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Spontaneous Atherosclerosis in Pigeons

A Model System for Studying Metabolic Parameters Associated with Atherogenesis

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The interpretation of metabolic studies related to early changes in spontaneous atherosclerosis has been hampered by the focal nature of the disease and by the lack of a well-defined model system of the disease process. Gross, histologic and ultrastructural observations of lesion development at the celiac bifurcation of the aorta in atherosclerosis-susceptible White Carneau and atherosclerosis-resistant Show Racer pigeons are compared and discussed in terms of hemodynamics, muscular aggregation and altered metabolism of smooth muscle cells. Detailed knowledge of the morphologic sequence of events in lesion localization makes the celiac bifurcation in White Carneau and Show Racer pigeons a useful model for genetic comparisons of arterial wall metabolism and for investigating metabolic alterations occurring with atherogenesis. (Am J Pathol 67:1–22, 1972)

CORRELATION OF THE MANY FACTORS reportedly influencing atherosclerosis has proven difficult in experiments with intact animals. Diet, blood lipids, environmental stress, hemodynamics, alterations in arterial structure, and metabolism are but a few of the factors implicated in initiating atheromatous changes. Moreover, interpretation of reports associating metabolic alterations in the intact aorta with atherosclerosis has been hampered by the focal nature of the disease and by an inability to follow these alterations through transition stages in lesion development.[1] In an effort to control these variables, much research has been done with experimental atherosclerosis as a model system.

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However, spontaneous atherosclerosis has been shown to differ from the experimentally induced disease in many respects [2], and there are no well-defined model systems for study of the spontaneous process.

Clarkson, Lofland, Prichard and co-workers [3–8] have described spontaneous aortic atherosclerosis in White Carneau pigeons and pointed out its close resemblance to the human disease. Cooke and Smith [9] subsequently described ultrastructural aspects of normal and diseased pigeon aortas. Differences in susceptibility between inbred strains of pigeons have been utilized to study relationships between various metabolic patterns and atherosclerosis.[10–12]

This communication extends previous work by describing the sequential development of atheromatous lesions in the celiac bifurcation of aortas from atherosclerosis-susceptible White Carneau and atherosclerosis-resistant Show Racer pigeons. Lesion development is related to histology, topography, hemodynamics, and intimal thickening. Spontaneous atherogenesis in the celiac bifurcation is presented as a model system for the study of metabolic parameters in the arterial wall that may be associated with the disease.

Materials and Methods

Subjects

Forty-three White Carneau (WC) and 30 Show Racer (SR) pigeons were examined. The majority were between 1 and 6 years of age with a few embryos and post-hatch squabs included. Sexes were nearly equally represented. All birds were derived from inbred lines maintained by the Palmetto Pigeon Plant, Sumter, SC. Most of the birds older than 1 year were housed in fly-coops, and the 1-year-old birds were reared in battery cages with approximately 2 sq ft of floor space per bird. The pigeons were fed either a mixture of yellow corn, wheat, peas, kafir and health grit or Purina pigeon pellets and similar health grit.

Light Microscopy

All birds were sacrificed by exsanguination. The entire aorta from the arch down to, but usually not including the trifurcation, was removed, washed in warm (37 C) buffered saline, pH 7.4, cleaned of excess connective tissue, and fixed at room temperature for 30–90 minutes in 3% phosphate-buffered glutaraldehyde, pH 7.4. After fixation, aortas were rinsed briefly in saline, immersed in Tissue-Tek O.C.T. Compound (Ames Company, Miles Laboratories, Inc, Elkhart, Ind) and quick-frozen at −18 C. A serial survey of each aorta was made by cutting two or three 8-μ cross sections every 700–800 μ; the sections were stained with hematoxylin-eosin, hematoxylin–oil red O (ORO), and sometimes Alcian blue–safranin O.

A severity index was obtained for each aorta by comparing hematoxylin-ORO stained sections with a series of 6 grades ranging from 0 to 5 (see Fig 7–11). The grading system was based on the following five criteria:

1. Amount of lipid present.
2. Extent of proliferation.
3. Amount of luminal circumference affected.

4. Degree of luminal occlusion.
5. Extent of necrosis, calcification and vascularization.

Although the entire aorta was surveyed, the celiac bifurcation demonstrated the most severe lesions and most important breed differences at 6 years. For this reason the detailed study of lesion development in this segment was undertaken.

