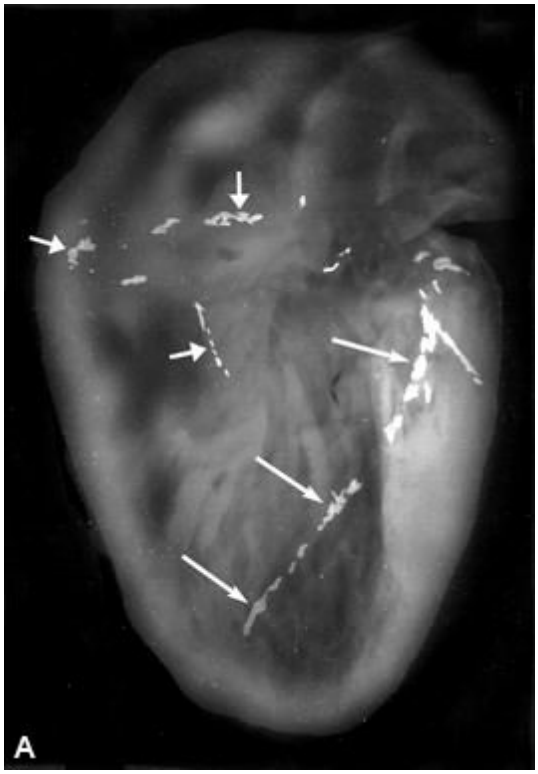
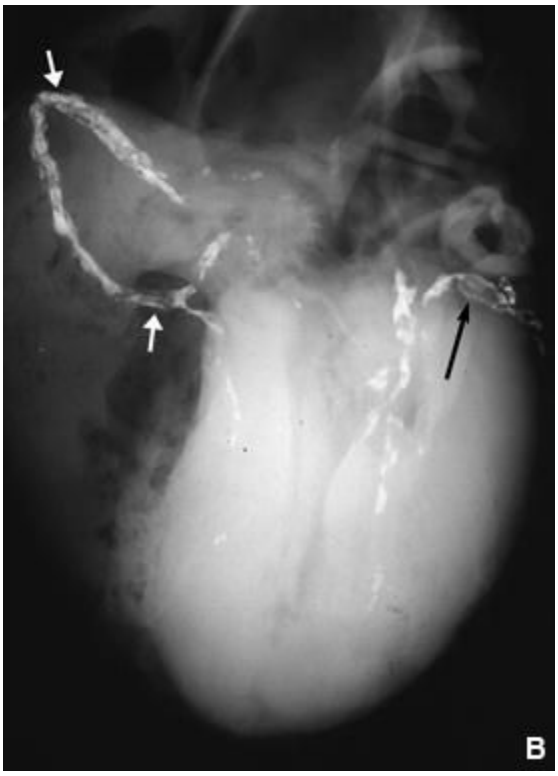
5. Calcification: A Physiologic Defense

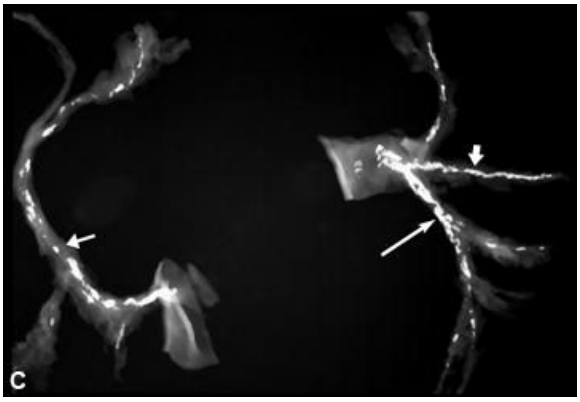
"Atherosclerotic calcification is an organized, regulated process similar to bone formation that occurs only when other aspects of atherosclerosis are present."
L Wexler, et al., American Heart Association Writing Group, [74]

Considerable insight into the nature and character of the IA causing atherosclerosis can be gained from the study of coronary calcification. Figure 9 shows postmortem X-rays illustrating different patterns of coronary calcification in intact human hearts (Figures 9A,B,D) and one case of dissected, uninjected coronary arteries (Figure 9C), in four patients of differing age and sex who died of ACD. Calcific deposits may be small, discrete, widely separate foci, measuring only a few mm, or large, confluent masses measuring several cm in length (Figures 9A-C). Calcification does not occur in a normal vessel wall [74] and, when present, provides objective evidence of arterial wall injury and the presence, location, and extent of atherosclerotic plaque formation within the coronary tree [75]. Widely separate foci of calcification, as shown in Figure 9, confirms that atherosclerosis is multi-centric in origin, with the IA being present and simultaneously active at multiple separate sites within the coronary tree.

Confluent, uninterrupted blocks of calcification (Figures 9A-C), reflect active longitudinal growth with fusion of adjacent plaques [76], as reported in Chapter 2. The most extensive calcification is often found in the proximal half of the main epicardial coronary arteries, particularly the Left Anterior Descending (LAD) artery [75], (Figures 9A,C,D), suggesting hemodynamic forces contribute to wall injury and plaque growth. Extensive calcification, however, is often present in the distal half of a coronary artery as well (Figures 9A-C), implicating additional factors besides hemodynamic stress in the pathogenesis of injury and calcification. This chapter will show that vascular calcification is a physiologic defense against active, progressive atherosclerotic disease, that it is produced by physiologic mechanisms similar to those required for normal bone formation [74,77,78], and that it is potentially reversible [79,80].







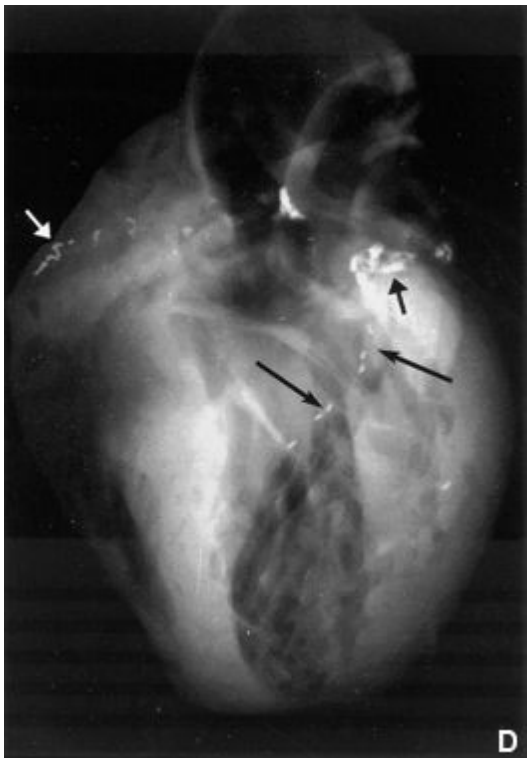


Figure 9: Post-mortem X-rays of 3 hearts before injection of barium gelatin mass (**A**, **B**, **D**) and the dissected coronaries of one heart not injected (**C**). **A**, 42-year-old white male who died of cardiogenic shock shows extensive, confluent calcification of the LAD coronary artery (long arrows) and focal discrete calcium deposits of the RCA (short arrows). **B**, 54-year-old white male who died of SCD outside the hospital. Extensive calcification of all three coronary arteries, particularly the RCA (white arrows), with diffuse confluent calcification. Note the circumferential calcification of the CIRC (black arrow). **C**, Coronary arteries of a 62-year-old white male who died SCD outside the hospital. The RCA (short thin arrow)shows focal calcification extending into the posterior descending artery. Extensive, confluent calcification is seen in the LAD coronary artery (long arrow), and the intimedius branch (fat arrow). **D**, 93-year-old white female who died as a result of cardiogenic shock secondary to a large anterior myocardial infarction. Relatively minimal calcification is present in the RCA (short white arrow), the LAD coronary artery (long black arrows), and CIRC (short black arrow) in this elderly patient.

