

# Lipid and macrophage accumulations in arteries of children and the development of atherosclerosis<sup>1-3</sup>

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**ABSTRACT** About one-half of infants in the first 6 mo of life have small collections of macrophages and macrophages filled with lipid droplets (foam cells) in susceptible segments of the coronary arteries. In subsequent years, fewer children have foam cells but around puberty (12–15 y) foam cell accumulations mostly larger than those in infants occur in 69% of adolescents. Lesions that represent the previously missing link between foam cell accumulations and atheromas have now been identified in a subgroup of highly susceptible locations. Such “preatheroma” lesions contain small pools of lipid droplets and dead cell remnants (extracellular lipid) in addition to macrophage foam cells. Atheromas, which emerge in some adolescents and young adults in the same locations, have a lipid core in which increased extracellular lipid displaces structural smooth muscle cells and the normal extracellular matrix. As soon as lipid cores form, calcium granules appear in some smooth muscle cells and among the extracellular lipid of the core. The degree of calcification is variable and, in youth, generally small. In the age group of 16–19 y, 15% of persons have either preatheromas or atheromas in coronary arteries; foam cell accumulations only are present in an additional 53% of 16–19-y-olds. Because the lipid cores of atheromas may be an underlying cause of lesion rupture, hematomas, and thrombosis, and because their development begins soon after puberty, it would be prudent to attempt to lower the influx of excessive atherogenic lipoproteins into the arterial wall by that age. *Am J Clin Nutr* 2000(suppl);72:1297S–306S.

**KEY WORDS** Infants, children, young adults, coronary arteries, atherogenesis, thrombosis, lipid regression, calcium deposits, atheroma, preatheroma, foam cells

## INTRODUCTION

This article describes accumulations of lipid and the associated cell reactions in the arteries of infants and children and the sequence of cell and tissue changes whereby some of these initial and minimal changes develop into lesions that occlude arteries, most often at and after late middle age. I hope to clarify the hitherto disputed question of the development of clinical disease from minimal accumulations such as those in children. Closely related to this issue and also addressed is the dispute about the cause and origin of the great number of intimal smooth muscle cells present from birth in locations in which lipid and foam cells

tend to accumulate. Other topics include the frequency and amount of calcium and thrombotic deposits in young persons and the feasibility of regression of lesions.

Although advanced histologic techniques are very time consuming when used in large-scale population studies, my laboratory nevertheless chose to use such techniques in a systematic study of coronary arteries and aortas to try to clarify these questions. Overall, this part of our study included the coronary arteries of 691 persons (and the aortas of 648 of the 691 cases), whose ages spanned the first 4 decades of life (ie, from full-term birth to 39 y) and who mostly died suddenly of causes other than disease.

The methods we used were described in other publications (1–3). Briefly, the improvements made over other studies included preserving the structure of the coronary arteries as it had been in life (or close to it) by perfusing and fixing the arteries with glutaraldehyde under physiologic (or near physiologic) pressure rather than examining the arteries as they collapsed and contracted after death. We then prepared entire cross-sections of the pressure-perfused arteries of 691 cases at a thickness of only 1  $\mu\text{m}$ . The 1- $\mu\text{m}$  thick sections revealed much higher resolution of structure and composition by light microscopy than do conventional 5–6- $\mu\text{m}$  thick sections. Additional detail of selected smaller regions or cells was obtained by electron microscopy. Histologic processing of aortas (4) was similar to that of coronary arteries except that aortas were opened and fixed flat by immersion in formalin rather than by pressure-perfusion fixing in glutaraldehyde.

The segments of arteries we sectioned and evaluated in every case included the highly susceptible locations of coronary arteries and aortas in which atheromas predictably make their first appearance in young adults with such lesions. We characterized the intima and lesions in these precisely defined locations in infants and children and then studied the same sites in adolescents and young and middle-aged adults. Multiple 1- $\mu\text{m}$  thick cross-sections from along the highly susceptible segments enabled us to reconstruct the three-dimensional structures and compositions of these segments and of the changes and lesions they contained.

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**TABLE 1**  
Terms used to designate the normally thicker segments of arteries

Terms for thick arterial wall segments present from birth and histologically normal	Additional terms used for identical and probably identical intimal structures
Adaptive intimal thickening: eccentric type (eccentric intimal thickening)	Intimal cushion, intimal pad, spindle cell cushion, smooth muscle mass, mucoïd fibromuscular plaque, focal intimal hyperplasia
Adaptive intimal thickening: diffuse type (diffuse intimal thickening)	Musculoelastic intimal thickening, intimal hyperplasia

We found that the intima was thicker in highly susceptible locations from birth and that macrophages containing lipid droplets (macrophage foam cells) and other changes developed more strongly in these than in other locations. The various terms that have been applied to the thicker intima segments are listed in **Table 1**. The contiguous nature of the histologic changes and the time of life at which a specific change predominates indicate that each represents a gradation or stage in a temporal sequence. The characteristic histologic changes were therefore arranged as a numbered sequence of lesion types. Shown in **Figure 1** is an overview of the sequence and a comparison with the more limited conventional classification developed mainly by viewing arteries with the unaided eye.

In the histologic sequence, lesions that precede advanced atherosclerosis are classified as types I, II, and III. Lesion types I and II are macrophage foam cell accumulations that do not disorganize the normal structure of the intima, deform the artery, or become clinically overt. In themselves, they are harmless, and the term *lesion* when applied to them should not be taken to indicate mandatory treatment. Type I and II lesions are included in the natural history of atherosclerosis because the sequence of histologic changes indicates that, in symptomatic individuals, the clinical lesions develop from these initially harmless changes. Subjects likely to develop histologically advanced disease from the initial changes develop the still relatively benign type III lesion, which represents the histologic link between type II and atheroma (type IV lesion).

