Human Aortic Fibrolipid Lesions

Progenitor Lesions for Fibrous Plaques, Exhibiting Early Formation of the Cholesterol-Rich Core

THOMAS M. A. BOCAN, PhD, and JOHN R. GUYTON, MD

From the Departments of Medicine and Cell Biology, Baylor College of Medicine, Houston, Texas

The early development of the lipid-rich core and other features of artherosclerotic fibrous plaques has been elucidated by examining discrete, small regions of raised intima in human aorta, which often bear a resemblance to both fatty streaks and fibrous plaques. Approximately one-fourth of small raised lesions (<16 sq mm of surface area) contained little or no stainable lipid, while three-fourths had a characteristic appearance, which included a superficial layer of foam cells, a core of noncrystalline and/or crystalline lipid, and a developed or developing collagenous cap. Total intimal volumes of the lipid-containing lesions, termed "fibrolipid lesions," ranged from 3 to 43 μl, with the majority less than 16 μl. Core lipid in the smallest lesions was located in the musculoelastic layer of the intima. In larger lesions the core extended luminally into the elastic hyperplastic layer, and cholesterol crystals were found more frequently. Total cholesterol concentration in fibrolipid lesions was similar to that in fatty streaks; however, the ratio of unesterified to total cholesterol was relatively high, similar to that found in fibrous plaques. It is concluded that 1) the formation of a lipid-rich core and cholesterol crystallization are early events in the development of many raised lesions; 2) the consistent association between the superficial layer of foam cells and the deep-lying lipid-rich core raises the possibility of an influence, possibly indirect, of foam-cell lipid metabolism on core formation; and 3) the fibrolipid lesion may represent one stage in a potential transitional morphologic sequence between fatty streak and fibrous plaque. (Am J Pathol 1985, 120:193–206)

IN THE DEVELOPMENT of clinically significant atherosclerotic lesions, two processes of major importance are encountered. One is fibrocellular proliferation, which adds to intimal bulk and eventually leads to chronic ischemic syndromes via gradual constriction of the arterial lumen. The other major process is the combination of necrosis and lipid deposition in the core of raised lesions. Enlargement of this core tends to erode viable tissue toward the luminal surface,[1] eventuating in plaque rupture, exposure of circulating blood to highly thrombogenic material, and sudden ischemic episodes such as myocardial infarction.[2,3] In contrast to current understanding of fibrocellular proliferation, very little is known about the development of the lipid-rich core region. In particular, no previous morphologic study has focused on the early stages of core region development. The present study addresses this issue by examining lipid accumulation in small distinct raised lesions in human aorta. Preliminary investigation indicated that a very high percentage of these lesions contain a lipid-rich core region.

Atherosclerotic lesions are defined on the basis of their appearance upon gross examination. Fatty streaks are flat, minute, round or oval yellowish patches which may become arranged in rows of different lengths. Fatty streaks are often most evident along the dorsal surface of the descending thoracic aorta. On light-microscopic examination, the fatty streak contains a superficial layer of lipid-filled foam cells and sometimes a layer of diffuse sudanophilic material along the internal elastic lamina. Fibrous plaques are defined as elevated lesions and often have an opaque, "pearly white" appearance. In the aorta these lesions tend to involve the ostia of the intercostal and lumbar arteries.[4] Microscopically, fibrous plaques contain a fibrocellular cap which commonly covers a core region composed of cholesterol crystals

Supported by Grants HL-29680, HL-07282, and HL-07090 from the National Institutes of Health.

Accepted for publication March 21, 1985.

Address reprint requests to John R. Guyton, MD, Dept. of Medicine, A-601, The Methodist Hospital, 6565 Fannin Street, Houston, TX 77030.

as well as other forms of lipid. The thickness of the cap was found to be inversely related to the size of the lipid-rich core.[1] Controversy still exists as to whether fatty streaks, or a subset of fatty streaks, develop into fibrous plaques. A closer examination of the smallest discernible raised lesions, with particular attention to those containing lipid, should increase our knowledge of potential transitional morphologies. Such information will aid in understanding the overall development of the fibrous plaque.

Several studies have focused on the lipid composition of lesions believed to be intermediate between fatty streaks and fibrous plaques. Katz et al,[5] on examining the lipid composition of fatty streaks, found a subset of lesions with a high content of unesterified cholesterol. Cholesterol crystals were found in approximately one-third of these lesions when examined by polarizing light microscopy. Other than polarizing light microscopic examination of tissue minces, no histologic, locational, or dimensional analysis was performed on these lesions. Smith and co-workers[6] also examined a few small, raised fatty nodules and plaques to find that they contained a marked increase in the proportion of amorphous lipid, free cholesterol, and cholesteryl linoleate. While these studies[5-7] have suggested the presence of lesions intermediate between fatty streaks and fibrous plaques, very little is known about the size of the lesions, their morphologic appearance, the lipid-rich core, and the particular location of the lesions within the aorta.

Thus, the present study aims to examine discrete, small, lipid-rich regions of raised intima for their size (volume), morphologic appearance, and lipid composition. The human aortic lesion that we have studied possesses characteristics of both fatty streak and fibrous plaque. We have called this particular lesion a fibrolipid lesion because it contains dense lipid infiltrates and a developed or developing fibrous covering. Small nodules without a lipid-rich core were also noted but were much less frequent and were not consistently found around the ostia of the intercostal or lumbar arteries. By focusing on small, raised fibrolipid lesions, we may elucidate the early stages in the formation of the lipid-rich core found in fibrous plaques and obtain morphologic evidence consistent with a fatty streak origin for many fibrous plaques.

Materials and Methods

Selection of Fibrolipid Lesions

One hundred forty-seven human aortas (132 from men, 15 from women) obtained at autopsy were examined for the presence of small, raised lesions using oblique lighting and stereomicroscopy to establish luminal surface elevation. The aortas were from individuals aged 18–71 years, 85% of the individuals aged 45 or younger. Trauma was the major cause of death in about 90% of the cases. The descending aorta, from thorax to abdomen, was examined closely for the presence of any raised patches of intima. Two types of raised lesions, based upon gross examination, were found. One lesion resembled a fibrous plaque, in that it contained a "pearly white" fibrous cap. The second, smaller lesion, though also raised, had an appearance similar to a fatty streak in that the lesion appeared as a round yellowish patch. It was decided to examine the smallest lesions detectable with reasonable frequency. Lesions covering no more than 16 sq mm were dissected from the aorta and serially sliced transversely at 1-mm intervals with the use of a McIlwain Mechanical Tissue Chopper (Mickle Laboratory, Brinkmann Instruments, Westbury, NY). Tissue slices were examined under ultraviolet light (365 nm) with a WILD M5 stereomicroscope (WILD Heerbrugg, Switzerland). Lipid deposits showed yellow-orange autofluorescence, while collagen and elastin were perceived as a dull blue-gray. The internal elastic lamina was consistently visualized as a relatively bright, wavy, blue-gray line dividing the very autofluorescent intima from the dim autofluorescence of the media. This was a useful point in the sampling strategy, because the presence of lipid could be established before further analyses were performed.

Determination of Lesion Volume

Once a lesion was found, the outline of the slices making up the lesion were traced using a camera lucida attachment. The lateral boundaries of raised lesions were distinct and easily recognized. The traced images were digitized on an electronic digitizing board (Houston Instruments, Austin, Texas) interfaced with an Apple II Plus microcomputer. The volume of the entire lesion was estimated by summing intimal areas and multiplying by 1 mm the slice thickness. The volume of the lesion above the level of surrounding normal intima (volume of lesion thickening) was also determined. The 1-mm slices were also used in determining the intimal thickness. The distance from lumen to internal elastic lamina was measured on two adjacent slices from the center of the lesion. Intimal thickness measurements were taken from at least three equidistant points across the lesion slice, and the mean was calculated. Similar thickness values were obtained from measurements made on frozen sections.