Aging and Vascular Calcification

The presence of widely spaced calcified plaques in a young patient, shown in Figure 9A, indicates atherosclerotic injury occurred early in life and at multiple locations, leading to premature, accelerated atherosclerosis and to early death from ACD. We

can assume, based on the multiple foci of calcification, that the IA was active at multiple locations within the coronary tree, spreading proximally, distally, and circumferentially, with fusion of adjacent plaques.

Extensive calcification in young persons, Figure 9A, and less extensive calcification in older persons, Figure 9D, demonstrate calcification is not, per se, an aging or age-dependent process [81], even though the extent and amount of vascular calcification tend to increase with age. If calcification were an aging process, then all older persons would show calcification, and the amount and extent of calcification would be directly related to age. Calcification reflects and is related to atherosclerotic injury, caused by an IA that can and does occur at any age. The increase in extent and severity of calcification that occurs with increasing age is due to the patient's harboring and surviving an active, spreading, IA for a long period of time. Age, per se, plays no direct role in the pathogenesis of coronary calcification.

Calcification and Luminal Stenosis

Table 3 compares the relationship between coronary calcification and luminal stenosis in the same 83 patients who died of ACD, shown in Table 1, Chapter 3, and Table 2, Chapter 4. The amount of calcification was determined histologically by viewing each microscopic slide and estimating the circumferential extent of calcification. Each coronary segment was divided into four 90° quadrants and graded according to the presence or absence of calcification in each quadrant. Grade I indicated one quadrant showed calcification whereas Grades II, III, and IV indicated the number of additional quadrants with calcification.

Table 3:. Comparison between the frequency and circumferential extent of calcification and luminal stenosis, determined histologically, in 83 patients who died of acute coronary disease.

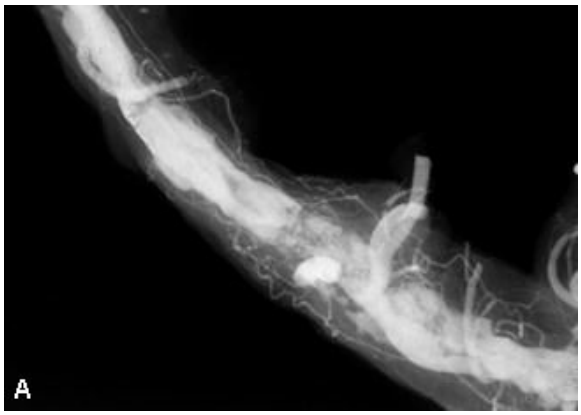
			% Calcification		Amount of Calcification					
					None		I–II		III–IV	
Degree of Stenosis (%)	# of Sections	%	#	%	#	%	#	%	#	%
<50	3221	46	574	18*	2647	82	548	17	26[a]	1
50–80	2458	35	1196	49	1262	51	1046	43	150	6
>80	1377	19	877	64*	500	36	654	47	223[a]	16
Totals	7056		2647	38	4409	62	2248	32	399	6

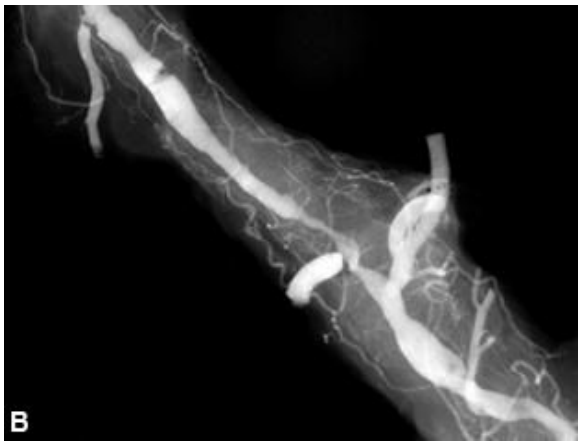
* = p =<0.001; a = p =<0.001

The results: 2,647 (38%) of 7,056 coronary segments contained calcification. Of these segments, 2,073 (78%) were associated with >50% luminal stenosis. Calcification was significantly more common, P=<0.001, in segments with >80% stenosis than in those with <50% stenosis. The frequency of calcification is directly related to the severity of luminal stenosis or to plaque size. Plaque size, as discussed previously, is directly related to the magnitude of the injury caused by the IA. Therefore, calcification is similar to adventitial inflammation and to the presence of a necrotic core in that all three basically reflect the magnitude, extent, and severity of injury caused by the IA. Calcification is a component and another consequence of active atherosclerotic disease, not a specific pathologic complication of the disease process [82,83] that can or should be prevented, apart from preventing atherosclerotic disease itself.

Further examination of Table 3 shows extensive calcification of a given segment, Grades III/IV, is significantly more common in those segments with >80% stenosis than it is in those with <50% stenosis, $p<0.001$. These results confirm our previous observations, Chapters 1 and 4, that the IA tends to spread in a circumferential direction, and that calcification of the injured tissue produced by the IA follows in the wake of this expanding injury [Figure 9B].

Figure 9B, and Figures 10A and 10B, illustrate, in one patient, the direct relationship between the magnitude of atherosclerotic injury, the extent of calcification, and the severity of luminal stenosis. The entire right coronary artery, Figure 9B, is involved with what appears to be a confluent block of calcification, presumably formed by the growth, enlargement, and fusion of many adjacent plaques. The calcification is so severe that the lumen, even though filled with injection mass, cannot be detected in Figure 10A. In Figure 10B, after decalcification, it is evident the patient has severe and extensive obstructive disease throughout the course of this artery, although the channel itself is not completely obstructed. The amount and extent of calcification reflect the plaque burden as well as the extent and severity of atherosclerotic injury. Further, no acute lesions such as thrombosis and/or UPs were present in this artery, so extensive calcification is not synonymous with nor indicative of acute or unstable lesions [74].