Histologically advanced atherosclerotic lesions are classified as types IV, V, VI, VII, and VIII. By histologic criteria, atherosclerotic lesions are considered advanced when the structure of the intima is disorganized and changes in the outer or inner contour of the arterial segment are present. Type IV lesions, which begin to appear in the second half of the second decade of life, may narrow the arterial lumen only minimally and may not be visible by angiography. However, type IV lesions can become clinically overt by developing a fissure at their surface, a hematoma, or a thrombus. The sequence of changes that produce a type IV lesion and the sequences that may follow once a type IV lesion is present are shown in **Figure 2**.

Subsequent sections of this article describe the characteristic types (or developmental stages) of lesions. However, these are preceded by a description of the structure of the vascular wall and of the fluid mechanical forces at sites where atherosclerosis preferentially develops. Without an appreciation of the special nature of these sites, the development of the disease cannot be understood.

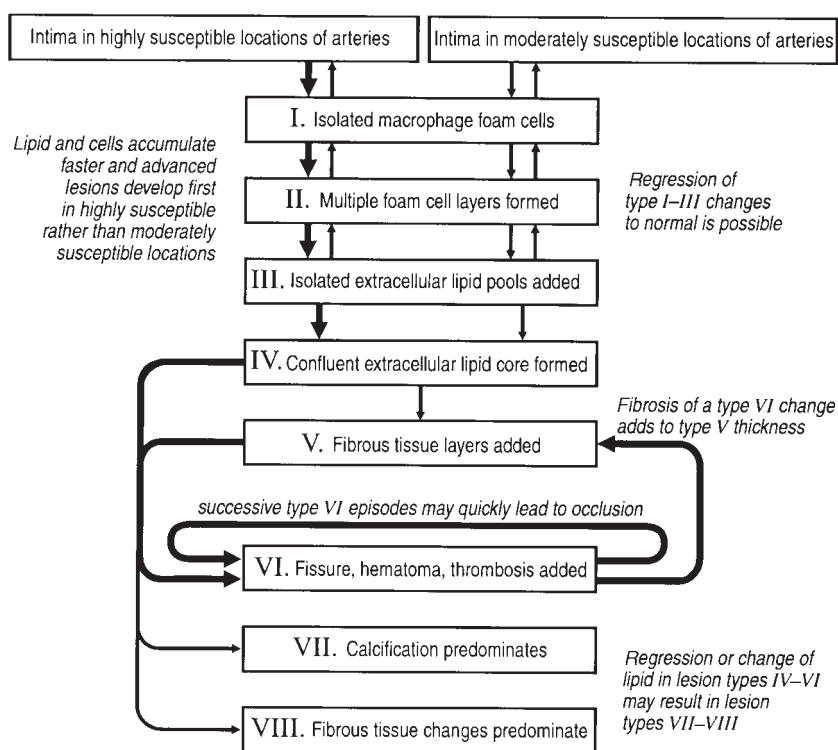
### ADAPTIVE THICKENING AND ARTERIAL SITES SUSCEPTIBLE TO LIPID ACCUMULATION

An artery adjusts to normal asymmetries in fluid mechanical forces along its course and around its circumference through adjustments in the thickness of the intima (and thus the lumen) in individual segments or regions. The aim is to maintain an optimal flow equally at all points along the artery's course. Thus, arteries normally have both thin and thick segments. Increases in thickness occur through the activation of a subgroup of native intimal smooth muscle cells. Increases begin to develop in fetal life (6) and, although variable in degree, are found in everyone at birth (1, 7). A thick intima is inevitably seen at and near bifurcations of arteries and at the mouths of even the smallest branch vessels, where it is focal and eccentric. A thick intima is also found at some sites that are not obviously related to a branch vessel, where it is more diffuse. The thick segments are called *adaptive intimal thickening*, although many other terms have also been used (Table 1).

In cross-sections of a properly distended medium-sized vessel such as a coronary or carotid artery, adaptive thickening appears

Histologic classification and terms used for atherosclerotic lesions		Additional terms used and often based on appearance with the unaided eye	
Type I lesion	Initial change	Fatty dot, fatty streak	Early lesion, minimal lesion
Type II lesion	IIa: Progression-prone IIb: Progression-resistant		
Type III lesion	Preatheroma	Intermediate lesion, transitional lesion	
Type IV lesion	Atheroma	Fibrolipid plaque, fibrous plaque, plaque	Advanced lesion, raised lesion
Type V lesion	Fibroatheroma		
Type VI lesion	Fissured lesion, ulcerated lesion, hemorrhagic lesion, thrombotic lesion	Complicated lesion	
Type VII lesion	Calcific lesion	Calcified plaque	
Type VIII lesion	Fibrotic lesion	Fibrous plaque, plaque	

**FIGURE 1.** Classifications of atherosclerotic lesions used in pathology.



**FIGURE 2.** Pathways of atherosclerosis progression and regression. The order in which distinct lesion types usually develop is designated with the numerals I to VIII. The diagram lists the main histologic characteristics of each sequential step (lesion type). Thick or thin arrows differentiate the relative ease with which lesions develop in specific locations or indicate the relative frequency and importance of a specific section of the pathway. Reproduced from reference 5 with permission.

as a crescent-shaped eccentric increase in the thickness of the outer wall of a bifurcation. The thickest part of the intima may be as much as twice as thick as the media from the time of infancy but, in the coronary arteries, considerable individual variation in degree has been found (1).

