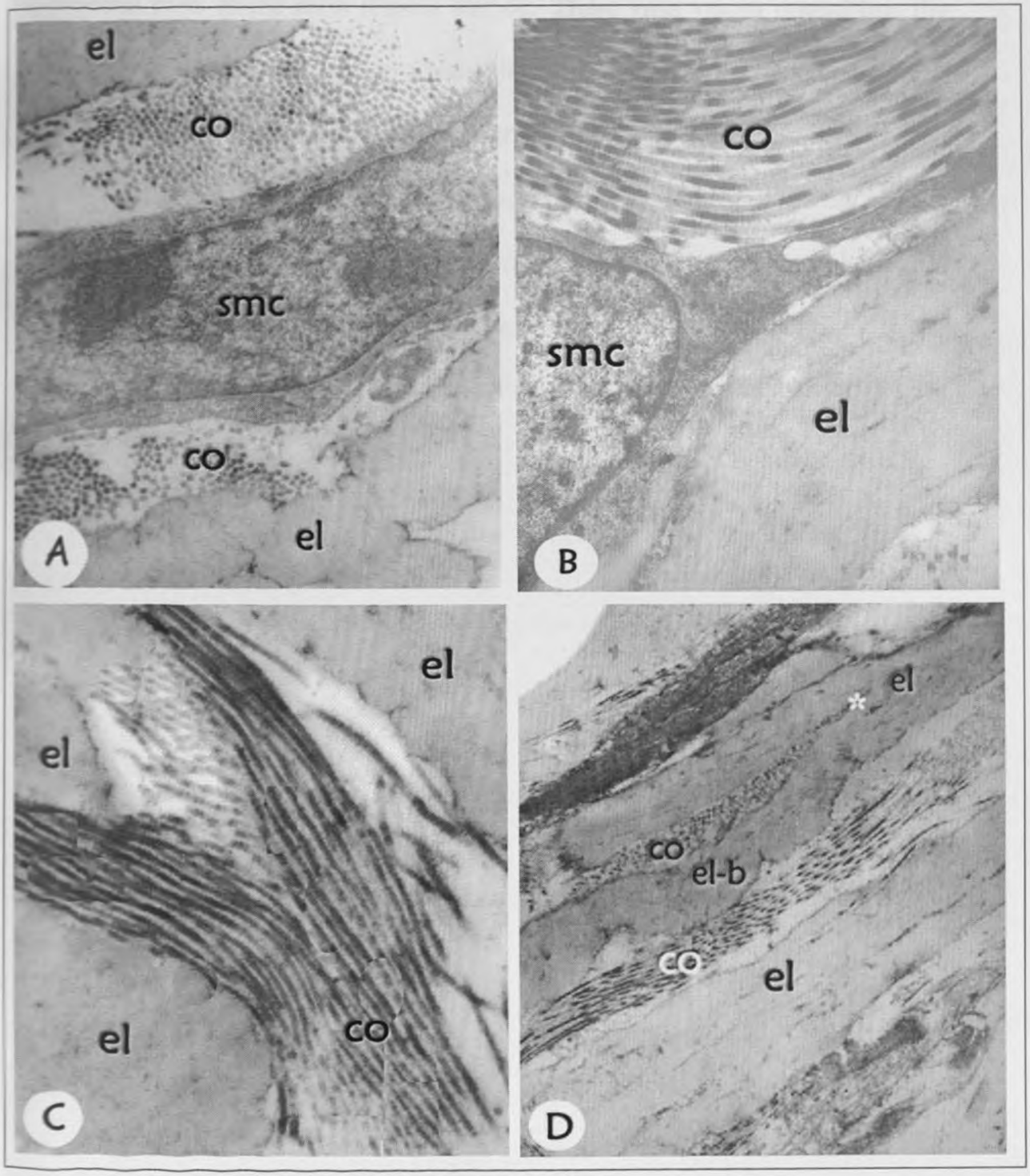
A: Electronmicrograph of the luminal zone of the tunica media o aorta showing a smooth muscle cell (smc) surrounded by b collagen (co). Note how both the cell and the surrounding co sandwiched by elastic lamellae (el). Magnification x63,400.

B: Electronmicrograph of the tunica media of the goat aorta s smooth muscle cell (smc) surrounded by collagen bundle (co) or and elastic lamella (el) on the other. Magnification x63,400.

C: Electronmicrograph of the luminal zone of the tunica media of showing collagen bundle (co) running perpendicular to elasti (el). Magnification x63,400.

D: Electronmicrograph of the tunica media of goat aorta showing a elastic lamella (el-b) sandwiched by bundles of collagen (co). collagen fibres running in different directions, the branching p of some of the elastic lamellae (el). Magnification x8,760.

Figure 17



The tunica media contains vasa vasora which penetrate into the inner half in the proximal parts of the aorta (Figure 18A,B). These vasa vasora extend into the tunica media from the tunica adventitia with a sleeve of connective tissue, perpendicular to the long axis of the artery then branch and ramify between the lamellae (Figure 18B,C). Frequently these vessels co-localise with the muscle islands (Figure 18D). In the distal segments of the thoracic aorta and abdominal aorta, vasa vasora are still present in the tunica media, but are confined to the outer one third of the tunica media, or the media-adventitial border (Figure 18E,F).

Figure 18A-F: Vasa vasora in the tunica media of the goat aorta.

A: Photomicrograph of transverse section of the goat ascen showing vasa vasora (stars) in the luminal half of the tun Mason's trichrome stain. Magnification x100.

B: Photomicrograph of transverse section of goat aortic arch show vasorum (vv) projecting into the tunica media at right ang sleeve of connective tissue. Note a circumferential branch (cv the lamellae. Mason's trichrome stain. Magnification x250.

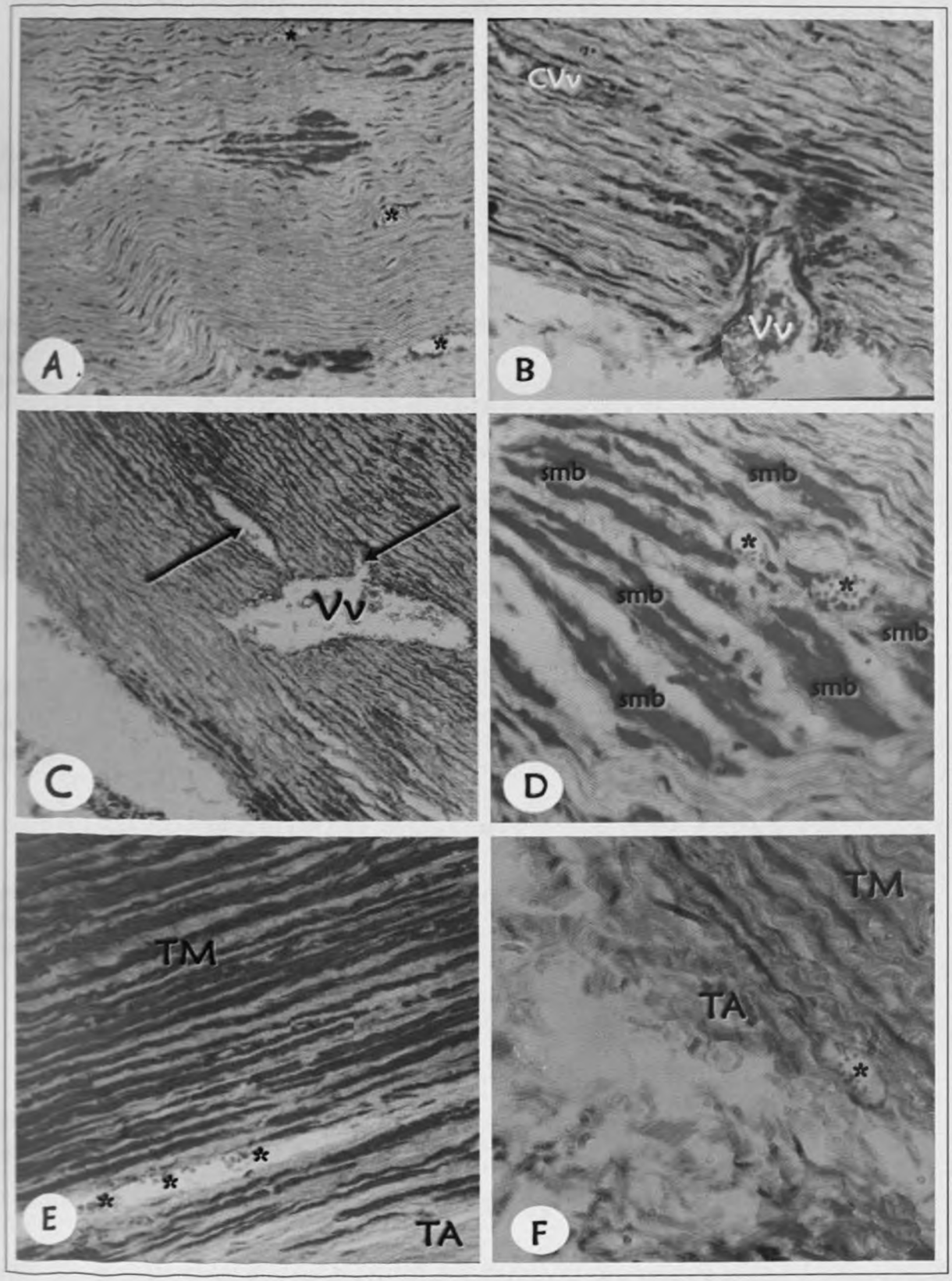
C: Photomicrograph of goat thoracic aorta showing a perpendi vasorum (vv) and its branches (arrows) running circum Mason's trichrome stain. Magnification x100

D: Photomicrograph of the tunica media of the goat thoracic aort vasa vasora (stars) co-localised with muscle island in whicl surrounded by smooth muscle bundles (smb). Mason's trichr Magnification x250.

E: Photomicrograph of longitudinal section of goat distal thor showing vasa vasora (stars) in the outer one third of the tur (TM) adjacent to the tunica adventitia (TA). Mason's trichr Magnification x250.

F: Photomicrograph of longitudinal section of goat abdom showing vasa vasorum (star) between Tunica media(TM) a adventitia (TA) Mason's trichrome stain. Magnification x250.

Figure 18



3.2.3 The tunica adventitia

Tunica adventitia, the outermost coat of the aorta, is made of dense irregular fibro elastic connective tissue rich in cells, contains vasa vasora and merges with the subjacent connective tissue (Figure 19A). The connective tissue of the tunica adventitia comprises elastic and collagen fibres, organised as bundles and fibres of variable sizes (Figure 19B,C). Elastic tissue consists of elastic fibres interweaving with collagen (Figure 19D), and microfibrillar material which surround some of the cells, separating them from the adjacent collagen bundles (Figure 19E,F).

Cells of the tunica adventitia comprise a heterogenous population. One category of the cells are elongated with long cytoplasmic extensions in all directions and a large euchromatic nucleus. Their long slender processes course between bundles of collagen, and contain prominent rough endoplasmic reticulum (Figure 19D,E).

A second category of cells has a large euchromatic eccentric nucleus, and several pseudopodia-like extensions (Figure 20A). Some of the extensions have vacuoles, while others seem to enclose parts of the extracellular matrix fibres. The nuclear free part of the cell cytoplasm is characterized by prominent rough endoplasmic reticulum, lysosome-like structures, and vacuoles, some of which contain dark particulate matter, resembling residual bodies. The third category

comprises cells with long cytoplasmic extensions in all directions, and a relatively poor organelle disposition. Their long slender processes extend between bundles of collagen, and contain rough endoplasmic reticulum. Their nuclei are, however, large and euchromatic filling most of the cell (Figure 20B).

A fourth category of cells are observed in the neighbourhood of vasa vasora (Figure 20C-E). Some cells have flask shaped cell bodies, large euchromatic nuclei which fill most of the cell, leaving only a thin rim of cytoplasm. These cells have their own basal lamina, and several processes, some of which are situated between the endothelial cells of the vasa vasora, reaching the lumen, while others extend into the space between the vessels (Figure 20D,E). The processes which extend between endothelial cells share a basal lamina with the endothelial cells, like pericytes. Other perivascular cells embrace both the endothelial cell and the former perivascular cell, and do not bear remnants of basal lamina. This latter type of cells have a large euchromatic nucleus (Figure 20D). Between the vasa vasora there are other large perivascular cells with huge euchromatic nuclei and numerous cytoplasmic extensions (Figure 20E).

Figure 19A-F: Fibres and cell types in the tunica adventitia of the goa

A: Photomicrograph of proximal thoracic aorta of the goat sh tunica adventitia (TA) with vasa vasora (vv). Note the mus (MI) in the tunica media (TM). The tunica adventitia me subjacent connective tissue (CT). Mason's trichrome stain. Ma x100.

B: Photomicrograph of the tunica adventitia (TA) and outer tur (TM) of the goat abdominal aorta showing collagen fibr haphazardly arranged. Among them are various types (arrowheads). Mason's trichrome stain. Magnification x 400.

C: Photomicrograph of the goat abdominal aorta showing t adventitia (TA) and a small part of tunica media (TM). irregularly arranged collagen fibres (stained pink) mixed w fibres (stained black). Weigert's elastic stain. Magnification x40

D: Electronmicrograph of the tunica adventitia of the goat abdon showing a fibroblast (Fb) surrounded by collagen (co) a fibres(ef) running in various directions. Magnification x8,760.

E: Electronmicrograph of the tunica adventitia of the goat abdon showing a cytoplasmic extension of fibroblast containing abunda endoplasmic reticulum (arrowheads). Note also the microf adjacent to the cell. Magnification x63,400.

F: Electronmicrograph of the tunica adventitia of the goat abdon showing a cell with large euchromatic nucleus, and cy extensions (arrows). Note that the cell is surrounded by collage one side and microfibrils (ox) on the other. Magnification x27,80

Figure 19

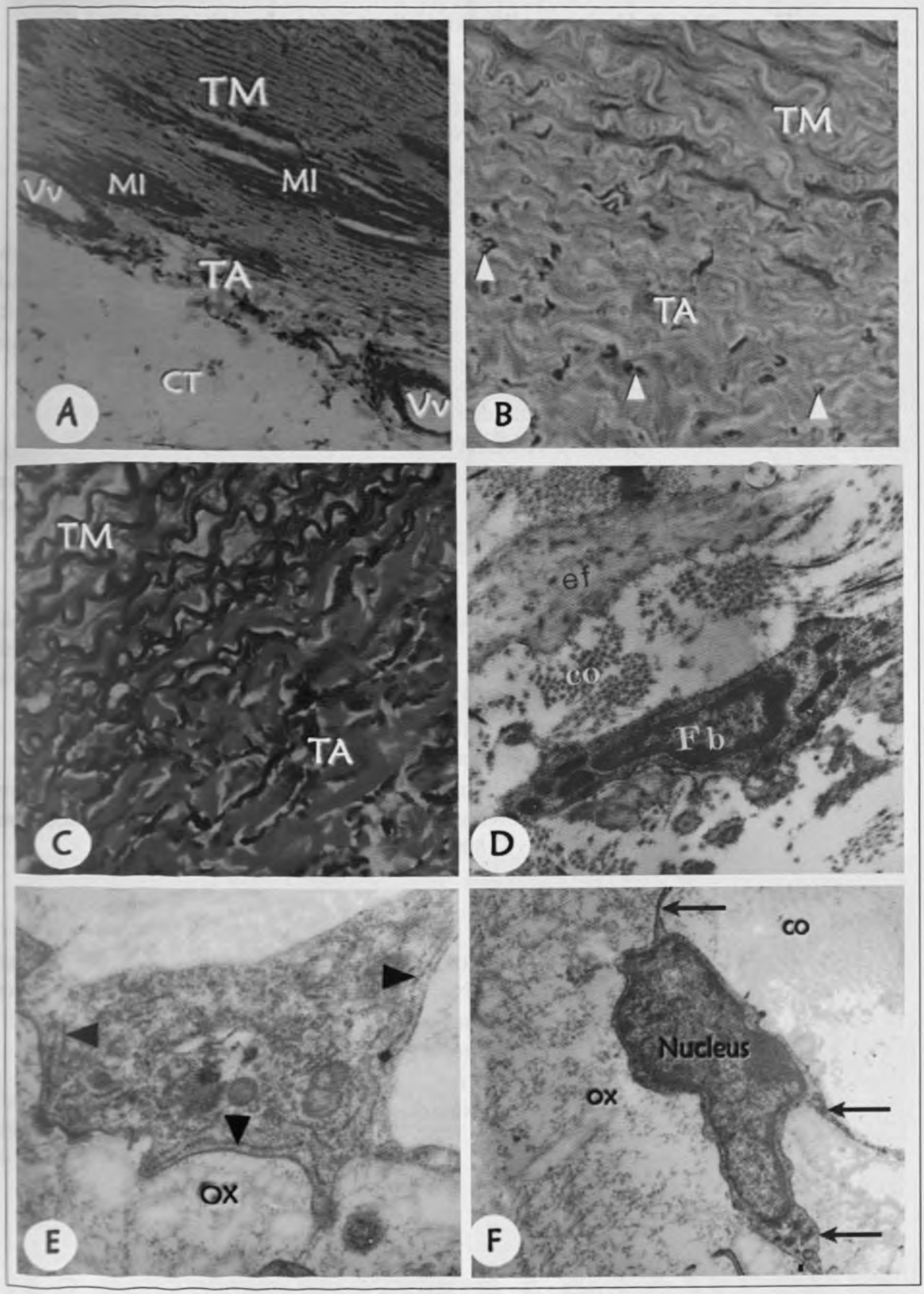


Figure 20A -F: Various cell types in the tunica adventitia of the goat a

A: Electronmicrograph of goat aortic tunica adventitia showing a euchromatic nucleus (Nu), rough endoplasmic reticulum vacuoles (v) some of which contain dark material (star). Note s cell processes engulfing elastic fibres (ef). Magnification x27,800.

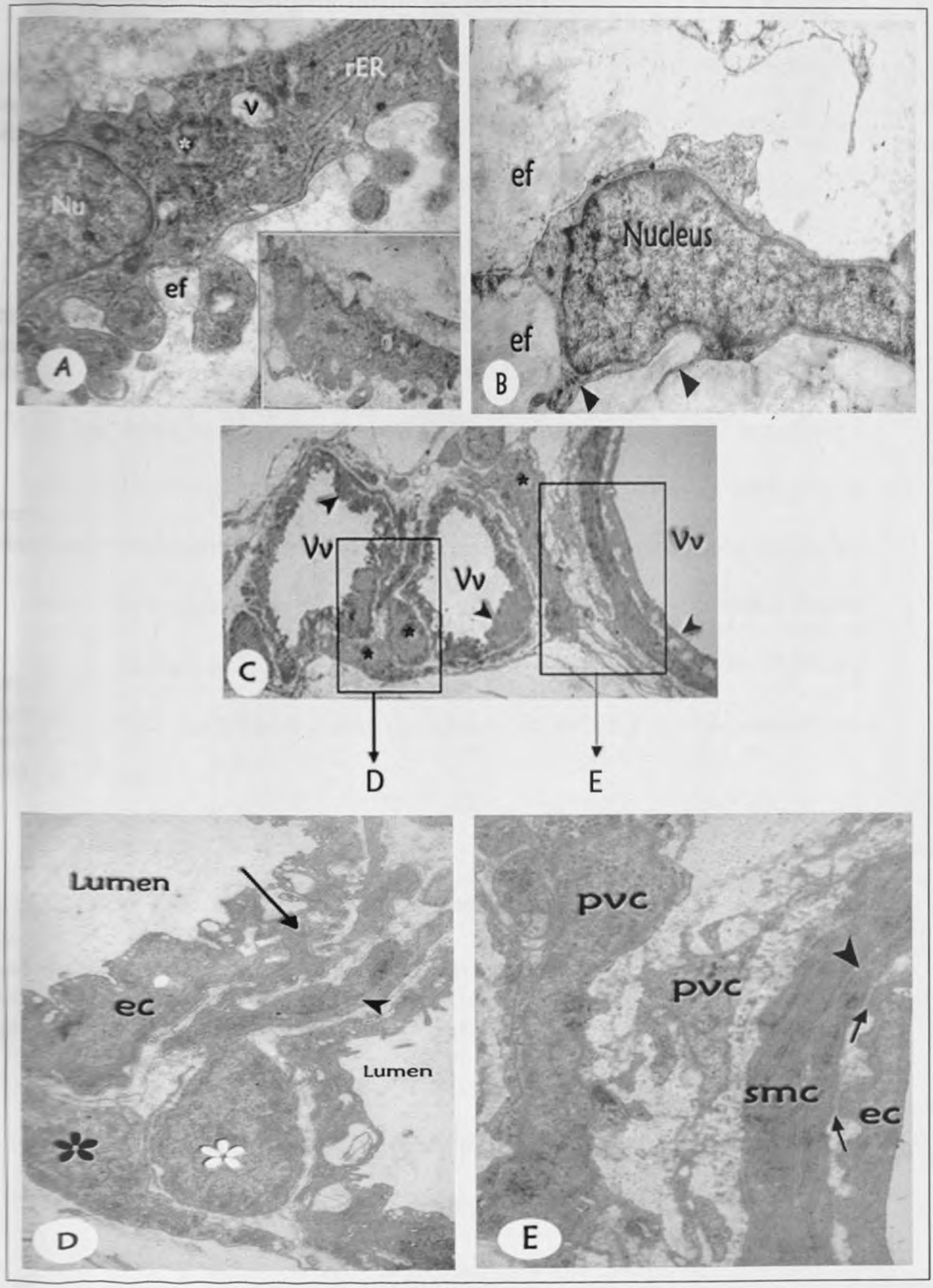
B: Electronmicrograph of goat aortic tunica adventitia showing a large euchromatic nucleus, cytoplasmic extensions (arrowhe relatively poor organelle content. Note the elastic fibres (ef) a cell. Magnification x8,760.