Electron Microscopy

Advanced plaques were fixed in 3% glutaraldehyde—0.1 M cacodylate buffer, pH 7.3, washed in cacodylate-buffered 6% sucrose, postfixed in 2% osmium-Veronal, pH 7.3, dehydrated in a graded series of alcohols and embedded in Epon. Adjacent thick (1 μ) and thin sections were cut on a Porter-Blum MT-2 ultramicrotome. Thin sections were stained with saturated uranyl acetate solution and Reynolds lead citrate, and examined with a Philips EM200 electron microscope.

Results

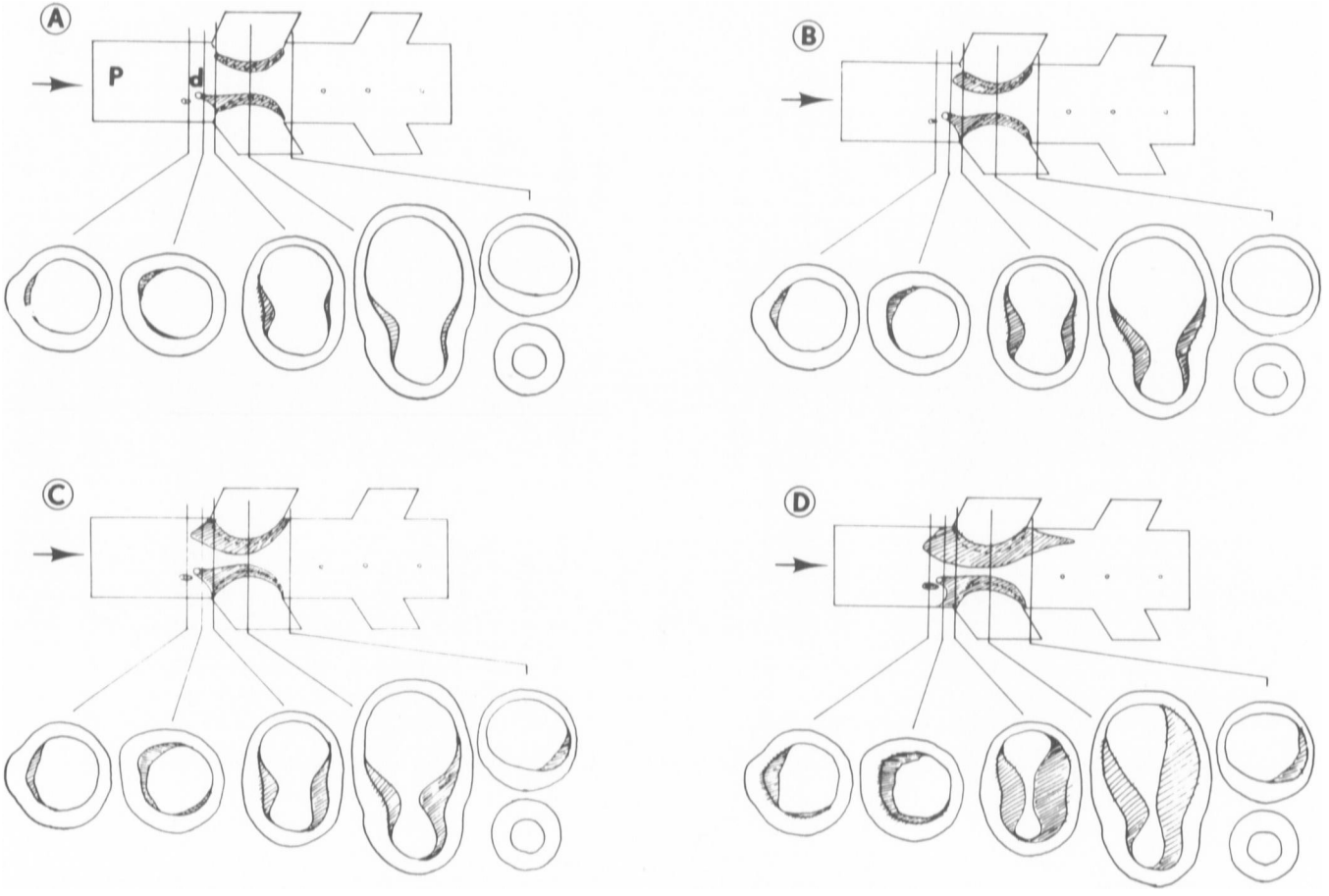
Gross Observations

In the aortas of squabs, prior to the appearance of lipid, ridge-like thickenings were seen arising from the lateral edges of the celiac orifice and extending diagonally in a proximal direction (Fig 1). At 4–6 months, the earliest visible lipid accumulation appeared as a fine white stippling on the surface of these cushions. As lesion development continued, the lipid became yellow and the surface of the area was raised and was either rough or smooth. At later stages, the main plaque, projecting well into the lumen, had a nodular, pearly appearance and was usually surrounded by a roughened, yellow skirt of involved tissue, sometimes fan-shaped and extensive in the proximal aspect (Fig 2). In advanced lesions, ulceration sometimes was evident, particularly in centrally depressed regions of the largest plaques.