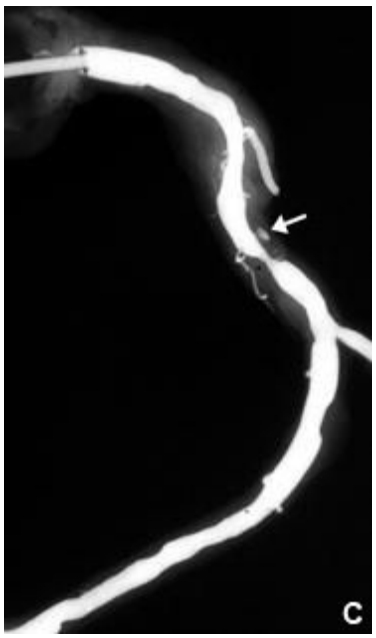


Figure 10: Radiographs of dissected coronary arteries in 2 patients, taken before (**A**, **C**) and after (**B**, **D**) decalcification. **A** & **B**, Themid-RCA of the same patient shown in Figure 9B. Removing the calcium deposits in **A** reveals extensive obstructive disease of the artery in **B**. **C** & **D**, The RCA from a 63-year-old white male who died in hospital of cardiogenic shock, secondary to a large anterior myocardial infarction. This artery showed small flecks of calcification,one area located just proximal to a 90% luminal stenosis (white arrow). The artery beyond this obstruction shows no significant angiographic stenosis, but histologic examination showed multiple areas with 70% stenosis.

Luminal Stenosis without Calcification

Table 3 shows that 51% of all coronary segments with 50–80% stenosis, and 36% of segments with >80% stenosis did not show calcification. Atherosclerotic plaques can become quite large, significantly occluding the lumen, and still not show calcification, confirming that not all plaques become calcified and that the absence of calcification does not exclude significant stenosis [74]. These observations limit the usefulness of electron beam computed tomography (EBCT) to predict plaque volume or the severity of stenosis at a specific site in the coronary tree based on the amount and location of calcification [84,85].

The primary purpose of EBCT is to identify the presence and extent of atherosclerotic involvement of the artery wall and the overall “plaque burden” [86,87]. However, calcific deposits must be >2 mm to be seen by EBCT, and the reproducibility of the scans is low [88], making it difficult to follow the progression of the disease at a specific site in the coronary tree. The identification of coronary calcification by EBCT has broad implications for increased risk (89), but EBCT cannot detect the current activity of the IA [74]. It is not possible to distinguish an actively growing, progressive plaque from an inactive, stable plaque on the basis of coronary calcification. Therefore EBCT is of limited value in determining or predicting acute events [90].

Figures 10C, 10D, are examples of diffuse, obstructive atherosclerotic disease of the right coronary artery, with one tiny fleck of calcium at the point of a 90% stenosis in a 63-year-old, insulin-dependent, diabetic patient. Multiple, widely separated areas of 70% stenosis were present throughout the length of this coronary artery without associated calcification, but no acute lesions, thrombosis, or UPs were present in the artery. These observations emphasize the difficulty of predicting the extent and severity of stenosis on the basis of coronary calcification, even in high-risk patients.

However, a previous study from this laboratory showed virtually all acute coronary lesions to be associated with calcification at the site of the acute lesion [57]. The absence of calcification, even at points of significant stenosis, is strong evidence against the presence of an acute lesion at such sites [57]. The absence of calcification at the site of significant stenosis suggests a stable, non-dangerous, non-vulnerable lesion. At the same time, the consistent presence of calcification at the site of UPs, with or without thrombosis [57], means that

calcification must play some role, discussed below, in the pathogenesis of PU and the development of ACD.

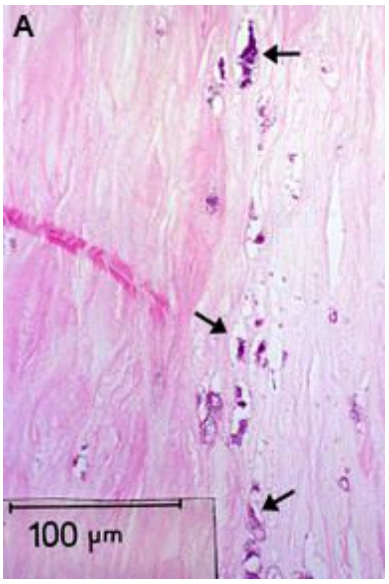
Calcification Is Similar to Bone Formation

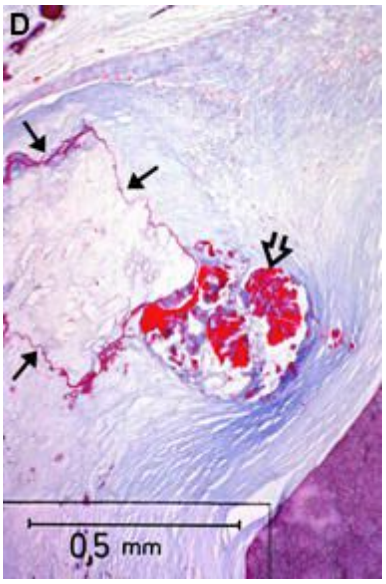
Vascular calcification is now recognized as an active, organized, complex, highly regulated process that is similar, if not identical, to normal bone formation [60,74,77,91]. It is NOT the result of passive precipitation of calcium salts in or on degenerating fibrous tissue [74]. Vascular cells are induced to calcify by the same set of genes as those expressed during bone formation [92]. Specifically, the calcification of atherosclerotic plaques involves virtually the same biologic reactions as normal physiologic bone formation and is not the result of a pathologic biochemical calcification process. The calcium deposits in vascular calcification exist primarily in the form of hydroxyapatite (HA) that is identical to the HA in bone [77,93]. Although the sequence of events leading to normal bone formation is well known, the sequence of events leading to vascular calcification is not completely understood. Various mechanisms may be involved [74,91,94].

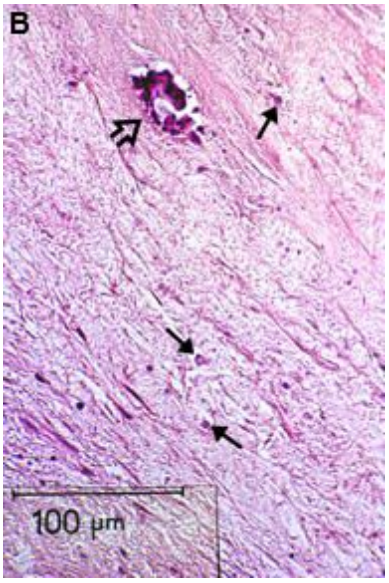
Matrix Vesicles

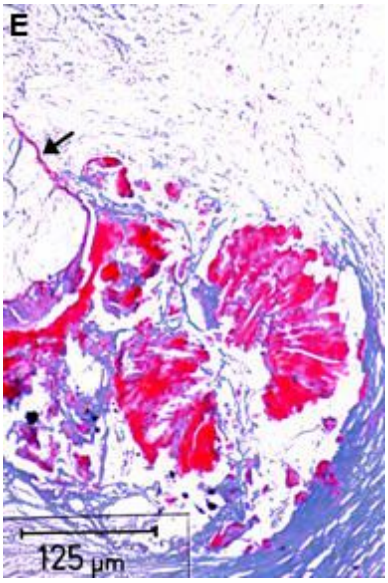
Figures 11, 12 and 13 illustrate the similarity between vascular calcification and normal bone formation. Vascular calcification is believed to begin with the formation of matrix vesicles within degenerated SMCs [25,82,95], Figures 11A–11C, while in bone formation matrix vesicles are formed within degenerating chondrocytes (Figure 13D). Matrix vesicles are tiny, round structures of variable size and density, containing microcrystalline calcium derived from mitochondria, other cell organelles, or from influx of calcium from the extracellular space [25,74,95,96]. Matrix vesicles are believed to serve as nucleators for vascular calcification and may be present within degenerating SMCs or be excytosed into the extracellular space (Figures 11A, B), the way matrix vesicles pinch off from chondrocytes in bone formation (Figure 13D) [74,93,97]. Matrix vesicles tend to clump and fuse together causing a breakdown of the matrix vesicle membrane, exposing their contents to intra- or extracellular fluid containing bone matrix proteins (Figures 11A, B), followed by the formation of HA. Matrix vesicles are an objective sign of cellular injury, death or apoptosis [25,96,98], are an essential element required for calcification, and are commonly present in most atherosclerotic plaques.