C: Electronmicrograph of goat aortic tunica adventitia show adjacent vasa vasora (vv). Note endothelial (arrowhea perivascular cells (stars). Magnification x4000.

D: Electronmicrograph of goat aortic tunica adventitia showing fla perivascular cell (white star) with one process (arrow) extendin a gap in the endothelium (ec), and the other process extendin the two vessels (arrowhead). The other cell (black star) embrace one, Magnification x27,800.

E: Electronmicrograph of goat aortic tunica adventitia showing tl one of the vasa vasora. Note the two layers of smooth muscle sharing a basal lamina (arrowhead). Note also the endothelia with basal membrane irregularities. Endothelial cells share bas with smooth muscle cell (arrows). The perivascular cells (pvc) l nuclei and cytoplasmic extensions. Magnification x27,800.

Figure 20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3.3 Regional variations in the aortic wall

Regional variations in the structure of the goat aorta have been observed in all three layers. They involve cellular morphology, fibre composition and disposition, and are both qualitative and quantitative.

3.3.1 Regional variations in the tunica intima

In the tunica intima, the frequency of round endothelial cells varies such that they are most frequent in the ascending aorta. They show progressive craniocaudal reduction till in the abdominal aorta, they are only scattered (Figure 21A-D). The internal elastic lamina in the ascending aorta and aortic arch is not readily distinguishable from the elastic lamellae of the tunica media (Figure 22A). It displays a craniocaudal increase in prominence and degree of folding (Figure 22B,C), such that it is most prominent and folded in the abdominal aorta (Figure 22D).

3.3.2 Regional variations in the tunica media

Definite muscle islands in the adventitial zone of the tunica media are only found in the ascending aorta, aortic arch and thoracic aorta down to T9 (Figure 23A-C). At T10 and T11, irregular patches of muscle cells occupy only the outer one third of the tunica media (Figure 23D). In the most distal part of the thoracic aorta at T12-T13, the tunica media shows a homogenous structure in which the elastic

lamellae are uniformly arranged without any interruption by muscle islands (Figure 23E). At this level, the elastic lamellae are a little further apart than in the proximal segments. The abdominal aorta comprises a uniform structure of elastic lamellae without interruption by muscle islands (Figure 23F).

Morphometric analysis by point counting shows that the proportion of the tunica media occupied by the muscle islands is about fifty per cent in the ascending aorta, then declines craniocaudally (Figure 24). T10-T11 constitutes a transitional zone between the cranial musculoelastic and the caudal elastic parts of the descending thoracic aorta. The number of concentric elastic lamellae displays craniocaudal decrease, with a maximum of 50 ±5 in the ascending aorta. This number gradually decreases to 13±3 in the lower abdominal aorta (Figure 25). The width of the interlamellar spaces increases caudally, such that it is narrowest in the ascending aorta, and widest in the abdominal aorta (Figure 26A-D).

Electron microscopy shows that in the ascending aorta, arch of the aorta, and proximal thoracic aorta, the interlamellar spaces are narrow and generally contain a single layer of smooth muscle cells (Figure 27A). In the middle and distal thoracic aorta, some of the spaces contain more than one cell layer (Figure 27B,C). In the abdominal aorta, the interlamellar spaces were much wider, and some of them contain more than two layers of smooth muscle cells (Figure 27D).

Figure 21A-D: Frequency of round endothelial cells in the goat aorta

A: Photomicrograph of the goat ascending aorta showing the tu and part of the tunica media. Note the round endothelial ce red) projecting into the lumen. Mason's trichrome stain. Ma x400.

B: Photomicrograph of the goat aortic arch showing the tunica part of the tunica media. Note the fewer round endothelial ce red) compared to A) above. Masons's trichrome stain. Ma x400.

C: Photomicrograph of the goat middle thoracic aorta showing intima and part of the tunica media. Note that only a f endothelial cells (stained red) are round. Mason's Trichr Magnification x400.

D: Photomicrograph of the goat abdominal aorta showing the tur and part of the tunica media. Note that very few of the endot (stained red) are round. Mason's trichrome stain. Magnificatio

Figure 21

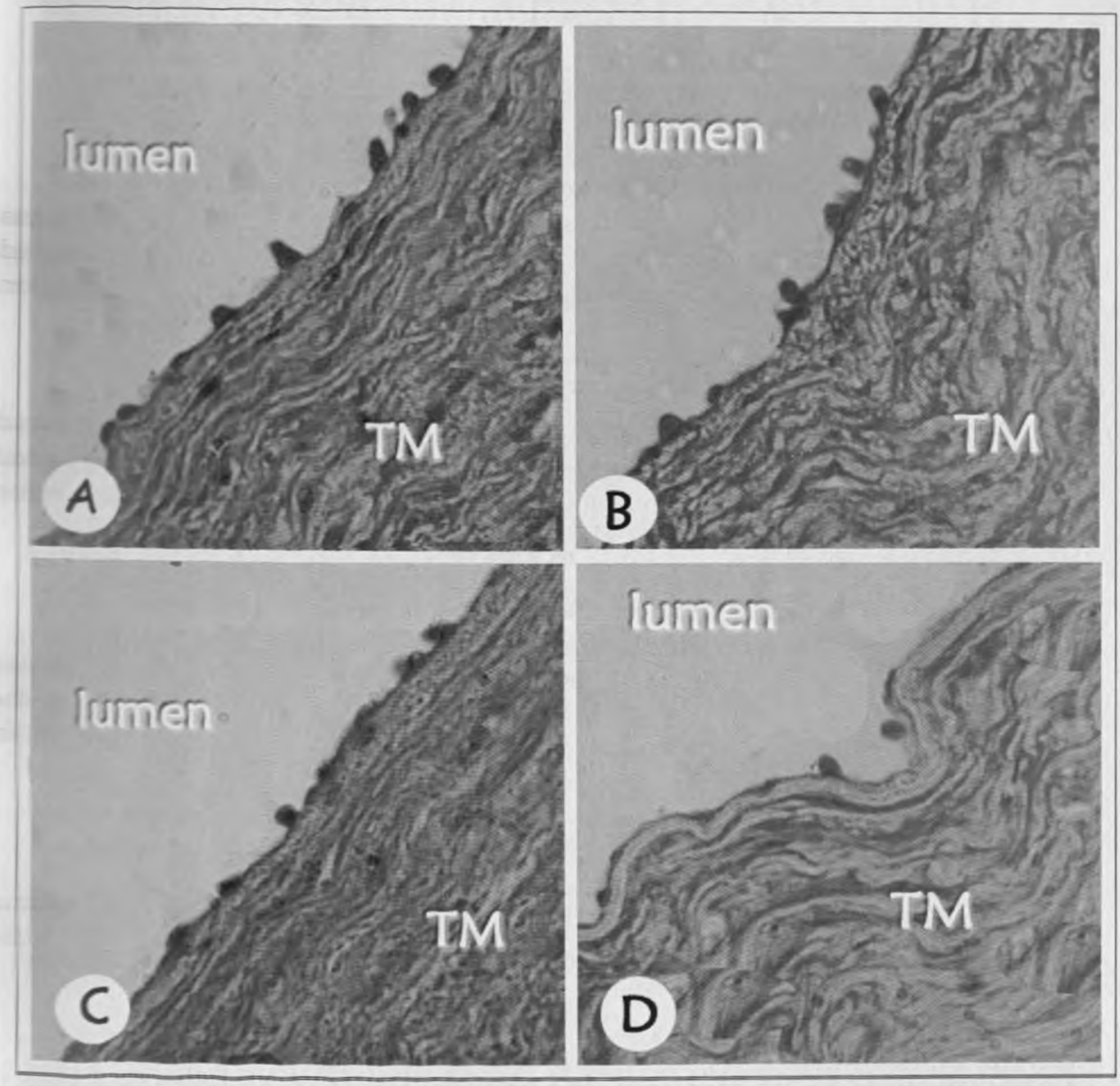


Figure 22 A-D: Internal elastic lamina of the goat aorta

A: Photomicrograph of the goat ascending aorta showing the tun and luminal zone of tunica media (TM). Note that the inter lamina (star) is difficult to distinguish from the elastic lamel tunica media. Weigert elastic stain. Magnification x400.

B: Photomicrograph of the goat proximal thoracic aorta showing intima and luminal zone of tunica media (TM). Note the distin elastic lamina (star). Weigert elastic stain. Magnification x400.

C: Photomicrograph of the goat distal thoracic aorta showing intima and luminal zone of the tunica media (TM). Note disti internal elastic lamina (star). Weigert elastic stain. Magnificatior

D: Photomicrograph of the goat abdominal aorta showing the tun and the lumina zone of the tunica media (TM). Note the disti folded internal elastic lamina (star). Weigert elastic stain. Ma x400.

Figure 22

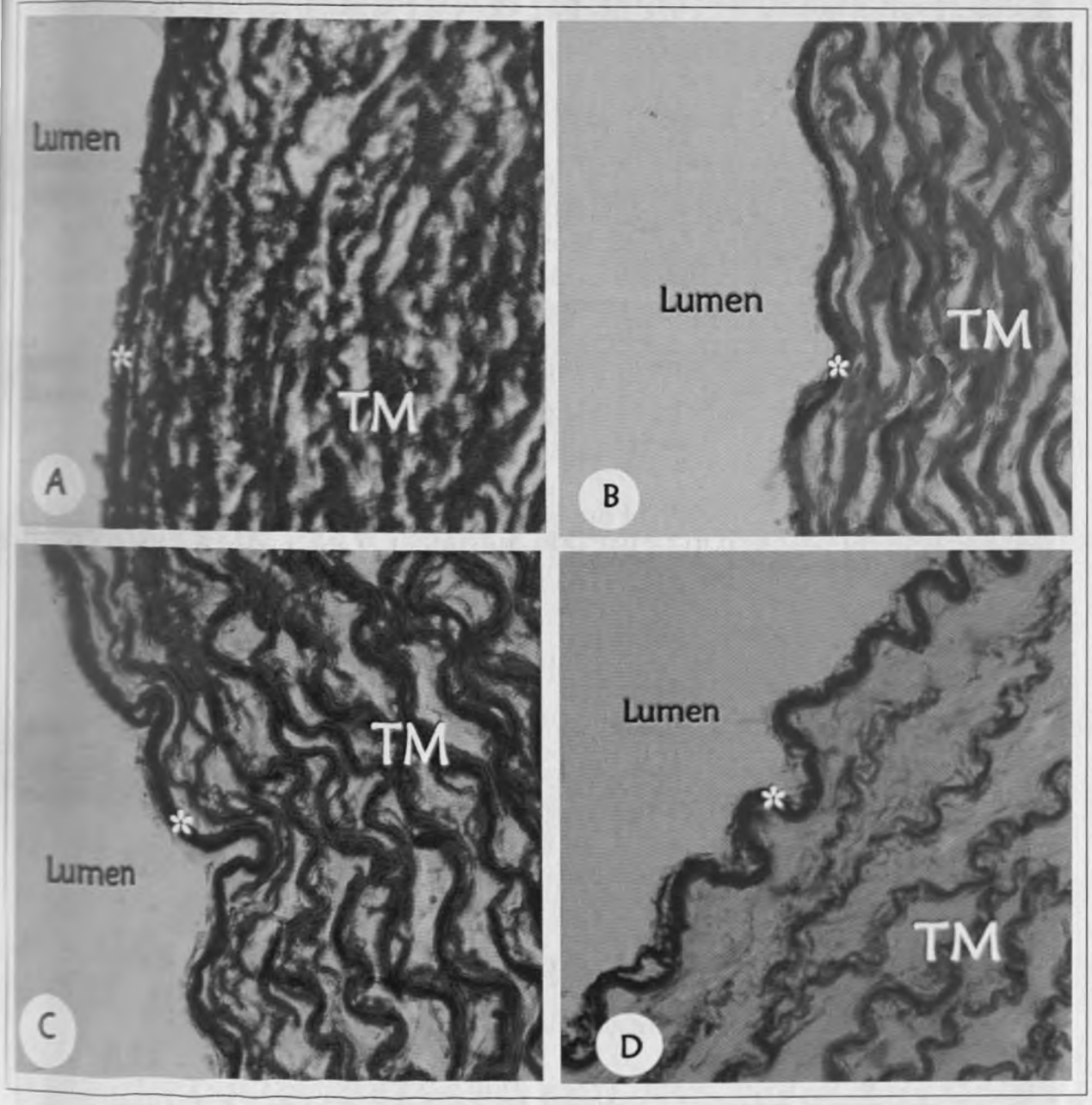


Figure 23 A-F: Regional variations in the tunica media of the goat aor

A: Photomicrograph of the goat ascending aorta to sho islands (MI) in the adventitial zone of the tunica media (TM) elastic stain. Magnification x100.

B,C: Photomicrographs of the goat thoracic aorta at T7(B) and T9(C muscle islands (MI) in the adventitial zone of the tunica me Weigert elastic stain. Magnification x100.

D: Photomicrograph of the goat thoracic aorta at T10, showing a si of muscle (MI) in the adventitial third of the tunica media (TM the tunica adventitia (TA). Weigert elastic stain. Magnification x1

E: Photomicrograph of the goat thoracic aorta at T12 showing a ho tunica media (TM) without muscle islands. Note the tunica (TA) surrounding the tunica media. Weigert elast Magnification x100.

F: Photomicrograph of the goat abdominal aorta showing a ho tunica media (TM) without muscle islands. Note the thi adventitia (TA). Weigert elastic stain. Magnification x100.

