

AHA Medical/Scientific Statement

Special Report

A Definition of the Intima of Human Arteries and of Its Atherosclerosis-Prone Regions

A Report From the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association

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This report is a concise review of current knowledge of the structure and function of the intima of the aorta and the major distributing arteries. The main purpose of the review is to delineate normal arterial intima from atherosclerotic lesions and, in particular, to distinguish physiological adaptations from atherosclerotic increases in intimal thickness. To characterize normal intima, including the adaptive intimal thickenings, some of which represent locations in which atherosclerotic lesions are prone to develop, the structure, composition, and functions of the arterial intima in young people as well as in laboratory animals not subjected to known atherogenic stimuli are reviewed.

This report on arterial intima is the first in a series of four. The second report will review and define initial, fatty streak, and intermediate types of atherosclerotic lesions, and the third report will review all types of advanced (i.e., potentially clinical and clinical) lesions. The overall objective is to define arterial intima and all types of atherosclerotic lesions, and then to postulate, in a fourth and final report, a valid and up-to-date pathobiological nomenclature and classification of atherosclerotic lesions.

Role of Laboratory Technique in Evaluation of the Arterial Intima

The Committee on Vascular Lesions reviewed findings obtained by methods that include macroscopic, light and electron microscopic, histochemical, immunohistochemical, and chemical techniques applied to whole-artery segments or tissue samples as well as cell and tissue culture methods. Differences in the manner

of tissue sampling and preparation are responsible for some discrepancies among the many studies. Artifacts of technique may be acceptable and discrepancies reconciled if the associated changes are recognized, evaluated, compensated, and standardized.

For example, when dimensions and structure of arterial intima and atherosclerotic lesions are to be assessed, fixation of vessels while distended at mean arterial pressure is essential to approximate as closely as possible, and under standard conditions, the *in vivo* state. Configurational distortions and tissue disruptions associated with collapse and retraction of an artery or with improper tissue handling have been misinterpreted as abnormalities in many studies. Some of the artifacts have been reported in the literature as lesions. Focal physiological adaptations in intimal thickness have, for example, been thought to represent arterial stenoses or occlusions because of protrusion of the intima into the lumen when arteries were studied in the collapsed and contracted state (Figure 1). When elastic arteries are released and removed from their supporting tissues, they contract by about one third.

Because intimal structure is in part related to arterial geometry, samples for study should be obtained in a standardized manner that is reproducible and representative. The sampling should have a defined and consistent relation to anatomic landmarks such as the origins of specific branch vessels. However, the size of the samples taken for study should not be too small to be representative, for small shifts in vessel geometry with respect to branches and with regard to circumference influence both the extent and location of adaptations of the intima. Standardization of quantitative techniques for evaluating tissues is especially important with regard to the current development of automatic or semiautomatic measuring devices. For example, it is essential that adequate methodological details are

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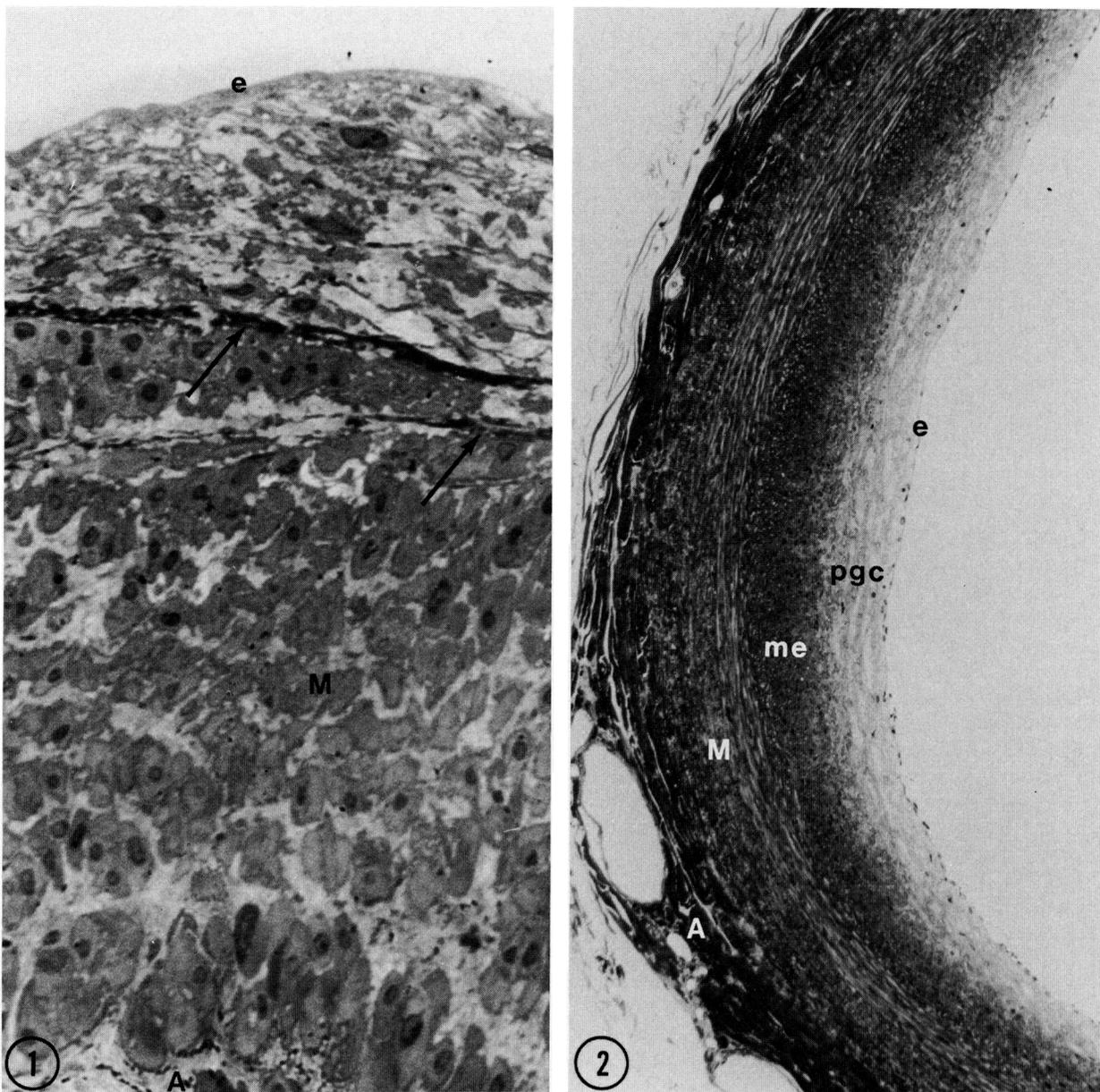


FIGURE 1 (left). Light micrograph of a 1- μ m-thick section through the wall of the distal left main coronary artery of a healthy young adult rhesus monkey that had been eating food low in cholesterol (serum cholesterol 130 mg/dl). An adaptive intimal thickening of the eccentric pattern protrudes into the lumen. The thickening appears greater after death than it was in life because the artery was fixed in the collapsed state. Endothelial cells (*e*) line the intimal thickening at the lumen. Arrows, internal elastic lamina; *M*, media; *A*, adventitia. Magnification $\approx \times 740$.