Cytochemical Methods

After the volume measurements were made, alternating contiguous aortic slices were flash-frozen in n-hexane chilled by a CO_2–ethanol slurry and stored at

−30 C until sectioned. The flash-frozen samples of the small fibrolipid lesion were sectioned at 10 μ with the use of an AO cryostat (American Optical, Buffalo, NY). The nature of the anisotropic structures was distinguished by examining sections under polarized light and determining the melting point of anisotropic structures with the use of a Bailey TS-2ER heating stage element (Bailey Instruments Inc., Saddle Brook, NJ). This method[5] entailed heating the sections (20 degrees per minute) to 43–100 C. Because arterial cholesteryl esters melt to an isotropic state at or before 43 C, any remaining bright birefringence is probably due to either phospholipid-rich lamellar crystals or free cholesterol monohydrate crystals.[5] Vibratome-prepared sections, 100 μ thick, of fresh unfrozen aortic tissue were also examined under polarized light for assessment of the possible effect of freezing on the birefringent crystal formation. Contiguous lesion slices were fixed with Karnovsky's fixative, dehydrated in a graded series of ethanol, cleared in propylene oxide, embedded in Epon 812, sectioned at 2 μ, and stained with toluidine blue-basic fuchsin for verification of the presence of crystalline clefts, suggesting free cholesterol within the lesion. Fresh-frozen aortic sections were also stained with oil red O in 60% isopropyl alcohol to demonstrate the distribution of lipid.[8] Control sections, pretreated in chloroform prior to oil red O staining, showed very little staining. Tissue sections were also stained with hematoxylin and eosin (H&E), with toluidine blue, and with van Gieson's stain.[8] The von Kossa technique was used for demonstration of calcium.[9] The α-naphthyl acetate method for nonspecific acid esterase was used to stain selectively for cells with a high content of lysosomal enzymes.[10]

In order to describe the location of lesion features with respect to depth in the aortic intima, we identified intimal layers according to their description by Geer and Haust.[11] The layers and their origins are as follows: 1) Soon after birth a portion of the internal elastic lamina "splits" on its luminal aspect, forming what is referred to as the inner limiting membrane. Smooth-muscle cells migrating through the internal elastic lamina intermingle with the elastic fibers, forming the first layer of the intima, namely, the musculoelastic layer. 2) Coarse elastic fibers which have separated from the inner limiting membrane on its luminal aspect, along with smooth-muscle and occasional macrophagelike cells, form the second layer, namely, the elastic hyperplastic layer. The inner limiting membrane forms a dividing line between the two layers. 3) In the middle decades of life a third connective tissue layer develops beneath the endothelium, assuming a somewhat circumferential, segmental, or even focal distribution.

Chemical Methods

Slices from fibrolipid lesions, normal intima, fatty streaks, and small fibrous plaques were assayed for their total and free cholesterol and phospholipid content. The media was stripped away from the intima at the internal elastic lamina. This was confirmed on frozen sections. The surrounding normal intima was cut away from the fibrolipid lesion. The slices were minced, and the lipids were extracted from the minced slices in chloroform/methanol (2:1) by the procedure of Folch et al.[12] Two successive extractions were performed, and 95–98% of the measured lipid appeared in the first extraction. Lipid extracts were evaporated to dryness and redissolved in 1 ml of isopropyl alcohol. Total and free cholesterol were assayed in duplicate with the use of a modification of the enzymatic, colorimetric method of Cooper and co-workers.[13] This method entailed incubating the lipid extract in a reaction medium containing cholesterol esterase, cholesterol oxidase (Boehringer Mannheim, Indianapolis, Indiana, 393924 and 393916) horseradish peroxidase (Sigma, St. Louis, Mo, P-8250), 4-amino-antipyrene (Sigma) and dichlorophenol (Research Organics, Inc., Cleveland, Ohio). The colored reaction product, a quinoneimine, was stable and was linearly related to the total cholesterol level over a range of 0.78–50 μg ($r = 0.996$). Standards of cholesterol dissolved in isopropyl alcohol (Sigma, St. Louis,

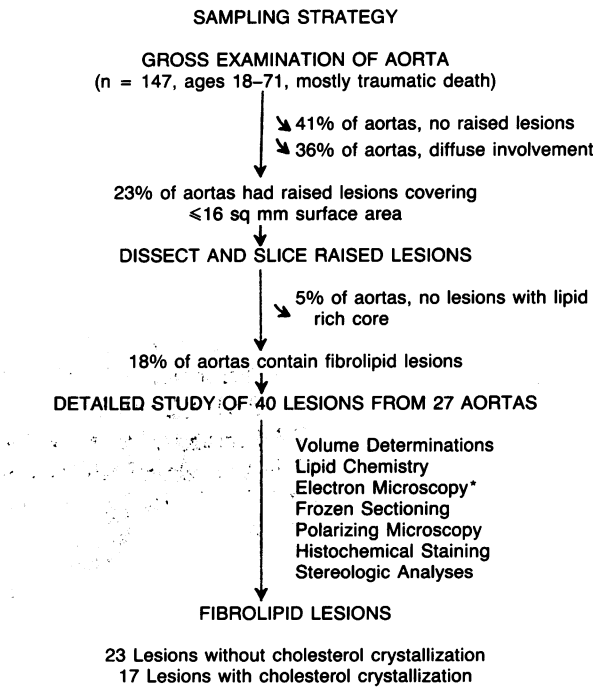

Figure 1—Sampling strategy for the selection of fibrolipid lesions. The electron microscopic analyses will be reported elsewhere.

Mo) were run in triplicate with an intraassay coefficient of variation of 4.8%. Free cholesterol concentration was measured with the same reaction medium without the addition of the cholesterol esterase. Total phospholipids were measured in triplicate by the method of Baginski et al.[14] In this method nitric acid and trace amounts of calcium were used to digest the organic compounds. After the nitric acid was removed by heating, the extracts were incubated with 1% ammonium molybdate tetrahydrate. After 15 minutes the colored reaction product was stable and linear in the range of 0.5–5 μg phosphate ($r = 0.973$). Standards of KH_2PO_4 were run in triplicate with an intraassay coefficient of variation of 3.9%. Total phospholipids were determined by multiplying the phosphate concentration by 25. Lipid concentrations for each sample were corrected for the recoveries (mean 70%) of ^{3}H-cholesterol, which was added to the samples at the beginning of the first extraction step. Tissue dry defatted weight was determined after lipid extraction and drying to constant weight at 70 C. Lipid concentrations and free/total cholesterol ratios were compared among lesion types with the use of analysis of variance and application of the Bonferroni inequality for multiple comparisons.[15]

Results

Distribution and Gross Appearance of the Small Fibrolipid Lesions

The sampling strategy adopted for finding early fibrolipid lesions is illustrated in Figure 1. The aortas were first examined for determination of the extent of atherosclerotic involvement. Forty-one percent of the aortas received had no atherosclerotic involvement or only fatty streaks. An additional 36% had either extensive involvement with large fibrous plaques and complicated lesions, or a few large fibrous plaques. In these aortas discrete small fibrolipid lesions, circumscribed by normal intima, could not be found. The remaining 23% of the aortas showed discrete, small, raised areas of intima. However, 5% of the aortas contained only fibrous lesions without a lipid-rich core. In these lesions the yellow-orange autofluorescence associated with the lipid-rich core was absent. Histologic examination of selected tissue sections also revealed an absence of lipid deposition. The remaining 18% of the aortas were found to contain appropriately sized lesions with a lipid-rich core. A total of 40 lesions was found; the volumes of 31 were measured. Half of these aortas contained more than one lesion.

The lesions were found predominantly distal and lateral to the ostia of the intercostal and lumbar arteries (Figure 2). These lesions were found in persons ranging in age from 27 to 71. In younger individuals (aged 27–40) the lesions were found primarily around the lumbar ostia, with a few exceptions (Figure 3). In older individuals the lesions were found associated with the T4–T12 intercostal ostia. The correlation between lesion location (vertebral level) and age was significant at $P < 0.02$.

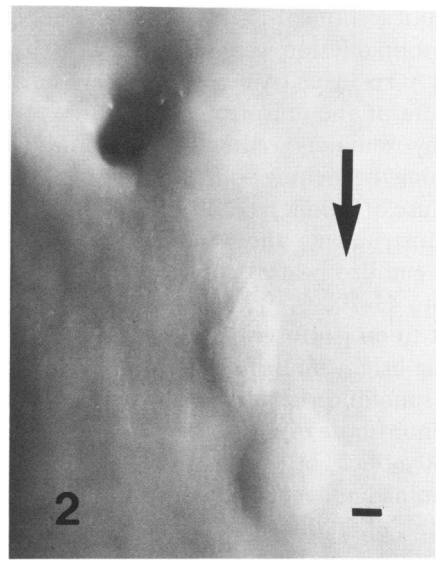

Figure 2—Gross appearance of the fibrolipid lesion. The two lesions are 3–4 mm in length and 3 mm distal to the ostia of the intercostal artery. The *arrow* indicates the direction of blood flow. (×4; bar = 1 mm)

The gross surface appearance of the fibrolipid lesions varied according to lesion size. The smaller lesions, viewed directly from above, showed either a cluster of small yellow dots or a homogeneous yellowish cover. A distinct yellowish rim was present around the perimeter of the whole lesion. Fingerlike projections of a white fibrous material, oriented primarily longitudinally, sometimes appeared to overlie the lipid-rich core. The larger fibrolipid lesions, which approached 16 sq mm in area, were different only with respect to the extent of fibrous material. A few lesions contained a rim of fibrous material surrounding a yellowish center. Several of the larger lesions had a complete "pearly white" cap over a core of lipid, which was only perceptible upon slicing.