Figure 23

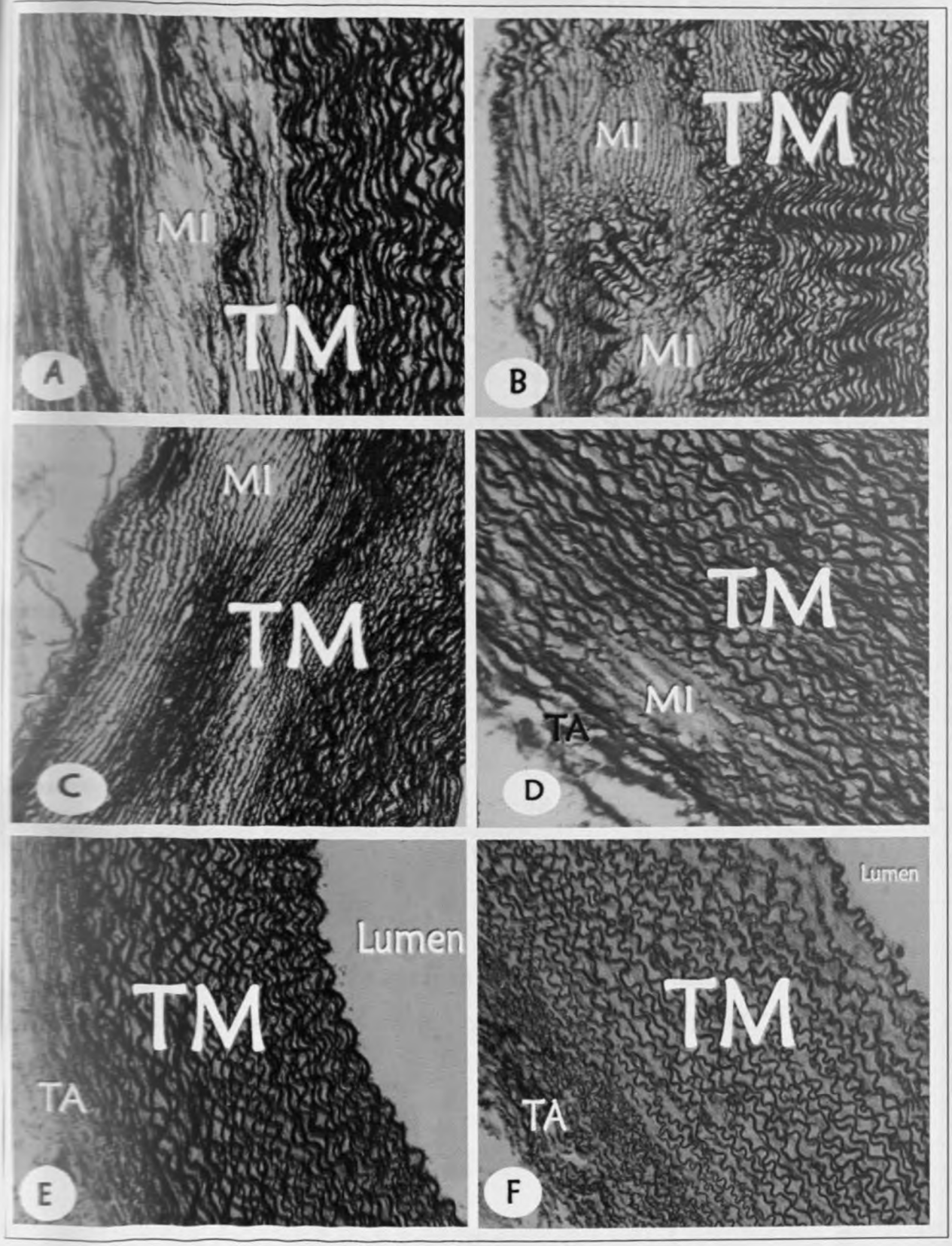
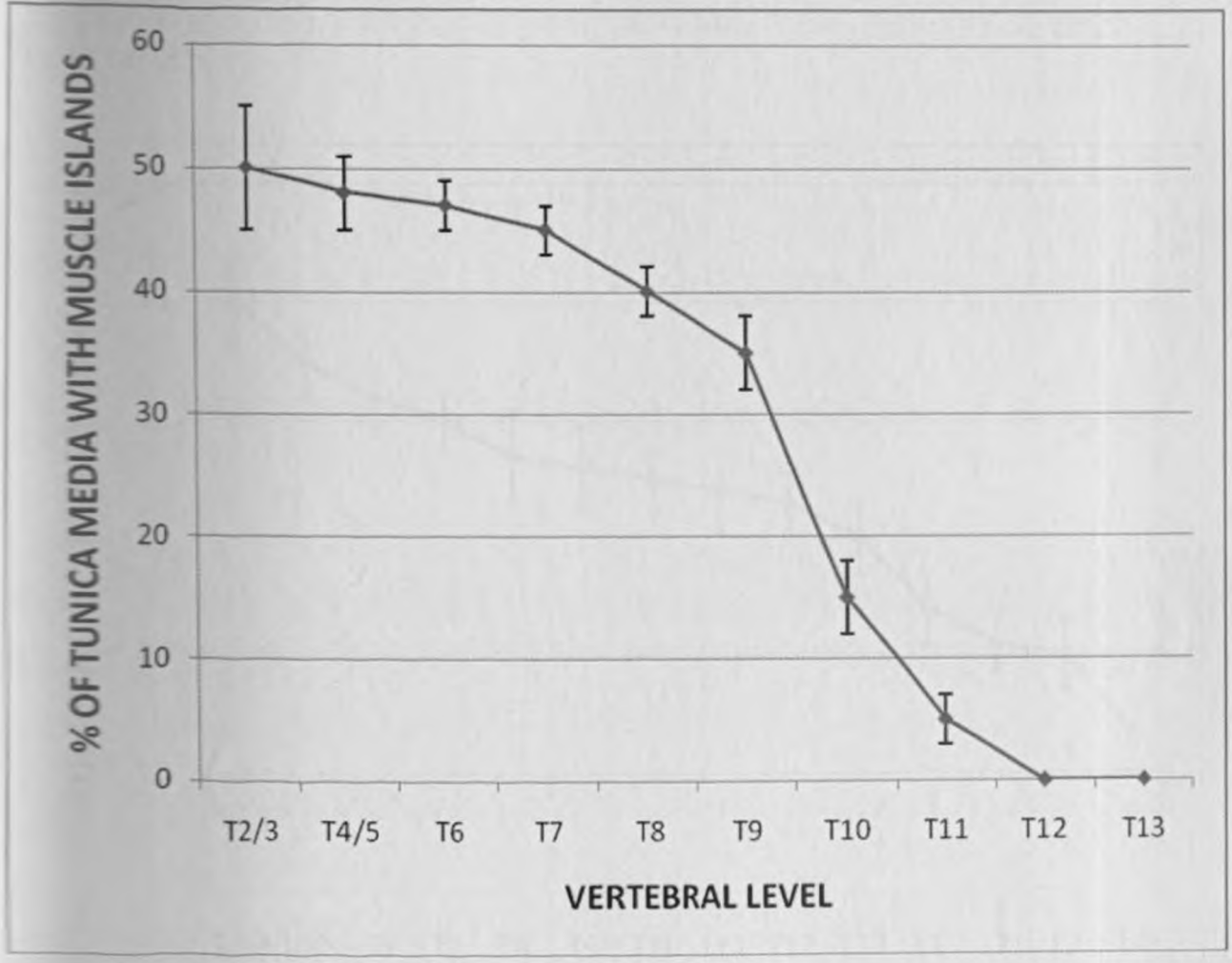
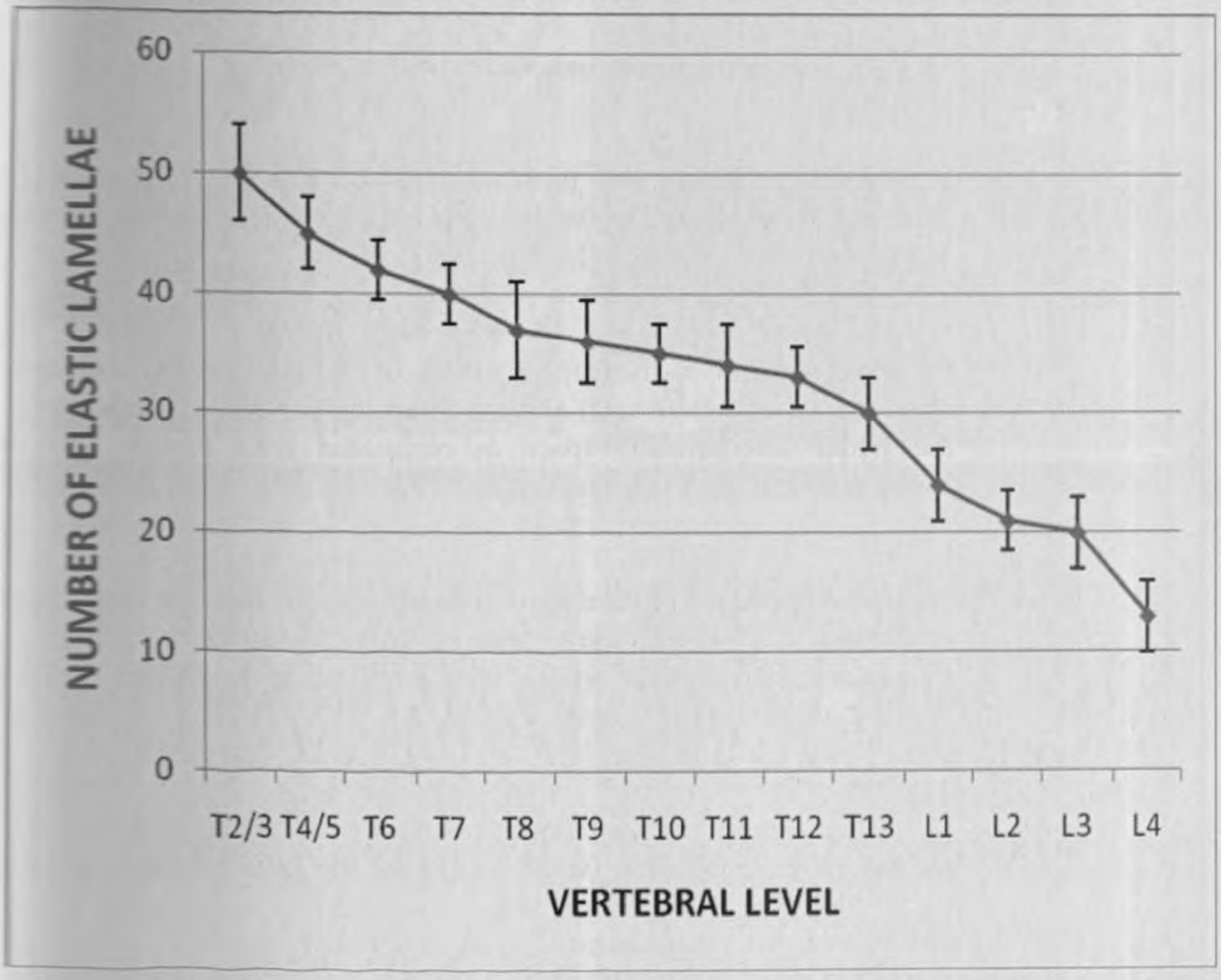


Figure 24



Graph showing the percentage of tunica media of the goat aorta with muscle islands. Note the craniocaudal decline in muscle islands down to T12.

Figure 25



Graph showing the average number of elastic lamellae at various vertebral levels of the goat aorta. Note the craniocaudal decline in the number of lamellae.

Figure 26A-D: Regional changes in the width of interlamellar spaces ir goat aortic tunica media.

A: Photomicrograph of the tunica media of the goat ascendir showing elastic lamellae (white star) and the interlamellar spa star). Weigert elastic stain. Magnification x400.

B: Photomicrograph of the tunica media of the goat aortic arch, elastic lamellae (white star) and the interlamellar space (black sta the wider interlamellar space as compared with Figure 26A Weigert elastic stain. Magnification x400.

C: Photomicrograph of the tunica media of the goat thoracic aorta, elastic lamellae (white star) and the interlamellae space (black sta the wider interlamellar space as compared with Figure 26I Weigert elastic stain. Magnification x400.

D: Photomicrograph of the tunica media of the goat abdomin showing elastic lamellae (white star) and the interlamellar spa star). Note the wider interlamellar space as compared with Fi above. Weigert elastic stain. Magnification x400.

Figure 26

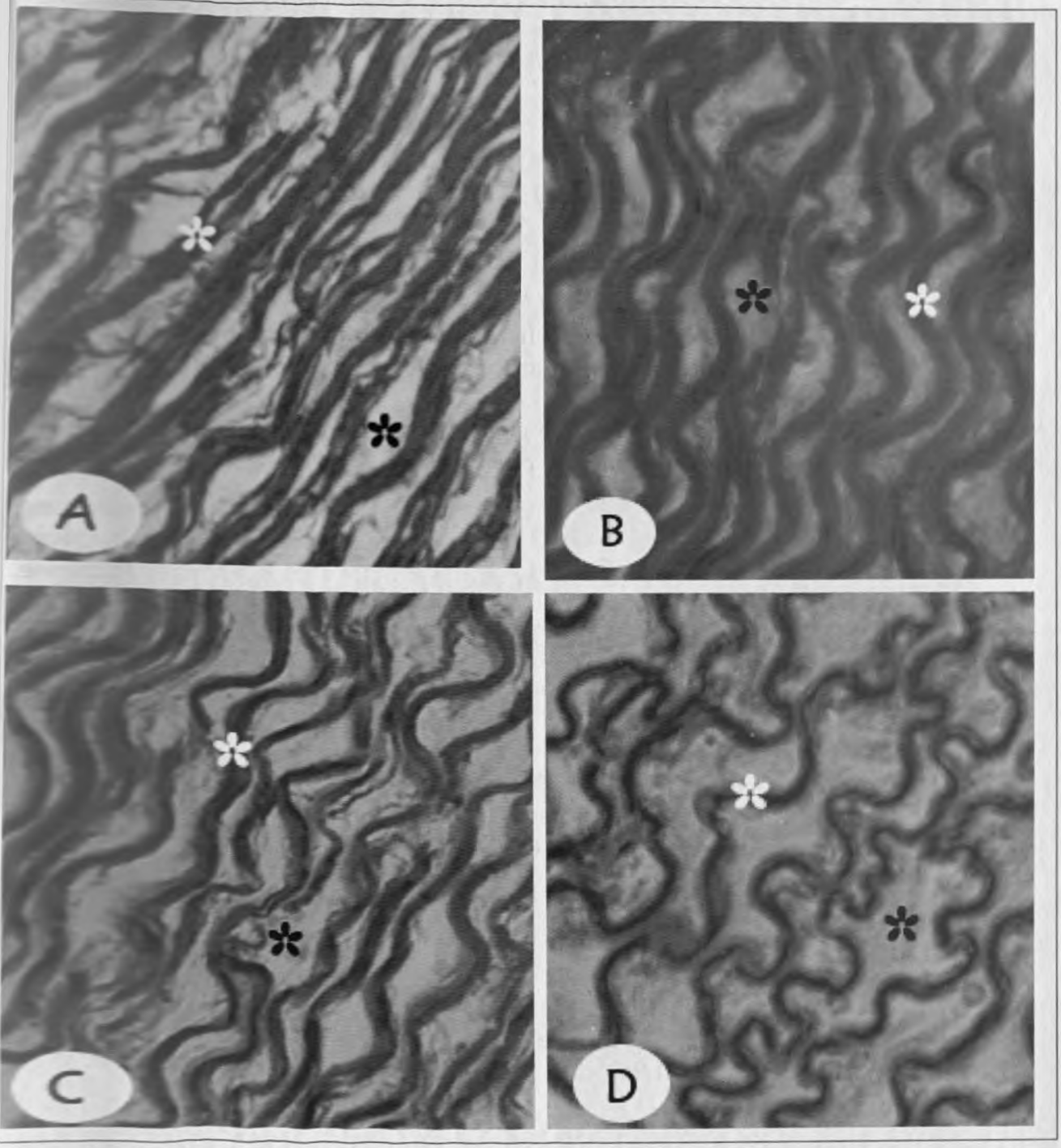


Figure 27A-D: Regional changes in the number of smooth muscle cell in the interlamellar spaces of the goat aortic tunica me

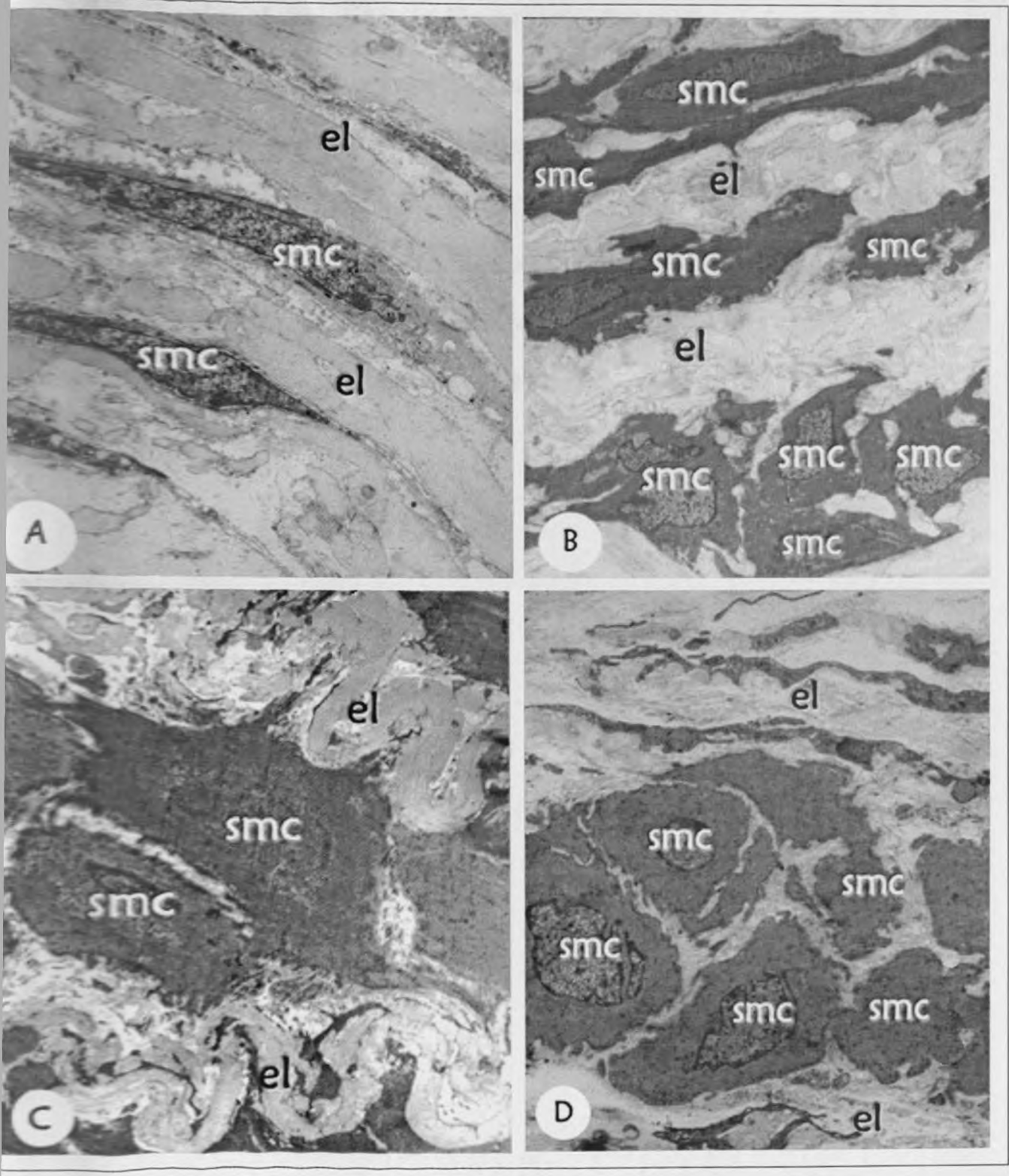
A: Electronmicrograph of the goat proximal thoracic aorta showin layer of smooth muscle cells (smc) between the elastic lam Magnification x1,950.

B: Electronmicrograph of the goat middle thoracic aorta at T9, occasional two layers of smooth muscle cells (smc) betwe lamellae (el). Magnification x1,340.

C: Electronmicrograph of the goat distal thoracic aorta at T12 sho layers of smooth muscle cells in the interlamellae space. Mag x4000.

D: Electronmicrograph of the goat abdominal aorta showing more layers of smooth muscle cells (smc) between elastic lame Magnification x1,950.

Figure 27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3.3.3 Regional variations in the tunica adventitia

The tunica adventitia displays a craniocaudal increase in thickness and elastic fibre content (Figure 28 A-D). In the ascending aorta, aortic arch and proximal descending aorta, the tunica adventitia is thin, and consists predominantly of collagen fibres, with a few scattered elastic fibres running in different directions (Figure 28A-C). In the abdominal region, it is much more prominent, compact and contains more elastic fibres than in the thoracic part (Figure 28D).

Figure 28 A-D: Regional variations in the tunica adventitia of the goa

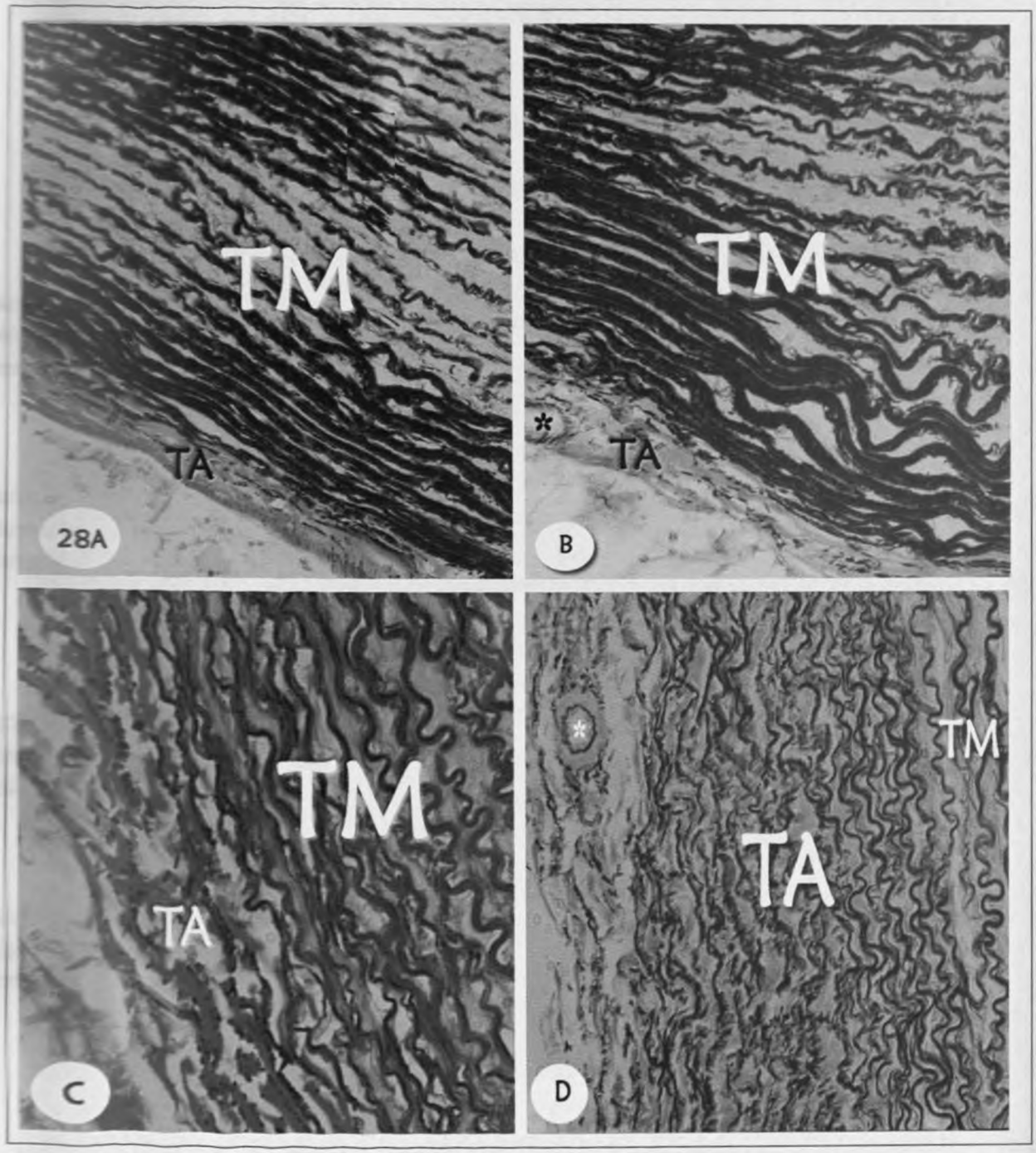
A: Photomicrograph of the goat ascending aorta showing t adventitia (TA) and tunica media (TM). Note that tunica ad thin and predominantly collagenous. Weigert elastic stain. Ma x250

B: Photomicrograph of the goat proximal thoracic aorta showing adventitia (TA) and tunica media (TM). Note the few scatte fibres (black) and a vasa vasorum (star) in the tunica adventiti elastic stain. Magnification x 250.