FIGURE 2 (right). Light micrograph of a 1- μ m-thick section through the outer wall of the proximal left anterior descending coronary artery of a 16-month-old boy. The eccentric intimal thickening present consists of a proteoglycan-rich (*pgc*) layer and a musculoelastic (*me*) layer and is without evidence of lipid accumulation or other atherosclerotic change. The eccentric thickening is crescent-shaped (concave) because the artery was fixed by perfusion at physiological pressure. Although a clear-cut internal elastic lamina is not present in this section, the media (*M*) can be distinguished from the intima by following the media around the circumference of the artery to a point (not seen in this picture) where the internal elastic lamina is present. *e*, Endothelial cells at lumen; *A*, adventitia. Homicide was the cause of death. Magnification $\approx \times 170$.

included in descriptions of image analysis systems and the data they produce.

Artifacts of cell and tissue morphology have been caused by changes that occur during the interval between death and fixation. The various cell types,

and the integrity of cell organelles and matrix components, are affected differently. Conditions such as temperature and fluid balance modify the rate at which tissue alterations develop. Endothelial cells and some organelles such as mitochondria of any cell

type deteriorate especially rapidly after death. Such considerations must be taken into account in interpretations of studies on the ultrastructural and molecular level.

Definition of Arterial Intima

The intima is defined as the region of the arterial wall from and including the endothelial surface at the lumen to the luminal margin of the media. The internal elastic lamina, generally considered part of the media, denotes the border between intima and media. However, a well-defined internal elastic lamina is absent in some parts of geometric transitions of arteries such as bifurcations, branch vessels, and curvatures. Thus, in these regions, recognition of the demarcation between intima and media may be difficult.

The thickness of arterial intima is not uniform. The criteria for normal thickness should recognize a broad range conveniently expressed quantitatively as the intima:media ratio. The ratio may vary from about 0.1 to 1.0 or more in normal arteries of humans.¹

Thick segments of intima exist in arteries obtained from healthy human subjects of all ages and from many other species. The thick segments may be focal (eccentric) or they may be more extensive (diffuse). They represent physiological adaptations to changes in flow and wall tension.

At vascular transitions such as bifurcations or trifurcations, normal structural reorganizations of the arterial wall, themselves localized thickenings, may overlap or fuse with intimal thickening caused by physiological adaptation. Structural reorganizations involve thickening of the deep (musculoelastic) intima layer and of the adjacent inner media. In regions of vascular transitions, the internal elastic lamina is partly or completely absent, and the intima and media may appear as a unit, indistinguishable from each other. Although it is sometimes difficult to distinguish how much of a particular thickening is intima or media and how much is of the adaptive or reorganizational type, the structures are composed of normal elements, differing clearly from atherosclerotic changes and other conditions. The difference between normal and pathological structure is clear when vessels are studied with appropriate methods.

The arterial intima is composed of two layers (Figure 2). The layers may be absent or barely visible by light microscopy in segments of arteries with a very thin intima. In segments with adaptive thickening of the eccentric or diffuse type, the layers are clearly visible. The inner layer, subjacent to the lumen, has been called the *proteoglycan layer* because it contains an abundance of finely reticulated nonfibrous connective tissue identified as proteoglycan ground substance by electron microscopy.^{2,3} Elastic fibers are scarce here. Smooth muscle cells are of both the rough endoplasmic reticulum-rich (synthetic) (Figures 3 and 4) and myofilament-rich (contractile) phenotypes. They occur as widely spaced single cells rather than in layers. Smooth muscle cells of the

rough endoplasmic reticulum-rich type may dominate in the fetal and immediate postnatal period. The part of the proteoglycan layer near the endothelium contains isolated macrophages.

The thicker layer underlying the proteoglycan layer (and adjacent to the media) has been called the *musculoelastic layer* because of the abundance of smooth muscle cells and elastic fibers. This lower intima layer also contains more collagen than the upper layer. Smooth muscle cells are of the myofilament-rich phenotype and arranged in close layers.

The individual cell and matrix components are reviewed in more detail under separate headings in subsequent sections of this report.

Physiological Adaptations in Intimal Thickness: Eccentric and Diffuse Intimal Thickening

Definition of Adaptive Intimal Thickening

The regions of thick but undiseased intima discussed here represent physiological adaptations to mechanical stresses secondary to variations in flow, wall tension, or both. Some authors have used the terms *eccentric* and *diffuse* to differentiate between two patterns of adaptive intimal thickening, although the two are generally contiguous, run into one another, and sometimes cannot be clearly distinguished from each other.

Eccentric intimal thickening is a relatively abrupt and focal increase in the thickness of the intima associated with branches and orifices. At an arterial bifurcation, the thickening involves about half the circumference (i.e., the outer wall, that opposite the flow divider) of the parent and daughter vessels and extends for a short distance along the length of the artery proximal and distal to the flow divider. In arteries fixed under physiological pressure, the structure is a crescent-shaped increase in intimal thickness (Figure 2). Eccentric thickening has been seen in coronary, carotid, cerebral, and renal arteries, although the extent and thickness were not described precisely in some arteries. In human coronary arteries, eccentric thickening has been observed from the first week of life and thereafter, although considerable individual variation in degree is found.¹ Its three-dimensional extent has been graphically outlined.⁴

Diffuse intimal thickening is a spread-out and often circumferential pattern of adaptive intimal thickening not clearly related to specific geometric configurations of arteries. In coronary arteries the degree of thickening is less than that of eccentric thickening, although more extensive.