Lesion Volume

The lesions examined were divided into two groups according to intimal volume measurements. Larger volumes correlated well with the presence of a "pearly white" cap on gross inspection, while smaller lesions

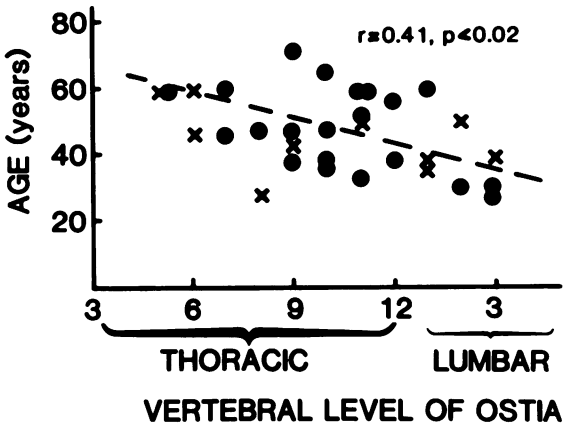

Figure 3—Plot of age versus fibrolipid lesion location (vertebral level). The two parameters have a correlation coefficient of 0.41, which is significant at $P < 0.02$. Not shown by this plot is the observation that with age fibrous plaques are found about the lumbar ostia, whereas the fibrolipid lesions are present in the thoracic aorta. Lesions with cholesterol crystals are represented by X, whereas O represents lesions without crystals.

tended to have a yellowish, fatty cap. The two groups were further subdivided into those lesions with and without cholesterol monohydrate crystals (determined microscopically). Lesions without a well-developed "pearly white" fibrous cap ranged in volume from 2.7 to 16.0 μl (Table 1). Such volumes correspond to a mean intimal thickness of 742 ± 126 μ for lesions without cholesterol crystals and 648 ± 123 μ for lesions with cholesterol crystals. Lesions with a fibrous cap ranged in volume from 21.6 to 42.8 μl. Due primarily to the presence of the cap, the mean intimal thickness of these larger lesions was 1278 ± 179 μ.

Because the mean thickness of normal intima surrounding the lesions of both categories was approximately the same (344 ± 114 μ), a second parameter of lesion size was measured, namely, the volume of the fibrolipid lesion above the level of normal intima (volume of thickening, Table 1). In the lesions examined with a total volume less than 16 μl, approximately one-third of that volume was due to the region above the level of the surrounding normal intima. In the larger lesion category—ie, lesions with fibrous caps—the volume of thickening comprised one-half the total volume.

Histologic Examination of the Small Fibrolipid Lesions

Despite the differences in sizes of the fibrolipid lesions, several consistent histologic features were observed. All the fibrolipid lesions contained superficial foam cells, generally in clusters, overlying a deep lipid-rich core. In the smaller lesions (approximately 3–16 μl), the foam cells generally formed a continuous layer over the entire lesion (Figure 4). A clear boundary, based on luminal contour and extent of the foam-cell infiltrate, was present between the fibrolipid lesion and the surrounding normal intima. In the lesions greater than 16 μl, the superficial layer of foam cells tended to be fragmented and discontinuous (Figure 5). The foam cells tended to occur in the shoulders of the lesions; these cells were not seen to extend beyond the edges of the raised lesion into the surrounding normal intima. In lesions of both sizes, monocytelike cells identified by the presence of their indented round nuclei and highly eosinophilic perinuclear cytoplasm were frequently found scattered among the foam cells (Figure 4). The superficial foam cells and monocytelike cells stained positively for nonspecific acid esterase activity. Esterase-positive cells were not found in the intimal musculoelastic layer, beneath the inner limiting membrane. Cells in the musculoelastic layer appeared to be predominantly smooth-muscle cells, based on spindle-shaped nuclei, scant perinuclear cytoplasm, and esterase negativity. In a few esterase-negative cells in the musculoelastic layer, considerable quantities of lipid appeared in the form of large intracellular droplets.

In addition to the superficial layer of foam cells a prominent lipid-rich core was present in every lesion. Thus, the ability to discern lipid by viewing slices under ultraviolet light was confirmed. The core region sometimes contained cholesterol crystals, but more often did not. The lipid-rich core occurred consistently in the musculoelastic layer of the intima and sometimes

Table 1—Total Volume, Volume of Thickening, and Intimal Thickness of the Fibrolipid Lesions With and Without Cholesterol Crystallization

Crystals present	Number of lesions	Total volume (μl)	Volume of thickening* (μl)	Maximum lesion thickness (μ)	Maximum lesion thickening† (μ)
≤16 μl					
–	18	9.6 ± 4.5‡	3.3 ± 1.6	742 ± 126	391 ± 92
+	4	9.1 ± 6.5	3.5 ± 2.3	648 ± 123	437 ± 127
>16 μl					
–	3	27.3 ± 6.4	13.6 ± 3.5	1068 ± 212	624 ± 221
+	6	29.9 ± 7.7	13.2 ± 5.6	1126 ± 169	745 ± 176

* Volume of the fibrolipid lesions above the level of normal intima.
† Thickness of the fibrolipid lesion above the level of normal intima.
‡ Mean ± SD.

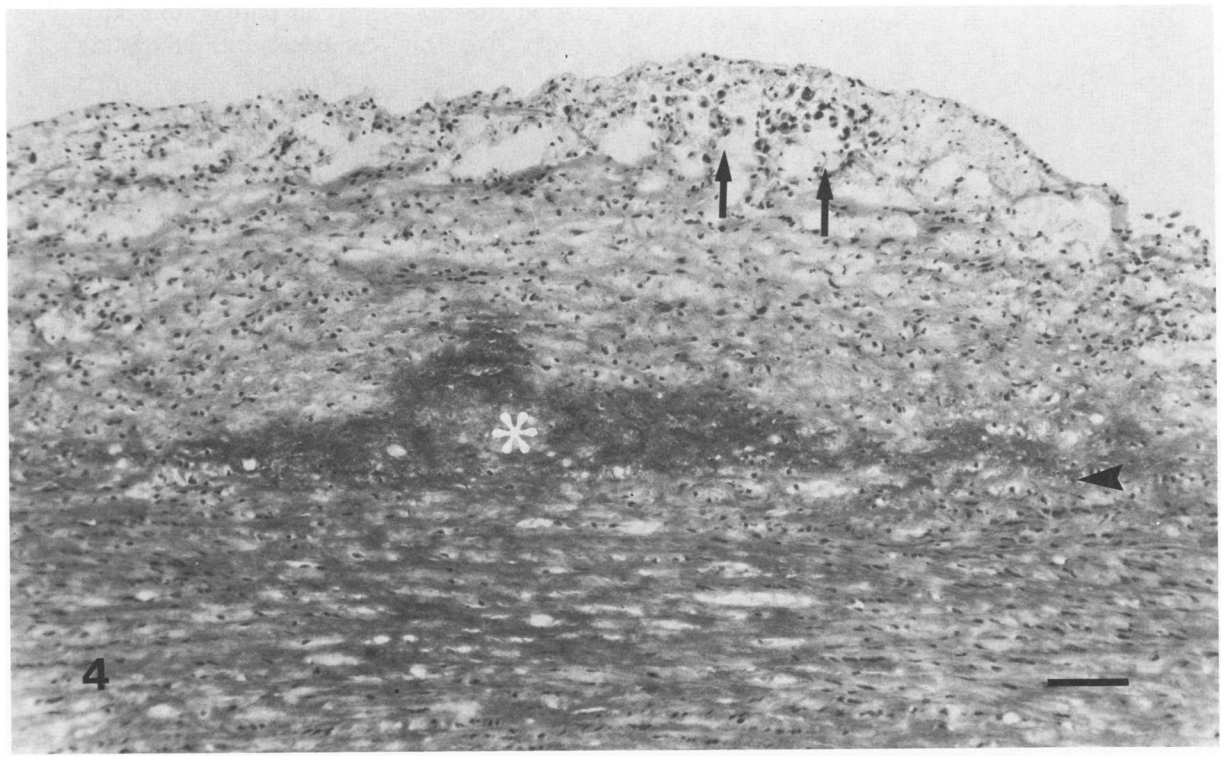

Figure 4—Microscopic appearance of a small fibrolipid lesion. The lesion was found in a 47-year-old white woman lateral to the tenth intercostal artery. The total lesion volume equaled 3.1 μl, and the mean intimal thickness was 603 μ. A definite core region, with intense basophilia which appears dark in this micrograph, is present (*asterisk*) just above the internal elastic lamina (*arrowhead*). Foam cells, which appear as clear spaces, are superficial in location. Scattered among the foam cells at the luminal aspect are monocytelike cells (*arrows*). Fresh-frozen 10-μ section. (H&E, × 100; bar = 100 μ)