From the initial appearance of minimal lipid and macrophage foam cell accumulations in early life, the accumulations are more prominent in a subset of adaptive intimal thickenings. The sites also contain more albumin and fibrinogen. The fluid mechanical forces in these regions give rise to adaptive thickening and also independently enhance the influx of plasma proteins. A distinct fluid mechanical force in such locations is low shear stress (8, 9). In regions of low shear, circulating plasma particles are in longer contact with the endothelial surface. This enhances the frequency with which particles enter the intima. When plasma is too rich in lipoprotein, it accumulates most in these locations. If atheromas are present in later life, they are found here first.

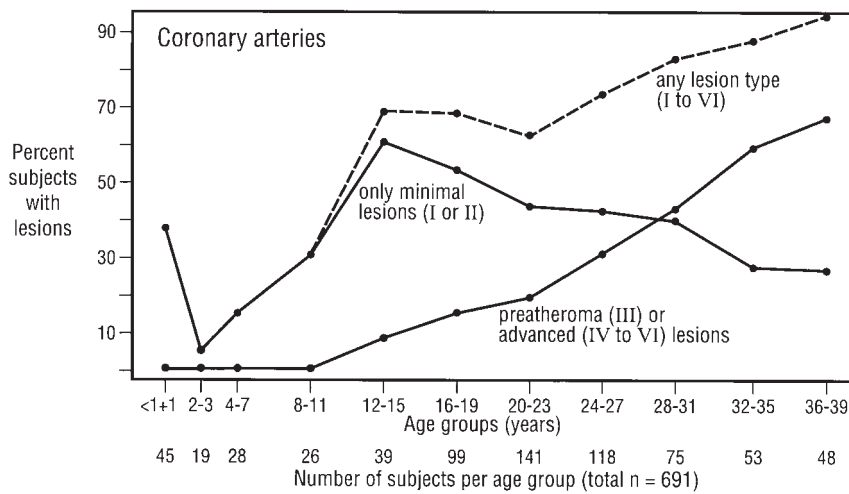
Because the degree and precise nature of the fluid mechanical forces vary for different arteries and different segments and regions of arteries, the rate at which lipid influx (and in hyperlipidemia, lipid accumulation) occurs also varies. Simplified, 3 degrees of susceptibility to lipid influx, accumulation, and lesion formation can be distinguished:

1) Segments of arteries in which lipid does not accumulate except when atherogenic lipoproteins are extremely high (as in persons homozygous for familial hypercholesterolemia). These generally include segments without adaptive thickening, for example, the ventral part of the descending thoracic aorta.

2) Moderately susceptible locations. Such locations often contain macrophage foam cell accumulations (lesion types I and II), but progression to atheromas, if it occurs, is relatively slow and late. The accumulations are generally in or at the periphery of adaptive intimal thickenings, for example, the thickenings at the orifices of the intercostal branches in the dorsal part of the descending thoracic aorta.

3) Highly susceptible locations. These sites contain more foam cells than other locations at the same time. Atheromas and more advanced lesions appear here first and these locations are generally where the greatest degree of adaptive thickening is seen in certain arteries. Examples include the eccentric adaptive thickening at the main bifurcation of the left coronary artery, the bifurcation of the common carotid artery, and the 2 opposing dorsolateral walls of the abdominal aorta near to where it bifurcates to form the common iliac arteries.

The nature and significance of adaptive intimal thickening have been disputed. Some authors view adaptive thickening as atherosclerosis because it is present where atheromas are typically found, because it is mostly circumscribed and eccentric, and because it projects into the lumen of vessels when these are collapsed after death. Others have speculated that adaptive thickening, although not a lesion itself, is a prerequisite for retention and accumulation of lipid and thus for lesion formation. Neither assumption is likely because when atherogenic lipoprotein concentrations are extremely high, macrophage foam cells and lipid do accumulate and advanced lesions do develop also at locations without adaptive thickening.



**FIGURE 3.** The percentages of all subjects with only minimal lesions or with preatheromas or advanced lesions, plotted for successive 2-y or 4-y age groups. The data were obtained by microscopy of 1- $\mu$ m thick sections from the left coronary artery. Note that 69% of the subjects at puberty and 95% of the subjects by the end of the fourth decade had some type of coronary artery lesion. The years after puberty are marked by a rise in lesions of types III–VI, which, as the microscopic data indicate, develop from minimal lesions. Lesion types VII and VIII were not found in the coronary arteries of this relatively young population. Reproduced from reference 5 with permission.

### MACROPHAGE FOAM CELLS IN INFANTS

Evidence of focal lipid accumulation in arterial intima was found in human fetal aortas by Napoli et al (10). By using immunocytochemistry, these investigators showed focal oxidized lipoprotein accumulations and their colocalization, in most instances, with macrophage foam cells. The changes were present more often when the mothers had high plasma cholesterol concentrations. These changes in fetuses appear to be identical to those we found in infants (1, 11). The lesions in infants consist of small, isolated groups of macrophages both with and without lipid droplets. In these initial lesions, the latter are twice the number normally present (3). The initial accumulations of lipoprotein in the intima do not disrupt the structure of the intercellular matrix. In fact, high resolution light and electron microscopy without the help of immunostaining for LDL does not detect the increase in lipoprotein between the intimal cells.