There are only incomplete data on the range of eccentric and diffuse intimal thickening in arteries. What is known is based on the study of sample regions of vessels. The eccentric variant has been relatively well mapped in some arteries, but the extent of diffuse thickening and its relation to anatomic landmarks in vessels is not clear. In humans, adaptive intimal thickening of one or the other pattern may involve much of

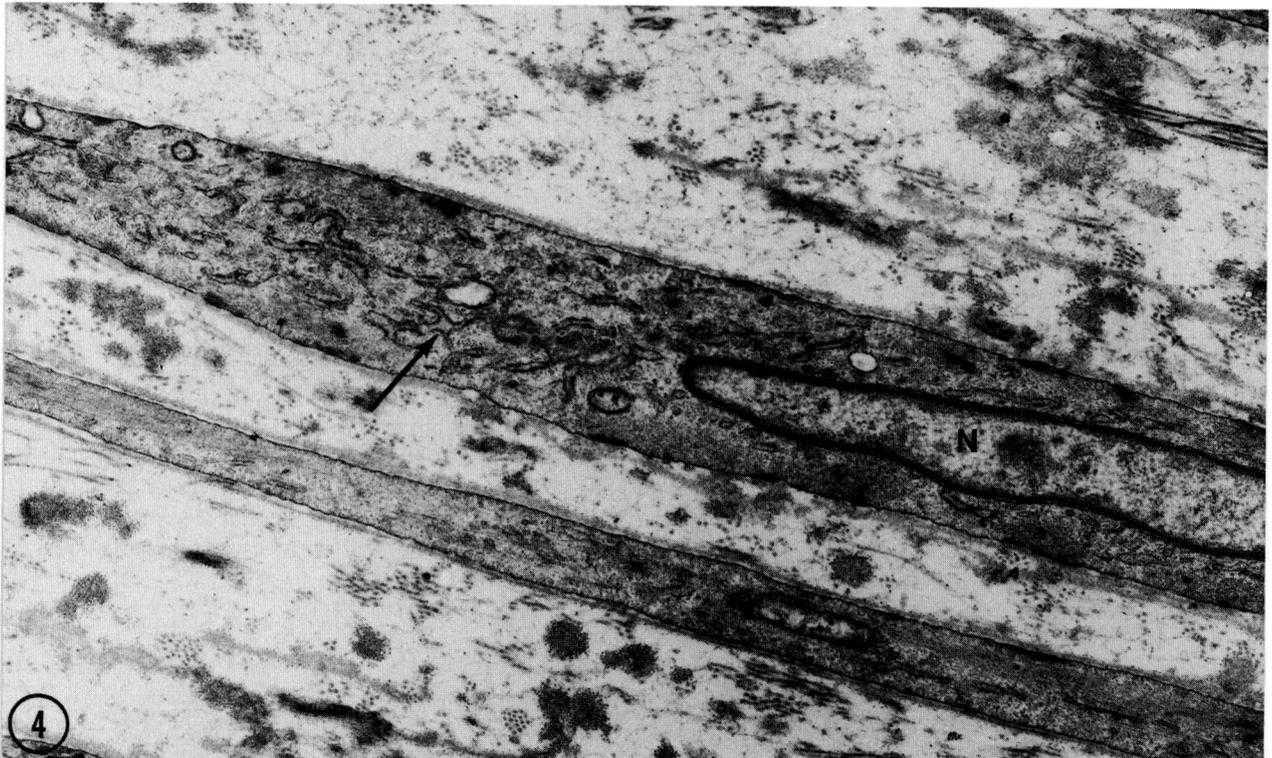
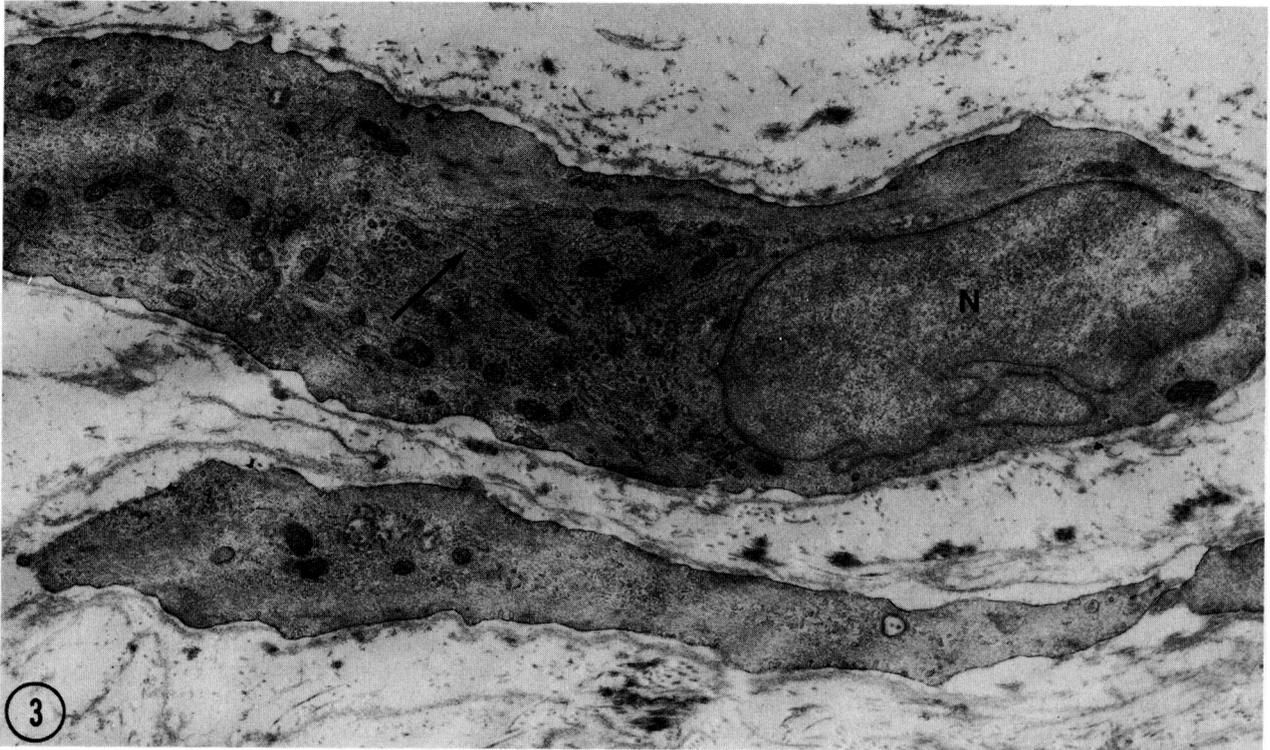


FIGURE 3. Electron micrograph of rough endoplasmic reticulum-rich smooth muscle cell in an eccentric intimal thickening at the bifurcation of the left coronary artery of a healthy young adult rhesus monkey that had been eating food low in cholesterol (serum cholesterol 160 mg/dl). Arrow, rough endoplasmic reticulum; N, nucleus. Magnification $\approx \times 18,000$.

FIGURE 4. Electron micrograph of rough endoplasmic reticulum-rich smooth muscle cell in the proteoglycan layer of an eccentric intimal thickening. The section is from the bifurcation of the left coronary artery of a 17-year-old boy. The coronary arteries were without evidence of lipid accumulation or other atherosclerotic change. Arrow, rough endoplasmic reticulum; N, nucleus. Suicide was the cause of death. Magnification $\approx \times 14,000$.

the extent of some arteries. More information could be acquired through microscopic studies; however, an inordinate number of microscopic sections would be required to map, for example, the intima of the human aorta precisely. Variation between persons and possible changes with age complicate the mapping.