extended to more superficial intimal layers. In a few lesions (n = 4), the lipid-rich core was found above the inner limiting membrane with no core in the musculoelastic layer. The lipid in the core appeared to be predominantly extracellular on the basis of oil-red-O–stained sections counterstained with hematoxylin. The lipid stain in this region appeared mostly in fine grains and less frequently in small droplets (2–13 μ), which were not specifically associated with cell nuclei (Figure 6). This appearance is in contrast to the foam cells, which were seen as large masses of stain, encompassing nuclei. When the tissue sections were stained with H&E, the core was a well-demarcated region containing small basophilic granules (see Figures 4 and 5). There was a close correspondence among the area of basophilic granules, the negative metachromatic area (green) seen upon toluidine blue staining, and the lipid-rich core visualized by oil red O staining and polarized light microscopy. Formalin fixation and/or chloroform pretreatment of tissue sections resulted in an almost complete removal of the core basophilia. Most of the core regions (85%) were hypocellular, relative to the surrounding normal intima. A small percentage (4%) of the lesions had lipid-rich cores which were acellular, while another group of lesions (11%) had core regions with normal cellularity relative to the surrounding intima. The area around the core region appeared hypercellular relative to the surrounding normal tissue. In 10% of the lesions, foam cells and monocytelike cells from the shoulders of the lesion were observed in close association with the lateral aspects of the presumed developing core.

In 43% of the lesions, generally in the larger lesions, the region of basophilia was observed to extend above the inner limiting membrane in the center of the lesion (Figure 5). In a few cases this extended region of basophilia appeared to be a distinct secondary focus. In histologic appearance, this region of basophilia was somewhat different from the core found in the musculoelastic layer. Bundles of collagen were surrounded by fine basophilic grains. Scattered throughout the collagen bundles were foam cells and smooth-muscle cells. Small (2–8 μ) eosinophilic spheroids were found in this second core region (Figure 7). The larger lesions appeared to have a greater number of these eosinophilic structures, whereas lesions without the secondary focus of basophilia contained very few of these structures. The eosinophilic structures appeared to be calcium deposits by von Kossa staining.

Thirty-one lesions were examined by polarizing light microscopy. Of these, 37% contained cholesterol mono-

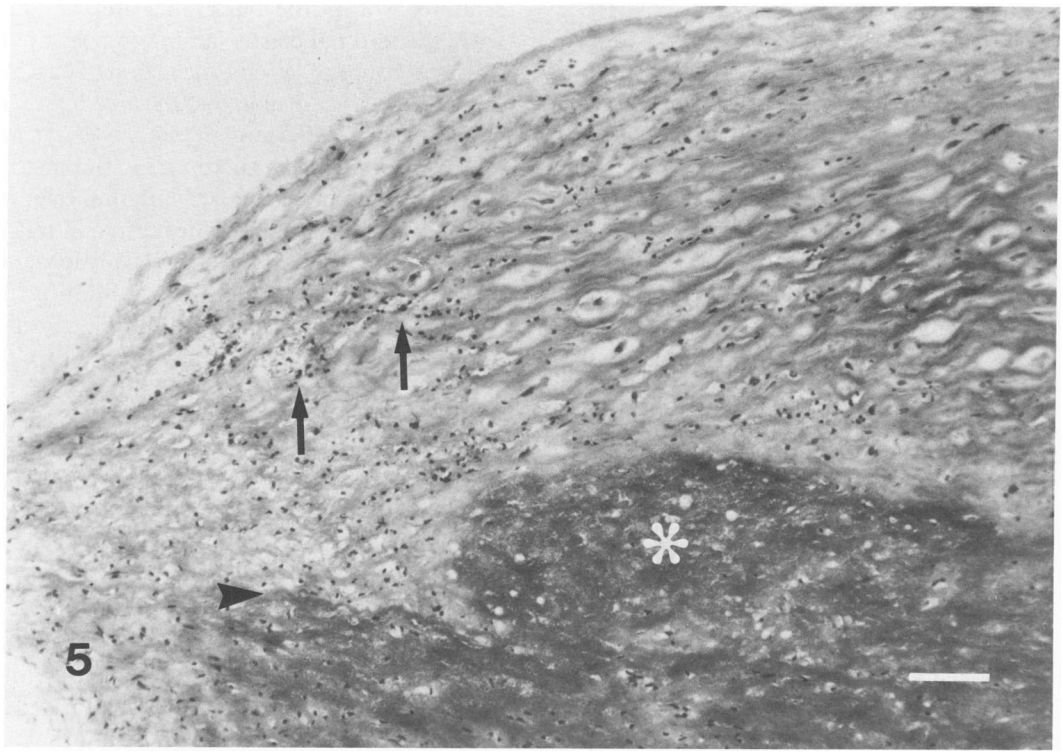

Figure 5—A fibrolipid lesion with a well-developed connective tissue cap and lipid-rich core, found in a 27-year-old white man lateral to the third lumbar artery. Total lesion volume was 31.8 μl, and the mean intimal thickness was 1560 μ. The core region (*asterisk*) extends from the musculoelastic intimal layer into the more superficial intima above the inner limiting membrane (*arrowhead*). Foam cells and small mononuclear cells (*arrows*) are found in close association with the lateral aspect of the lipid-rich core. No foam cells are observed at the top surface of the lesion. Fresh-frozen 10-μ section. (H&E, ×78; bar = 100 μ)

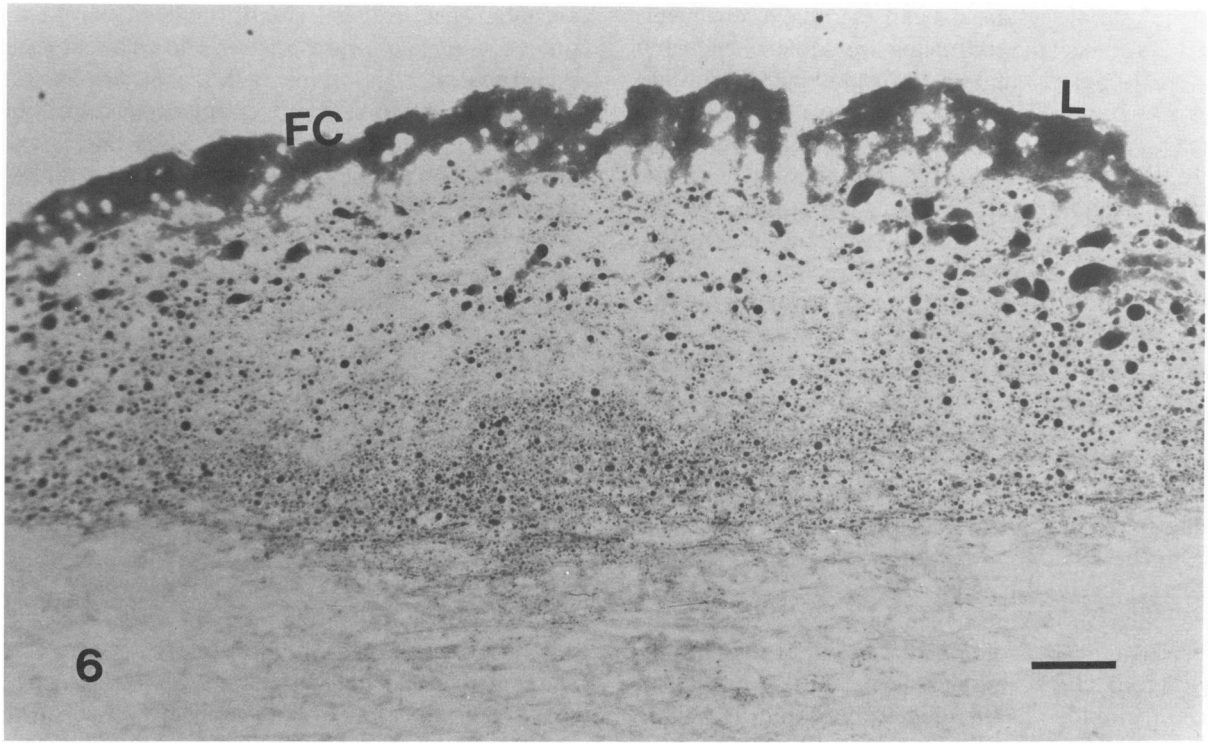

Figure 6—Distribution of lipid within the small fibrolipid lesion. A layer of foam cells (*FC*) is present at the luminal surface (*L*). The foam cells appear as a large mass of oil red O stain. Deep within the lesion small oil-red-O–stained lipid droplets and grains of stain make up the core region (*arrows*). This section was adjacent to that shown in Figure 4. Fresh-frozen 10-μ section. (Oil red O in 60% isopropyl alcohol, ×122; bar = 100 μ)