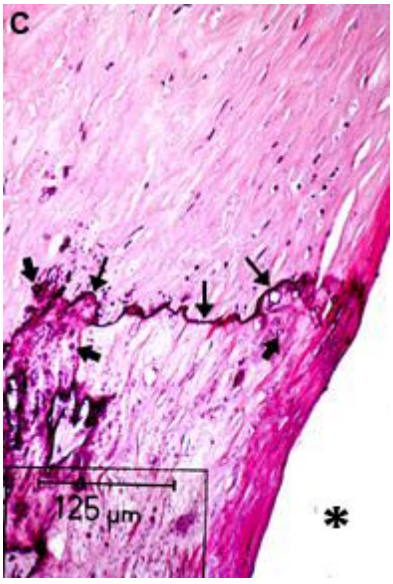
The tiny focus of calcification shown in Figure 11B is composed of many matrix vesicles that have fused together in a rather random, disorganized fashion in an area of tissue degeneration containing many other isolated matrix vesicles. In contrast Figure 11C, the calcification process appears to be quite organized with the formation of a relatively uniform calcification front [93,94], surrounded by many matrix vesicles. The calcification front identifies the dividing line between viable and nonviable tissue. Note how the degenerating fibrous tissue in Figure 11C serves as scaffolding for calcium deposition and how the basic fibrous architecture is maintained after calcification. Why an organized calcification front develops in some plaques while in others tiny foci of calcification are scattered throughout the plaque is not clear.

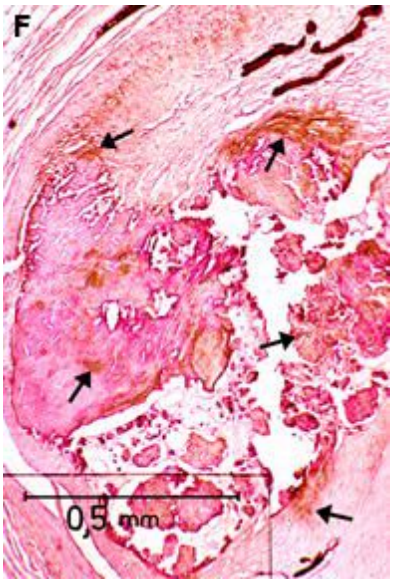

A
100 µm

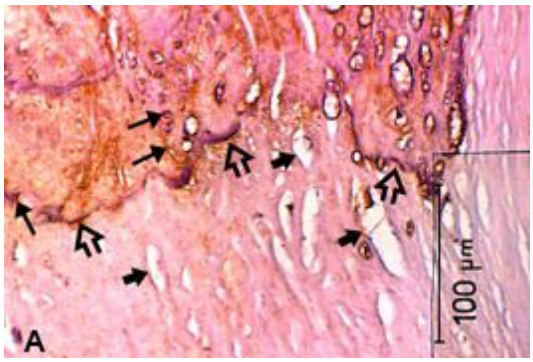

D
0,5 mm

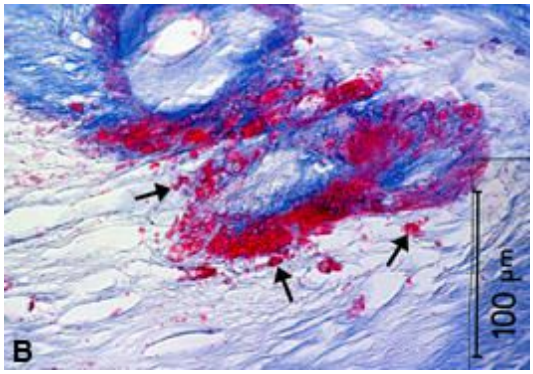

B
100 µm

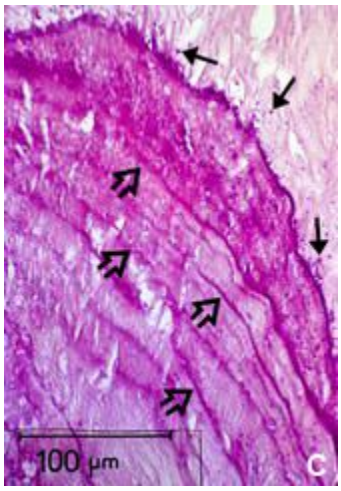

E
125 µm


C
125 µm
*









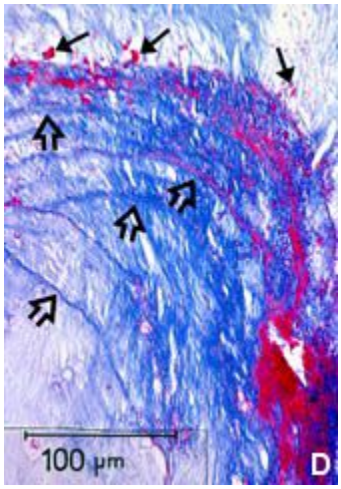
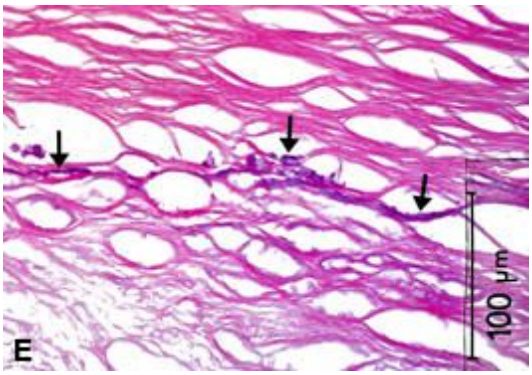
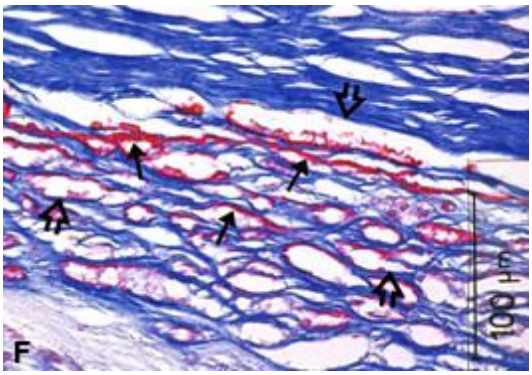
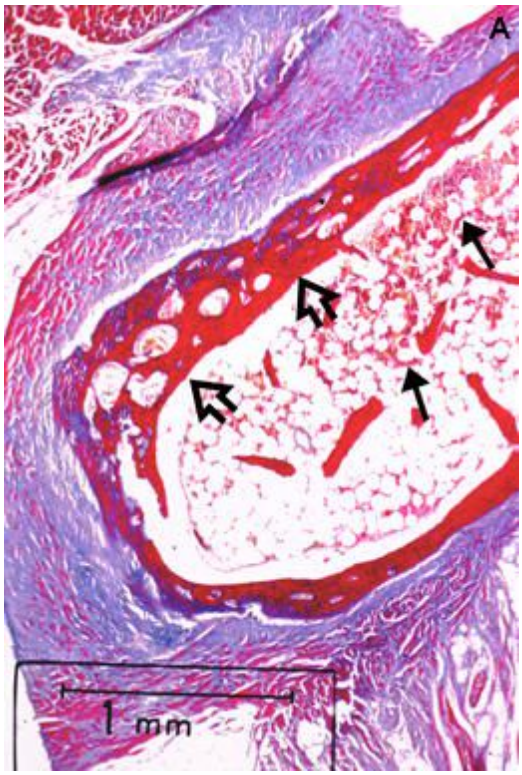