Adaptive increases in intimal thickness do not obstruct the vascular lumen, although they may appear to do so in improperly fixed vessels (see "Role of Laboratory Technique in Evaluation of the Arterial Intima" and Figure 1). In particular, adaptive thickenings of the eccentric pattern appear as localized bulges into the lumen in arteries that were allowed to collapse and contract. Some adaptive intimal thickenings coincide with locations at which advanced atherosclerotic lesions develop early (atherosclerosis-prone locations). The relation between adaptive intimal thickening and atherosclerosis is discussed in a subsequent section of this report. Adaptive thickening can be clearly recognized by light microscopy of sections 1 μm thick. The microscopic composition is that of arterial intima in general (described in "Definition of Arterial Intima"), except for proportional increases in thickness. In thickenings of the eccentric pattern, the two normal layers of the intima are distinct and prominent.

Regions of the intima with adaptive increases in thickness differ functionally from adjacent, thinner regions. The turnover of endothelial cells⁵ and smooth muscle cells⁶ and the concentrations of low density lipoproteins^{7,8} and other plasma components are increased in adaptive intimal thickening compared with adjacent segments of intima without thickening. These increases should not be considered abnormal unless they enter a range associated with tissue damage.

In some laboratory animals (dogs,⁹ rabbits,^{10,11} pigs,^{12,13} and rats¹¹), physiological differences in aortic intima have been demonstrated by injection of the protein-binding azo dye Evans blue. Blue-staining areas of the intima correspond to areas of relatively enhanced permeability of the endothelial lining to, and intimal accumulation of, plasma macromolecules. It is not clear to what extent blue-staining areas of arterial intima overlap with physiological increases in intimal thickness in animals. Because studies with Evans blue have not been made in humans, it is not known whether or not blue areas would coincide with arterial locations known to have adaptive intimal thickening or locations prone to the development of clinical atherosclerotic disease.

Intimal thickening lacking the features of atherosclerosis or other disease processes was described in the human aorta in 1883 by Thoma,¹⁴ who assumed it to be a universal feature in human arterial development.¹⁵ In the early 1920s, Wolkoff^{16,17} described such intimal thickening in the coronary arteries of infants, children, and adults and in several animal species. Many authors have described similar intimal thickening in human coronary arteries^{1,18–30} and in the coronary arteries of normocholesterolemic ba-

boons,³¹ howler monkeys,³² rhesus monkeys,³³ and vervet monkeys.³³

Various terms have been used for intimal thickening of the eccentric pattern: *intimal cushion*,^{19,28,34} *spindle-cell cushion*,³⁵ *intimal pad*,^{30,35–38} *musculoelastic plaque*,³¹ *localized fibrous plaque*,²⁶ *mucoïd fibromuscular plaque*,³⁹ *normal intimal cell mass*,^{40,41} and *focal intimal hyperplasia*.⁴² Another term for the diffuse pattern is *diffuse intimal fibrosis*.²⁶ Many authors have not distinguished between the eccentric and diffuse patterns. General terms used for adaptive intimal thickening include *musculoelastic intimal lesion*,⁴³ *musculoelastic intimal thickening*,²³ *musculoelastic layering*,⁴⁴ and *fibromuscular intimal thickening*.⁴⁵

Although the terms attached to adaptive intimal thickenings and the significance attributed to them have varied, the microscopic descriptions and illustrations published by these authors resemble each other closely and doubtless represent either one or the other, or both, of the patterns of adaptive intimal thickening.

Hemodynamic Causes of Adaptive Intimal Thickening

Adaptive intimal thickening may be thought of as the consequence of a range of physiological stimuli, constituting an attempt by the tissue to maintain normal conditions of flow, wall tension, or both. Intima, as a living, reactive tissue, adapts in thickness to changes in pulse rate, blood pressure, arterial geometry, flow rate, and resistance to flow in distal vascular segments and in supplied organs.

Arterial locations with eccentric and diffuse intimal thickening correspond to regions of altered mechanical stress.^{46,47} In these locations, wall shear stress is reduced or wall tensile stress is elevated, or both. These findings indicate that the intima may thicken in response to reduced wall shear, reducing lumen diameter to elevate flow velocity and thereby restore wall shear to baseline values.⁴² In response to increased tensile stress, intimal thickening would appear to strengthen the arterial wall to maintain normal values of tensile stress. Thus, the intima may participate in adaptive remodeling processes in adjusting to altered local mechanical stresses. In relatively straight arterial segments, such mechanical stresses are evenly distributed, causing diffuse thickening. The thickening would be eccentric in regions of bifurcations and branches where both shear and tensile stresses are not uniformly distributed.

Relation Between Adaptive Intimal Thickening and Atherosclerosis

When atherosclerosis develops, advanced lesions form first in some regions with adaptive intimal thickening. In humans, the topographic distribution of eccentric intimal thickening and of advanced atherosclerotic lesions is similar in the coronary arteries,⁴ renal arteries,^{34,36} the internal carotid artery at the level of the carotid sinus,⁴⁶ and the aorta.^{48,49} In the aorta, intimal thickening is most prominent in the abdominal segment,^{48,49} particularly in the dorsal

rather than in the ventral aspect^{48,50}—that is, in locations where atherosclerosis is most severe in later life. The susceptible regions have been called the atherosclerosis-prone areas of arteries. Because of the colocalization of advanced atherosclerotic lesions with some intimal thickening (particularly with that of the eccentric pattern) and because both are more or less focal, the question of whether eccentric intimal thickening should be considered atherosclerotic has been much debated. If eccentric thickening is accepted as a physiological adaptive process, then the development of a lesion refers only to changes superimposed on it. The available evidence indicates that specific mechanical stresses, present in locations of the arterial tree with adaptive intimal thickening, cause the thickening whether high concentrations of atherogenic lipoproteins are present or not. When atherogenic lipoproteins exceed certain critical levels, the same mechanical forces may enhance lipoprotein deposition in the same regions, leading to transformation into atheromatous lesions.

Although some intimal thickenings mark regions of increased susceptibility to formation of advanced atherosclerotic lesions, the fact remains that in severely hypercholesterolemic humans and in several species of animals subjected to severe hypercholesterolemia, nearly all regions of the aorta and of many arteries may ultimately be the sites of advanced lesions. Thus, advanced lesions are not confined to regions with adaptive intimal thickenings. The latter simply mark locations where, under the influence of atherogenic stimuli, lesions form earlier and more rapidly than elsewhere, and where, therefore, symptomatic lesions tend to occur.