Figure 7—Histologic appearance of the lipid-rich core region in an extended (midintima) focus in a larger fibrolipid lesion. Scattered throughout the area of basophilia are small eosinophilic spheroids (*arrows*). Note the sparse presence of nuclei within the core region. Fresh-frozen 10-μ section. (H&E, ×653; bar = 10 μ)

hydrate crystals, which were identified in histologic section by their shape and their constant, bright birefringent properties when viewed at temperatures of up to 100 C. Characteristic formée cross figures were not present within the area of birefringence. Figures 8–13 compare the appearance of three different lesions examined with polarized light at 25 C and 43 C. Most lesions without cholesterol crystals lost considerable birefringence at 37 C and became completely isotropic when heated to 43 C (Figures 8 and 9). In lesions with cholesterol crystals, foam-cell lipids melted to isotropic droplets, whereas the crystals remained brightly birefringent even at 100 C (Figures 10 and 11). In the lesions with core regions extending into the elastic hyperplastic intimal layer, the core regions maintained a faint birefringence which did not change over the temperature range 43–100 C (Figures 12 and 13). This last group of lesions did not contain any morphologic evidence of free cholesterol monohydrate crystals. In Vibratome-prepared sections, heating the samples produced similar responses, suggesting that freezing did not induce the formation of birefringent crystals.

The location of cholesterol crystals within lesions appeared to be related to overall lesion volume. The smaller lesions (<16 μl) contained cholesterol crystals arranged in plates closely associated with the inner limiting elastic membrane (Figure 14). Lesions with volumes between 16 and 27 μl contained crystals in the basophilic region found in the musculoelastic layer. The inner limiting membrane was fragmented and somewhat diffuse in these lesions, so that one was unable to clearly associate the crystals with this membrane. Lesions with volumes between 28 and 43 μl contained many crystals scattered within the basophilic area which extended from the internal elastic lamina to near the lumen. In all three groups of lesions, the region of cholesterol crystallization was acellular, and the area immediately surrounding the cholesterol crystals was hypocellular.

The normal intima surrounding the small fibrolipid lesion and the media underlying the lesion contained varying amounts of lipid. The normal intima contained fine sudanophilic particles specifically located at the internal elastic lamina and the inner limiting membrane. Very little oil-red-O–positive staining was observed above the inner limiting membrane. In chloroform-pretreated tissue sections this staining was absent, which suggests that the stained material was indeed lipid. With respect to the media, some of the lesions revealed varying degrees of lipid staining, but most contained very little medial lipid staining.

Lipid Composition

The lipid composition of a sample of fibrolipid lesions, normal intima, fatty streaks, and fibrous plaques examined morphologically are summarized in Table 2. Sufficient tissue was available on 27 of the 31 fibrolipid lesions with volumetric determinations. The total cholesterol concentration was similar for all fibrolipid lesion groups except for the large lesions with volumes greater than 16 μl and with cholesterol monohydrate crystals. These larger lesions contained two to three times the concentrations of free and total cholesterol observed in smaller lesions. In the lesions with volumes less than 16 μl, a greater proportion of the total cholesterol was in the free form, even when no histologic evidence of free cholesterol monohydrate crystals was present. Lesions (<16 μl) which contained cholesterol crystals upon microscopic observation contained an increased proportion of free cholesterol. Sixty-two percent of the total cholesterol was free in these lesions. Lesions with volumes greater than 16 μl contained free/total cholesterol ratios similar to those of fibrous plaques, namely, 0.38. The total cholesterol concentration of the fibrolipid lesions was 50% lower than typical fatty streaks; however, the free cholesterol concentration was 10–200% higher than levels found in fatty streaks. It is noteworthy that in the lesions with cholesterol crystals and volumes greater than 16 μl, the total and free cholesterol concentrations approached those found in typical fibrous plaques. The phospholipid concentrations of the new fibrolipid lesions were about the same as those of fibrous plaques.

Statistical analysis of lipid concentrations and free/total cholesterol ratios revealed that the fibrolipid lesions as a group had values significantly different ($P < 0.01$) from those of normal intima, fatty streaks, and fibrous