The topography of lesion development in the celiac bifurcation was quite specific (Text-fig 1). In the young WC pigeon, lesions appeared earliest in the left lateral cushion lying below the ductus arteriosus. In older birds, lesion development in the right lateral cushion progressed more rapidly, and, by 6 years, involvement was greatest in this cushion. No consistent differences in lesion development between right and left cushions were seen in the SR. In both breeds, involvement of areas surrounding the cushions progressed mainly upstream and laterally.

Light Microscopy

A prominent feature of the celiac bifurcation in embryos and very young birds was the presence of paired muscular intimal thickenings corresponding to the ridge-like thickenings observed grossly (Fig 3). The smooth muscle cells in these cushions were oriented longitudinally and the laminar organization of elastica present in the media is



TEXT-FIG 1—Diagrammatic representation of lesion progression in the cushions of the celiac orifice. In *A* and *B*, the left cushion (*lower*) beneath the ductus arteriosus (*d*) is the most highly involved. The more rapid progression of the right cushion (*upper*) is depicted in *C* and *D*. Lesions progress mainly in the lateral and proximal direction (*P*). The extent of luminal occlusion can be followed in the cross-sectional diagrams. Cross-hatching represents proliferative growth with lipid accumulation. Direction of blood flow is indicated by arrows.

interrupted here (Fig 4). The muscular nature of these cushions was emphasized because only in these smooth muscle cell aggregations did significant lipid accumulate leading to the production of advanced plaques.

The earliest evidence of atherosclerotic change in 4- to 6-month-old birds was the appearance of fine lipid droplets in the endothelium and subendothelium of the cushion (Fig 5). Later, find lipid droplets became more prominent, appearing in the deeper regions of the cushion. Some proliferation of smooth muscle cells could also be seen at this time (Fig. 6). By 1 year, this proliferative response could be a distinctive feature (Fig 7A), and lipid involvement was frequently more extensive with some larger lipid pools (probably extracellular) accumulating (Fig 7B). Fragmentation of elastic laminae was also evident (Fig 7A).

With further development, luminal protrusion of the lesion was amplified, producing partial occlusion of the vessel. Large amorphous pools of lipid were found in necrotic centers of these plaques (Fig 8A and B). In this and later stages, lesions expanded not only luminally, but laterally and medially as well, by muscular transition of normal elastic areas.

Beyond this stage, most lesions developed a fibrous cap of modified smooth muscle cells. In regions underlying the fibrous cap, fatty degeneration and formation of cholesterol clefts were frequently seen. Luminal occlusion was a striking feature at this stage (Fig 9A and B). In the most advanced stages, a variety of features were commonly encountered: massive fibrosis (Fig 10A), sometimes nearly complete luminal occlusion, necrosis and further lipid accumulation, vascularization (Fig 10B), ossification (Fig 10C) and ulceration. No thrombi and very few lymphocytes or macrophages were seen. Accumulation of acid mucopolysaccharide was also commonly seen in advanced plaques.