Gender Differences in Adaptive Intimal Thickening

Dock¹⁹ found variation in the intimal thickness of coronary arteries of human fetuses. He described a higher degree of intimal thickening in the arteries of males than of females. Gender differences in adaptive intimal thickening were also observed by others.^{27,48,51,52} However, Stary¹ calculated the ratio between the cross-sectional areas of the intima and the media of coronary arteries of young children and found no difference between young male and female children in the intima:media ratio.

Ethnic Differences in Adaptive Intimal Thickening

Neufeld⁵³ presented the hypothesis that ethnic differences in coronary heart disease mortality were related to differences in structural changes in coronary arteries. This hypothesis was prompted by the autopsy examination of 211 infants and children up to 10 years of age who belonged to three ethnic groups: Jews of Central and East European origin (Ashkenazi), Bedouins, and Yemenite Jews.⁵⁴ The thickness of the intimal and internal muscular layers was significantly greater in Ashkenazi boys than in boys from the two other ethnic groups. The same measurements in girls yielded few differences. Intimal thickening in the coronary arteries of children has been reported to be

greater in Eastern than in Western Finland.^{51,55} The differences paralleled differences in coronary heart disease incidence and mortality between West and East Finland. Daoud et al,⁵⁶ who compared intimal thickening (referred to as preatheromatous proliferative changes) in the coronary arteries of young men from New York with those in young men from East Africa, found less intimal thickening and less atherosclerosis in the East Africans.

The Cells of Arterial Intima

General Comments

Endothelial cells and smooth muscle cells are the principal cellular components of human arterial intima. Isolated macrophages are also always present. These cell types are discussed in detail under separate headings.

The presence of mast cells in normal human arterial intima has been reported by several authors,^{57–62} but intimal mast cells are relatively rare and are not found in every person. They are particularly rare in young children, although there is electron microscopic evidence that mast cell granules may be small in young children, that mast cells may be degranulated, and that they are therefore often impossible to detect by light microscopy.⁶² Therefore, mast cells may be present in arterial intima more often than present studies indicate. The possible functions of mast cells in arterial intima have been reviewed recently.^{63,64}

Lymphocytes have been found in atherosclerotic intima, but whether they occur in normal intima is not yet clear. Jonasson et al⁶⁵ used immunocytochemistry to search for T lymphocytes in normal intima of human aortas and uterine arteries but failed to find any. However, a few T cells were found in nonatherosclerotic intima adjacent to lesions of the atheroma type in carotid arteries. Munro et al⁶⁶ did not find T lymphocytes in normal aortic intima adjacent to lesions of the fatty streak type, although the lesions contained them. Lymphocytes have been reported in the intima of nonatherosclerotic rat aortas.^{67,68} The possible significance of lymphocytes in arterial disease has been reviewed.⁶⁹

Endothelial Cells

Most data on endothelial cells come from animal models and in vitro experiments. Studies of endothelial cells in intact human arteries are difficult because the interval between death and study of tissues is often too long in autopsied human beings. The endothelial cells of normal muscular and elastic arteries form a continuous layer of flattened, elongated, polygonal cells. With the exception of cells situated in areas of turbulent flow and reduced shear, the long axes of endothelial cells are oriented in the direction of flow.^{70–72}

At the luminal surface, endothelial cells are coated by a glycocalyx consisting primarily of free polysaccharides and glycosaminoglycans plus glycoprotein

and glycolipid side chains emanating from the plasma membrane.⁷³ Endothelial cells also synthesize and secrete extracellular matrix components, such as fibronectin, and components of the endothelial-cell basement membrane, such as type IV and type V collagen, laminin, and proteoglycans.^{74,75}

The luminal surface of the plasma membrane of arterial endothelial cells contains a mosaic of microdomains, charged in part because of the distribution of anionic proteins.⁷⁶ There are also membrane-associated glycoproteins that specifically bind lectins as well as proteoglycans such as heparan sulfate proteoglycans that may be instrumental in binding lipoprotein lipase to the endothelial surface.^{77,78} In addition, endothelial cells contain a variety of plasma membrane receptors such as those for native and modified LDL,⁷⁹ insulin,⁸⁰ and histamine.⁸¹

Endothelial cells contain a normal complement of organelles, a large number of plasmalemmal vesicles (also called *caveolae* or *transcytotic vesicles*), and an extensive cytoskeleton of microfilaments, microtubules, and intermediate filaments. Microfilament bundles, commonly referred to as stress fibers, are the most abundant fibrous protein component of endothelial cells. They contain F-actin, myosin, α -actin, tropomyosin, and vinculin.⁸² Characteristic of endothelial cells are Weibel-Palade bodies, rod-shaped structures that contain factor VIII-related antigen and von Willebrand factor.^{83,84}

Endothelial cells have a variable turnover rate. The thymidine labeling index for the aortic endothelium of normal mature rats is less than 1.0 (i.e., less than 1.0% of the total population of cells enter the S phase of the cell cycle in a 24-hour period), and there is a tendency for higher labeling on the dorsal than on the ventral surface of the thoracic aorta.⁸⁵ Labeling is higher around the mouths of branches originating from the aorta in guinea pigs⁵ and higher in areas of Evans blue staining in pig aortas.⁸⁶ Sade et al⁸⁷ found that labeling of rat aortic endothelial cells decreased greatly with age.

Arterial endothelial cells contain a complex system of interendothelial cell junctions that include both tight and gap junctions.⁸⁸ The majority of endothelial cell-smooth muscle cell contacts are via gap junctions, where cytoplasmic bridges pass through fenestrations in the internal elastic lamina.⁸⁹

Arterial endothelium is permeable to all plasma proteins. The final concentration of these proteins in the intima is apparently dependent on the degree of retention (trapping) and rates of degradation and efflux.⁹⁰ Transcytosis and passage through intercellular junctions are both probable pathways for the transport of macromolecules across the endothelium.⁹¹ The rates of transport of lipoproteins and other macromolecules across the endothelium appear to be dependent on the plasma concentration and on the size and charge of the particles or proteins⁹² as well as on the location along the arterial tree and the age, blood pressure, and vascular tone of the individual.^{93,94}

Under normal conditions, the endothelium does not support the adherence of platelets or the formation of thrombi. This thromboresistant property appears to be dependent on the endothelial cell membrane content of thrombomodulin and its ability to bind thrombin.⁹⁵ The thrombomodulin-thrombin complex in turn activates protein C, which complexes with protein S and inactivates coagulation factor Va.⁹⁶ In addition, endothelial cells may derive thromboresistance from their ability to metabolize the platelet aggregating agents adenosine diphosphate, serotonin, angiotensin, and prostaglandin F₁ and to synthesize and secrete plasminogen activator⁹⁷ and prostacyclin.^{71,98}