C: Photomicrograph of the goat distal thoracic aorta showing adventitia (TA) and tunica media (TA). Note the increase in and the definite amount of elastic fibres (black) in the tunica Weigert elastic stain. Magnification x 250.

D: Photomicrograph of the goat abdominal aorta showing t adventitia (TA) and tunica media (TM). Note the marked t preponderance of elastic fibres (black) and presence of a vasa (star). Weigert elastic stain. Magnification x 250.

Figure 28



3.4 Adrenergic Innervation of the aorta

Adrenergic nerve terminals are present in the tunica media, in the ascending aorta, aortic arch and proximal thoracic aorta (Figure 29A-D). These terminals are confined to the outer half of the tunica media where they co-localise with the muscle islands (Figure 29B,C). Nerve terminals appear as discrete varicosities and are distinguishable from the fluorescence of the elastic lamellae and fibres by their greater intensity and nodular appearance.

Whereas in the proximal parts of the aorta, the fluorophores are numerous and penetrate into the outer half of the tunica media (Figure 29A), in the distal thoracic aorta, they are fewer, and confined to the outer one quarter of the tunica media (Figure 29D). In the abdominal aorta, the fluorophores are very few if any, and are confined to the media-adventitial border (Figure 29E).

Figure 29A -E: Adrenergic innervation of the goat aorta

A: Fluorescent photomicrograph of the goat ascending aorta, fluorophores (arrow) distinguishable from elastic fibres (arrov their greater fluorescence and nodular appearance. Mag x1,100.

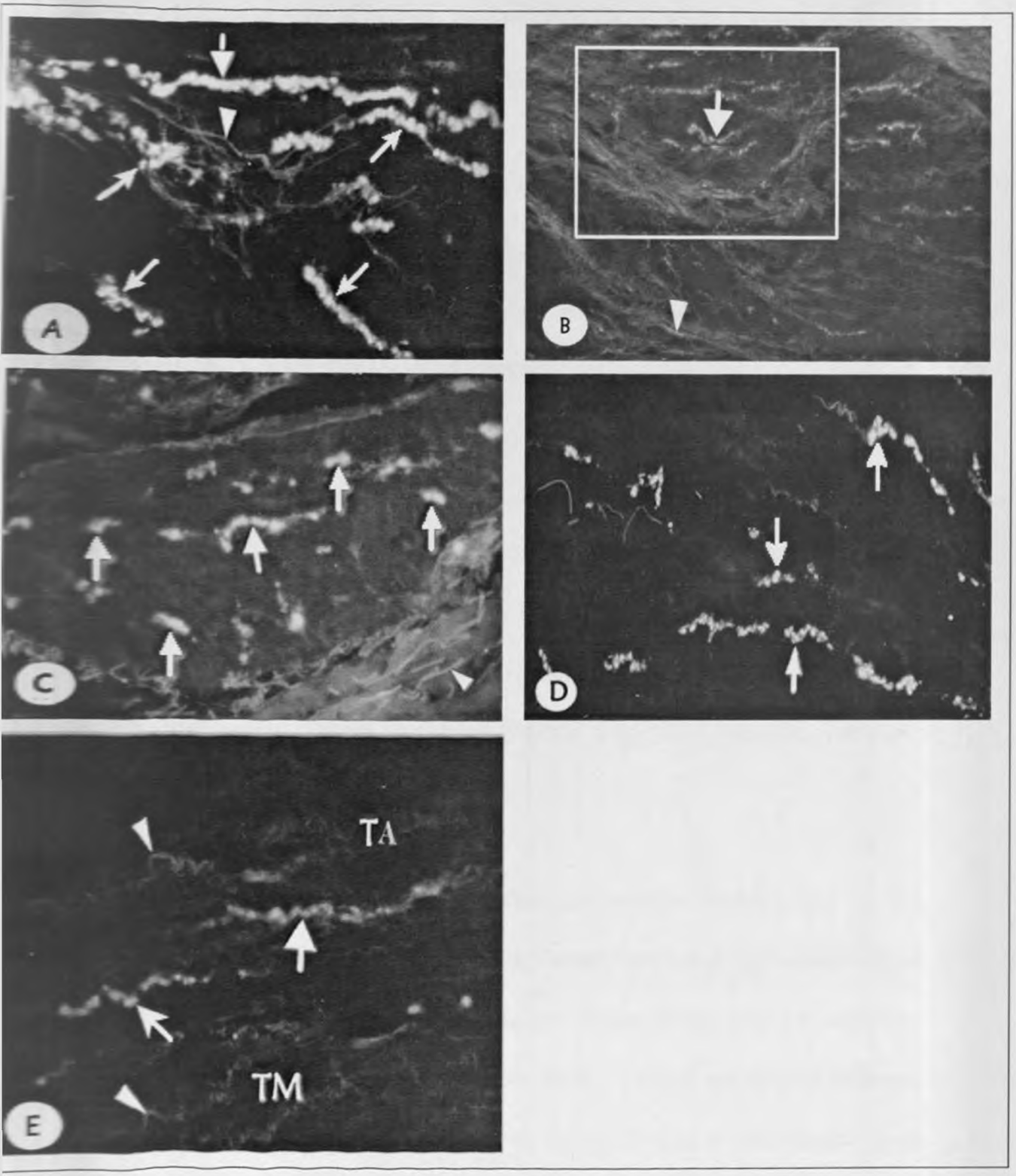
B: Fluorescent photomicrograph of the goat proximal thora showing the adrenergic fluorophores co-localised with the islands. The fluorophores (arrow) are distinguishable from ela (arrowhead) by greater fluorescence. Magnification x440.

C: Fluorescent photomicrograph of the goat proximal thora showing adrenergic fluorophores (arrow) co-localised with island. The elastic fibres (arrowhead) are shown outside th Magnification x1,100.

D: Fluorescent photomicrograph of the goat distal thoracic aorta, diminution of fluorophores (arrow) as compared with Figure 29 Magnification x1,100

E: Fluorescent photomicrograph of the goat abdominal aorta, fewer fluorophores (arrows), as compared with Figures 29A a and their relative confinement between tunica media (TM) an adventitia (TA). Note the elastic fibres (arrowheads) in the tuni and adventitia. Magnification x1,100

Figure 29



3.5 Age related changes in the aortic wall

Aging in the aortic wall affects all the components of tunica intima, media and adventitia. They involve both cells and extracellular matrix.

3.5.1 Age related changes in the tunica intima

In the tunica intima, aging affects the endothelium, subendothelium and internal elastic lamina. In goats older than five years, the predominantly flat endothelial cells in the ascending aorta and the aortic arch are separated by wide gaps (Figure 30A,B). Some of the endothelial cells are coated by amorphous material resembling elastin, part of which passes through the gap between endothelial cells (Figure 30C). Other cells are round, with large irregular nuclei, dendrite like extensions, and variable lysosome-like structures (Figure 30D). Some extensions of these cells show unusual pear shaped structures which project towards the lumen. These structures contain rough endoplasmic reticulum, (Figure 30D).

The subendothelial zone consistently circumferentially thickens and in the proximal segments comprises two layers, namely an inner one consisting of vacuolated loose tissue that stains poorly for elastic fibres, and an outer one containing bundles of elastic fibres (Figure 31A). There are cells of different staining ability, shapes and sizes in both layers of the subendothelial zone (Figure 31B,C). Some of the cells are round with blunt cytoplasmic extensions,

and a slightly dented nucleus that almost fills the whole cell (Figure 31C), while others are elongated with extensions (Figure 31C,D). The internal elastic lamina appears fragmented and disrupted (Figure 31C,D)

Figure 30A-D: Age related changes in the goat aortic endothelium

A: Electronmicrograph of tunica intima of the goat ascending aorta discontinuity (star). Magnification x1,950

B: Electronmicrograph of tunica intima of the goat ascending aorta endothelial discontinuity (star). Note the basement membrane closely following the basal contour of the endothelial Magnification x8,760

C: Electronmicrograph of tunica intima of the goat ascending aorta a gap between endothelial cells (ec) filled with elastin-like mater Magnification x4000.

D: Electronmicrograph of tunica intima of the goat ascending aorta a cell in the endothelial layer, with an irregular nucleus, extensions (arrowheads) containing rough endoplasmic reticulum lysosome-like structures (L) and phagocytic vacuoles (arrow). Magnification x8,760.

Figure 30

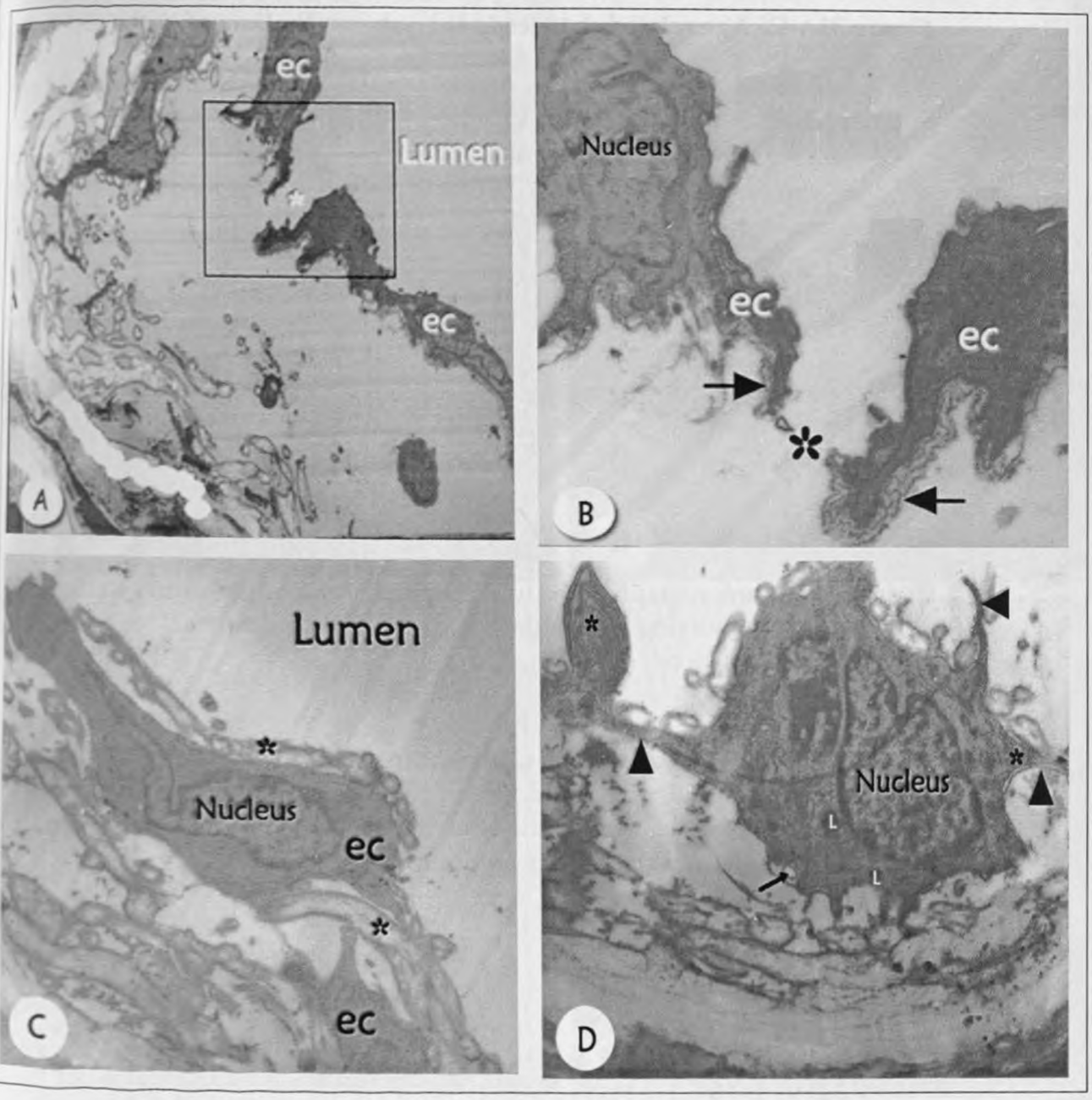


Figure 31A-D: Age related changes in the subendothelium of in the g

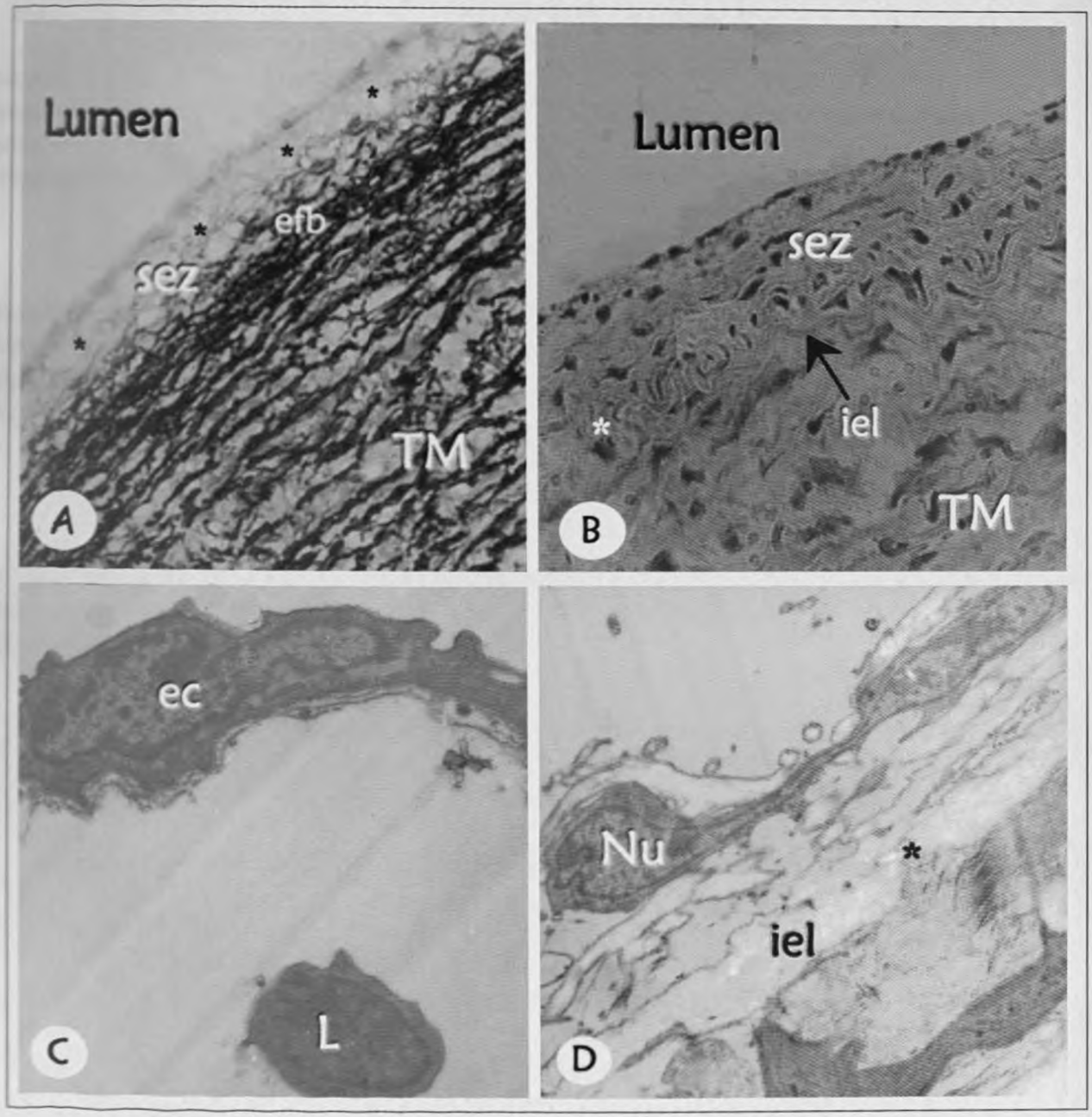
A: Photomicrograph of tunica intima of ascending aorta in a 7 yea showing two layers of the subendothelial zone (sez): an inner v (stars), and an outer one containing bundles of elastic fibres (e the fragmentation of elastic lamellae in the tunica media (TM elastic stain. Magnification x 250.

B: Photomicrograph of tunica intima of ascending aorta in a 7 yea showing the cellular nature of the subendothelial zone (sez). internal elastic lamina (iel) disrupted in some parts (star) intima from the tunica media (TM). Masons trichrome stain. Ma x 250.

C: Electronmicrograph of the tunica intima of ascending aorta in a old goat, showing a subendothelial cell with a dented nucleus th fills the entire cell (L), below the endothelial cell (ec). Magnificati

D: Electronmicrograph of the tunica intima of ascending aorta in a old goat, showing a subendothelial cell with an irregular nucleu luminal to the internal elastic lamina (iel). Note a disruption in t internal elastic lamina(star). Magnification x4000.

Figure 31



3.5.2 Age related changes in the tunica media

The age related changes in the tunica media affect smooth muscle cells, elastic lamellae and collagen fibres. In the luminal zone of the ascending aorta and aortic arch, the elastic lamellae are discontinuous and fragmented (Figure 32A). Between the pieces of the fragmented lamellae there are collagen fibres running in various directions (Figure 32B). Some of the collagen is tangled (Figure 32C) and in some instances joins the fragments (Figure 32D). Concomittant with these connective tissue fibre changes, is cellular degeneration, whereby smooth muscle cells become randomly oriented and may assume bizarre shapes (Figure 32E), with some becoming binucleated (Figure 32F). The smooth muscle cells in the adventitial zone are disorganized, and there is infiltration of small cells ressembling inflammatory ones (Figure 32H).

These aging changes display zonal and regional variations. In the ascending aorta and aortic arch, the elastic lamellae of the luminal zone are thin, discontinuous and fragmented (Figure 33A,C). In the adventitial zone, on the other hand, the elastic lamellae between the muscle islands are thin but continuous with relatively less fragmentation (Figure 33B,D). These aging changes show a progressive craniocaudal diminution such that in the transitional region around T10, the elastic lamellae are mildly fragmented (Figure 33E) with minimal change in the adventitial zone (Figure 33F). In the most distal thoracic aorta extending between T11 - T13, and the abdominal aorta, the tunica media

comprises continuous fairly well preserved wavy elastic lamellae, in both zones (Figure 33G,H).

3.5.3 Age related changes in the tunica adventitia

In the caudal part of the thoracic and in the abdominal aorta, aging change is associated with appearance of muscle strips in the tunica adventitia (Figure 34A). These strips run longitudinally, are present mainly on the ventral aspect and each of them is surrounded by elastic fibres that also run longitudinally (Figure 34B).

Figure 32A-H: Age related changes in the tunica media of the goat ao

A: Electronmicrograph of a 7 year old goat ascending aortic tu showing fragmentation of elastic lamellae (el). Magnification x8,760.

B: Electronmicrograph of a 7 year old goat ascending aortic tu showing increased amount of collagen fibres (co) running directions, between elastic lamellae (el) and smooth muscle Magnification x8,760.

C: Electronmicrograph of a 7 year old goat ascending aortic tu showing tangling and knotting of collagen fibres (arrow) fragments of elastic lamellae (el) close to smooth muscle Magnification x27,800.