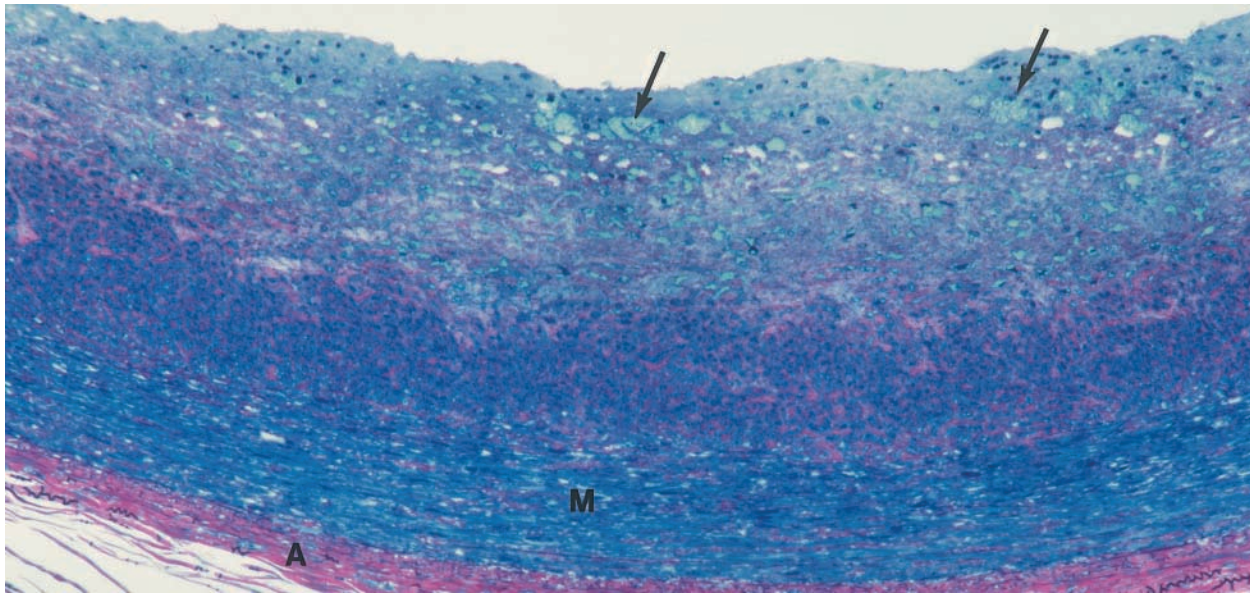
We designated such minimal changes as type I lesions. The segments in which we found type I lesions in the coronary arteries were the highly susceptible locations discussed earlier. Most type I lesions are not visible to the unaided eye. Although such minimal changes are most frequent in infants (in our studies) or in intrauterine life (10), we also found them in older children and adults, particularly in the moderately susceptible locations of arteries (highly susceptible segments may then have more than minimal changes).

In the population we studied, 38% of infants who were <2 full years old had type I lesions in their coronary arteries. The incidence was greatest (50% of infants) in the first 6 mo after birth. The incidence declined in young children after infancy but then increased again in older children (Figure 3). Because our regression studies showed that most macrophage foam cells have a maximum life span of 6 mo (see the section "Regression and prevention of progression"), it is possible that the type I changes we found in the first 6 mo of life had formed in fetal life and may reflect risk factors of the mother. However, when found in children beyond infancy, lipid accumulations should reflect a child's own constitution or nutrition.