Figure 11: **A**, This section was taken from the proximal LAD coronary artery and shows “dead” SMCs containing intracellular matrix vesicles, clumped together and forming tiny foci of calcification. The cell spaces are of different size, suggesting adjacent cells have fused together (arrows). H & E stain. **B**, A small focus of calcification composed of multiple matrix vesicles that appear to have fused together (open arrow) in an area of cellular and tissue degeneration. Cell loss and degeneration of fibrous structure have occurred with many matrix vesicles in the area (solid arrows). H & E stain. **C**, Calcified plaque lying adjacent to the lumen of a 68-year-old female. Note the relatively uniform calcification front (thin arrows) and the presence of numerous matrix vesicles (fat arrows) surrounding the front. Viable tissue is above the front and calcified tissue below. The tissue architecture is preserved and serves as a scaffolding for the advancing front. Asterisk = lumen. H & E stain. **D**, Low-power view of a calcified plaque (solid arrows) and an adjacent mass of amorphous globular material (open arrow), staining red with MSB stain. **E**, Higher-power view of the material in **D**, showing its “knobby” appearance and attachment to the calcification front (arrow). **F**, Monoclonal antibody staining for osteopontin of the osteoid-like tissue shown in **D** & **E**. A brown, positive staining reaction for osteopontin (arrows), is present throughout this tissue, suggesting active calcium deposition.







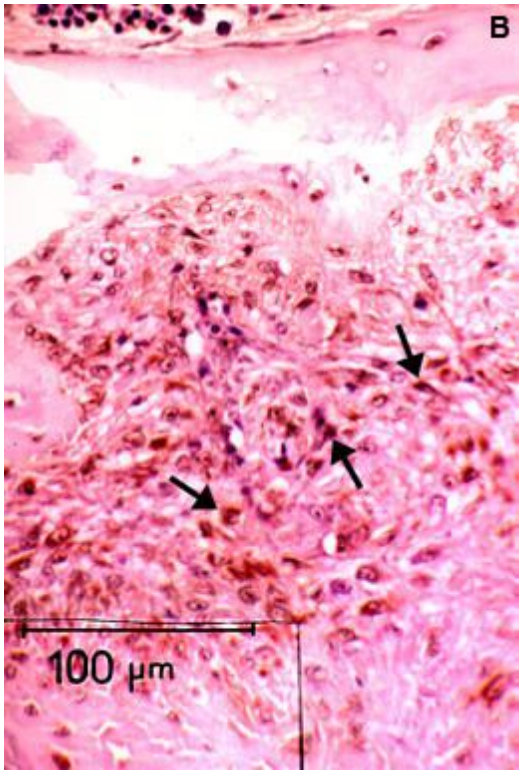


Figure 12: **A**, A calcified plaque stained for osteopontin (brown reaction product), illustrates positive staining of the calcification front (open arrows), the surrounding matrix vesicles (thin arrows), and lipid laden SMCs (fat arrows). **B**, A focus of calcification showing a large number of matrix vesicles (arrows) congregating and fusing along a calcification front. The matrix vesicles stain red, in the same manner as they do in degenerating cartilage in Figure 13D, with the MSB stain. A calcified plaque shows multiple calcification layers (open arrows), suggesting appositional growth. **C** (H&E stain) and **D** (MSB stain). Clumps of matrix vesicles (thin arrows) along the outer layer suggest an active, advancing calcification front. **E**, High-power view of a calcification front (arrows) with matrix vesicles within SMC spaces. H & E stain. **F**, Same area as in **E** with MSB stain, demonstrating positive MSB staining material within the degenerated SMCs (solid arrows), as well as fusion and enlargement of the lacunar spaces (open arrows).