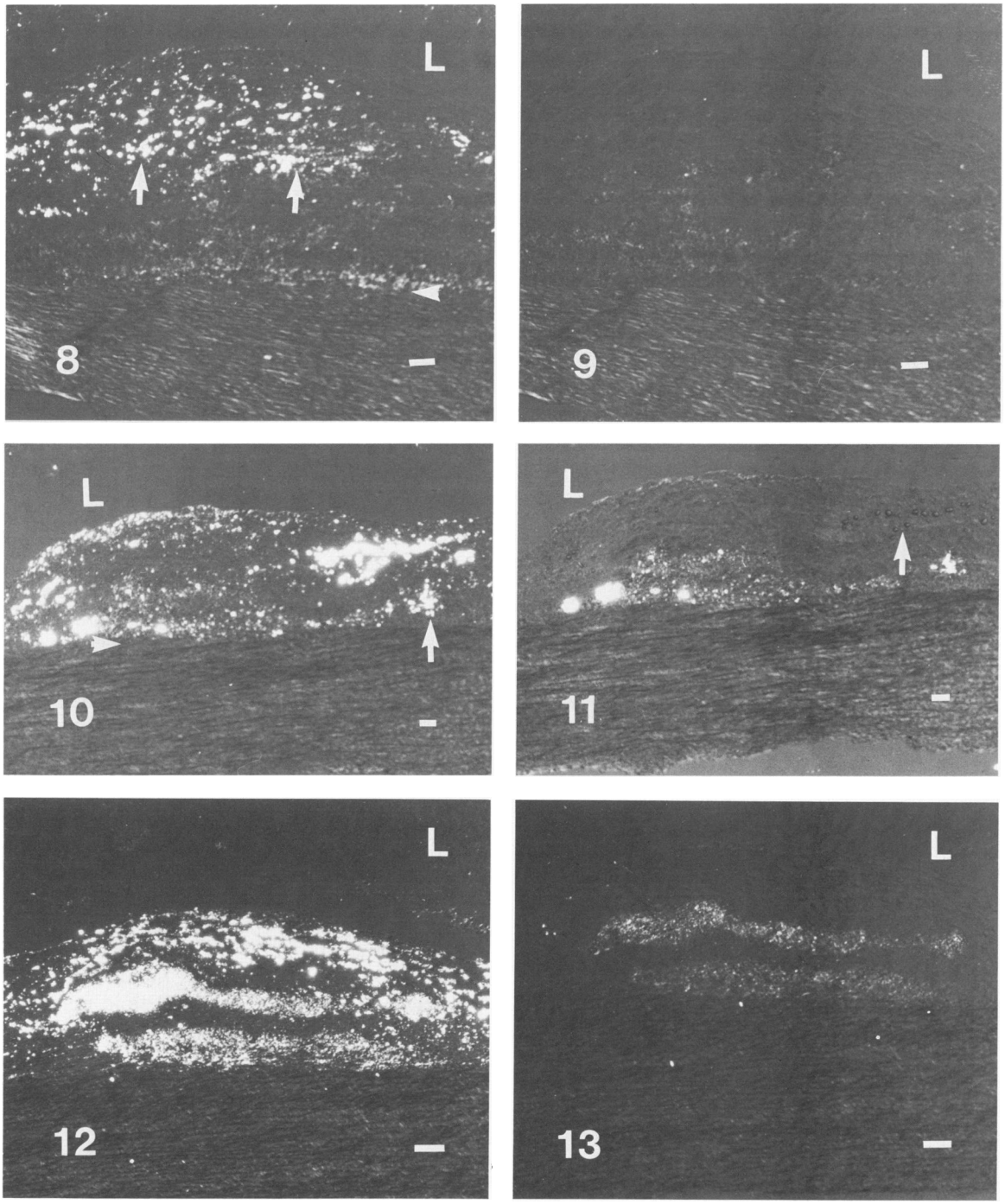

Figure 8—A fibrolipid lesion without cholesterol crystals viewed under polarized light at 25 C, from a 38-year-old white man distal to the tenth intercostal artery. The total intimal volume was 12.9 μl. Note the faint birefringence associated with the lipid-rich core about the internal elastic lamina (*arrowhead*). The brightly anisotropic structures are the foam cells (*arrows*). *L*, lumen. (Fresh-frozen 10-μ section, ×40; bar = 100 μ) **Figure 9**—Appearance of the lesion seen in Figure 8 after being heated to 43 C. Note the absence of birefringence associated with the foam cells and lipid-rich core, while the fainter birefringence associated with collagen and elastin fibers remains. *L*, lumen. (Fresh-frozen 10-μ section, ×40; bar = 100 μ) **Figure 10**—A fibrolipid lesion with cholesterol crystals viewed under polarized light at 25 C. The lesion was found in a 38-year-old white man lateral to the first lumbar artery. The total intimal volume was 16.2 μl. Brightly birefringent crystalline structures (*arrow*) are found just above the internal elastic lamina (*arrowhead*). A cluster of foam cells are found near the lumen (*L*). (Fresh-frozen 10-μ section, ×27; bar = 100 μ) **Figure 11**—The lesion seen in Figure 10 after being heated to 43 C. The foam cells have melted to isotropic droplets (*arrows*), whereas the crystals of cholesterol are brightly birefringent. *L*, lumen. (Fresh-frozen 10-μ section, ×27; bar = 100 μ) **Figure 12**—A fibrolipid lesion with two distinct foci of lipid deposition viewed under polarized light at 25 C. The lesion was found in a 56-year-old white man lateral to the 12th intercostal artery. The total intimal volume was 11.9 μl. One focus of birefringence is found along the internal elastic lamina (*arrowhead*), and a second area is found in the center of the lesion. Note that the whole lesion is covered by a layer of foam cells. *L*, lumen. (Fresh-frozen 10-μ section, ×48; bar = 100 μ) **Figure 13**—Microscopic appearance of the lesion seen in Figure 12 after being heated to 43 C. The superficial layer of foam cells have become isotropic, and the two foci of lipid deposition remain faintly birefringent. No morphologically distinct cholesterol crystals were found in this lesion. *L*, lumen. (Fresh-frozen 10-μ section, ×48; bar = 100 μ)

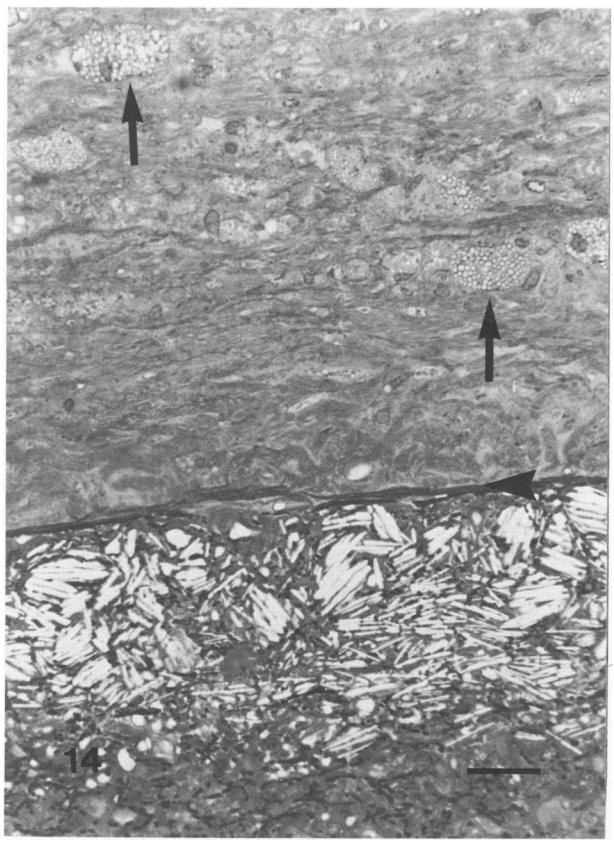

Figure 14—Localization of the cholesterol monohydrate crystals within a small fibrolipid lesion. The crystals are found in the musculoelastic layer of the intima in close association to the inner limiting membrane (*ILM, arrowhead*). The foam cells (*arrows*) are found predominantly above the ILM in the elastichyperplastic layer. No foam cells are found in the musculoelastic layer. (Two-micron-thick section of an Epon-embedded tissue slice, toluidine blue and basic fuchsin, ×658; bar = 2 μ)

plaques, with the following exceptions. The total cholesterol concentration in fibrolipid lesions was not significantly different from that in fatty streaks. Fibrous plaque and fibrolipid lesion phospholipid concentrations were also not significantly different. Free/total cholesterol ratios were much higher in fibrolipid lesions, compared with fatty streaks ($P < 0.001$), but only marginally higher in fibrolipid lesions, compared with normal intima ($P = 0.03$) or fibrous plaques ($P = 0.05$).

Discussion

The human aortic lesions that we have studied possess characteristics of both fatty streaks and fibrous plaques, and we have referred to these lesions as fibrolipid lesions. The major results of this study can be outlined as follows: 1) A defined procedure has been developed for selecting small, raised, lipid-rich lesions on the basis of gross appearance and examination of lipid autofluorescence at low magnification. 2) Fibrolipid lesions ranging in volume from 3 to 48 μl are found infrequently in human aorta but when present, have a typical location just lateral or distal to the ostia of intercostal and lumbar arteries. 3) The fibrolipid lesion characteristically contains a superficial layer of foam cells and a deeper core of extracellular lipid, sometimes with crystalline deposits. In the smallest lesions cholesterol crystallization was observed in the intimal musculoelastic layer in a setting of extensive deposition of noncrystalline, extracellular lipid. The lipid-rich core without crystals probably represents an earlier stage of core formation than that with crystals, because crystals were uncommon in the smallest lesions but were present in the majority of larger lesions. 4) The fibrolipid lesion has total cholesterol concentrations similar to those of fatty streaks; however, the ratio of unesterified to total cholesterol is greater than that found in typical fatty streaks. The present study is unique in that it presents a morphologic and chemical description of a lesion which probably is an early form of the atherosclerotic fibrous plaque. In addition, this study focuses on the formation of the lipid-rich core.

The most critical problem in a morphologic study of human atherosclerotic lesions is the sampling of le-

Table 2—Lipid Composition

Lesion type	Crystals present	Number of lesions	Total cholesterol*	Free cholesterol*	Free/total cholesterol	Phospholipid*
Normal Intima	–	6	14.0 ± 4.3	4.5 ± 1.5	0.33 ± 0.12	35.3 ± 18.6
Fatty Streak	–	6	82.0 ± 27.0	15.1 ± 5.2	0.19 ± 0.02	54.8 ± 20.3
Fibrolipid						
≤16 μl	–	14	47.5 ± 23.8	20.9 ± 9.5	0.48 ± 0.16	100.0 ± 32.0
	+	4	42.7 ± 28.0	25.9 ± 14.9	0.62 ± 0.13	88.2 ± 38.0
>16 μl	–	3	55.4 ± 10.9	16.5 ± 4.7	0.34 ± 0.10	90.3 ± 20.5
	+	6	121.4 ± 42.9	46.2 ± 18.1	0.38 ± 0.06	116.6 ± 22.8
Fibrous plaques						
Small	+	9	224.7 ± 86.4	83.6 ± 40.8	0.36 ± 0.07	92.8 ± 28.2

* Mean ± SD in micrograms per milligram dry defatted tissue.

sions of consistent size, location, and appearance. After rapid gross and low-power microscopic examination, we eliminated more than 75% of the aortas, because of the absence of small fibrolipid lesions. A valuable feature of this sampling strategy is that despite their infrequent occurrence, remarkably consistent lesions can be identified, prior to time-consuming processes such as frozen sectioning and paraffin or plastic embedding. The sampling technique also facilitates the characterization of lesions with respect to topographic location and size.

The topographic location of the fibrolipid lesion is similar to that of more advanced fibrous plaques. We have found these lesions primarily associated with the ostia of intercostal and lumbar arteries, locations with a distinct predilection for fibrous plaque formation.[4] It should be noted that the fibrolipid lesion does not have the same appearance as physiologic intimal pads or cushions at branch vessel sites. The latter have the appearance of being molded by hemodynamic or mechanical forces and have smooth, indistinct contours, but the fibrolipid lesion is clearly a pathologic nodule with distinct boundaries, often situated a few millimeters from the branch orifice. This same localization applies to fibrous plaques. Comparison of lesion location with age revealed that in younger individuals fibrolipid lesions were found predominantly in the abdominal aorta, whereas fatty streaks were present in the thoracic aorta. In older individuals the abdominal aorta contained large fibrous plaques, whereas the fibrolipid lesions were found in the more proximal segments of the thoracic aorta. These observations suggest that like fibrous plaques, fibrolipid lesions first develop at sites distal to the diaphragm and then with age assume a more proximal localization. Because the lesions also possess the morphologic characteristics of elevation and core region development, similar to those of fibrous plaques, it would appear that these lesions represent an early or precursor stage for the atherosclerotic fibrous plaque.