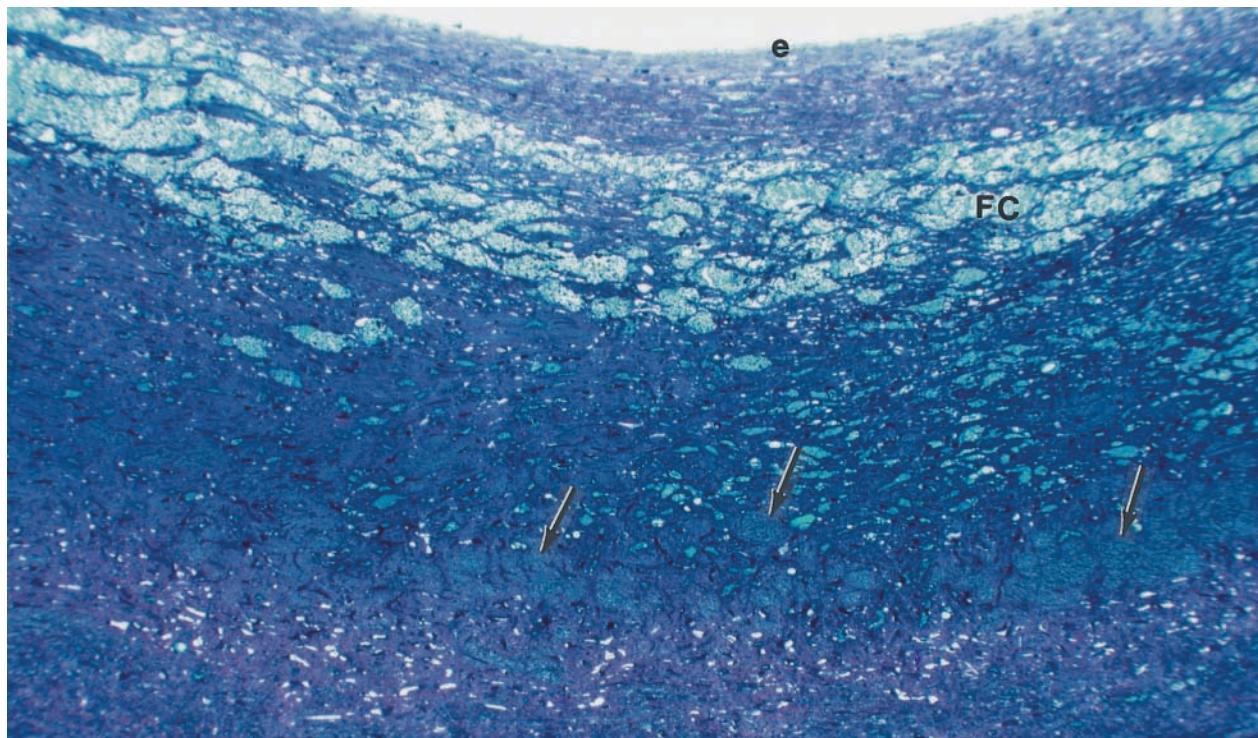
### MACROPHAGE FOAM CELL ACCUMULATIONS TYPICAL AROUND PUBERTY

After a temporary decline after infancy, macrophage foam cells, now more numerous and stratified in layers (type II lesions), are present in most children around the age of puberty at the susceptible sites of arteries (Figure 4). The intimal smooth muscle cells of the locations now also contain lipid droplets. Some extracellular lipid (as defined in the next section) may now be thinly scattered in the spaces between the cells. Because of the slight amount of extracellular lipid, structural smooth muscle cells are not displaced as when large amounts are present subsequently. Type II lesions also contain more macrophages that are not foam cells than do type I lesions or normal intima. Lymphocytes have been identified in such minimal lesions (12, 13), but are less numerous than macrophages. Isolated mast cells may also be present. Within these limits, the composition of type II lesions varies, possibly because of fluctuations in lipoprotein influx. Sometimes the cells that contain the most fat droplets are intimal smooth muscle cells and few macrophages are present. Experimental evidence indicates that the turnover of macrophage foam cells is relatively rapid whereas intimal smooth muscle cells die less readily or quickly. Smooth muscle cells also form and degrade lipid droplets more slowly than do macrophages. The lipid in type II lesions (predominantly intracellular) is cholesterol and its esters, primarily cholesteryl oleate (14–16).

Type II lesions predominate in children. Even in adults type II lesions may be the predominant, and in some the only, change. Of children aged 12–15 y, 69% have mostly type II lesions (a few have only type I and some have lesions advanced beyond type II) in coronary arteries (Figure 3) and all have mostly type II lesions in the aorta (4). The extent to which the surface of the arteries is involved in individual children with lesions varies greatly and may be related to risk factors (17). However, the factors that increase the extent of or give rise to type II lesions above all at puberty have not been identified. An increase in blood lipids is not associated with puberty. Factors other than, or in addition to,



**FIGURE 4.** Type II lesion in adaptive intimal thickening in the anterior descending coronary artery  $\approx 1$  cm beyond the main bifurcation. Adjacent layers of lipid-laden cells (arrows) constitute the lesion. In this location the intima is always thicker than in other locations, giving the illusion of a lesion more advanced than type II. M, media; A, adventitia. (From a 10-y-old male victim of homicide. Fixation by pressure perfusion with glutaraldehyde. Maraglas embedding. 1- $\mu$ m thick section. Toluidine blue and basic fuchsin stain. Case no. P-1880.)



**FIGURE 5.** Type III lesion in the anterior descending coronary artery  $\approx 1.5$  cm beyond the main bifurcation. Extracellular lipid (remnants of dead foam cells including small droplets) (arrows) is pooled in the deep intimal layer, whereas intact macrophage foam cells (FC) occupy the region between the pools and the endothelial cell layer (e) at the lesion surface. (From a 16-y-old girl who died of pulmonary atelectasis. Fixation by pressure perfusion with glutaraldehyde. Maraglas embedding. 1- $\mu$ m thick section. Toluidine blue and basic fuchsin stain. Case no. P-2179.)

relatively high serum cholesterol concentrations apparently come into play. One relevant factor that somewhat increases at puberty is blood pressure (18).

The locations in the arterial tree in which type II lesions develop include the highly susceptible locations in which the symptom-producing lesions of adults are found. We discovered that macrophage foam cell accumulations at the highly susceptible sites contain more foam cells than do other locations but that, because such intimal segments are normally thicker, foam cells accumulate at a greater depth and their lipid may not be visible when only the surface is examined.

#### **LINK BETWEEN MINIMAL AND ADVANCED DISEASE: FORMATION OF EXTRACELLULAR LIPID POOLS**

In addition to lipid-laden cells, lesions (except type I) contain a mixture of vesicular cell remnants and small lipid droplets. The cell remnants and droplets vary in size, are readily visible, are mostly derived from macrophage foam cells that died, and are traditionally referred to as extracellular lipid. Although extracellular lipid is thinly scattered in type II lesions, some lesions from adolescence onward contain extracellular lipid accumulations that are densely packed into the spaces between some of the normally adjacent smooth muscle cells of the deep intima. Lesions containing isolated pools of densely packed extracellular lipid are referred to as type III lesions or preatheromas (Figures 5 and 6). The pools are the direct precursor of the confluent and more extensive accumulation of extracellular lipid known as a lipid core and the hallmark of lesions referred to as type IV lesions or atheromas (Figure 7).

As early as the beginning of the 20th century, some authors considered the minimal type II lesions (referred to then as *fatty streaks*) the precursors of future, potentially symptom-producing atherosclerotic lesions (19, 20). But the manner in which the minimal lesions progressed was not clarified and the assumption that clinical disease developed from them was frequently questioned (21–24).

Several reasons account for such skepticism. According to the traditional view of minimal and advanced lesions, they differ too greatly from each other both histologically and grossly, and a precise topographic correspondence between the 2 types of lesions appeared to be lacking; furthermore, some older individuals had many minimal lesions but no advanced lesions whereas others had many advanced lesions but few that were minimal.

The apparent lack of conformity between locations with minimal accumulations and those with atheromas is, in part, the consequence of relying on the unaided eye to evaluate blood vessels. This means of examination often cannot reveal accumulations (and can never reveal that more foam cells are present) at the highly susceptible locations where atheromas develop.