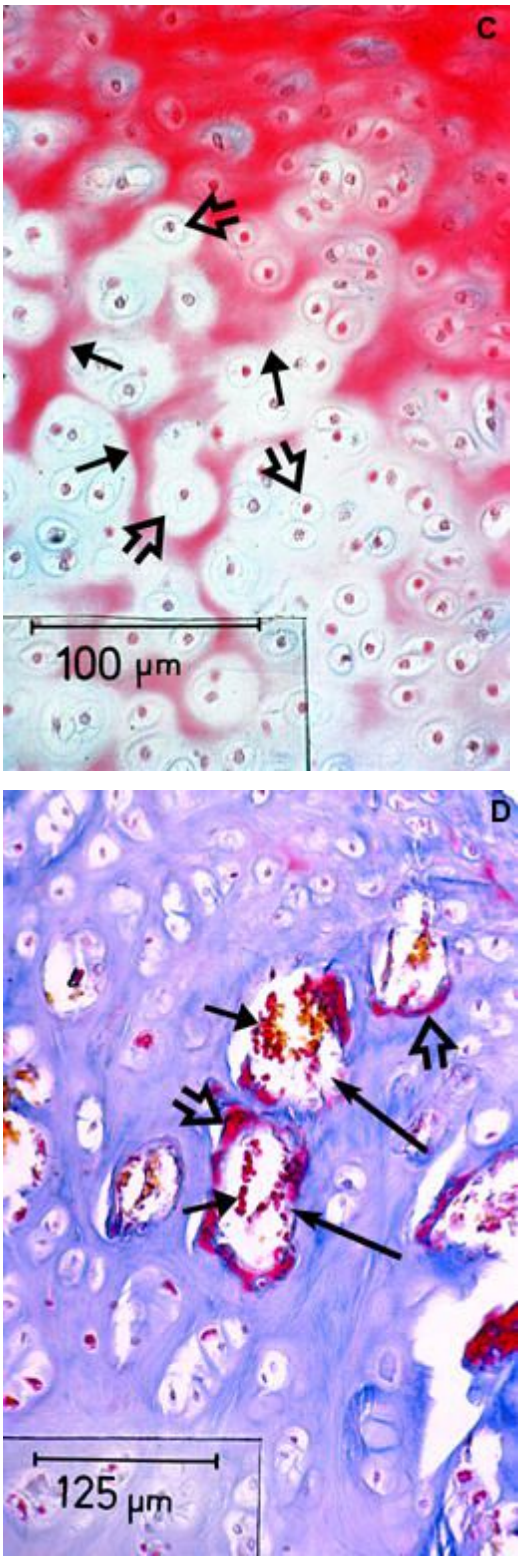


Figure 13: All sections in this figure were taken from the bone of the sheep heart. **A**, Low-power view showing marrow elements (short arrows) and bright red staining of cancellous bone (open arrows). MSB stain. **B**, High-power view of the periosteal layer of the bone shown in stained for osteopontin, showing osteoblasts contain large amounts of osteopontin (arrows). **C**, Interface between cartilage and bone Note chondrocytes (open arrows) and surrounding cartilage do not stain red with the MSB stain, but the encroaching osteoid, cancellous bone, stains deeply red (solid arrows) similar to the red staining matrix vesicles in SMCs in Figure 12E & 12F. **D**, Degenerated chondrocytes (long arrows) containing matrix vesicles (short arrows) that stain red with the MSB stain suggest that osteoid and bone matrix proteins (open arrows) line the margins of the lacunae.

Osteoid

Normal bone formation requires osteoid, the organic, unmineralized matrix of bone produced by osteoblasts. Osteoid is composed of matrix vesicles, osteopontin, collagen, and other non-collagenous bone matrix proteins. It often has a knobby appearance. The deposition of calcium salts in both normal bone formation and in vascular calcification is believed to begin and progress under the influence of osteopontin and other bone matrix proteins, which act as mediators of the calcification process [74,93,94,99]. Once osteoid is formed, calcification is believed to progress rapidly, within one or two weeks, with rapid disappearance of the osteoid [99]. Although osteoblasts involved in bone formation have not been identified as such in atherosclerotic plaques, several investigations have shown pericytelike cells, possibly originating in SMCs, within the plaque. These function much like bone osteoblasts [77,78,92,100]. It is reasonable to believe these pericytelike cells have the ability to produce osteoid.

Osteoid and Atherosclerotic Calcification

Calcification begins with the exposure of HA in the matrix vesicles to osteoid [99] and to bone matrix proteins that are found in atherosclerotic plaques, but not in normal vessel wall [93]. Osteopontin is synthesized by vascular SMCs, macrophages, and endothelial cells [100], has calcium-binding sites, is

regulated by cytokines, binds readily to HA, and is found at the sites of early calcification [74,99,101]. If atherosclerotic vascular calcification and bone are similar in formation and composition, then osteoid or osteoid-like tissue should be present in atherosclerotic plaques. Demer [99] believes some of the atherosclerotic matrix resembles osteoid, but osteoid, per se, has not been identified in calcified atherosclerotic plaques.

Figures 11D–11F, are examples of tissue in the wall of a calcified coronary artery that has features consistent with osteoid. These deposits consist of an amorphous, relatively acellular mass of tissue, staining bright red with MSB stain, knobby in appearance, and located adjacent to a calcified plaque. Immunohistochemistry staining of this tissue with osteopontin monoclonal antibodies shows intense staining, (Figure 11F), suggesting this material may be osteoid. Connected to the amorphous mass is a calcification front, also staining bright red with MSB (Figures 11D, 11E), and also with osteopontin antibody, Figure 12A. The amorphous mass and the calcification front may be related to and composed of the same tissue. If this amorphous tissue is osteoid, then it may be a site of active calcification and of an active expanding, spreading calcification front.

Figures 12C, 12D illustrate layering, apparently produced by an advancing calcification front. This appositional growth of the calcified plaque is similar to the appositional growth of bone. Note that the leading edge of the calcification front and associated matrix vesicles stain red with MSB, but those residual calcification fronts behind the leading edge, not surrounded by matrix vesicles, do not stain red with MSB. Therefore, a calcification front staining red with MSB and surrounded with similarly staining matrix vesicles, (Figures 12 B-F), may indicate active calcification is occurring, and the calcification front is advancing. If these features are absent, active calcification may have ceased. The MSB stain may be a convenient method of identifying osteoid in the artery wall. To further explore the possibility that this amorphous tissue in the artery wall may be osteoid, we performed similar stains on normal bone.

Normal Bone and Cartilage

Figure 13 illustrates the staining features of normal bone, cartilage, osteoid, and matrix vesicles in the bone and cartilage found within the sheep heart [102]. Cancellous bone, Figure 13A, is immature bone, recently formed from osteoid, that stains bright red with MSB like the amorphous masses in Figures 11D, 11E. Figure 13B shows osteoblasts at the periosteal surface, the site of new bone formation, stain strongly with osteopontin antibody, consistent with active bone matrix formation. Normal cartilage, (Figure 13C), does not stain red with the MSB stain, confirming that normal cartilage does not contain osteoid. However, osteoid-like tissue appears to be advancing into and replacing the cartilage. Finally, in Figure 13D, the matrix vesicles, like the matrix vesicles in Figure 11A, are contained within degenerating chondrocytes, and stain bright red with MSB. This is strong evidence that the amorphous mass, staining bright red with MSB stain in Figures 11D and 11E, is osteoid.

Evolutionary Purpose

What is the significance or evolutionary purpose of bone or bone-like deposits in the wall of an atherosclerotic artery?

Why is it necessary that these calcific deposits be bone, formed by a very complex series of biochemical reactions, rather than, say, by the passive precipitation of salts [99]? Is there a larger purpose here than just the calcification of injured, degenerating tissue? Is vascular calcification a physiologic

defense against the action of the IA or is it a pathologic process, a complication, of atherosclerosis, that should be prevented, as theorized by some investigators [82,83]? If vascular calcification is the same as normal bone, formed by the same biochemical reactions as normal bone, it is difficult to understand how this physiologic process suddenly becomes a pathologic process, a complication that must be prevented.

Calcification, in general, is often regarded as an important component of the healing process, generated as a physiologic defense designed to contain, wall off and stabilize injured or damaged tissue and/or foreign agents [99]. Calcification of a ghon complex is an example. Atherosclerotic calcification, however, appears to have a different purpose because the IA is not sequestered or walled off. In fact, calcification appears to have little if any direct influence on the activity of the IA because the disease continues to progress. The preservation of the fibrous tissue architecture behind the calcification front, as illustrated in Figures 11C and 12A, indicates the injured and degenerated tissue was calcified quickly, before the onset of necrosis. If there is prompt formation of pericytes or osteoblast-like cells and these cells produce osteoid, calcification of degenerated fibrous tissue by an advancing calcification front could occur rapidly, utilizing the same biochemical mechanisms as normal bone formation that are already present. The rapid calcification of degenerating fibrous tissue may be viewed as a physiologic defense that can be mobilized relatively quickly and efficiently.