The fibrolipid lesions occur infrequently. We have found them in only 18% of aortas, each showing one, two, or, rarely, more lesions. This is despite the fact that the case population was well suited to this study, being mostly male young adults. Possible explanations for the rarity of the lesion are as follows: 1) Fibrolipid lesions may progress to fibrous plaques very rapidly. 2) Our insistence on distinct, small lesions, surrounded by normal intima, may have caused us to miss lesions arising within confluent areas of fatty streaking. 3) Other lesions, including nodular foci without lipid, or perhaps gelatinous lesions,[16] may serve as precursors to fibrous plaques. The data presently do not allow the first two hypotheses to be distinguished clearly. We are examining confluent fatty streaks more closely to determine whether any contain discrete foci of lipid deposition between the inner limiting membrane and the internal elastic lamina. The third explanation really does not answer the question of rarity, because, among small raised lesions, the fibrolipid lesion was more than three times as common as the others combined. Furthermore, our preliminary observations suggest that neither nodules without lipid nor gelatinous lesions are specifically associated with branch ostia. For the gelatinous lesion, nonspecific aortic localization, including both ventral and dorsal surfaces, has been reported,[16,17] and our observations concur. Haust[17] has suggested that fibrous plaques may arise via multiple morphologic sequences. However, the proof of this statement must be based upon careful consideration of lesion frequencies, epidemiology, topography, the demonstration of appropriate transitional morphologies and the demonstration of pathogenetic mechanisms in animal and *in vitro* models that resemble human disease. The criteria of frequency, topography, and transitional morphology appear to be met by the fibrolipid lesion but remain to be shown for other candidate precursors.

A remarkable feature of the fibrolipid lesion is the appearance and location within the lesion of the lipid-rich core. The core is brightly birefringent at 25 C, metachromatic upon toluidine blue staining, and very basophilic. The core region birefringence is not an artifact of freezing and thawing, because we found it also in 100-μ slices of fresh tissue. The marked diminution of core region birefringence on warming to 43 C indicates a phase transition of an anisotropic substance. This suggests the presence of cholesteryl ester contained in nonlipoprotein deposits, because even concentrated cholesteryl ester-rich lipoproteins exhibit minimal birefringence (J. Morrisett, personal communication). At temperatures between 43 and 100 C, core region birefringence was constant and had two appearances. Bright focal birefringence was associated with clusters of objects identified as cholesterol crystals. A faint, yet distinct birefringence was sometimes found extending throughout the core region. The chemical basis for this latter appearance at high temperatures is obscure. In addition to free cholesterol, certain phospholipid structures are known to remain birefringent at those temperatures.[18] It is possible that the residual birefringence observed at 100 C could be the counterpart of the extracellular filipin-stained particle identified by Kruth[19] in more advanced lesions. If this proves to be true, then it suggests that these birefringent particles may indeed be free cholesterol. The supposition that cholesteryl ester accounts for birefringence in the core region is not contradicted by the lack of formée cross figures. Most of the lipid deposits in this region had diameters less

than 2 μ, which is the minimal size for the appearance of formée cross figures.[20] Because the lipid-rich core was examined in sections, connective tissue elements above and below the droplets may also interfere with the appearance of distinct formée cross figures.

The intense negative metachromasia (a shift in the light transmittance of the chromophore toward longer wavelengths) of the lipid-rich core when stained with toluidine blue suggests that the components of the core are structurally distinct from the surrounding normal intima. Sulfate groups in tissue glycosaminoglycans such as chondroitin sulfate are known to impart very strong metachromasia with basic dyes.[21] Several investigators have shown that with lesion development there is an alteration in the fractions of glycosaminoglycans, most notably chondroitin sulfate.[22,23] Thus, the negative metachromasia could be a histologic demonstration of the altered glycosaminoglycan composition, although other explanations are possible.

The well-demarcated area of basophilia associated with the lipid-rich core is further evidence supporting the hypothesis that the core region is both chemically and structurally distinct from the surrounding intima. Cellular necrosis or calcium deposits could account for some of the basophilia observed in the lipid-rich core. However, the extent of basophilia did not correlate with the extent of calcium observed in von Kossa-stained tissue sections. The observations that either formalin fixation or lipid extraction greatly diminished core basophilia are of some interest; however, the chemical nature of the basophilia remains enigmatic.

The lipid-rich core has a definite location within the fibrolipid lesion. The consistent site of core lipid deposition, observed in all but four lesions, including the smallest, was in the musculoelastic layer between the internal elastic lamina and inner limiting elastic membrane. Crystalline cholesterol, when present in the smaller lesions, was found in this same layer. Only when the lesions were larger did an extended or secondary focus of lipid deposition appear above the inner limiting membrane. Several other investigators have observed the deposition of lipid in distinct layers of the intima. Smith et al,[24] examining normal intima, have found that with aging, lipid is deposited along deep intimal elastic membranes and along nearby elastic fragments. They have referred to these lipid deposits as perifibrous lipid. Robertson and co-workers[25] have observed lipid in the elastic hyperplastic layer of the developing intima in coronary arteries. Daoud and co-workers[26] have also observed minute areas of necrosis characteristically at the base of what they described as a preatheromatous coronary lesion. It can be speculated that the elastin-rich composition of the deeper intimal layers favors the accumulation of lipid extracellularly. Support for such a theory can be found in the work of Kramsch and co-workers,[27] who have observed that 37% of the total lipid in severe plaques can be recovered with intimal elastin.

The core region does not appear to initiate from necrosis and transformation of a densely packed foam-cell infiltrate. Neither dense foam-cell infiltrates nor esterase-positive foam cells were found in the musculoelastic intimal layer, where the core region commonly originates. Individual lipid-containing cells were found in this region but were sparse. Thus, it is difficult to postulate cycles of cellular lipid accumulation and necrosis as a major mechanism for core lipid deposition, although this mechanism cannot be ruled out. Tracy and co-workers[28] have recently shown that in human aorta foam-cell infiltrates are scarce at distances greater than 300 μ from the endothelium. Bayliss High and Adams[29] have found monocyte-macrophages, identified histoenzymatically, to be absent from deep intimal layers in fibrous plaques, as long as capillaries had not grown into the base of the lesion. The hypothesis that core lipid is derived directly from lipoproteins permeating the arterial wall, without an intermediate step of intracellular lipid accumulation, has been advanced by Smith,[7] largely on the basis of cholesteryl ester fatty acyl patterns.

The question remains what, if any, is the relationship between the superficial, esterase-positive foam cells and the generally deeper-lying core lipid. These lesion features occur concomitantly in fibrolipid lesions. Their potential relationship could be either coincidental or causal. A coincidental relationship might arise, for example, if increased lipoprotein permeation from endothelial leakiness led to foam cells in the superficial intima and to core lipid in the deep intima. The other possibility, that foam cells could somehow cause or contribute to lipid deposition at some distance away from the cells, is only speculative, but it is worthwhile to note that these cells are likely to be metabolically active in many ways besides lipid accumulation. Thus, the role of the foam cells in the process of core lipid deposition is still unclear; however, their relative absence in the lipid-rich core but their presence in superficial layers of the lesions examined would be consistent with an indirect role in core region growth and development. Further studies are needed to substantiate such a role.

Comparison of the lipid compositions of the fibrolipid lesions with the lipid compositions of normal intima, fatty streaks, and fibrous plaques assayed along with them would tend to indicate a possible chemical transition from fatty streak to fibrolipid lesion to fibrous plaque. We have found that the total cholesterol concentration is similar to that of fatty streaks in all fibrolipid lesions except the lesions greater than 16 μl and with perceptible cholesterol monohydrate crystals.

These larger lesions have total cholesterol levels approaching that of the fibrous plaque. Despite the low total cholesterol levels, the content of free cholesterol was found to be intermediate between fatty streaks and fibrous plaques. Comparison of the lipid concentrations of normal intima, fatty streaks, and fibrous plaques obtained here with that of work done by Smith and coworkers[7,23,31] reveals similar results. Minor discrepancies may be due in part to the extent to which the lesions were dissected from surrounding tissue. We did not microdissect the lesion, and the lipid concentrations, therefore, were lower because of dilution by the connective tissue elements. Our fibrolipid lesions with cholesterol crystals and volumes greater than 16 μl have a lipid composition similar to that of the few fatty nodules examined by Smith.[7] These data would suggest that most of the lesions examined in this study are at an earlier stage of development than those previously reported.