The evidence that there is in fact a strong correlation between the locations with minimal accumulations containing more foam cells in children and the sites of atheromas of adults comes from extensive studies of 1- $\mu$ m thick sections of lesions (2, 3). As discussed previously, wherever the proteoglycan-rich layer of the intima is thick, foam cells accumulate in the deeper parts and the upper intima may remain foam cell free. In contrast, when the intima is thin, foam cells are, of necessity, confined to the narrow space immediately below the endothelial cell surface. Thus, the lipid of foam cells can be seen through the intimal surface when the intima is thin but not when it is thick, even though thick segments contain more foam cells. Thick intimal segments (with or

without initial foam cells) are not thick enough to be seen as raised plaques, although they may be visible as a gray-white patch.

There are no insurmountable differences between the morphologies of minimal and advanced lesions when the nature of the intima at sites where advanced lesions tend to develop is considered. Highly susceptible locations of the intima always contain layers of smooth muscle cells, which are cells that have been considered a product of advanced disease. The observations that small pools of extracellular lipid at such locations may follow or accompany foam cell accumulations and that pools may enlarge and fuse to form a lipid core provide the necessary link between minimal and advanced lesions. A lack of the correct combination of factors, such as the concentration of plasma lipoproteins, blood pressure, and fluid mechanical forces, may explain why many minimal accumulations reach a point of equilibrium beyond which they do not progress.

#### **ATHEROMA: THE ADVANCED LESION OF YOUNG ADULTS**

By the second half of the second decade of life, the first potentially clinical lesions may be found at highly susceptible arterial locations; these are known as type IV lesions or atheromas. The hallmark of atheromas is a lipid core, a large and well-delineated region of deep intima where the normal structural elements of this part of the arterial wall are replaced by densely packed extracellular lipid. A lipid core develops through an increase in the quantity of extracellular lipid and the confluence of separate pools of this lipid.

Thus, the compositions of extracellular accumulations of type III and IV lesions are identical except that lipid cores almost always also contain cholesterol crystals and calcium particles whereas the lipid pools of type III lesions rarely do. Smooth muscle cells normally resident in the region of the lipid core are decreased and sometimes absent. The packed particles and droplets that replace the normal intercellular matrix at the core presumably hinder the function and existence of the smooth muscle cells of this region. Any remaining smooth muscle cells become widely dispersed and have attenuated and elongated cell bodies and often unusually thick basement membranes. The cell organelles may be calcified and calcium particles of variable size and extent may also be found within the extracellular lipid of a core (see the section "Calcium deposits").

At the developmental stage designated a type IV lesion, the thickness of the tissue layer overlying the core does not substantially exceed the usual thickness of the intima at that location. The layer is composed of a proteoglycan-rich intercellular matrix, smooth muscle cells with and without lipid droplet inclusions, macrophages, and macrophage foam cells. Lymphocytes are also present, and their proportion to macrophages may be much increased compared with their proportions in lesion types II and III. Components such as newly formed fibrous connective tissue layers, surface disruption, hematomas, or thrombosis are not part of type IV lesions. However, once type IV lesions have formed, any of these components may then develop relatively readily.

#### **ADVANCED LESIONS WITH A LARGE PROPORTION OF REPARATIVE FIBROUS CONNECTIVE TISSUE**

The time of development of atheromas can be somewhat predicted from the degree of plasma atherogenic lipoprotein elevations and blood pressure. Progression to lesion types beyond

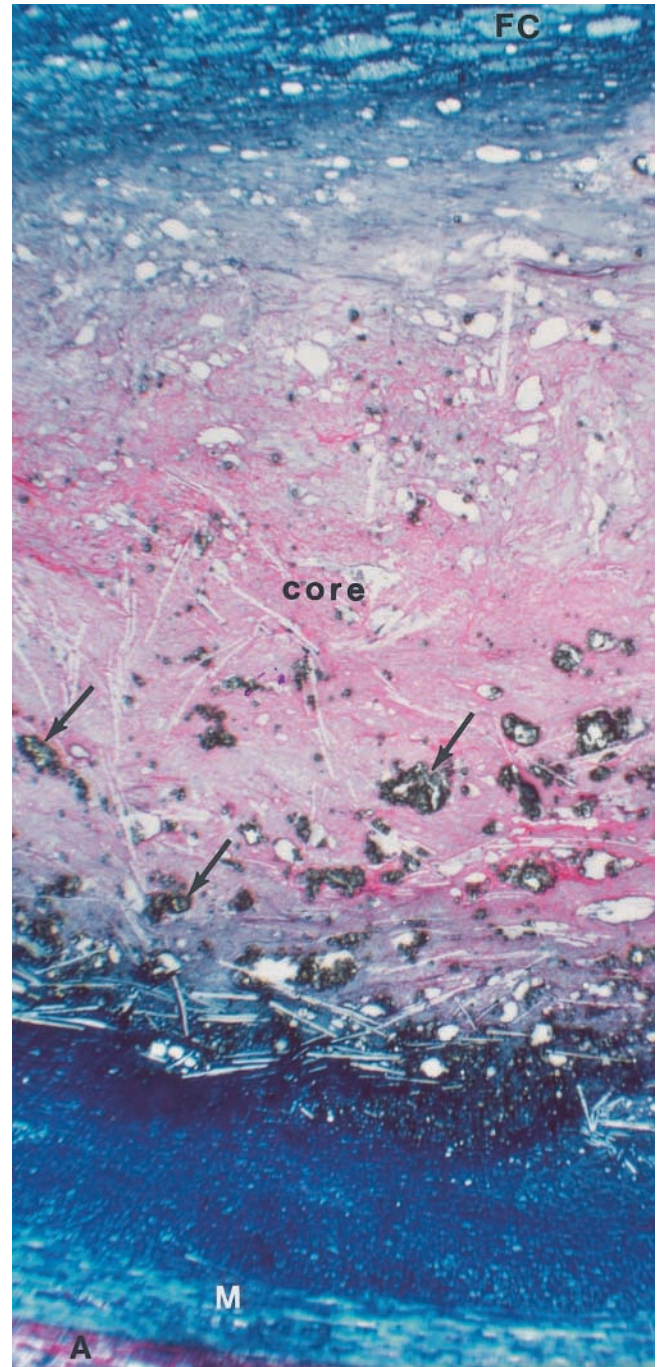




**FIGURE 6.** Detail of the type III lesion shown in Figure 5. Smooth muscle cells that normally are aligned in adjacent, parallel layers in this part of the intima are dispersed where extracellular lipid is packed between them (arrows). (1- $\mu$ m thick section. Toluidine blue and basic fuchsin stain.)

atheromas comes under the influence of additional risk factors that may or may not be present in an individual. Lesions of diverse composition may develop from atheromas at unpredictable rates.