D: Electronmicrograph of a 7 year old goat ascending aortic tur showing collagen (co) joining the fragments (star) of elastic la Magnification x8,760.

E: Photomicrograph of tunica media of aortic arch of a 6 yea showing disoriented smooth muscle cells (star). Mason's trichr Magnification x400.

F: Photomicrograph of tunica media of aortic arch of a 6 yea showing binucleated cells (arrowheads). Masons trichro Magnification x250.

G: Photomicrograph of tunica media of proximal thoracic aorta o old goat showing distortion of smooth muscle islands (sta adventitial zone. Mason's Trichrome stain. Magnification x400.

H: Photomicrograph of tunica media of proximal thoracic aorta o old goat showing inflammatory-like cells (square) between smoo cells (star). Mason's Trichrome. Magnification x 400.

Figure 32

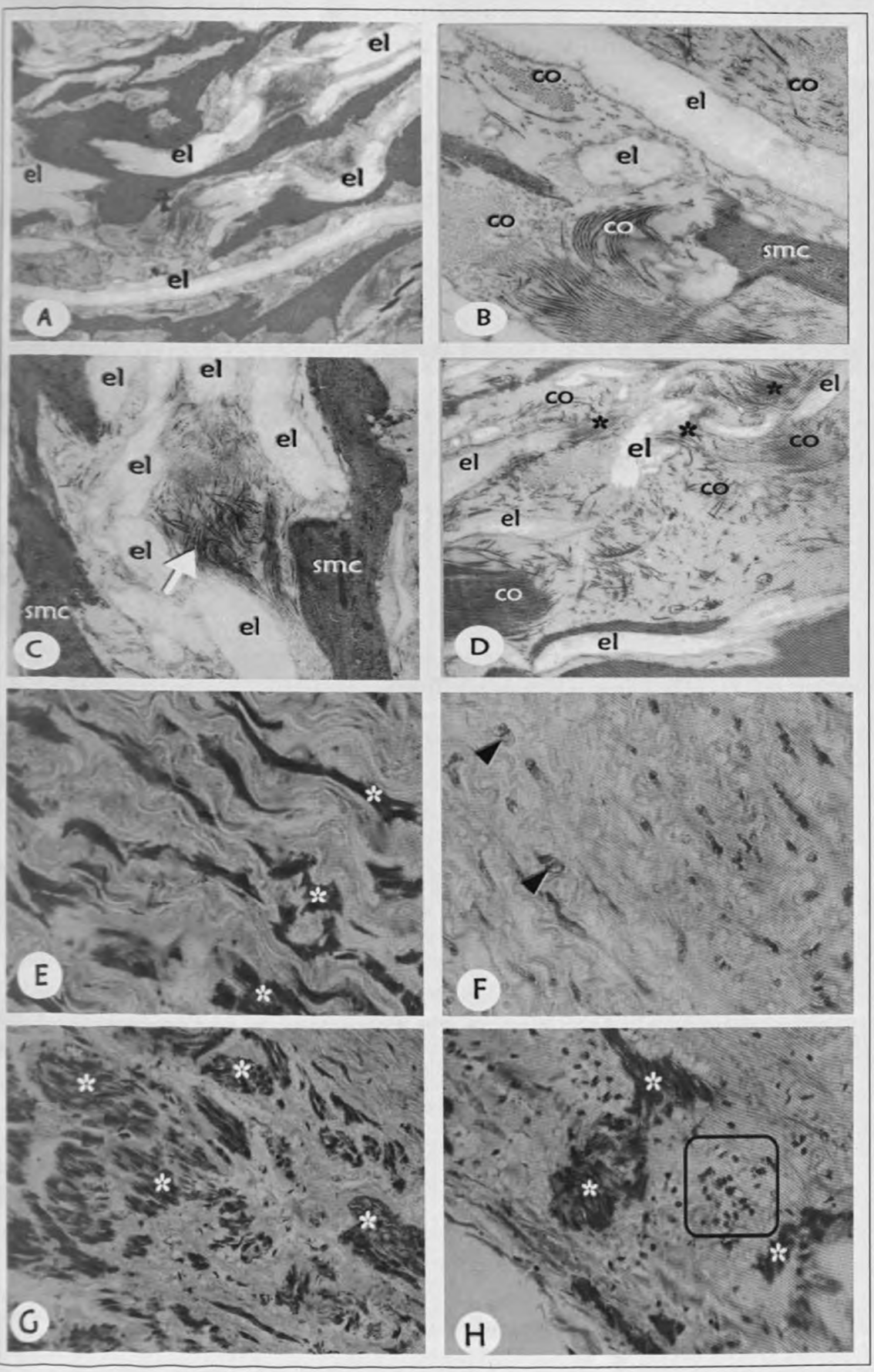

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Figure 33A-H: Zonal and regional variations of age related changes in tunica media of the goat aorta

A: Photomicrograph of a 7 year old goat ascending aorta sho luminal zone of the tunica media. Note the fragmentation lamellae (stained black). Weigert elastic stain. Magnification x400

B: Photomicrograph of a 7 year old goat ascending aorta sho adventitial zone of the tunica media. Note the continuous elasti (arrows) next to bundles of smooth muscle (smc). Weigert ela Magnification x400.

C: Photomicrograph of a 7 year old goat proximal thoracic aorta, the luminal zone of the tunica media. Note the fragmentation lamellae (stained black). Weigert elastic stain. Magnification x40

D: Photomicrograph of a 7 year old goat proximal thoracic aorta, continuous elastic lamellae (arrows) next to smooth muscle bund Weigert elastic stain. Magnification x400.

E: Photomicrograph of a 7 year old goat distal thoracic aorta sho luminal zone of tunica media. Note areas of elastic lamella fragı (star) among continuous elastic lamellae (arrows). Weigert ela Magnification x400.

F: Photomicrograph of a 7 year old goat distal thoracic aorta sho adventitial zone of the tunica media. Note the thick continuo lamellae (square), next to a muscle island (MI). Weigert elas Magnification x400.

G: Photomicrograph of a 7 year old goat abdominal aorta sho luminal zone of the tunica media. Note the continuous wa lamellae (stained black) separated by wide interlamellar space Weigert elastic stain. Magnification x400.

H: Photomicrograph of a 7 year old goat abdominal aorta sho adventitial zone of the tunica media. Note the continuous wa lamellae (stained black) separated by wide interlamellar space Weigert elastic stain. Magnification x400

Figure 33

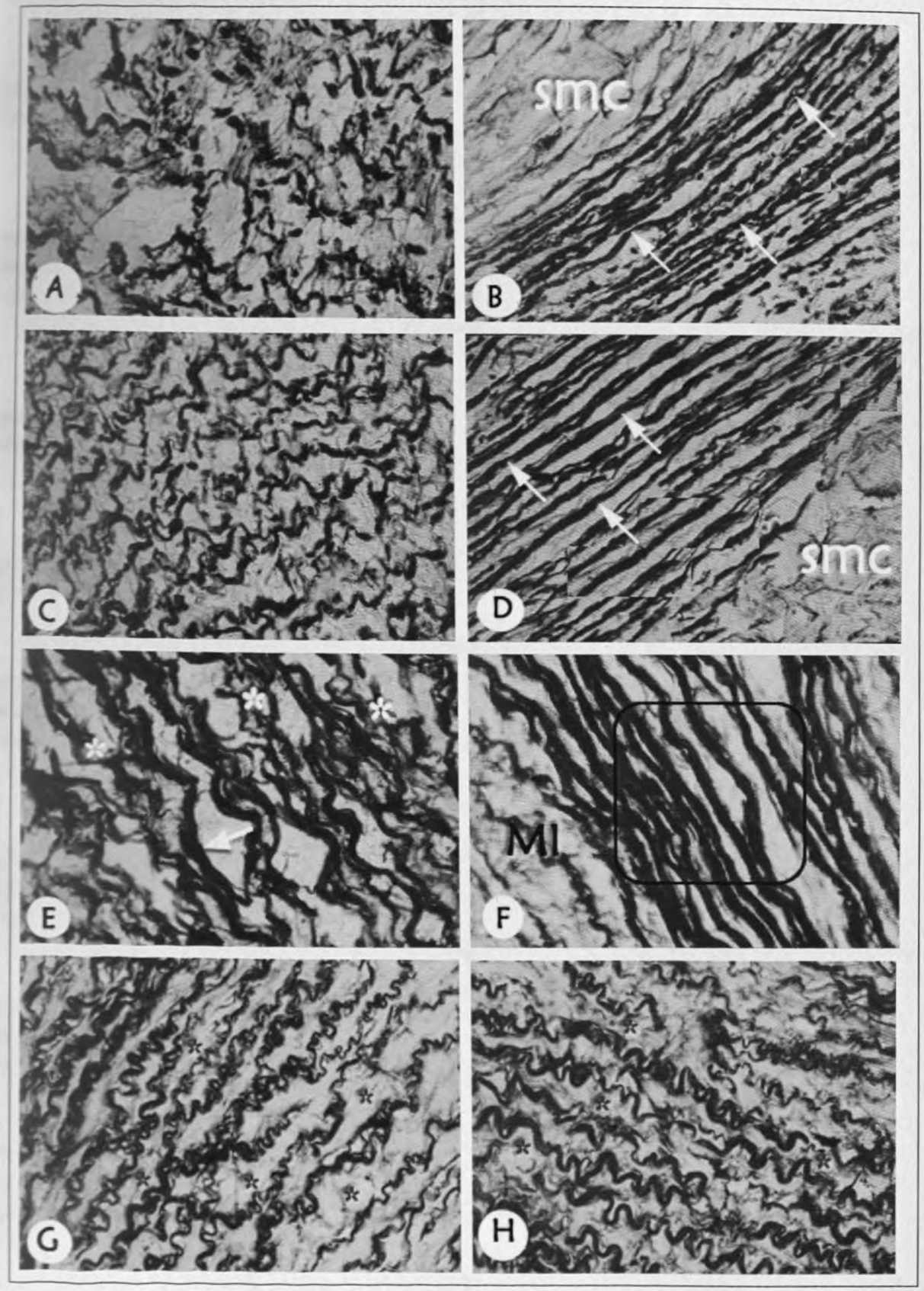
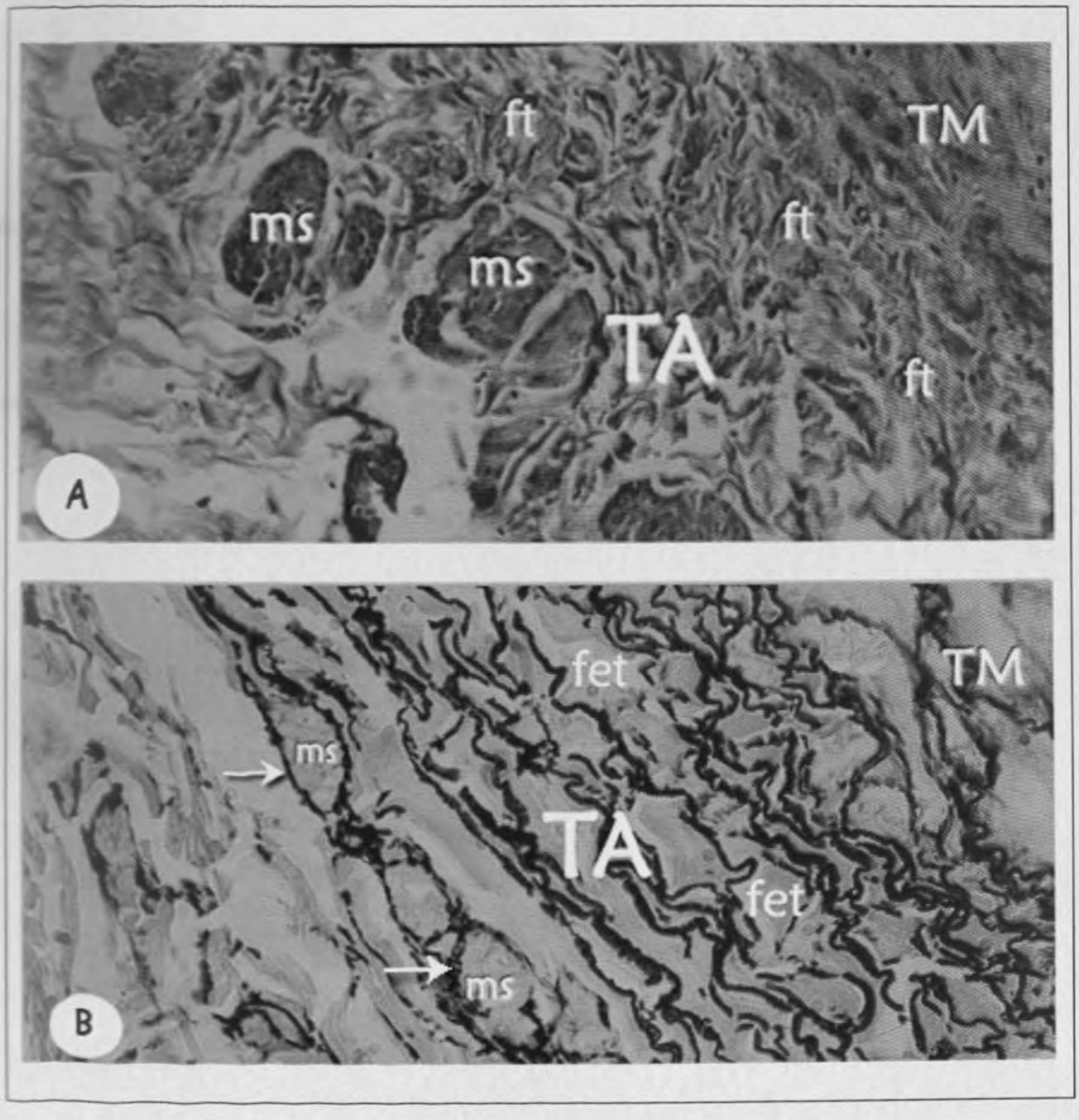


Figure 34 A,B: Muscle strips in the tunica adventitia of the aorta in a

A: Photomicrograph of tunica adventitia (TA) in the abdominal aor year old goat. Note the muscle strips (ms) separated from the tu media (TM) by fibrous tissue (ft) Mason's trichrome stain. Mag x 250.

B: Photomicrograph of tunica adventitia (TA) in the abdominal aor year old goat. Note the muscle strips (ms) surrounded by elastic (arrows). The strips are separated from the tunica media (TM) b fibroelastic tissue (fet). Weigert elastic stain. Magnification x 250

Figure 34



CHAPTER FOUR

DISCUSSION AND CONCLUSIONS

In the goat, the ascending aorta gives rise to the brachiocephalic trunk, then continues as the aortic arch. This shows that the goat aorta, in a way, resembles that of other ruminants, and equines, in which the aortic arch gives rise to only one branch the brachiocephalic trunk (Ghoshal, 1975; Kimani, 1979). In most other mammals, however, the aortic arch gives rise to either two branches namely brachiocephalic trunk and left subclavian artery (Ghoshal, 1975; Vladova *et al.*, 2005) or three branches namely the brachiocephalic trunk, left common carotid and left subclavian (Gabella, 1999). These variations in the pattern of branching of the aorta may have a developmental basis (Wheaton *et al.*, 1984; Sadler, 1988). Although it has been reported that arterial walls are remodelled in response to haemodynamic forces (Langille, 1996; Chesler *et al.*, 1999), it is not known whether the difference in branching patterns of the aorta is reflected in its histomorphology.

4.1 The structure of the aorta

The general structure of the goat aorta resembles that of other mammals (Gabella, 1999; Ross *et al.*, 2003). Details of the organization of cell types and matrix fibres as well as their ultrastructure, fibre interconnections and cell-matrix

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linkages, however, display some features which are hitherto unreported. Many of these features are common to all parts of the aorta, and are distributed in the three layers.

4.1.1 Tunica intima

The tunica intima comprises the endothelium, subendothelial zone and an internal elastic lamina. In all parts of the aorta studied, the endothelium comprises morphologically flat and round cells, as reported for the aorta of the rabbit (Hineck and Rosnowski, 1975; Hineck and Konsinski, 1975), rat (Baryshnikova *et al.*, 1989) and human (Kolpakov *et al.*, 1996). Morphological differences, reported from cell to cell, and over short distances within the vessel wall (Barbee *et al.*, 1994), may be determined by the interplay of haemodynamic microstimulation and surface geometry (Davies *et al.*, 1995, 1999, 2001; 2003).

Changes in shape of endothelial cells may arise from reorganization of the cytoskeleton as cells adapt to shear stress (Dewey *et al.*, 1981; Galbraith *et al.*, 1998) and also from rapid displacement and deformation of cytoskeletal filaments in response to a change of flow (Wang *et al.*, 2001; Stamenovich *et al.*, 2002; Helmke and Davies, 2002). In addition, it has been proposed that maintenance of shape of endothelial cells depends on the state of tension generated through anchorage of the cytoskeleton to focal adhesions (Burridge *et*

al., 1988, Ingber, 2002), and to neighbouring cells (Takeichi, 1988; Osawa *et al.*, 2002). Accordingly, the heterogeneity of endothelial cells found in the present study may reflect levels of microstimulation to which the different sites are subjected during pulsatile blood flow.

Caveolae of varying depth were observed on the luminal surface of endothelial cells, in agreement with other reports (Oli and Schitzer, 1998; Anderson, 1998; Boyd *et al.*, 2003). It is currently believed that apart from transporting molecules (Okamoto *et al.*, 1998), these endothelial caveolae are flow sensors which convert mechanical stimuli into chemical signals, adapting the endothelium to augmented laminar flow (Park *et al.*, 1998; Rizzo *et al.*, 1998; Sun *et al.*, 2002; Spisne *et al.*, 2003). It is probable that their prominence is related to high blood flow that occurs in the aorta. Pertinent to this suggestion are reports stating that chronic shear induces caveolae formation (Boyd *et al.*, 2003).

Endothelial cells have been observed to be connected to each other by finger like extensions where the cell membranes are characterized by high electron density. Endothelial cells are known to be laterally joined by tight junctions (Fishman, 1982; Gabella, 1999; Yeh *et al.*, 2000). Accordingly, the interdigitation between finger-like cell extensions may be designed to increase the surface area between the cell membranes for tighter mechanical coupling, thus preventing detachment in the wake of high shear stress.

Endothelial cells were seen to attach to the subendothelial extracellular matrix through a lamellated basement membrane, similar to those in the aorta of chicken (Moss and Bendit, 1970) rat (Laver-Rudich *et al.*, 1978), rabbit (Kramer, 1985) and mouse (Davies, 1994). These workers proposed that this type of basement membrane is made of microfibrils which play a role in cell anchorage, and thus may be involved in maintaining the structural integrity of the endothelial cell layer. A similar mode of attachment has been described in arteries subjected to elevated pressures, where it is thought to constitute adaptation to luminal shearing (Limas *et al.*, 1980; Kimani, 1981a). In the present study, fibrillar basement membrane has been observed in all the goats, suggesting that it is part of the normal structure of the aorta in these animals.

Endothelial cells, in some parts, are anchored onto the basal lamina by focal areas of high electron density, as observed in rabbit arterial intima, which revealed two modes of basal endothelial attachment, namely the continuous basement membrane complex, and the localized points of tight attachment to underlying elastic elements at hemi-desmosome-like contacts (Bierring and Kobayashi, 1963; Stehben, 1966; Tsao and Glagov, 1970). It is probable, as suggested by Tsao and Glagov (1970) and emphasized by Zarins *et al.*, (2004), that these forms of attachment prevent slippage and endothelial detachment by elevations of shear stress or other mechanical forces.

The mode of endothelial attachment is not uniform for the whole cell, or for different cells. This may be consistent with reports that endothelial cell-extracellular matrix interaction is dynamic (Hallman *et al.*, 2005). Accordingly, it will depend on the type of endothelium and the prevailing haemodynamic conditions at a particular time. The heterogeneity of endothelial basal attachment suggests that different parts of the cell, and of the aortic lumen experience different haemodynamic stresses.