Therefore, one evolutionary purpose of vascular calcification may be to preserve the fibrous tissue architecture by rapidly calcifying this tissue to maintain wall integrity, delaying the onset of necrosis. It is important to remember that necrotic tissue, per se, does not undergo calcification, primarily because necrotic tissue has no structure or scaffolding for the deposition of calcium salts. A second evolutionary purpose of calcification may be to delay the longitudinal spread of the IA by creating barriers between adjacent plaques, preventing or delaying the formation of cleavage planes between plaques and adjacent necrotic cores. Atherosclerotic calcification is an abnormality, but that does not prove it is pathologic in nature. Seen from the standpoint of a threatening, advancing, destructive, IA, calcification may be an essential physiologic defense, in delaying destruction of the artery wall.

Reversibility of Coronary Calcification

Several histologic observations suggest coronary calcification is reversible. Patients who died from malignant disease have a very low frequency of coronary calcification, indicating that calcification may be reversed in wasting conditions [75]. Clinical studies of patients receiving HMG-CoA reductase inhibitors to lower blood cholesterol show a reduction in calcium score on serial testing with EBCT [79,80]. Apparently the activity of the IA can be slowed or arrested, and calcification can be reversed. Figures 14A–14D, are typical calcified plaques from two patients of different ages. In Figure 14A and B the central portion of the calcified plaque contains many lacunae, of different size, apparently formed from dead SMC, that appear to be fusing or coalescing as a result of degeneration, destruction, or resorption of the calcified fibrous septa between them. In Figure 14C and 14D, these lacunae have fused to form a small lipid lake presumably formed by the fragmentation, erosion, and dissolution of the calcified, lipid-rich, fibrous tissue. Resorption, destruction, or dissolution of the central portion of calcified plaques may have important implications for plaque progression.

What is the mechanism of this apparent resorption? Several possibilities can be considered. First, Jeziorska, et al [103], found multinucleated giant cells in close apposition to carotid calcification that demonstrated all the normal features of bone formation and resorption. Vascular calcification may seemingly undergo resorption in the same manner as bone. Second, bone undergoes resorption and avascular necrosis when deprived of its blood supply. The same may be true of vascular calcification that has no nutrient blood supply [104]. Third, SMC produce and contain MMPs. These enzymes may remain active after the SMC dies and be a significant factor in the degradation and resorption of calcified vascular tissue [94,105], particularly when resorption originates within the central part of calcified plaque. Vascular calcification may not be a permanent or inert deposit, but an active, dynamic, changing structure capable of being reversed, removed, and/or remodeled [99]. If this is correct, then all calcified plaques have the potential to undergo reversal and resorption.

Figure 15A shows a large, circumferential calcified plaque containing a similar circumferential, structure-less, necrotic core. Figure 15B, in contrast, shows a very small, calcified plaque with similar findings, showing that such a structure-less central core can develop in calcified plaques of any size. Figure 15C illustrates a large necrotic core with a tiny rim of focal calcification that has an irregular, moth-eaten border facing the necrotic core. This calcified rim appears to be undergoing digestion or destruction, presumably by substances contained within the core. This calcific deposit does not stain bright red with the MSB stain, suggesting osteoid formation and active calcification are not taking place. This rim of calcification may be receding, not forming.

Support for this view is provided in Figure 15D, another calcific deposit at the rim of a necrotic core, but stained with osteopontin monoclonal antibody. Again, we have the irregular moth-eaten border consistent with degeneration and resorption, and this degeneration is associated with free-floating fragments within the necrotic core, also staining positive for osteopontin. The calcific deposit appears to be breaking up rather than forming. The rim of calcification commonly found at the edge of a necrotic core may be a remnant of a previously calcified plaque and the necrotic core may have originated and been formed by the resorption and dissolution of the calcified plaque. If this is correct, then the chemical agents and biologic compounds that are by-products of calcification resorption will be added to all the other compounds present within the necrotic core. Since HA is very irritating when injected into joints [96] it is possible that it may also be very irritating and toxic when present in the necrotic core.

What role, if any, does plaque calcification play in the formation of the necrotic core? What is the sequence of events in the growth and development of the calcified plaque? What comes first, the necrotic core or calcification? How can necrosis and the formation of an atheroma occur before calcification, as suggested by Stary, et al. [15], when matrix vesicles form at the time of SMC death, fuse, form calcific deposits, and calcify degenerated fibrous tissue well before the onset of actual necrosis? The presence of microcrystalline calcium within a necrotic core [15] suggests calcified tissue was digested in the formation of the core and that calcification preceded the development of necrosis.

Evidence presented shows that calcified plaques may be reversible. This reversal appears to result in the destruction and dissolution of the lipid-rich scaffolding of fibrous tissue, and leads to the formation

of a structureless central core that, in time, may lead to the formation of a necrotic, atheromatous core.

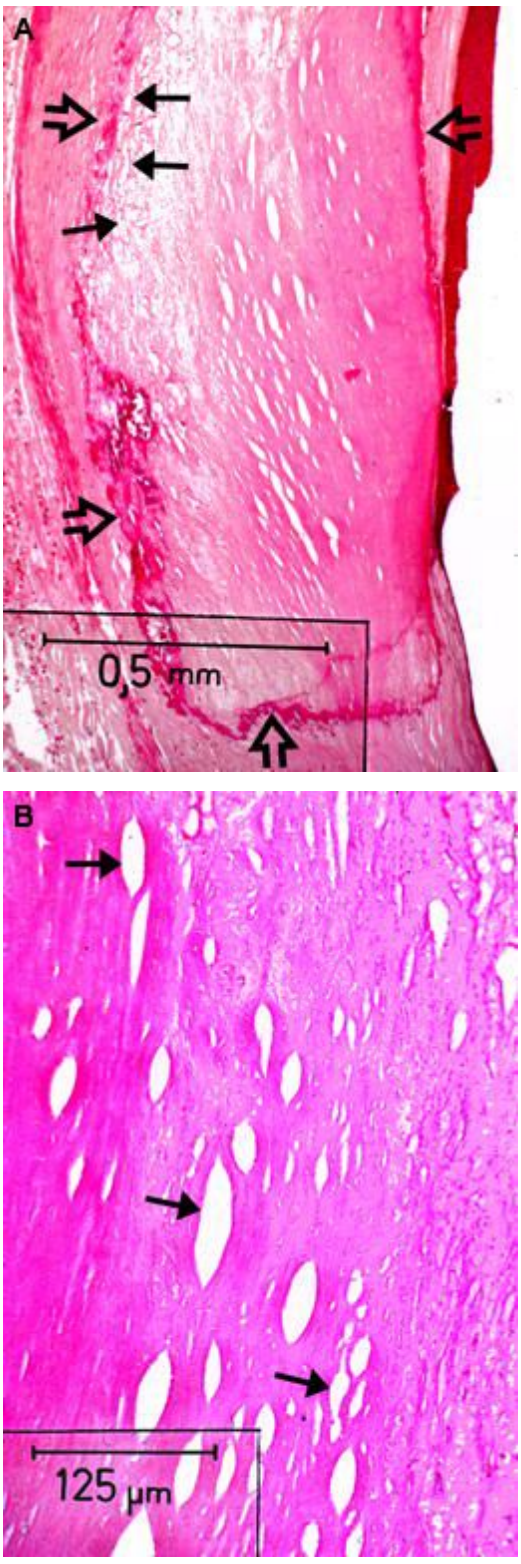
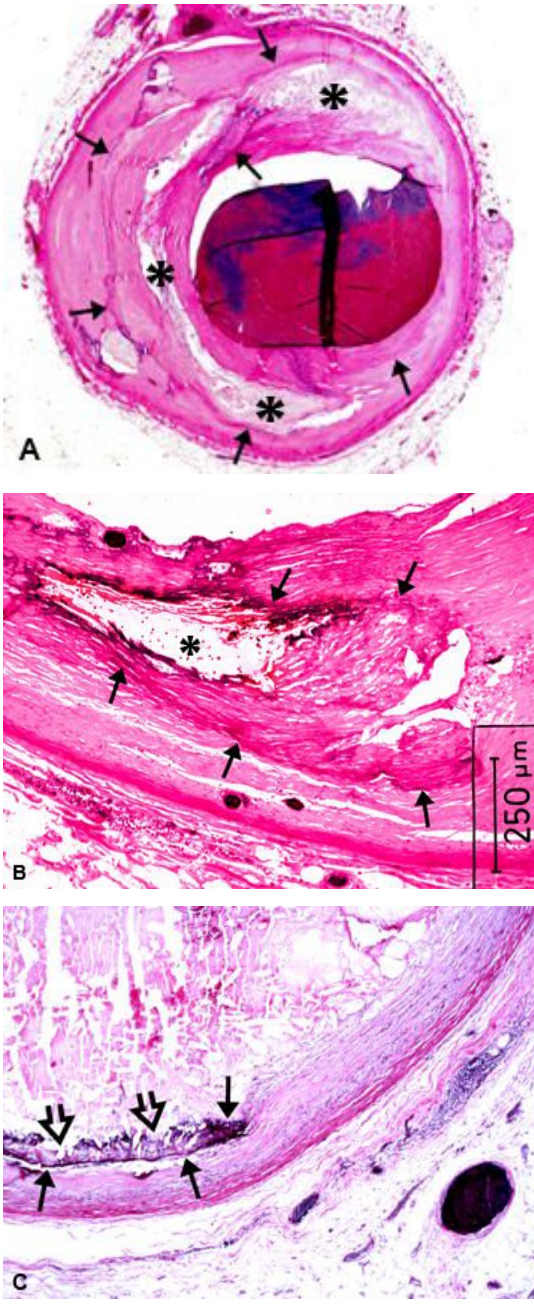