The normal arterial endothelium also does not support the adherence of large numbers of leukocytes. However, *in vitro* studies have shown that upon activation of endothelial cells from human umbilical veins with cytokines, such as interleukin-1 and tumor necrosis factor, or oxidized LDL, there is a large increase in leukocyte adherence that may be due to the expression of leukocyte-specific adherence molecules. These are referred to as endothelial-leukocyte adhesion molecules and intercellular adhesion molecules, which are specific for monocytes/neutrophils and lymphocytes respectively.⁹⁹⁻¹⁰¹ Endothelial cells from large arteries of hypercholesterolemic rabbits express monocyte-specific endothelial-leukocyte adhesion molecules.¹⁰² Endothelial cells can express chemotactic proteins,^{103,104} hematopoietic factors,¹⁰⁵ and major histocompatibility antigens.¹⁰⁶

Normal endothelial cells may be extremely important in regulating vascular tone and contraction.¹⁰⁷ Human and porcine aortic endothelial cells and smooth muscle cells synthesize and secrete prostacyclin, which prevents platelet aggregation and causes vascular relaxation by its capacity to stimulate the production of cAMP.^{108,109} Endothelial cells also secrete another potent vasoactive agent called endothelium-derived relaxing factor.¹¹⁰ Through a mechanism that at least in part includes nitric oxide, endothelium-derived relaxing factor stimulates vascular smooth muscle cell production of cGMP and phosphorylation of several proteins involved in cellular contractility.¹¹¹ Endothelial cells may also stimulate vasoconstriction. A peptide of 21 amino acids has been isolated from cultured porcine aortic endothelial cells and shown to be an extremely potent vasoconstrictor. This peptide, called endothelin, stimulates vasoconstriction by activating voltage-dependent calcium channels in vascular smooth muscle cells.¹¹²

The main properties of endothelial cells are summarized in Table 1. For more complete discussions of specific topics on endothelium, the reader is directed to recent reviews.^{75,91,113-116}

Smooth Muscle Cells

Smooth muscle cells are generally recognized as a normal component of the intima in humans and in many other species, although some segments of arte-

TABLE 1. Functional Properties of Endothelial Cells in the Arterial Intima

Function	Examples of properties supportive of function
Permeability barrier	Surface charge ⁷⁶ and presence of glycocalyx ⁷³ Tight junctions ^{88,91,92} Pore size of transcytotic vesicles ⁹¹ Basement membrane ⁷⁵
Thromboresistance	Thrombomodulin content of plasma membrane ⁹⁶ Rapid metabolism of platelet aggregating agents ⁷¹ Synthesis and secretion of prostacyclin ⁹⁸ and plasminogen activator ⁹⁷
Mediation of vascular tone	Synthesis and secretion of prostacyclin, ¹⁰⁸ endothelium-derived relaxing factor, ^{110,111} and endothelin ¹¹¹
Inflammatory and immune response	Expression of leukocyte adhesion molecules, ⁹⁹ leukocyte chemotactic proteins, ^{103,104} growth factors, ⁷⁵ hematopoietic factors, ¹⁰⁵ major histocompatibility complex antigens, ¹⁰⁶ and scavenger receptor(s) ⁷⁹

rial intima can be without smooth muscle cells. The mere presence of smooth muscle cells in the intima does not define the tissue as atherosclerotic.

Two distinctive types of smooth muscle cells are evident in arterial intima of humans and other species by electron microscopy and immunocytochemistry. Myofibrillar-rich (*contractile*^{117,118} or *adult*¹¹⁹) smooth muscle cells predominate in the intima. These cells occupy mainly the musculoelastic intima layer and resemble medial smooth muscle cells. Rough endoplasmic reticulum-rich (*synthetic*,^{117,118} *modulated*,¹²⁰ *immature*,¹¹⁹ or *ergastoplasm-rich*⁴¹) smooth muscle cells (Figures 3 and 4) occur in the proteoglycan-rich (upper) intimal layer. Rough endoplasmic reticulum-rich smooth muscle cells contain few myofibrils, and sometimes none are visible. Although there is more rough endoplasmic reticulum than there are myofibrils, rough endoplasmic reticulum does not fill the cytoplasm, and much of the cytoplasm consists of an electron-microscopically nondescript matrix. In cell culture, rough endoplasmic reticulum-rich smooth muscle cells can synthesize a wide variety of intercellular matrix components.¹²¹ When studied by immunocytochemistry, rough endoplasmic reticulum-rich smooth muscle subtypes differ in cytoplasmic fiber proteins.^{122,123} Further support for the synthetic role of rough endoplasmic reticulum-rich smooth muscle cells derives from tissue culture experiments in which arterial smooth muscle cells are subjected to cyclic stretching. In such cells collagen synthesis is markedly increased compared with stationary cells¹²⁴ as the cells are altered from the myofibrillar-rich to the rough endoplasmic reticulum-rich morphology.¹²⁵

In normal human,¹ nonhuman primate,¹²⁶ pig,⁴¹ and rabbit³⁸ intima, the proportion of rough endoplasmic reticulum-rich smooth muscle cells is greatest in the subendothelial portions of the proteoglycan-rich intima layer. In rats, smooth muscle cells in the eccentric intimal thickenings at arterial branch points were identical to smooth muscle cells elsewhere in the arterial wall.¹²⁷

The precise age at which smooth muscle cells appear in the intima has not been determined, but smooth muscle cells were described by Thoma¹⁴ in the aortic intima of two human fetuses at 30–33 weeks of gestation. Langhans¹²⁸ described cells in the aortic intima of a human infant dead 4 days after birth only as spindle-shaped cells. Wolkoff,¹⁶ who studied the coronary arteries of nine human subjects ranging in age from 8 months to 50 years, also recognized the cells in intimal thickening to be smooth muscle cells. Stary¹ stated that smooth muscle cells were present in the coronary arteries of each of the 63 infants and children under the age of 5 years whom he studied.