The ratios of free to total cholesterol for all lesions examined are similar to those reported by Katz and coworkers[5] and Smith.[7] In fibrolipid lesions with a total intimal volume of less than 16 μl, the free/total cholesterol ratio for lesions with and without cholesterol crystals is 0.62 and 0.44, respectively. The free/total cholesterol ratio of lesions with a total intimal volume greater than 16 μl is similar to that of typical fibrous plaques, namely, 0.38. These data suggest that a marked increase in the concentration of cholesterol may be an important step in core development. Malcolm and coworkers[32] have inferred that the content and concentration of free cholesterol were substantially higher in aortic fatty streaks obtained from a group of young men (from New Orleans) predisposed to the subsequent development of fibrous plaques, as compared with fatty streaks from a control group (Guatemalans). Because of the relative absence of foam cells and the extensive extracellular lipid in the core region, it does not seem likely that cell death could account for this marked increase in free cholesterol. It can be speculated that an interaction of the permeating lipids and lipoproteins with intimal collagen and elastin, with perhaps an indirect effect of overlying foam cells, may predispose certain regions of the aorta to fibrolipid lesion formation and eventual fibrous plaque development.

The transition from fibrolipid lesion to fibrous plaque seems clear, and whether the former should be regarded as nosologically distinct is perhaps debatable. However, in some prior work, it seems likely that fibrolipid lesions have been regarded as fatty streaks. The amount of elevation in the smaller fibrolipid lesions was only about one-third of a millimeter; oblique lighting was necessary to distinguish this feature. An occasional fatty streak, without deep-lying lipid, could be elevated to a lesser degree. These observations, plus the consistent finding of foam cells in the cap of the fibrolipid lesion and the appropriate biochemical transition, suggest that a complete transitional sequence of fatty streak, fibrolipid lesion, fibrous plaque might be demonstrated eventually. However, the notion of transition from fatty streak to fibrolipid lesion needs to be bolstered by the demonstration of extracellular lipid deposition in the musculoelastic intimal layer in typical fatty streaks, specifically localized beneath superficial foam-cell infiltrates. This would elucidate the formation of the core region, which was already present in all of the lesions we identified.

Perhaps the most important implication of this study with regard to atherogenesis is that the formation of the lipid-rich core region is disclosed to be a very early event in the development of the raised lesion. Small raised lesions with this histology are found with considerably greater frequency than small raised lesions without lipid. The present results do not exclude the formation of some fibrous plaques as masses of smooth-muscle cells with little lipid.[33] However, the morphologic and chemical evidence of this study suggests that most small, raised lesions do contain lipid in distinctive deposits at their earliest recognizable stage.

References

1. Tracy RE, Strong JP, Toca VT, Lopez CR: Atheronecrosis and its fibroproliferative base and cap in the thoracic aorta. Lab Invest 1979, 41:546–552
2. Constantinides P: Plaque fissures in human coronary thrombosis. J Ultrastruct Res 1966, 6:1–17
3. Ridolfi RL, Hutchins GM: The relationship between coronary artery lesions and myocardial infarcts: Ulceration of atherosclerotic plaques precipitating coronary thrombosis. Am Heart J 1977, 93:468–486
4. Woolf N: Pathology of atherosclerosis, Atherosclerosis: Mechanisms and Approaches to Therapy. Edited by N Miller. New York, Raven Press, 1983, pp 1–27
5. Katz SS, Shipley GG, Small DM: Physical chemistry of the lipids of human atherosclerotic lesions: Demonstration of a lesion intermediate between fatty streaks and advanced plaques. J Clin Invest 1976, 58:200–211
6. Smith EB, Slater RS, Chu PK: The lipids in raised fatty and fibrous lesions in human aorta: A comparison of the changes at different stages of development. J Atheroscler Res 1968, 8:399–419
7. Smith EB: The relationship between plasma and tissue lipids in human atherosclerosis. Adv Lipid Res 1974, 12:1–49
8. Chayen J, Bitensky L, Butcher RG: Practical Histochemistry. New York, John Wiley & Sons, 1973, pp 80–88
9. Thompson SW: Selected Histochemical and Histopathological Methods. Springfield, Ill, Charles C. Thomas, 1966, pp 581–584; 790–792; 658–660
10. Gomoro G: Microscopic Histochemistry: Principles and Practice. Chicago, The University of Chicago Press, 1952, pp 184–186; 193–194; 203–206; 210–212; 323–335
11. Geer JC, Haust MD: Smooth muscle cells in atherosclerosis. Monogr Atheroscler 1972, Vol 2
12. Folch J, Lees M, Sloane Stanley GH: A simple method

for the isolation and purification of total lipids from animal tissue. J Biol Chem 1957, 226:497–509
13. Cooper GR: Cholesterol: enzymic methods, Selected Methods for the Small Clinical Chemistry Laboratory. Vol IX. Edited by WR Faulkner, S Meiters. Washington, DC, American Association of Clinical Chemists, 1982, p 165
14. Baginski ES, Foa PP, Zak B: Microdetermination of inorganic phosphate, phospholipids, and total phosphate in biologic materials. Clin Chem 1967, 13:326–332
15. Wallenstein S, Zucker CL, Fleiss JL: Some statistical methods useful in circulation research. Cir Res 47:1–9
16. Smith EB: Identification of the gelatinous lesions, Atherosclerosis. Vol VI. Edited by G Schettler, AM Gotto, G Middlehoff, AJR Hobenickt, KR Jurukova. Berlin, Springer-Verlag, 1983, pp 170–173
17. Haust MD: The morphogenesis and fate of potential and early atherosclerotic lesions in man. Hum Pathol 1971, 2:1–29
18. Small DM: The physical state of lipids of biological importance: Cholesteryl esters, cholesterol, triglyceride, Advances in Experimental Medicine and Biology. Vol 7, Surface Chemistry of Biological Systems. Edited by M Blank. New York, Plenum Press, 1970, pp 55–83
19. Kruth HS: Localization of unesterified cholesterol in human atherosclerotic lesions: Demonstration of filipin-positive, oil red O-negative particles. Am J Pathol 1984, 114:201–208
20. Hata Y, Hower J, Insull W, Jr: Cholesteryl ester-rich inclusions from human aortic fatty streak and fibrous plaque lesions of atherosclerosis: I. Crystalline properties, size, and internal structure. Am J Pathol 1974, 75:423–456
21. Ruthmann A: Methods in Cell Research. Ithaca, NY, Cornell University Press, 1970, p 148
22. Velican C, Velican D: Heterogeneity in the composition and aggregation patterns of coronary intima acid mucopolysaccharides (glycosaminoglycans). Atherosclerosis 1978, 29:141–159
23. Stevens RL, Colombo M, Gonzales JJ, Hollander W, Schmid K: The glycosaminoglycans of the human artery and their changes in atherosclerosis. J Clin Invest 1976, 58:470–481
24. Smith EB, Evans PH, Downham MD: Lipid in the aortic intima: The correlation of morphological and chemical characteristics. J Atheroscler Res 1967, 7:171–186
25. Robertson WB, Geer JC, Strong JP, McGill HC, Jr: The fate of the fatty streak. Exp Mol Pathol 1963, Suppl 1:28–39
26. Daoud A, Jarmolych J, Zumbo A, Fanc K: Preatheroma phase of coronary atherosclerosis in man. Exp Mol Pathol 1964, 3:475–484
27. Kramsch DM, Franzblau C, Hollander W: The protein and lipid composition of arterial elastin and its relationship to lipid accumulation in the atherosclerotic plaque. J Clin Invest 1971, 50:1660–1677
28. Tracy RE, Kissling GE, Devaney KO, Lopez CR, Toca VT, Gandia M: Spatial dispersion of foam cells in the human thoracic aorta. Lab Invest 1983, 49(6):693–701
29. Bayliss High OB, Adams CWM: The role of macrophages and giant cells in advanced human atherosclerosis. Atherosclerosis 1980, 36:441–447
30. Rothblat GH, Phillips MC: Mechanism of cholesterol efflux from cells. Effects of acceptor structure and concentration. J Biol Chem 1982, 257:4775–4782
31. Smith EB: The influence of age and atherosclerosis on the chemistry of aortic intima. J Atheroscler Res 1965, 5:224–240
32. Malcom GT, Strong JP, Restrepo C: Atherosclerosis and lipid composition of the abdominal aorta: Comparison of autopsied New Orleans and Guatemalan men. Lab Invest 1984, 50:79–86
33. Schwartz SM, Ross R: Cellular proliferation in atherosclerosis and hypertension. Prog Cardiovasc Dis 1984, 26:355–372

Acknowledgments

We would like to thank Mr. Thomas A. Schifani for technical assistance and Ms. Ruth Collins for her help in manuscript preparation.