In the regions where the subendothelial zone is discernible, and in intimal thickenings there is a heterogenous population of cells. Most of these cells are typical smooth muscle cells or their modified form. Similar cell types have been reported in other animals (Tsao, 1968; Babaev, 1988; Nicosia and Villaschi, 1995). The presence of these smooth muscle cells in the normal rabbit aorta, and not in the inferior vena cava led to the suggestion that they play a role in repair of aortic endothelium damaged by high and pulsatile pressure in this vessel (Still and Dennison, 1967; Tsao, 1968). Indeed, it has been shown that smooth muscle cells can de-differentiate into endothelial cells (Tsao, 1968; Arciniegas *et al.*, 2000a,b). Further support for the repair role of these cells is drawn from the observation that balloon induced injury is usually followed by intimal hyperplasia characterized by smooth muscle proliferation (Dimayuga *et al.*, 2005; Wakamatsu *et al.*, 2005; Shimazawa *et al.*, 2005; Lau *et al.*, 2006).

Observations of the present study reveal that some of the subendothelial cells have features of the synthetic smooth muscle phenotype, and are intimately associated with elastic and collagen fibres which in some cases appear to be emanating from them. It is possible therefore, that these cells are involved in the synthesis of extracellular matrix of the subendothelial zone. Pertinent to this suggestion is the observation that after mechanical de-endothelialization of blood vessels early de-differentiation occurs in some smooth muscle cells in the tunica media to activate them so that they are able to migrate and participate in intimal thickening (Shinohara *et al.*, 2005; Louis *et al.*, 2006).

The other cells observed are fibroblast-like, similar to those reported in the aortic intima of monkeys (Rhodin, 1962); rats (Gerrity and Cliff, 1975); swine (Thomas *et al.*, 1979) and in human (Orekhov *et al.*, 1984; Rekhter *et al.*, 1991; Frid *et al.*, 1992; Glukhova and Koteliansky, 1995). Like the synthetic smooth muscle counterparts, these cells are intimately associated with connective tissue fibres. Functionally, such fibroblast-like cells have been implicated in intimal remodeling by synthesis and secretion of extracellular matrix (Whorhley *et al.*, 1995; Seidel 1997; Sartore *et al.*, 1997).

Monocyte-like cells are also present in the subendothelial zone. Similar cell types were reported in the subendothelium of the aorta in rabbits (Duff *et al.*, 1957); human (Geer, 1965; Navab *et al.*, 1988), rats (Joris *et al.*, 1979), swine (Gerrity *et al.*,

1979), pig, man and sheep (Kim *et al.*, 1985; Massman *et al.*, 1989), where they have been associated with atherosclerosis (Gerrity *et al.*, 1979; Ross, 1986). The consistent presence of these cells in young free ranging goats, in the present study suggests in agreement with Kim *et al.*, (1985) and Massman *et al.*, (1989), that they are normal physiological components of the subendothelial zone. They probably transform into vascular dendritic cells (Bobryshev and Lord, 1995) which are involved in the maintenance of immune surveillance in the normal arterial intima.

The subendothelial zone contains elastic and collagen fibres oriented in various directions. The presence of the connective tissue fibres in this layer is important in anchoring the endothelium to the internal elastic lamina, yet allowing its free play during the rhythmic contraction and dilatation of the vessel (Sato *et al.*, 1994; Dingemans *et al.*, 2000; Ross *et al.*, 2003). Notably, there are more abundant elastic than collagen fibres. Because of their long range reversible stretchability (Sandberg, 1976; Kielty *et al.*, 2002), it is probable that this greater quantity of elastic fibres adapts the intima to shear stress to which the vessel is subjected.

A hitherto undescribed feature of the subendothelial connective tissue is that elastic and collagen fibres attach onto the smooth muscle cells, and that the two fibres are interconnected with each other. These are features which have only been associated with the tunica media and adventitia, for purposes of

distributing and withstanding mechanical forces (Bezie *et al.*, 1998, 1999; Shadwick, 1999; Snowhill *et al.*, 2004). The tunica intima is thought to yield little if any, contribution to the structural mechanics of the vessel (Humphrey and Na, 2002; Greenwald, 2007). The physical inter-linkage between the components of the subendothelial zone, observed in the present study, suggests that this layer has structural features similar to those of the tunica media and adventitia, and contributes significantly to the mechanical properties of the aorta.

In most parts of the aorta, the dorsal aspect displays diffuse intimal thickenings (DIT). Similar enlargements of the subendothelial space have been described in the aorta of rabbits (Kurozuni *et al.*, 1978), swine (Scott *et al.*, 1979), rats (Kolpakov *et al.*, 1996) and in monkeys (Sato *et al.*, 1994). These areas of intimal thickening are believed to represent potential sites of future atherosclerosis (Kablak-Ziembika *et al.*, 2005; Rohani *et al.*, 2005). Considering, that this feature was observed in all age groups studied, it may constitute a repair process consequent to shear stress to which the endothelium is constantly subjected.

The predominance of the DIT on the dorsal aspect is similar to that reported in humans by Long *et al.*, (2000) and Bonert *et al.*, (2003), who proposed that, aortic curvature causes lower wall shear stress (WSS) on the posterior wall than on the anterior. There may be an inverse relationship between WSS and intimal thickening (Pedersen *et al.*, 1997, 1999; Bonert *et al.*, 2003). It is possible that

similar haemodynamic circumstances prevail in the goat, thus contributing to the distribution of DIT.

The present study further reveals that branching points are characterized by intimal thickenings called bifurcation cushions, with a prepondence of elastic fibres running almost perpendicular to the elastic lamellae of the tunica media. Such cushions have been described in cerebral arteries of dogs, pigs and man (Nanda and Getty, 1972; Lee, 1995; Ial'tsev and Shormanov, 2003), coronary arteries of various animals (Yohro and Burnstock, 1973; Sjoblom *et al.*, 1989; Shormanov, 1989; 1990; Whelan, 1996), lingual arteries (McLeod and Soames, 1985), ear arteries (Ninomiya, 2000) and renal arteries (Ial'tsev and Shormanov, 2003). At these points, the general postulation is that they constitute an adaptation to local haemodynamic stress.

It has been proposed that elevated haemodynamic stress at the branching points stimulates smooth muscle cells to proliferate and produce smooth muscle derived extracellular factor (SMEF) (Potvliege and Bourgain, 1980). This factor in turn induces synthesis of elastin and collagen by endothelial cells (Stenmark *et al.*, 1988). More recently, it has been suggested that these cushions are also involved in regulation of blood flow (Whelan *et al.*, 1996; Ninomiya, 2000; Ial'tsev and Shormanov, 2003). It is probable, therefore, that the bifurcation cushions of

the aorta, described in the present study, contribute to regulating blood flow into the branches.

Similar to the aorta of other animals, the internal elastic lamina of the goat aorta shows fenestrations which allow communication between the tunica intima and the tunica media (Yuan *et al.*, 1991; Huang *et al.*, 1994, 1997, 1998). Another important feature of the internal elastic lamina, is that it is connected to the smooth muscle cells, elastic and collagen fibres both on the luminal and the abluminal side. These observations, hitherto unreported, suggest that the tunica intima and media are physically interlinked. This linkage may be responsible for the structural integration which permits the endothelium, smooth muscles, fibroblasts and extracellular matrix to act in concert, thus producing a structure that is capable of withstanding the stresses to which the vessel wall is subjected (Snowhill *et al.*, 2004).

The structural linkage between the components of the tunica intima and tunica media suggest that the internal elastic lamina has a more significant mechanical role than previously appreciated. Pertinent to this suggestion are the results of numerical simulations of the internal elastic lamina which have revealed that the shear stress surrounding a smooth muscle cell near a fenestration can be 100 times higher than that recorded away from the fenestrations (Tada and Tarbell,

2000, 2001). Recent studies have suggested that the internal elastic lamina does support longitudinal forces (Farand *et al.*, 2007).

In the thoracic and abdominal aortae, the internal elastic lamina is thick and highly folded, similar to that reported in the aorta of rabbits (Svendsen and Jorgesen 1977; Svendsen and Tindal 1983; Svendsen, 1985) and dogs (Orsi *et al.*, 2004). The folding of the internal elastic lamina and hence the intima, may contribute to the resistance of the aorta to dilatation during ventricular ejection of blood. Secondly, the folds may constitute a "reserve" of intima which is stretched during systole to accommodate the increased luminal circumference (Tindal and Svendsen, 1982).

The splitting of the internal elastic lamina, observed in the present study, displays circumferential asymmetry, being found mainly in the neigbourhood of the diffuse intimal thickenings. Similar splitting has been reported in the rat and rabbit aorta during hypertension (Hadjiisky and Peyri, 1982; Lesauskaite *et al.*, 1999), and is thought to constitute an adaptation to the elevated haemodynamic stress (Tada and Tarbell, 2004). In muscular arteries such as hepatic, it may be due to synthesis of new elastin (Krus *et al.*, 2000). Accordingly, it is possible that the multiplication of the internal elastic lamina, observed in the present study, is part of the repair process of DIT.

4.1.2 Tunica media

The goat aortic tunica media comprises smooth muscle and other cells, elastic lamellae, collagen and elastic fibres. In the proximal segments there is a transmedial zonation, into a luminal zone of continuous elastic lamellae and an adventitial zone in which muscle islands interrupt elastic lamellae. Similar organization has been reported in the thoracic aorta of the cow (Knieriem, 1967). These observations are however at variance with the conventional medial lamellar unit structure proposed by Wolinsky and Glagov (1967) and consistently reported in aorta of mammals including laboratory rodents (Berry *et al.*, 1974; Awal *et al.*, 1995; Mello 1999); pigs (Tanigawa *et al.*, 1985; Sokolis *et al.*, 2002)), rabbits (Viegas *et al.*, 2001) and dogs (Orsi *et al.*, 2004). They are also at variance with transmedial zonation in terms of distribution and form of extracellular matrix fibres in the aorta of human (Fieldman and Glagov, 1971; Sans and Moragas, 1993; Dingemans *et al.*, 2000), sheep (Roach, 1983), fin whale (Gosline and Shadwick, 1996), and in cellular composition as in the aorta of the human embryo (Minorov *et al.*, 1995) or orientation of smooth muscle as in the rat (Fujiwara and Uehara, 1992).

The functional significance of this transmural zonation is unclear. It may, however, be related to the pattern of regulation of blood flow to the forelimbs and the cranial structures. This suggestion is supported by the proposal that in the birds, transmural zonation into a luminal elastic and an adventitial muscular

zones permits changes in vessel radius to modify the effects of drastic circulatory changes in the posterior part of the body during flight (Berry *et al.*, 1974). Among the mammals, in the giraffe, the muscular zone of the brachiocephalic and bicarotid trunks has been related to regulation of blood flow (Kimani, 1987; Kimani and Opole, 1991).

The transmedial zonation may also be related to the distribution of strain. Studies have shown that the adventitial zones of the tunica media experience higher levels of strain (Vaishnav and Vassoughi, 1987; Guo *et al.*, 2005) and residual circumferential stress may contribute to vascular remodeling (Fung, 1991). It is plaucible, therefore, that the increased contractile muscle mass in the adventitial zone of the tunica media, by myogenic response (Johanson, 1989; Civelek *et al.*, 2002; Fanchaouy *et al.*, 2007), increases the mechanical strength of the aortic wall.

Dobrin (1984) demonstrated that active vascular smooth muscle does resist distention upto 150 - 250 mmHg. Further, aortic smooth muscle may change its contractile state to keep intramural strain distribution uniform against temporary changes in blood pressure (Matsumoto *et al.*, 1996). Coincidentally, some of the smooth muscle cells of the goat aortic tunica media are in-series with the matrix, a feature which may enable the aorta to achieve greater mechanical strength, especially required at higher strains. In these circumstances, behaviour of the

vessel wall has been shown to be consistent with an in-series arrangement of collagen and smooth muscles (Silver *et al.*, 2003a).

The smooth muscle islands in the proximal aortic segments appear to be part of the normal growth and development of the thoracic aorta, in some animals, since they appear in prenatal life (Fukuda *et al.*, 1984, Stenmark *et al.*, 1993). Stenmark *et al.*, (1993) further demonstrated that in late gestation, bovine fetus tropoelastin mRNA was expressed by cells throughout the tunica media of the aorta. However, the strongest signal was expressed in outer medial smooth muscle cells which were separated by dense tropoelastin mRNA negative foci, that were also devoid of well developed elastic lamellae. The present study has demonstrated these islands using different stains, by light and electron microscopy in animals of various ages, suggesting that they are indeed part of the normal anatomy of the aorta.

Within these muscle islands, the smooth muscle cells bear features usually attributed to contractility (Clark and Glagov, 1979; Dingemans *et al.*, 1981; Tagani *et al.*, 1986). In addition, areas of high electron density in the smooth muscle cells such as those demonstrated in the present study represent points of anchorage of contractile filaments (Bezie *et al.*, 1998; Ross *et al.*, 2003; Junqueira and Carneiro, 2003), and their abundance on the sarcolema may therefore also support a contractile role. Together with the spiraling elastic fibres within the islands,

smooth muscle contraction may synergise the windkessel function of the aorta of these animals; a function hitherto attributed to elastic lamellae alone (Robert *et al.*, 1995). This probable role derives from the myogenic response of vascular smooth muscle (Johanson, 1989; Noda *et al.*, 1994; Civelek *et al.*, 2002; Fanchaouy *et al.*, 2007). Accordingly, during ventricular contraction, and rapid ejection of blood into the aorta, because of the in-series arrangement of the smooth muscle cells and matrix fibres, stretching of elastic fibres pulls the muscle islands, thus causing them to stretch as well. The smooth muscle cells then respond to this stretch by contracting, which in consonance with the elastic recoil, at the onset of diastole, facilitates the onward blood flow. In this way, the outer zone of the tunica media of the proximal parts of the aorta may constitute an "auxillary pump".

The present study further shows that smooth muscle cells in the tunica media display morphological heterogeneity where some cells are spindle shaped, or elongated but most are highly irregular. These findings concur with those of previous studies on other arteries (Carlson *et al.*, 1982; Komuro *et al.*, 1982; Todd *et al.*, 1983). They are however at variance with the view generally held that vascular smooth muscle cells are spindle shaped with a central nucleus oriented in the long axis of the cell (Rhodin, 1980). The surface irregularities associated with the vascular smooth muscle cells are thought to increase the surface areas to volume ratio (Gerrity and Cliff, 1975; Osborn Pelligrin, 1978; Fujiwara and

Uehera, 1992) for attachment of connective tissue elements. This view is supported by the observed high frequency of electron dense bodies in the smooth muscle cells, and cell-matrix contacts. It is plaucible therefore that the irregularity and hence higher cell matrix interaction constitutes a strengthening device that enables the aortic smooth muscle cell to endure the high systolic pressure, as has been suggested in the cerebral arteries of hypertensive rats (Fujiwara *et al.*, 1990).

The smooth muscle cells observed in the goat aortic tunica media, are generally oriented transversely, although some run longitudinally and obliquely. Studies on the orientation of smooth muscle cells in the wall of the aorta have previously yielded equivocal results, with some reporting oblique (Keech, 1960; Bierring and Kobayashi, 1963; Cliff, 1967; Berry *et al.*, 1974), circumferential (Clark and Glagov, 1979; Rhodin 1980; Todd *et al.*, 1983; Dingemans *et al.*, 2000), and others longitudinal (Wasano and Yamamoto, 1983; Clark and Glagov, 1985). In cases where smooth muscle is oriented in various directions (Osborne-Pelligrin, 1978), it is possible for tension to be developed in different directions in the vessel wall. The forces produced by each muscle bundle or sheet may be integrated into forces directed transversely with respect to the long axis of the vessel (Fujiwara and Uehara, 1992).

Longitudinal smooth muscles in the aortic wall is thought to strengthen the vessel wall (Yohro and Burnstock, 1973; Osborn-Pelligrin, 1978; Schimid *et al.*, 1982; Wasano and Yamamoto, 1983, Clark and Glagov, 1985) and also contribute to regulation of blood flow (Yohro and Burnstock, 1973). It is therefore possible that the longitudinally oriented smooth muscle cells support the arterial wall against longitudinal stress parallel to the muscles (Shiraishi *et al.*, 1986). Further, the various orientations and interdigitations of smooth muscles may interact with innervation, blood pressure, paracellular matrix or a combination to maintain vascular strength (Todd *et al.*, 1983).

Most of the smooth muscle cells of the goat aortic tunica media contain many subsarcolemal areas of high electron density. These electron dense areas are of two types, namely those associated with attachment of matrix fibres onto the cell, and those not associated with fibre attachment. The present observations support those on the normal aorta of various mammals (Keech, 1960; Clark and Glagov 1979, 1985; Dingemans *et al.*, 1981; Bezie *et al.*, 1998). The membrane associated dense plaques of muscle cells are major sites of anchorage of contractile apparatus to extracellular matrix (Clark and Glagov, 1979; Gabella 1984; Small and North, 1995). The mechanical link between muscle cells and elastic or collagen provided by the dense plaques, plays an important role in regulating contractile and elastic tension in stressed vessel. It has been shown that these focal attachments are stable and have great mechanical strength (Clark

and Glagov, 1979; McGufee and Little, 1996). Bezie *et al.*,'s (1998) observation that the percentage of cell surface occupied by dense plaques increased in hypertension suggests that these electron dense plaques are involved in strengthening the aortic wall.

The tunica media of the goat aorta contains smooth muscle cells of variable sizes, nuclear morphology, organelle disposition and density. These findings support those of reports that vascular smooth muscle cells exist in synthetic, intermediate and contractile forms, which although interchangeable, have different internal features (Chamley-Campbell *et al.*, 1979, 1983; Chamley-Campbell and Campbell, 1983; Schwartz *et al.*, 1986). The goat aortic tunica media also contains fibroblast-like cells, characterised by cytoplasmic processes, abundant rough endoplasmic reticulum, euchromatic nucleus, prominent nucleolus, and absence of myofilaments and basal lamina. The demonstration of these cells is at variance with the generally accepted view that the arterial tunica media consists of only the smooth muscle cells and their phenotypic variants (Wissler, 1968; Villaschi *et al.*, 1994; Bochaton-Pillat *et al.*, 1996; Frid *et al.*, 1997). Similar cells have been reported in the aortic tunica media of the dog by Geer *et al.*, (1961) and chicken by Moss and Bendit (1970), who called these cells interlamellar connective tissue cells, and postulated that they were involved in the synthesis of extracellular matrix. It is possible that similar cells differentiated within the muscle nests to specifically serve the function of matrix synthesis and secretion so that they

relieve the smooth muscle cells of this function. In this way, the smooth muscle cells, free of synthetic role, concentrate on contractility.

The other cell type in the tunica media is characterized by large dented nuclei, vacuoles with dark material, cytoplasmic extensions and lysosome-like structures. These features are usually associated with macrophages. The presence of these "macrophage-like" cells supports reports that macrophages, and vascular dendritic cells, exist in the tunica media of the healthy rabbit, and human aortae (Hineck and Rosnowski 1975; Hineck and Konsinski, 1975; Krupa *et al.*, 2002; 2004). The presence of these cells in healthy aortae supports the view that such cells, usually considered as typical of atherosclerosis, (Gerrity *et al.*, 1979; Ross, 1986) are normal constituents of the aortic wall. Their demonstration, by the present study, in young free-ranging goats further supports the view that they are constituents parts of the normal aorta, which are important in maintaining immune surveillance in the aortic wall by phagocytosis and antigen presentation (Weyand *et al.*, 2005; Galkina *et al.*, 2006).

Both elastic lamellae and elastic fibres are structurally linked to smooth muscle cells through electron dense areas. Similar physical linkages have been reported in the aorta of rodents and pigs (Berry *et al.*, 1974; Clark and Glagov 1979; Dingemans *et al.*, 1981), man (Dingemans *et al.*, 2000); and the spontaneously hypertensive rat (Bezie *et al.*, 1998, 1999). Functionally, two modes of adhesion

have been described between smooth muscle cells and the elastic fibres. These are strong focal and rigid cell to elastic fibre attachments which modulate elasticity and extensibility of the tunica media, and diffuse, pliable, pericellular fibrillary system which binds cells together to prevent cell separations and coordinate cell functions (Clark and Glagov, 1979). The two modes may be complementary to each other (Knox, 1981).