A progressive increase in the degree of intimal thickness has been described in the postnatal period.^{16,21,29,128} It is unknown whether this is caused by intramural division of cells or by migration of medial smooth muscle cells into the intima. Radioautographic studies with tritiated thymidine, conducted primarily in rabbits,^{6,129} suggest that an increase in the number of cells occurs through mitosis of existing intimal smooth muscle cells. On the other hand, migration of cells from the media into the intima is suggested by the observation of medial smooth muscle cells in gaps of the internal elastic lamina.¹³⁰ At present there is no proof, however, that this phenomenon represents transmigration of medial smooth muscle cells into the intima, although migration of smooth muscle cells clearly can occur under certain conditions.¹³¹

An increase in the number of smooth muscle cells in the developing intima by mitosis could be a response to growth regulatory molecules such as growth factors. To date, there is no information about the expression of growth factors or their accompanying receptors by normal intimal smooth muscle cells in either humans or animals. However, there is evidence that platelet-derived growth factor, platelet-derived growth factor receptors, and insulin-like growth factor-1 are expressed by smooth muscle cells from normal rat aorta.¹³² Smooth muscle cells can remove deposited lipoproteins by expression of LDL receptors, phagocytosis, or both.^{133,134}

The presence of autophagosomes in some smooth muscle cells and of small quantities of cell debris in the extracellular matrix of arterial intima (and media) have been reported in unmanipulated and apparently healthy rhesus monkeys,¹³⁵ rabbits,³⁸ and rats.^{136,137} Evidence of smooth muscle cell damage or death has not been reported for undiseased human arterial intima but, based on the animal evidence, it can be assumed to occur.

TABLE 2. Functional Properties of Smooth Muscle Cells in the Arterial Intima

Function	Examples of properties supportive of function
Contractility	Modulation of content of contractile proteins ¹¹⁷ Responsiveness to mediators of vascular tone ¹⁰⁷
Maintenance of structural integrity	Synthesis and secretion of connective tissues (collagen, elastin, and proteoglycan) ^{120,121} Capacity to proliferate via expression of growth factor receptors and synthesis and secretion of growth factors ¹³² Capacity to migrate ¹³¹
Lipid metabolism	Removal of deposited lipoproteins via expression of LDL receptors and/or phagocytosis ^{133,134}

The main functional properties of smooth muscle cells in the arterial intima are summarized in Table 2. For detailed information on recent developments in arterial smooth muscle research, the reader is referred to recent reviews.^{119,138,139}

Macrophages

Macrophages appear in the normal arterial intima as isolated cells at distant and irregular intervals. They have been seen in undiseased intima of rats,^{67,136,140} rabbits,¹⁴¹ pigs,¹⁴² and humans.^{1,143} Some authors have described the cells as monocytoïd or monocyte-like or as mononuclear leukocytes. Normal human coronary arteries contain isolated macrophages in the part of the intima adjacent to the endothelium from the first week of life.¹ The number of macrophages increases with age (adjusted for increase in artery size) until their number stabilizes in young adults at about two and a half times that in infants.⁶² Eccentric intimal thickenings contain three times as many intimal macrophages as the opposite wall of the artery without eccentric thickening.

The presence of macrophages in arterial intima is consistent with the presence of macrophages in many other normal tissues.¹⁴⁴ The functions of macrophages depend on the nature of their environment.¹⁴⁴⁻¹⁴⁶ There is speculation about the functions macrophages are required to perform in normal arterial intima. Data from animal and in vitro studies indicate that macrophages can elaborate collagenase,^{147,148} elastase,^{149,150} growth factors for smooth muscle cells and endothelial cells,¹⁵¹⁻¹⁵³ chemotactic factors for smooth muscle cells,¹⁵⁴ and angiogenesis factors,¹⁵⁵ and that they can be active in lipid metabolism¹⁵⁶⁻¹⁵⁸ and phagocytosis.¹⁴⁵

The functions of macrophages are summarized in Table 3. For extensive discussions of macrophage functions in host defense, the immune response, and lipid metabolism, the reader is referred to recent reviews.^{69,159-161}

TABLE 3. Properties of Macrophages That May Account for Their Presence in the Arterial Intima

Function	Examples of properties supportive of function
Remodeling of the intima	Synthesis and secretion of collagenase ^{147,148} and elastase ^{149,150} Synthesis and secretion of growth factors for smooth muscle cells and endothelial cells ¹⁵¹⁻¹⁵³ Synthesis and secretion of chemotactic factors for smooth muscle cells ¹⁵⁴ Synthesis and secretion of angiogenesis factors ¹⁵⁵
Inflammatory and immune responses	Direct phagocytosis and cytolysis of bacteria and tumor cells ^{144,145} Antibody-dependent cytolysis ¹⁴⁴ Activation of immune response (accessory and regulatory function for T cells, B cells, and natural killer cells; binding and presentation of antigens; cytokine and growth factor production) ⁶⁹
Scavenger functions	Lipid metabolism (receptor-mediated uptake of native and modified lipoproteins ¹⁵⁶ ; secretion of apoprotein E ¹⁵⁷ ; and secretion of lipoprotein lipase ¹⁵⁸) Phagocytosis and removal of dead cells and immune complexes ¹⁴⁵ Fibrinolysis and removal of mural thrombi and other deposited plasma proteins ¹⁴⁵

Matrix of Arterial Intima

General Comments

The extracellular matrix is a major component of normal arterial intima, particularly in areas of intimal thickening. It constitutes up to 60% of the volume of the intima. The matrix is important as the medium through which essential nutrients are transported across the intima, the site for accumulation of products elaborated by the various intimal cells, the site for accumulation of cell debris, and the avenue for migration of cells entering and traversing the intima.

Because the intima can consist of variable amounts of smooth muscle cells while the number of endothelial cells remains about the same, its extracellular matrix will consist of differing proportions of the matrix products secreted by the two cell types.

Proteoglycans

Proteoglycan molecules, because of their large physical size, concentration, and ionic properties, function in arterial permeability, filtration, ion exchange, transport and deposition of plasma materials, and regulation of cellular metabolism.

The nature, identity, and precise localization of proteoglycan types in the normal human intima has not been resolved. Ruthenium red staining of pig and rabbit aortas indicate that the subendothelial space contains large chondroitin sulfate proteoglycans that may or may not be similar to the chondroitin sulfate

proteoglycan in the intercellular space of the media.^{3,162} There is increased proteoglycan¹⁶³ in normal human arterial intima containing several smooth muscle cell layers (intimal thickening). In normal human coronary arteries, the contents of dermatan sulfate and chondroitin sulfate increase with age.¹⁶⁴ Much of the current information on intimal proteoglycans has been extrapolated from observations in cell culture. A number of studies have demonstrated that endothelial cells in culture produce primarily heparan sulfate-like proteoglycans along with minor amounts of dermatan sulfate proteoglycans.¹⁶⁵ Three species of heparan sulfate proteoglycan have been identified in cultured endothelial cells from bovine aortas,¹⁶⁶ one of which was associated with basement membranes. Based on studies of proteoglycan synthesis by cultured smooth muscle cells,^{167,168} one would expect dermatan sulfate proteoglycan to be a major component of the matrix around intimal smooth muscle cells, although studies to demonstrate the presence of this type of proteoglycan specifically in the intimal layer have not been completed. Preparations of human intima-media used to chemically isolate proteoglycans have demonstrated at least two distinct dermatan sulfate proteoglycan molecules.¹⁶⁹ Based on topographical distribution of proteoglycan types in normal human aortic media, Völker et al¹⁷⁰ described dermatan sulfate proteoglycan associated with collagen fibers, heparan sulfate proteoglycan associated with elastic fibers as well as the surfaces of smooth muscle cells, and chondroitin sulfate proteoglycan in the extracellular space.