Figure 14: **A**, Low-power view of a calcified plaque (open arrows) from the CIRC coronary artery of a 51-year-old male. The calcified fibrous tissue appears to be undergoing degeneration, and the lipid-laden lacunar spaces fusing together (solid arrows). **B**, High-power view of the same plaque as **A**.

Lacunar spaces vary in size, presumably formed by the fusion of adjacent lacunae formed from dead SMCs. H & E stain. **C**, A calcified plaque showing fusion and enlargement of lipid-laden lacunae (solid arrows) forming a small lipid lake (open arrow). **D**, High-power view of rectangle in **C** showing dissolution of the calcified fibrous tissue (solid arrows) and fragmentation of tissue at the edge of the lipid lake (open arrows). H & E stain.



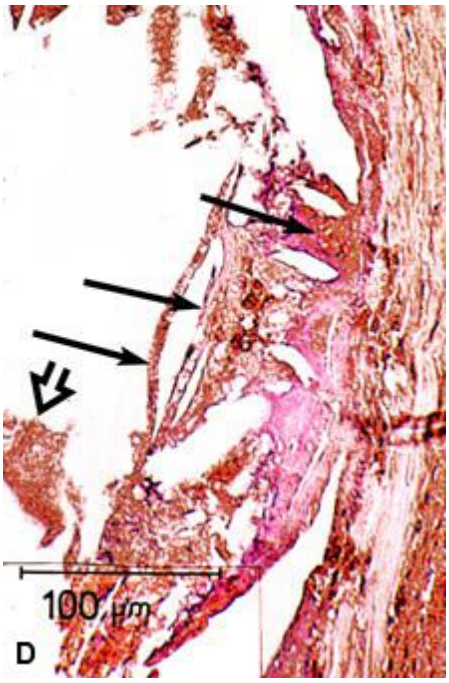


Figure 15: **A**, Section from the mid-LAD coronary artery in a 58-year-old male. A large circumferential calcified plaque (arrows) surrounds a large lipid rich necrotic core (asterisks). H & E stain. Magnification x11.5. **B**, Small calcified plaque (arrows) in the distal RCA of a 58-year-old male, showing a small structureless central core (asterisk). H & E stain. **C**, Large necrotic central core with small rim of calcification (solid arrows). Note the irregular, moth-eaten border (open arrows) that faces the core. MSB stain. **D**, Small calcium deposit at the rim of a necrotic core (solid arrows), immunostained for osteopontin (brown reaction product), showing that these calcium deposits stain intensely for osteopontin. Note that the loose fragment (open arrow) also stains positive for osteopontin.

Sequence of Events

Insight into the sequence of plaque development can be gained by reviewing Table 1 in Chapter 3, Table 2 in Chapter 4 and Table 3 in this chapter. These tables show 51% of all coronary segments have inflammatory cell infiltrates, Table 1; 38% show calcification, Table 3; and 32 % show the presence of a necrotic core, Table 2. The frequency of calcification in Table 3 is greater than the frequency of a necrotic core at all levels of stenosis, whether <50% stenosis, 50—80% stenosis, or >80% stenosis. Inflammation develops first following the injury, followed by calcification and then by

the formation of a necrotic core. This evidence supports our view that many atheromas have their origins within a calcified plaque. The reversal or resorption of plaque calcification may explain why EBCT studies are not reproducible, and why plaque volume cannot be quantitated from the amount of calcification present [88].

In Review

Calcification of the coronary arteries identifies the site and extent of atherosclerotic involvement. Calcification is a component not a complication of atherosclerosis. Uninterupted blocks of calcification indicates the IA spreads in a longitudinal direction and adjacent plaques fuse together. Age plays no direct role in the pathogenesis of calcification. The amount of calcification reflects the magnitude, extent and severity of the injury caused by the IA. The amount of calcification tends to reflect plaque size, but is not sufficiently accurate to predict the severity of luminal stenosis. Calcification does not detect the current activity of the IA or the site of currently active disease, limiting its clinical usefulness. The absence of calcification is strong evidence against the presence of active disease even with significant luminal stenosis. Calcification plays some role in PU.

Vascular calcification is believed to utilize the same biochemical processes and substances as in normal bone formation, and the composition of both bone and vascular calcification is similar. Matrix vesicles derived primarily from degenerating SMCs act as nucleators for the deposition of calcium salts in atherosclerotic plaques, with calcification commonly occurring on a scaffolding of degenerated fibrous tissue, in the same manner as bone forms on the scaffolding of degenerating cartilage. Calcification serves to preserve the integrity of the artery wall, delaying and/or retarding the spread of the IA. Calcification is reversible and may contribute to the formation of an atheroma by adding the byproducts of resorption to the necrotic core. The sequence of events in plaque development following injury is inflammation, followed by calcification of the damaged tissue, ending ultimately in the formation of a necrotic core.