Collagen fibres in the goat aortic tunica media appear to insert onto the surface of smooth muscle cells through the basal lamina and subsarcolemal regions of high electron density. Previous *in vivo* studies did not demonstrate such insertions in the aorta (Keech, 1960; Clark and Glagov, 1979, 1985; Dingemans *et al.*, 1981; 2000). *In vitro* studies have, however, demonstrated that bovine aortic tunica media smooth muscle cells may attach to collagen (Grotendorst *et al.*, 1981; Tosseli *et al.*, 1984; Kielty *et al.*, 1992). In their model of the vascular wall, Snowhill *et al.*, (2004) depicted collagen fibres connected to integrins on the smooth muscle cell surface.

In other arteries, collagen to smooth muscle cell contacts have been described (Rees, 1968; Kimani, 1981b; Komuro *et al.*, 1982), whereby collagen fibres were partially placed in series with the intracellular myofilaments at the specialized sarcolemmal dark areas. The observation that the frequency of electron dense

plaques which are thought to be the sites of cell-matrix anchorage, increases in hypertension suggests that these matrix-cell linkages have an important role in adapting the vessel to haemodynamic stress (Bezie *et al.*, 1998, 1999). Thus the collagen-cell linkages in the present study may constitute a mechanism of strengthening the aortic wall.

The present study further demonstrates that collagen and elastic fibres are intimately associated, and are in some cases structurally interlinked. Ultrastructural studies on the aorta of rodents, pigs, man and dog did not make reference to such linkages (Keech, 1960; Clark and Glagov, 1979; 1985; Dingemans *et al.*, 1981; 2000; Orsi *et al.*, 2004, Farand *et al.*, 2007). Some workers explicitly denied the existence of these linkages in the aortic wall (Wolinsky and Glagov, 1967a; Gosline and Shadwick, 1996; Shadwick, 1999). Snowhill *et al.*, (2004) however, inferred that there appeared to be connections between collagen and elastic fibres. Physical linkages between collagen and elastin have, however, been reported in the carotid arteries of rodents including porcupines (Rees, 1968; Kimani, 1993).

The view generally held is that the close association of collagen and elastic fibres in the aortic wall provides the anatomical basis for the functioning of the tunica media in a biphasic manner (Glagov and Wolinsky, 1963; Wolinsky and Glagov, 1964; Mcveigh *et al.*, 2002; Silver *et al.*, 2003a). In this arrangement, it is thought

that below physiological pressures, the strain is born by elastic fibres of long range reversible stretchability. At and above physiological pressure, the strain is taken up by collagen which has greater strength and resists stretch. It is conceivable, therefore, that the structural intimacy demonstrated in the present study is designed to confer higher mechanical strength to the aorta, while at the same time enabling it to function in the biphasic fashion.

The physical linkages between elastic and collagen fibres, may provide a morphological basis for functional integration between the two extracellular matrix fibres. It has been proposed that although the two fibre types have different physicomechanical properties, they always structurally coexist (Wolinsky and Glagov, 1964; Kimani, 1979; Walji, 1985). Elastic fibres, being highly extensible, permit long range reversible stretchability without energy input (Sandberg, 1976; Aaron and Gosline, 1980; Kielty *et al.*, 2002). Collagen fibres on the other hand, being about 1000 times, stiffer than elastin, are relatively indistensible and thus provide stiff reinforcement of the vessel wall (Shadwick and Gosline, 1981; Davison *et al.*, 1995; Mc Connell *et al.*, 1996; Shadwick, 1999).

It is probable therefore, as suggested by Kimani (1979), that at and above physiological pressures, the elastic elements in the aortic wall have not reached their elastic limit and would continue to stretch if it was not for the restraining action of the collagen fibres. Many of the collagen fibres in the goat aortic wall

exist in series with elastic lamellae and fibres. Similar in-series model has been reported in the wing ligaments of birds (Oakes and Bialkower, 1977; Brown *et al.*, 1994) and in the aortic arch of the fin whale (Gosline and Shadwick, 1996). These workers proposed that such an arrangement confers wider extensibility but with reasonable restraint. The association between collagen and elastic fibres allows a compromise between stretchability and stiffness, and provides an aorta that will distend but not develop aneurysm or rupture. In this way, elastic and collagen fibres may be acting to compliment the mechanical properties of each other (Kielty *et al.*, 2002). All these aspects of structural co-existence and functional integration may have their morphological basis in the collagen-elastin linkages described in the present study.

Smooth muscle cells in the tunica media of the goat aorta, and especially in the muscle islands of the adventitial zone, interdigitate and frequently form junctions with each other. This supports reports on the existence of simple appositions (Pearse and Paule, 1960; Paule, 1963; Bierring and Kobayashi, 1963), characterized by folds, invaginations and projections (Gabella, 1977), zonulae occludentes (Stein *et al.*, 1969) and gap junctions (Litwin, 1980; Berry and Sosa-Melgarejo, 1989; Sosa -Melgarejo and Berry, 1991; Sosa-Melgarejo *et al.*, 1991). These cellular interdigitations, may be areas of close intercellular apposition which represent various junctional structures, designed to provide both

electrotonic and mechanical coupling between the cells. Their greater abundance among the cells of the muscle islands suggests that they are part of the adaptation of these cells for powerful contraction.

The three components of the tunica media of the aorta are therefore physically interlinked. These interlinkages may confer plasticity, adaptability and flexibility to the aortic wall (Christ *et al.*, 1996). The interlocked structure of elastin, muscle and collagen together with the nerves enables the tunica media to function as a mechanically homogenous structure (Dobrin, 1999), which is subject to neurogenic influence (Dora, 2001). The observations of the present study have elucidated two aspects of vascular tissue structure that were hitherto unresolved, namely the arrangement and connectivity between the components of the extracellular matrix, and between smooth muscle cells and the matrix (Dingemans *et al.*, 2000; Midwood and Schwarzbauer, 2002; Snowhill *et al.*, 2004).

The linkage between the structural components could play a physiological role, leading to the maintenance of physicomechanical homeostasis. Mechanical stretching and relaxation of the integrin sites connecting cells and the matrix leads to the synthesis and inhibition of various components (Cremona *et al.*, 1994; Gerthoffer and Gunst, 2001). Some of the macromolecular components involved include: focal adhesion kinase (FAK), plasminogen activator inhibitor I (PAI-1), paxillin, vasodilator stimulated phosphoprotein (VASP), protein kinase C (PKC),

and tyrosine kinase (Hedin *et al.*, 1997; Haller *et al.*, 1998; Tanaka *et al.*, 2002; Scherberich *et al.*, 2002). Mechanical stretch of the cellular membrane between integrins has been reported to activate g-proteins that result in the phosphorylation of c-fos and c-jum, which make up and alter the activity of the transcription factor activating protein I (AP-I) in the nucleus (Li and Xu, 2000). In this manner the integrin mediated sites of cell-matrix linkages may be involved in mechanosensation and transduction (Silver and Siperko, 2003; Silver *et al.*, 2003b).

Many parts of the tunica media of the goat aorta comprise Medial Lamellae Units (MLU) as described in rats (Keech, 1960, Cliff, 1967, Wolinsky and Glagov 1967a, Berry *et al.*, 1974; Clark and Glagov, 1979; 1985; Dingemans *et al.*, 1981, 2000) sheep, dogs and cats (Roach, 1983; Song and Roach, 1985); pigs (Snowhill *et al.*, 2004) and rabbits (Farand, 2007). These workers proposed that the elastic lamellae and fibres provide sites of attachment for smooth muscle cells and uniformly distribute the forces across the aortic wall during systole. The concentric elastic lamellae are also believed to constitute a windkessel mechanism that helps to propel blood to the periphery during diastole, thus maintaining laminar flow (Roberts *et al.*, 1995; Orsi *et al.*, 2004; Snowhill *et al.*, 2004). In addition, the lamellae are thought to bear the circumferential forces while the branches and other fibres distribute the forces uniformly (Dingemans *et al.*, 1981; 2000; Tonar *et al.*, 2003). It is therefore possible that the elastic fibre

organization ensures mechanical strength, force distribution and the windkessel mechanism.

In some parts of the tunica media, the smooth muscle cells or their processes alternate in harmony with elastic lamellae and the two are then sandwiched by collagen, to conform with the Musculo Elastic Fascicle (MEF) unit proposed by Clark and Glagov (1985) in rats, rabbits and pigs. They, however, appear at variance with those of Davies (1993a) in the mouse, and in the human (Dingemans *et al.*, 2000). The former failed to recognize the unit proposed by Clark and Glagov (1985) and suggested that this organization may be a feature of the aorta of larger animals. Dingemans *et al.*, (2000) on the other hand, considered that MEFs may show age variation. It is possible that both Davies (1993) and Dingeman's *et al.*, (2000) examined different areas of the aortic tunica media. As Dingemans *et al.*, (2000) suggested, many observations regarding the organization of the extracellular matrix in the aorta are not controversial per se because they are derived from randomly sampled specimens.

The interlamellar spaces of the tunica media, contain not just branches but definite elastic fibres running in different directions, similar to those in human aortic media (Jiang *et al.*, 1995; Dingemans *et al.*, 2000). The interlamellar connections to the main lamellae may also determine distensibility properties of the aorta, (Nakashima *et al.*, 1990; Nakashima and Sueishi, 1992). The structural

orientation throughout the wall (Loree *et al.*, 1992), and the complex interlocked structure described in present study may be important in maintaining the mechanical properties of the aorta (Avolio *et al.*, 1998).

Observations of the present study further reveal that some of the elastic lamellae are arranged longitudinally and obliquely. It has been suggested that elastic lamellae and fibres, which form a complex meshwork, strengthen the aortic wall in such a manner that it can withstand multidirectional stresses imposed onto it by ventricular systole (Gosline and Shadwick 1996; Shadwick, 1999). Pertinent to this suggestion is the recent proposal that the array of tunica media elastic fibres perpendicular to luminal pulsatile flow gives additional strength to the elastic lamellae in supporting circumferential stress, while those arranged parallel to luminal flow are best adapted to sustaining longitudinal stress (Farand *et al.*, 2007). Accordingly, the final three-dimensional structure of elastic fibres, as well as their interaction with other elements of the extracellular matrix and smooth muscles, ultimately determines the mechanical properties of the aorta.

In most cases, the smooth muscle cells of the goat aortic tunica media are immediately surrounded by collagen which separates them from elastic lamellae. This observation is at variance with the description of Clark and Glagov (1985) among rabbits, rats and pigs in which the functional unit called the Musculo Elastic Fascicle (MEF) comprised smooth cells and elastic lamellae, which are

then bracketed by collagen. They are also at variance with previous reports on the human thoracic aortic tunica media in which a preferential localization of collagen fibrils immediately adjacent to the smooth muscle surface was not observed (Dingemans *et al.*, 2000). These observations, however, support those of Snowhill *et al.*, (2004), who suggested revision of the lamellar unit to fit collagen. Although Snowhill et al's (2004) study shows the same cellular layers bounded by elastin, these workers suggested that the lamellar unit should consist of collagen and smooth muscle bounded by a variable concentration of elastic fibres. The new unit would be smooth muscle cells bounded by collagen, in a 1:1 volume fraction ratio and then bounded by two elastic laminae one on the luminal side and the other on the abluminal side. Thus, observations of the present study suggest that collagen fibres play a more important role in the structure and function of the aorta than previously appreciated, and should be included in the definition of the structural unit of the aortic tunica media.

Vasa vasora of the goat aorta, extend into the inner half of the tunica media in the ascending aorta, the aortic arch, and proximal descending thoracic aorta. Vasa vasora have also been demonstrated in the tunica media close to the intima, in the horse (Woodruff, 1926), cow (Knieriem, 1967), sheep (Song *et al.*, 1985) dog (Stefanadis *et al.*, 1993), monkey (Heistad and Amstrong, 1986) and pig (Angouras *et al.*, 2000). These medial vasa vasora, considered to be more effective than adventitial ones (Webster and Heistad, 1985), supply a specific part

of the aortic media since their removal causes medial necrosis (William *et al.*, 1988, Angouras *et al.*, 2000).

The vasa vasora extend into the outer zone of the tunica media in the distal thoracic and abdominal aorta, which is at variance with reports that there are no vasa vasora in the abdominal aorta (Ramsey, 1936; Gerringer, 1951; Wolinsky and Glagov, 1969; Heistad, *et al.*, 1978). According to these workers, vasa vasora only penetrate the tunica media where the lamellae are more than 29, and wall thickness exceeds 0.5 mm. The presence of vasa vasora in the tunica media even where the wall thickness is less than 0.5mm, and there are less than 29 lamellar units suggests that other factors are important in determining the density and penetration of vasa vasora. Such factors may include the activeness of the vessel wall in regulating blood flow (Chuncher and Somana (2005); and metabolic activity (Kachlick *et al.*, 2007). The overall cellularity and activity of the cells in other functions of the vessel wall such as remodeling, mechanical strength and immune surveillance, may also be important.

The vasa vasora of the goat aorta in many places co-exist with the muscle islands. This co-existence may be designed to enable the vasa vasora deliver nutrients, thus supporting contractile activity of smooth muscle cells. This is supported by studies which suggested that the activeness of the vascular wall in varying the amount of blood flow to certain organs may be an important factor in the

existence, density and distribution of the vasa vasora (Chuncher and Somana, 2005).

4.1.3 Tunica adventitia

The tunica adventitia, which is fibroelastic especially in the distal segments comprises the outermost coat of the aorta. These findings show that the tunica adventitia of these parts of the aorta, in the goat, are similar to those of dogs (Hass *et al.*, 1990; Orsi *et al.*, 2004); rabbits (Viegas *et al.*, 2001) guinea pigs and albino rats (Mello *et al.*, 2004). The collagen content could confer higher tensile strength to the adventitia to enable it bear higher pressures, which the tunica media is unable to cope with while the elastic fibres allow reversible stretchability (Kielty *et al.*, 2002). After aortoiliac endarterectomy, in which the entire tunica intima and most of the tunica media are removed, the tunica adventitia is able to maintain the integrity of the vessel wall (Zarins *et al.*, 2004). Accordingly, it is plaucible, as has been suggested by Orsi *et al.*, (2004), that the fibro-elastic composition of the tunica adventitia enables it to maintain the structural and functional integrity of the aorta.

Between the cells and collagen or elastic fibres in the tunica adventitia, there were microfibrils, resembling oxytalan fibres, similar to those in the tunica media of the human aorta (Dingemans *et al.*, 2000). These fibres are connecting structures (Goldfischer *et al.*, 1983; Davis, 1993b), with considerable strength, and

they constitute the main supporting structure in the tissues of lower animal species (Isokawa *et al.*, 1989). The oxytalan fibres described in the present study may participate in anchoring the cells to the rest of the matrix, and also increase the mechanical strength of the tunica adventitia.

The tunica adventitia in the abdominal aorta, is characterized by the presence of multiple populations of cells. These findings support the reports that the tunica adventitia contains fibroblasts, mast cells and macrophages (Humphrey, 1995; Gabella, 1999); progenitor cells (Torsney *et al.*, 2005); and pericytes (Howson *et al.*, 2005). According to these workers, the progenitor cells may be a source of smooth muscle cells, and pericytes. Their presence in the tunica adventitia may have implications for cellular, genetic and tissue engineering approaches to vascular disease (Torsney *et al.*, 2005). These immature cells may represent an important source of pericytes during angiogenesis in physiological and pathological processes, and also provide a convenient supply of mural cells for vascular bioengineering applications (Howson *et al.*, 2005).

Many of the cells in the goat aortic tunica adventitia resemble fibroblasts, as shown by Gabella (1999) and Ross *et al.*, (2003), who stated that, fibroblasts are the major cells involved in the synthesis and degradation of connective tissue fibres in routine remodeling of the tunica adventitia. It is possible that the other relatively poorly differentiated cells constitute fibroblast precursors, namely

mesenchymal stem cells. Some of them, on the other hand, may be pericytes or myofibroblasts, both of which on their own, or by transformation into smooth muscle cells are involved in matrix synthesis (Canfield *et al.*, 2000; Torsney *et al.*, 2005; Howson *et al.*, 2005).

Other cells resemble those of the mononuclear phagocytic system, thus supporting reports that the tunica adventitia of the aorta contains vascular dendritic cells (Bobryshev and Lord, 1995; Krupa *et al.*, 2002, 2004; Weyand *et al.*, 2005). These workers suggested that, these cells are responsible for maintaining immune surveillance and therefore protecting the vascular wall. The resident macrophages and dendritic cells could comprise a large population of immune cells which may be involved in homeostatic regulation of immune responses within the aortic wall (Galkina *et al.*, 2006).

4.2 Regional variations of the aortic wall

The tunica intima, media and adventitia display regional variations, which are both qualitative and quantitative, and involve every component.

4.2.1 Regional variations in the tunica intima

The endothelium in the present study has been shown to display more round cells in the proximal than in the distal segments of the aorta. These results are hitherto unreported, but suggest that the differences are related to haemodynamic forces. They appear at variance with reports that parts of the aorta exposed to laminar high shear stress were covered by long fusiform cells, while those exposed to stagnated flow are covered by round cells (Okano and Yoshida, 1993, 1994). It has been suggested that endothelial morphology depends on the level of haemodynamic microstimulation (Davies, 2001, 2003). Accordingly, it is plaucible that the higher number of round cells in the proximal segments of the aorta, reflects the greater variability in microstimulation in these parts which receive the direct thrust of blood during systole.

The internal elastic lamina increases in thickness and folding craniocaudally. A prominent internal elastic lamina is usually associated with muscular arteries and not the aorta (Gabella, 1999; Ross *et al.*, 2003). A probable function of the prominent internal elastic lamina is to augument the windkessel mechanism,

usually attributed to medial elastic lamellae alone (Robert *et al.*, 1995). A pertinent observation of the present study, in support of this suggestion is that the number of elastic lamellae display a craniocaudal decline. It is plaucible that the medial lamellae, provide for the attachment of the increased smooth muscle number and may need supplementation regarding elastic recoil, to propel blood. Another possible explanation is that there is greater longitudinal load in the distal segments of the aorta in which the impact of systole is less marked. In this regard, it has been demonstrated that the internal elastic lamina is more important in bearing longitudinal than circumferential forces (Farand *et al.*, 2007).

4.2.2 Regional variations in the tunica media

The tunica media shows regional variations, on the basis of which it is divisible into three regions; a proximal one bearing muscle islands in the adventitial zone, a transitional region where occasional smooth muscle islands are confined to the adventitial third of the tunica media, and a distal predominantly elastic region without any muscle islands. Among mammals, this is the first report of qualitative regional variations characterized by the existence of muscle islands, in the tunica media of the aorta.

Why these muscle islands appear in the thoracic and not abdominal aorta is unknown. It is possible, however, that whereas the proximal aortic segments

function to actively supplement cardiac activity and also prevent excessive distension during systole, in the abdominal aorta, the windkessel mechanism of the remaining elastic lamellae is supplemented by a prominent internal elastic lamina and the elastic tunica adventitia. It is also possible that diversion of blood to the abdominal viscera (Charbon *et al.*, 1989) and the hind limbs creates a suction effect on the blood in the aorta. The difference may also have a genetic basis, since the abdominal and thoracic regions of the aorta have different gene expression patterns (Absi *et al.*, 2003), which may influence the extracellular matrix composition and organization (Behmoaras *et al.*, 2005).