Collagens

Collagen plays a role in the attachment of endothelial cells to the subendothelial matrix, thus contributing to endothelial cell integrity. The family of collagen molecules consists of at least two or more genetically distinct protein types. Based on current studies, there are at least 18 genes to code for the constituent alpha chains.¹⁷¹⁻¹⁷⁴ The major types in the artery wall are the two interstitial collagens, types I and III.

Gay et al¹⁷⁵ demonstrated immunologically, in the aorta of a 4-year-old child, that type III collagen was localized in the subendothelial space of the intima. Type I collagen was not detected in this location. Type III collagen localized in the subendothelial space of arteries of young persons may be synthesized by the endothelium, because bovine endothelial cells in culture can synthesize this collagen type.^{176,177}

With aging, there is a change in the intimal ratio of types I and III collagen in favor of type I. Increased amounts of type I may reflect the metabolic properties of an increased number of smooth muscle cells present in the intima, because smooth muscle cells in culture synthesize both type I and type III collagen.¹⁷⁸

Studies of human aorta intima-media, including the arch, the thoracic and abdominal aortas, and the carotid artery, have consistently indicated type I collagen as the major type.¹⁷⁸⁻¹⁸¹ The presence of

other collagen types has been described in human arteries but in smaller amounts than types I and III. These include types IV, V, and VI, which collectively compose only about 0.5-1.0% of the total arterial collagen.

Type V collagen is a pericellular collagen and may bind interstitial collagen to cells or basal laminae. This collagen has been localized immunologically in close association with the endothelial cells and the basal lamina of the smooth muscle cells and in the subendothelial basement membrane.¹⁸²⁻¹⁸⁵ Several studies have demonstrated that both arterial smooth muscle cells and endothelial cells synthesize type V collagen.^{176,177,184,186}

Type IV collagen, the collagen characteristic of basement membrane, has been isolated from human aorta,^{181,187} and immunolocalization studies^{183,184} have demonstrated its presence in the subendothelial basement membrane and the basal lamina of the smooth muscle cell. Cultures of smooth muscle cells¹⁸⁸ and endothelial cells^{176,177,182,184,189} have been shown to synthesize type IV collagen. Type VI, or "short-chain," collagen has been isolated from human aortic intima¹⁹⁰ and is thought to serve as a link between collagenous and noncollagenous structures.¹⁹¹

Elastin

By electron microscopy,¹⁹²⁻¹⁹⁴ the elastic fibers of the media include two distinguishable components: an amorphous component, elastin, which does not possess any regular repeating structure or banding pattern, and a microfibrillar component. In the musculoelastic layer of intima, and particularly in eccentric intimal thickening, elastic fibers are prominent and mimic those of the media. With increasing age there is a decrease in the elastin content (relative to collagen) in the entire grossly normal human aorta.¹⁹⁵

The precursor to the elastin component of the elastic fiber is tropoelastin, which is secreted from cells as a protein with a molecular weight of 72,000.¹⁹⁶⁻²⁰⁰ Both smooth muscle cells^{121,201,202} and endothelial cells^{203,204} have been reported to synthesize elastin.

Fibronectin and Laminin

Other components such as fibronectin and laminin are also present in the extracellular matrix of normal intima.²⁰⁵ Fibronectin is a high-molecular-weight, multifunctional, ubiquitous, adhesive glycoprotein found on cell surfaces, in extracellular matrixes, and in blood. The molecule functions in cell-cell adhesion and cell-substrate adhesion, cell motility, and specific binding of molecules through specialized domains in the molecule. Most notable are the collagen-binding domain, the heparin-binding domain, and the cell membrane-binding domain, through which cell-matrix interactions occur. Laminin, another major noncollagenous glycoprotein, along with heparan sulfate proteoglycan and type IV collagen, is a major component of the basement membrane underlying the endothelium. In vitro stud-

ies have demonstrated that laminin accelerates the attachment of endothelial cells to type IV collagen but not to other collagen types.

Plasma Components

Saline extracts of human aortic intima have been examined and in general show protein content (polyacrylamide gel electrophoretic patterns) very similar to those of plasma proteins.²⁰⁶ Soluble proteins extracted from grossly normal aortic intima with neutral buffered saline contain immunoglobulin A, immunoglobulin G, a trace of immunoglobulin M, c3-complement component, α_1 -antitrypsin, α_2 -macroglobulin, fibrinogen, albumin, LDL, HDL, α_1 acid glycoprotein, β_2 -glycoprotein, transferrin, and ceruloplasmin.²⁰⁷ In studies using nonionic detergents to isolate soluble proteins from human thoracic aortic intimas without visible atherosclerosis, major proteins identified were actin, tropomyosin-like proteins, and glycoproteins with molecular weights of 35,000 and 30,000, each consisting of more than one component differing in relative charge.²⁰⁶ Several proteins apparently originating from plasma were identified, including albumin, immunoglobulin G, α_1 antitrypsin, transferrin, haptoglobin β -chain, apo A-I, apo A-II, fibrinogen β -chain, α_2 heparan sulfate glycoprotein, and an α_1 -antichymotrypsin.

Concentrations of the plasma proteins in normal human intima have been measured by electrophoresis directly from the tissue into antibody-containing gels.^{207,208} For some cases the concentrations of LDL in intima were similar to, or higher than, the concentration of plasma LDL. For some proteins such as albumin, intimal concentrations were significantly lower than plasma concentrations. LDL concentration in lesion-free intima has been positively correlated with an individual's plasma lipid level.²⁰⁸ It appears that all plasma proteins are present in the intima in concentrations directly related to the protein's molecular weight and to the plasma concentration.²⁰⁹⁻²¹¹ In the normal artery, LDL is localized in the intima and is usually not detectable in the underlying inner media.²¹¹ Lipoproteins are increased in eccentric intimal thickening compared with adjacent areas without eccentric thickening in the human abdominal aorta.⁸

Normal intima contains soluble fibrinogen as well as fibrinogen cleavage products, suggesting that in addition to flux of fibrinogen in normal artery, there is continuous conversion of fibrinogen to fibrin and lysis of fibrin within the intima.²¹² Fibrinogen/fibrin-1 was detected with a specific monoclonal antibody in two of 12 specimens of grossly normal human aortas.²¹³

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