Generally, layers of fibrous connective tissue are added in the years or decades after atheromas form. The resulting morphology is referred to as the type V (fibroatheroma) lesion. Intimal smooth muscle cells, which may themselves be greatly increased in number, produce the new fibrous tissue. Various stimuli account for



**FIGURE 7.** Type IV lesion in the anterior descending coronary artery  $\approx$ 1 cm beyond the main bifurcation. Extracellular lipid, including cholesterol crystals and small aggregates of calcium granules (arrows), make up the lipid core. Besides thickening the wall of the artery, extracellular lipid may weaken the wall by dispersing the structural smooth muscle cells that are always present in this location. M, media; A, adventitia; FC, foam cells. (From a 23-y-old male victim of homicide. Fixation by pressure perfusion with glutaraldehyde. Maraglas embedding. 1- $\mu$ m thick section. Toluidine blue and basic fuchsin stain. Case no. P-1917.)

the different degrees of intercellular fibrous matrix production by the smooth muscle cells. Disruption of vascular wall structure by the accumulated and compacted extracellular lipid appears to induce a relatively sluggish response whereas a marked increase

in smooth muscle cells and fibrous tissue follows thrombosis (as discussed in the next section).

Some older adults have lesions consisting mainly of fibrous connective tissue that is sometimes hyalinized. Lipid cores are absent, other accumulated lipid is minimal, and sometimes there is apparently no lipid at all. Such lesions may be designated type VIII (fibrotic) lesions. Fibrotic lesions may be the result of one or more processes, including organization of thrombus, extension of the fibrous component of an adjacent fibroatheroma, and resorption (regression) of lipid cores.

Type VIII lesions must not be confused with focal and eccentric adaptive thickenings. The smooth muscle cells and fibrous connective tissue matrix of type VIII lesions have the characteristics of reparative reactions, whereas adaptive thickening has a predictably harmonious structure with smooth muscle cells present in normal proportions (7).

### THROMBOTIC DEPOSITS

When lesions (generally types IV and V) include surface disruptions, hematomas, thrombi, or (as is usually the case) all of these together, they are designated type VI. Occasionally, a lipid core is not present, and a thrombus with or without a fissure and hematoma occurs in association with lesion types II, III, or VIII. Small thrombotic deposits, often composed of only fibrin, are superimposed on some lesions as early as the third decade of life. The superimposed complications acutely accelerate the disease beyond the gradual rate of growth usually seen with lipid accumulation alone. Histologic evidence indicates that complicating episodes follow each other at variable intervals, often interspersed by months or years without additional episodes. Sometimes, recurrent superimpositions of layers of thrombi follow in quick succession and a fatal occlusion builds up within the arterial lumen within hours, days, or weeks. When episodes are not fatal, fissures are sealed and hematomas and thrombotic deposits are colonized by increasing numbers of intimal smooth muscle cells, which will produce collagen. The changes result in a lesion with type V morphology, larger and with greater obstruction of the arterial lumen than before the complicating episode.

When fissures of the lesion surface are only microscopic in size, the associated hematomas and surface thrombi are also small. Small thrombotic deposits, often composed only of fibrin, are also found in the absence of detectable disruption of the underlying lesion surface. Presumably, a succession of such deposits leads to incremental fibrous thickening of the underlying lesion, but at a rate that (although faster than lipid accumulation alone) is so gradual as to take decades before blood flow is severely obstructed.

Fibrin or fibrinogen accumulations forming a morphologic pattern suggesting the remnants of thrombi may be identified by immunohistochemistry on or within some lesions. We found this presumed evidence of earlier thrombosis in young and middle-aged adults in association mainly with some lesions of types IV to VI (5).

It has been proposed that platelet or fibrin deposits on the intima could be the initial event in atherogenesis (25, 26). We did not find platelet deposits or fibrin accumulations suggesting thrombosis on intima we considered normal. However, diffuse fibrinogen that does not suggest thrombi or their remnants can often be identified in adaptive intimal thickening and in lesions. The effect, if any, of this fibrinogen on the arterial wall or on lesion progression is not known.

### CALCIUM DEPOSITS

The first and smallest calcium deposits visible by electron microscopy and in the 1- $\mu\text{m}$  thick sections we examined were found in the (lipid) cores of histologically advanced lesions (mostly type IV) in young persons. The initial deposits consisted of calcium granules within some smooth muscle cells and outside cells. Extracellularly, granules were scattered among the small lipid droplets and vesicular remnants of the dead cells that form the lipid core of a lesion. Granules in the cytoplasm of smooth muscle cells measured from  $\approx 5$  to  $\approx 10$   $\mu\text{m}$ . Smooth muscle cells containing the granules were mainly cells trapped in the lipid cores of lesions—cells that had become dispersed, isolated, and encased among the vast masses of accumulated extracellular lipid. Smooth muscle cells existing in a so severely altered environment can be presumed not to function normally and often to be agonal.