In the tunica media, there is a craniocaudal decline in the number of elastic lamellae, similar to that described in pigs (Tanigawa *et al.*, 1985; Sokolis *et al.*, 2002; Mello *et al.*, 2004), fin whales (Gosline and Shadwick, 1996) and dogs (Orsi *et al.*, 2004). This diminution of elastic lamellae and presumably elastic tissue, may be related to the blood pressure profile (Awal *et al.*, 1999). The volume of blood leaving the heart during systole imparts greatest tension on the ascending aorta, aortic arch and proximal thoracic aorta. The pulsatile nature of the blood flow probably requires a large amount of elastic tissue to absorb it. Because of elastic recoil in the more proximal aortic segments, blood flow in the abdominal aorta may be less intermittent and imparts less tension (Katzberg 1966; Awal *et al.*, 1999; Orsi *et al.*, 2004). An alternative explanation for the craniocaudal decline

in elastic lamellae is that some of the lamellae are involved in the formation of small arteries such as intercostals which arise from the aorta (Roach, 1983).

Another important regional difference is the craniocaudal increase in smooth muscle cell density, similar to that reported in dwarf pigs (Tanigawa *et al.*, 1985) and in the hamster aorta (Yamanouchi *et al.*, 1995). This observation supports the view generally held that the further one moves from the heart, the less the elastic tissue, but the more the smooth muscle (Gabella, 1999). These differences have also been associated with blood pressure profile, and the need to regulate blood flow to peripheral organs, a function usually attributed to muscular arteries (Ross *et al.*, 2003; Junqueira and Carneiro, 2003).

4.2.3 Regional variations in the tunica adventitia

The tunica adventitia of the proximal parts of the goat aorta is thin, and collagenous. It begins to increase and become elastic in the distal thoracic aorta, such that it is thickest and most elastic in the abdominal aorta. These findings suggest that the tunica adventitia of the goat aorta is different from that of other animals in which it is thickest in the ascending, arch and thoracic aorta, and thin in the abdominal aorta as in the case of dogs (Hass *et al.*, 1990; Orsi *et al.*, 2004); and rabbits (Dabanoglu, 2000). These workers related the craniocaudal decrease in the adventitial size to the pattern of turbulent flow during ventricular systole and the influence of systolic pressure. The predominantly collagenous nature of

the tunica adventitia in the proximal segments described in the present study may, because of the mechanical properties of collagen, provide the structural basis for this function.

What is not clear is why the tunica adventitia should be thicker and more elastic in the abdominal aorta where the pressures are relatively lower than in the thoracic region. Recent studies suggest that smooth muscle plays a significant role in the mechanical properties of the aorta (Silver *et al.*, 2003a). It is possible, therefore, that in the goat the strength of the aorta even at high pressure is a function of both the tunica media, and the tunica adventitia. Accordingly, the thinness of the tunica adventitia may be compensated for by higher medial thickness.

In the proximal aorta, an additional mechanism for protecting the aortic wall from rupture or aneurysm formation may exist in some animals. This additional mechanism probably resides in the muscle islands described in the tunica media of the proximal aortic regions. Pertinent to this suggestion is Dobrin's (1984) observation that active vascular smooth muscle can markedly resist distension upto 150 - 250 mmHg. Further, studies of incremental stress-strain curves of porcine aortae suggest that high strain behaviour is consistent with an in-series arrangement of collagen and smooth muscle (Silver *et al.*, 2003a). Conceivably, the in-series arrangement of smooth muscle and collagen observed in the

adventitial zone of the tunica media of the goat aorta may constitute part of this mechanism.

Moreover, the tunica adventitia of abdominal aorta is fibroelastic. These findings are at variance with those of the aorta in rats (Keech 1960; Osborn-Pelligrin, 1978) rabbits (Wolinsky and Glagov, 1964); and in porcine (Snowhill *et al.*, 2004), where the tunica adventitia is predominantly collagenous. They, however, concur with those on dogs (Hass *et al.*, 1990; Orsi *et al.*, 2004); guinea pigs and albino rats (Mello *et al.*, 2004) where it has been demonstrated that there is a random pattern of collagen and elastic fibres. These later workers proposed that the adventitial connections between the fibrous elements and their different spatial orientations collectively contribute to the integrity of the vessel wall. Accordingly, it is possible that the composition of the tunica adventitia of the abdominal aorta, confers it with both strength and distensibility.

4.3 Adrenergic Innervation of the aorta

The proximal thoracic segments of the goat aorta are densely innervated. This is at variance with reports from laboratory animals that the adrenergic innervation of the aorta is sparse (Burnstock, 1975; Burnstock and Costa, 1975; Osborn-Pelligrin, 1978). This sparsity of aortic innervation may be due to abundance of elastic fibres with only a few smooth muscle cells. These workers suggested that the stretch-recoil properties of elastic fibres suffice in passively maintaining blood flow, as regulation by adrenergic fibres would produce too great latency in functional adaptation (Kienecker and Knoche, 1978). The observations of the present study suggest that whereas the luminal zone of the tunica media and the elastic bundles between the muscle islands function in this way, the areas with the muscle islands, by neurogenic muscular response, operate in a different manner that enables the aorta to act as an auxillary pump, and also participate in regulating blood flow.

Adrenergic nerves penetrate into the tunica media of the goat aorta, as shown in the aorta of fish and amphibians (Kirby and Burnstock, 1969), cat, fox and badger (Abraham, 1969) and dog (Abraham, 1969, Dolezel, 1972). These findings are, however, at variance with reports from laboratory animals that adrenergic nerves in the aorta are confined to the medio adventitial border (Burnstock, 1975; Bevan *et al.*, 1972; Luff and McLachlan, 1989). Penetration of nerves into the

tunica media is usually retained in those parts of the arterial tree where a predominantly sympathetic control would be of some physiological value (Furness, 1971; Bevan and Purdy, 1973), as in the case of the proximal arteries of diving mammals which undergo reflex vasoconstriction during diving (White *et al.*, 1973). A notable difference is that whereas in these studies a uniform pattern of distribution is described, observations of the present study reveal a circumferential asymmetry in which the nerve fibres occur in patches co-localized with smooth muscle islands. This implies that a close functional relationship exists between the sympathetic nerves and the smooth muscle nests.

The sympathetic nerves in the smooth muscle islands may serve to suppress the proliferation, differentiation or, retro-differentiation of the smooth muscle cells into morphogenic cells, thus maintaining the contractile phenotype. Observations of *in vitro* studies showed that sympathetic nerve fibres inhibit phenotypic modulation of smooth muscle cells, and their proliferation (Chamley *et al.*, 1973, 1974; Chamley-Campbell and Campbell, 1983; Hartley and Campbell, 1987) and also suppress their tendency to de-differentiate (Campbell and Chamley 1976; Kacem *et al.*, 1995)

A second function of the adrenergic nerves in the tunica media of the goat aorta may be to modulate its mechanical properties. The importance of neurohumoral influences on the mechanical properties of the aorta has been suggested in man

(Greenfield and Patel, 1962) and in experimental animals (Pieper and Paul, 1969; Nicolosi and Pieper, 1971; Gerova *et al.*, 1973). Increased sympathetic activity alters the aortic diastolic pressure diameter relationship, reducing the diameter for any given pressure in the dog (Pieper and Paul, 1969, Gerova *et al.*, 1973) and cat (Pangani, 1975). Neurogenic contraction of smooth muscle cells in the islands may contribute to aortic stiffness, thus constituting part of the mechanism for preventing aortic rupture in the wake of a diminished tunica adventitia in the thoracic aorta. A third function of the nerves co-localized with the smooth muscle nests may be to modulate the phenotypic characteristics of the cells by exerting their trophic effect as has been shown in other arteries (Bevan and Bevan, 1981; Mueller and Rusterholz, 1983; Dimitriadou *et al.*, 1988; Bevan, 1989; Tsuru *et al.*, 2002).

The density of adrenergic innervation decreases in the distal descending thoracic and abdominal aortae which are only sparsely innervated with nerves confined to the medio-adventitial border. Similar paucity of adrenergic innervation of the abdominal aorta has been reported in dogs (Gerova *et al.*, 1973), rats (Osborn Pellegrin, 1978) and rabbits (Cowen and Burnstock, 1980). In cases where nerves are confined to the medio-adventitial border, there is dual control of the vessel by the nerves, and circulating catecholamines which diffuse in an intimo-medial direction (Avakian and Gillepsie, 1968; Burnstock and Iwayama, 1971; Burnstock and Costa, 1975). Activation of nerves at the medio-adventitial border, indirectly

activates the other muscles which are electronically coupled. Since such coupling is precluded by elastic lamellae, in the aorta, it is possible that after the action of neurogenically and myogenically driven smooth muscle nests have augmented the elastic recoil in the thoracic region, the abdominal aorta may not need any special modifications, and continues to function mechanically.

4.4 Age related changes in the aortic wall

All three coats of the aortic wall showed age-related changes. In each of the coats, various components were affected. The extent of change in the tunica media displayed intimo-adventitial and craniocaudal variation.

4.4.1 Age related changes in the tunica intima

The endothelial cells of older animals are irregular and heterogenous with large intercellular discontinuities not observed in younger animals. Reduction of endothelial cells with age may be explained by the findings of Kunz and Klein (1975), who demonstrated that in rabbits, there is a decrease in the 3H - thymidine labeling index and in mitotic rate leading to an increase in mean generation time of endothelial cells with advancing age. Further, in the rhesus monkey aorta, apoptosis of endothelial cells has been suggested to contribute to the reduced cell density (Asai *et al.*, 2000). The discontinuities in the endothelium may result from this reduction in cell density and also from alteration of connexins (Connat *et al.*, 2001a). Such gaps may permit greater ingression of material, including lipids into the vessel wall and contribute to the higher incidence of atherosclerosis among the aged.

Some of the cells in the endothelium are large, irregular and bear lysosome-like structures and dendritic extensions. These are features associated with dendritic

cells of the mononuclear phagocytic system. These cells probably perform a phagocytic role, protecting the vessel wall from antigens which may gain access to the vessel wall through the enlarged discontinuities observed in the endothelial lining. The presence of phagocytic cells in the endothelium of aged aorta in rats and monkeys may be a result of loss of primitive intima which is replaced by a pseudoendothelium made of adhering leukocytes (Aliev *et al.*, 1995; Ye *et al.*, 2000; Connat *et al.*, 2001b).

In the proximal parts of the aged goat aorta, the subendothelial zone is uniformly thickened, and contains abundant cells, elastic and collagen fibres. These observations are similar to those of other animals (Jayer *et al.*, 1982; Kojimahara, 1985; Wang, 2003; Bonert *et al.*, 2003). The changes may represent a process of intimal remodeling, designed to enable the vessel cope with the increased haemodynamic stress that attends advancing age (Li *et al.*, 1999). Pertinent to this suggestion is the observation that sub-endothelial thickness does increase in hypertension (Gabbiani *et al.*, 1979; Ferrante *et al.*, 1994; Lesauskaute *et al.*, 1999) and that blood pressure is elevated in aged animals (Benetos *et al.*, 2002).

The presence of elastic fibre bundles in the deeper layer of the subendothelium may also constitute a shear stress buffering mechanism. This suggestion is based on the function of elastic tissue which is considered to be that of controlling the rate of change in the form of a structure by reversibly stretching without energy

expenditure (Sandberg, 1976; Kielty *et al.*, 2002). Therefore, its presence in the thickened subendothelial zone of aged animals may act to control the longitudinal shearing of the luminal surface of the vessel wall in systole, and its resumption of normal morphology in diastole.

The thickened subendothelial zone contains a heterogenous population of cells, some of which resemble smooth muscle cells and lymphocytes. Increased heterogeneity of subendothelial cells with age has also been found in the aorta in rat and monkey (Haudenschild *et al.*, 1981; Guyton *et al.*, 1983; Aliev *et al.*, 1995; Wang *et al.*, 2003). This cellular composition may enable extracellular matrix remodeling as well as intima protection by immunosurveillance, as in younger animals.

With age, the internal elastic lamina disintegrates, as shown by Connat *et al.*, (2006b) in rats. This disintegration is part of the general change that occurs in the tunica media, and may permit migration of medial smooth muscle cells to the subendothelial zone, contributing to thickening of subendothelial zone, (Glagov *et al.*, 1993), and development of atherosclerotic plaques (Ross and Rosenfeld, 1996).

4.4.2 Age related changes in the tunica media

In the tunica media, aging is associated with fragmentation of elastic lamellae, similar to that described in other mammals (Robert *et al.*, 1986; Dalessandri *et al.*, 1994; Ooyama and Sakamato, 1995 a,b; Zhu *et al.*, 2001; Connat *et al.*, 2001b). This degradation of elastic fibres has been attributed to continuous stimuli, including pulse movement with resultant fatigue failure (O'Rourke, 1976), binding of substances with strong affinity for elastin, such as lipoproteins, $ca^{2+;}$ antielastin antibodies and endogenous elastases (Spina *et al.*, 1983; Robert *et al.*, 1986), integrins and oxidative stress (Finkel and Holbrook, 2000; Shao *et al.*, 2006). This fragmentation of elastic lamellae with age has two consequences, namely transfer of mechanical load to collagen with consequent aortic stiffness (Ooyama, 1991; Ooyama and Sakamoto, 1995a,b; Reddy *et al.*, 2003), and weakness usually associated with aneurysm formation. Such aortic stiffness, and attendant elevation in blood pressure may constitute part of the explanation for higher cardiovascular morbidity and mortality including cardiac hypertrophy among the aged (Bulpitt *et al.*, 1999; Benetos *et al.*, 2002).

In the present study, aging is associated with deposition of more collagen in the interlamellar spaces, and also between the fragments of elastic lamellae, where it appears to join them. Increased secretion of collagen in the aorta with age has also been reported in man, rats and mice (Virmani *et al.*, 1991; Nicols and O'Rourke, 1998; Connat *et al.*, 2001a,b) and in rabbits (Ooyama and Sakamoto

1995a,b; Zhu *et al.*, 2001). The increase in collagen may synergise the fragmentation of elastic fibres in causing aortic stiffness in the aged aorta (Avolio *et al.*, 1985; Robert and Jacotot 1994; Benetos *et al.*, 2002). The increased cardiovascular morbidity and mortality consequent to this increase in collagen may parallel that due to elastic fibre degradation.

The age related elastic fibre degradation in the tunica media displays transmedial variation, such that the luminal zone is more affected than the adventitial one. The pattern of aging of the goat aorta follows a pattern similar to that in both human and animal models, where the medial elastic lamellae begin to straighten and fragment from the luminal side (Nakamura and Ohtsubo, 1992). This pattern of degradation appears to be commensurate with the transmural gradient of wall tension in which there is greater strain on the inner than on outer tunica media (Guo and Kassab, 2003). The transmedial zonation in pattern of aging may also be related to the active Matrix Metalloproteinase-2 (MMP-2), which positively correlated with age, and has highest activity in the intima (McNulty *et al.*, 2005).

Elastic lamellae degradation, decreases gradually towards the abdominal aorta, where the lamellae are almost entirely spared. Age changes are usually more pronounced where tension is highest in the proximal aorta (Reddy *et al.*, 2003). It is therefore probable that shear stress and wall tension are the critical factors

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which influence the pattern of age change. An alternative explanation for this zonal and regional variation is the intimo-adventitial and craniocaudal increase in cellularity of the tunica media. It is possible that short-term alterations in smooth muscle tone (Kawasaki *et al.*, 1987; Greenwald, 2007) off-loads haemodynamic strain from the elastic fibres, thus protecting them from fatigue fracture.

Besides, there is fragmentation of elastic lamellae which appear highly electron lucent and increase in collagen. This is probably a result of accretion of calcium salts referred to as medial elastocalcinosis (Dao *et al.*, 2005; Shao *et al.*, 2006). Similar sclerotic material could coat cells, including those of the endothelium, as observed in the present study.

The smooth muscle cells in both the luminal and adventitial zones of the tunica media in the proximal segments of the aged aortae appear distorted in shape and orientation. In some areas, the smooth muscle cells are binucleated, as found in aged aortae, with irregularily in shape (Toda *et al.*, 1980); cell necrosis (Kojimahara, 1985), degradation of cells (Ooyama and Sakamato 1995 a,b); increased proliferation (Moon *et al.*, 2003) and polypoidy (Jones and Ravid, 2004). These changes may represent both the degradation and attempted repair. The presence of "inflammatory" cells in old goat aortic tunica media could be due to age related cellular activation similar to what occurs in atherosclerosis (Hansson,

2005; Galkina, 2006). These inflammatory cells have been implicated in the production of matrix metalloproteinases (Palombo *et al.*, 1999) some of which may be involved in elastic fibre degradation.

4.4.3 Age related changes in the tunica adventitia

In the caudal descending and abdominal aorta of the aged goat, strips of longitudinal smooth muscles appear in the tunica adventitia. These findings support those of studies which demonstrated that conditioned medium from periaortic adipose tissue stimulated smooth muscle cell proliferation, in aged rats (Barandier *et al.*, 2005). The development of the muscle strips may be stimulated by periaortic adipose tissue, as part of normal aging. These strips are surrounded by elastic fibres suggesting that they constitute part of compensatory mechanisms for attempting to restore compliance of the aortic wall.

Longitudinal smooth muscle cells have been described in the adventitial side of the tunica media in the normal aortic wall of various mammals (Schmid *et al.*, 1982; Wasano and Yamamoto 1983; Clark and Glagov, 1985). They also have been found in the coronary arteries (Yohro and Burnstock, 1973), rat renal artery (Osborn-Pelligrin, 1978), monkey mesenteric arteries (Fujiwara and Uehara, 1982) and dog brain vessels (Shiraishi *et al.*, 1986). These longitudinal smooth muscle cells could serve to strengthen the wall against longitudinal stress. The muscle

strips seen in the present study may develop in response to increased stress on the vessel wall due to reduced elasticity, in order to strengthen the wall.

4.5 Conclusion

Results of the present study suggest that the structure and adrenergic innervation of the goat aorta are designed to confer unitary functional integrity, spatial stability, temporal adaptability, immunological homeostasis and an auxillary blood pumping mechanism. Unitary functional integrity is due to the interconnection between the tunica intima and the tunica media, both of which comprise an interlocked structure of their components as well a multidirectional orientation of smooth muscle cells, collagen and elastic fibres. Interlinkages between the structural components may further enhance spatial mechanical stability. The synthetic smooth muscle cells and fibroblasts may respond to tension by synthesizing extracellular matrix thereby permitting temporal adaptability to haemodynamic change. Presence of phagocytic cells could be responsible for maintaining immunological homeostasis, while innervated muscle islands in the proximal segments constitute an auxillary blood pump during diastole.

The zonal and regional variations in structure and adrenergic innervation of goat aorta suggest that different layers of its wall vary in their roles along the vessel. A thin tunica adventitia in the proximal segments where the thrust of systole is highest suggests that the smooth muscle islands in

the tunica media play a significant role in mechanical properties of the aorta.

Aging changes in the endothelium may predispose the aortic wall to atherosclerosis by allowing ingression of lipids. More pronounced aging changes in the tunica media of the proximal segments, implies that haemodynamic strain is a critical factor in their initiation. On the other hand, sparing of elastic lamellae in the outer zone and the abdominal aorta which have more smooth muscle suggests that changes in muscle tone influence the aging changes.

Parts of the aorta differ in organization of their mural components including innervation and vascularization. These regional variations may explain the differences in physicomechanical properties, pattern of aging changes and susceptibility to diseases. Thus, whereas in the proximal segments the tunica media may actively maintain blood flow and mechanical strength through myogenic and neurogenic contraction, in the distal segments blood flow is passive, and the tunica adventitia provides tensile strength.

4.6 Suggestions for further studies

Further studies are proposed first to determine the structural organization and adrenergic innervation of the aorta in other artiodactyls such as sheep, equines, bovines and antelopes which have common features with the goat. Secondly, to elucidate the age changes that occur in adrenergic innervation of the aorta in the goat and other artiodactyls. Thirdly, to compare the structure and innervation of the aorta between artiodactyls and birds. Fourthly to elaborate the pattern of non-adrenergic innervation of the aorta, and finally to study age changes in the aortae and their branches among various mammals to enhance understanding of the effect of arterial structural composition on pattern of aging.

CHAPTER FIVE

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