We did not recognize the precise nature of the (altered) calcified organelles because of the particles or crystals that were superimposed on the organelles. Mitochondria have often been suspected in studies of other conditions but it is likely that other organelles also calcify.

Intracellular calcium granules become extracellular when the cells die and break up. However, extracellular granules, which are scattered diffusely among the other extracellular components of the lipid cores of lesions, are not only cell components that have calcified intracellularly. Calcification of cell components also occurs only after their release into the extracellular environment and these granules in fact constitute the bulk of those present. In most lipid cores, extracellular granules outnumber intracellular calcium granules from the very beginning of granule formation. The size and density of extracellular granules is variable. They often exceed the size of intracellular granules and frequently form aggregates of many granules. The overall picture is that of a relatively fine salt-and-pepper-like distribution either throughout the lipid core or sometimes only, or more heavily, in the deeper parts of a lipid core (Figure 7).

The precise identity of the extracellular particles that became the *nidi* for calcium precipitation are as difficult to determine with certainty as the *nidi* in intracellular calcification. The most abundant extracellular particles in atherosclerotic lesions are vesicle-like structures that are sometimes outlined with calcium deposits. Vesicle-like structures have been seen by many authors in atherosclerotic lesions and other conditions and have been designated with various terms, most often as matrix vesicles (27). Our own observations agree with those of Ghadially (28), who concluded that the so-called matrix vesicles are part and parcel of matrical lipidic cell debris, which can take several forms (vesicles, vacuoles, granules, membranes, and larger obvious cell fragments), and that all or most of these structures can calcify when conditions are appropriate.

Altogether, the mineral might account for up to 10% of the lipid core of a type IV lesion in a person 20–29 y old. Although visible by high-resolution light microscopy, this degree and pattern of mineralization may remain undetected with available clinical imaging techniques. When the resolution of clinical imaging becomes high enough to detect calcium particles of microscopic size dispersed over a region  $< 5$  mm in diameter, then it will be possible to identify atheromas whether they narrow the arterial lumen or not. Mahoney et al (29) examined the coronary arteries of 384 subjects aged 29–37 y by electron beam computed tomography and detected calcification in 21%. In almost the same age group (30–39 y; Figure 4), we found that





63% of the subjects had coronary artery lesions that mostly contained a lipid core, and most of these contained at least microscopically detectable calcium particles.

The present observation that calcium deposition begins with the formation of a core of extracellular lipid is in agreement with the statement by Wexler et al (30) that the presence of calcium implies the association of lipid-rich (and presumably vulnerable) plaque. However, the amount of calcium varies greatly in the lesions of individuals of identical age even when the lesions and their lipid cores look similar otherwise. A systemic, individually variable predisposition to mineralization must therefore be present in addition to the local factors.

None of the calcium-granule-containing advanced lesions of young persons that we studied showed histologic evidence of osseous metaplasia. Nor were elastic fibers, either intact or altered, a nidus for the deposition of calcium granules. Lesions designated as types I and II did not have calcium deposits; type III lesions only rarely had calcium deposits and when they did only a few fine granules were visible.

With increasing age of subjects (or age, extent, and density of the extracellular lipid accumulation), the initial particles of calcium increase in size and then fuse into large aggregates. From middle age, large clumps of mineral may dominate the core of a lesion by replacing the extracellular lipid at the base or throughout the lesion core. Osseous metaplasia may appear at this time. When  $\geq 50\%$  of the cross-sectional area of a lesion consists of mineral, it can be called a type VII (calcified) lesion.

#### REGRESSION AND PREVENTION OF PROGRESSION


The present data confirm the assumption that clinically overt atherosclerotic lesions develop through a sequence of morphologic changes beginning as minimal type I and type II lesions. It must be remembered, however, that lesion types I and II may reach an equilibrium and stabilize at that morphology without progressing further in many individuals at some arterial locations and in some individuals at all sites. Furthermore, when circumstances (risk factors) change favorably, lesion types I and II, and probably type III also, can regress and disappear.

Histologic evidence of the ability of lesion types I and II to form, dissolve, and reform was obtained in children. Fewer children have lesion types I and II in the period between infancy and puberty than either in infancy or puberty (2, 5). A reduction in the number of lesions now histologically classified as types I–III was also noted by Aschoff (19) and other pathologists in Germany and Austria with the increasing duration of World War I and was attributed to wartime food shortages.

In monkeys, experimental lesions comparable to human lesion types I–III can disappear from arteries within 6 mo of blood cholesterol being reduced to very low concentrations through changes in diet (5, 31). The mechanism whereby these lesion types disappear consists of the gradual death of existing macrophage foam cells and cessation in the formation of new ones (5). Small amounts of extracellular lipid can also be removed from arteries within this time frame.

At susceptible arterial sites, influx of atherogenic lipoprotein into the intima may be so excessive as to induce formation of very large numbers of macrophage foam cells and, over time, of a core of extracellular lipid, the hallmark of lesion types IV and V. The studies in monkeys indicate that it takes far longer than a 6-mo period of very low blood cholesterol to substantially

reduce or remove cores of extracellular lipid. Besides, fibrocalcific remnants remain in locations formerly occupied by lipid cores. Preferable to the regression of lipid cores is prevention of their development because it will prevent the possibility of overt clinical disease and because prevention surely requires less drastic measures than regression.

In Figure 3, the ascending line for lesion types III–VI reflects mainly cases with lesions with lipid cores. Such lesions emerge around puberty and by the age of 27 y they are present in about one-quarter of the population. Therefore, measures that reduce risk factors responsible for the influx of excessive atherogenic lipoprotein into the arterial wall and thus the formation of lipid cores should be in place by the time of puberty. 

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