Electron Microscopy

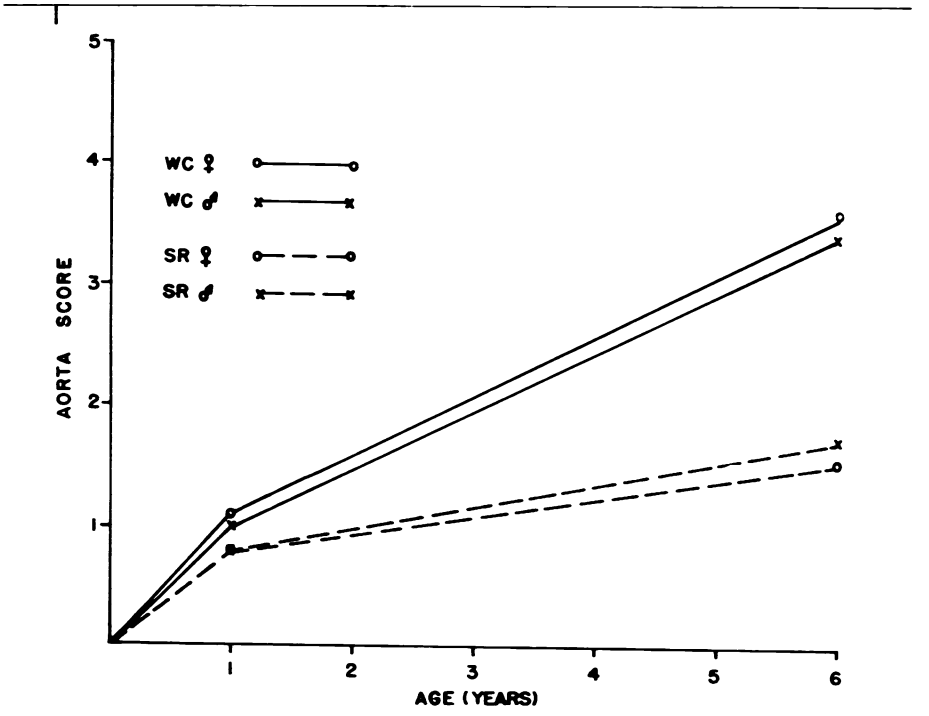
Ultrastructurally, the fibrous cap of advanced lesions contained long, spindle-shaped, modified smooth muscle cells, foam cells and prominent collagen bundles. Upper regions of the cap contained modified smooth muscle cells having few myofilaments with fusiform dense bodies, a patchy basement membrane envelope, typical pinocytotic vesicles along the cell membrane, numerous mitochondria, much granular endoplasmic reticulum and many polysomes and free ribosomes (Fig 11). Modified smooth muscle cells in deeper regions of the fibrous cap

contained a similar array of organelles, often including a prominent Golgi apparatus (Fig 12). However, these cells contained many more myofilaments with fusiform and marginal dense bodies, as well as a distinct basement membrane envelope. Many of the cells contained darkly stained inclusions. In both regions, the extracellular space contained little or no elastin, much collagen and a large amount of vesiculated material perhaps representing cell debris and extracted lipid.

Lesion Scores

Based on the severity index, there was little difference in atherosclerotic involvement between the two breeds up to 1 year of age. However, by 6 years, lesions in the celiac bifucation averaged 2 grades higher in the WC than in the SR (Text-fig 2). Furthermore, 100% incidence of some degree of histologic lipoidal involvement was found in both breeds of pigeons at 1 and 6 years. The developmental history and histopathology of atheroclerotic plaques of a given grade were similar for both breeds.

While there were no significant differences in severity between sexes



TEXT-FIG 2—Atherosclerotic grade in the celiac bifurcation of male and female White Carneau and Show Racer pigeons as a function of age. Lines between 1 and 6 years are least-square regression plots.

in either breed, the sample size was too small to be conclusive. A trend toward higher grades in the WC female and the SR male was found at 6 years (Text-fig 2).

Discussion and Conclusions

As McGill *et al*[1] pointed out, the lack of data on site-specific lesion development in humans has made it difficult to follow transition stages in atherosclerotic involvement. Our observations in the celiac bifurcation of the pigeon aorta show that fatty streaks can develop into fibrous plaques in a manner similar to that suggested for humans.[13,14]

Our findings are essentially similar to those in original reports on spontaneous atherosclerosis in pigeons by Clarkson, Lofland, Prichard, and co-workers.[3–8] However, several important distinctions were noted. While they reported a difference in incidence of atherosclerosis between the two breeds[4,7] our birds exhibited 100% incidence of some degree of involvement in the celiac bifurcation at both 1 and 6 years. We found the major difference between breeds to be a much lower severity index in the SR than in the WC at 6 years. No difference were found in incidence, location, developmental history or histopathology of WC and SR lesions, as has been reported.[4,7] These similarities suggest that the resistance of the SR may result from subtle metabolic factors.

The pattern of lesion localization and development at the celiac bifurcation is quite predictable. Similar findings have been reported for atherosclerosis in human cerebral and coronary arteries[15,16] and for arteriosclerosis of the lower extremities.[17] Hemodynamics undoubtedly plays a role in this localization as suggested by numerous workers.[18–23] The work of Caro *et al*[24] is most consistent with our observations. As predicted by their model, we find atheroma localized lateral to the celiac orifice and progressing upstream, thus suggesting the presence of low shear rates and poor nutrition due to decreased exchange of materials in these regions. However, hemodynamics is probably not the sole factor causing localization since unique metabolic capabilities have been associated with susceptible regions of the aorta in other animals.[25,26]

Another very important factor, apparently acting synergistically with hemodynamics to localize lesion development, is the presence of raised muscular cushions representing a normal feature of vascular architecture at the celiac bifurcation and elsewhere.[27–29] Muscular aggregations exhibit the earliest enzymatic changes associated with atherogenesis[30] and proliferative reactions induced by cholesterol feeding are also prominent there.[31] In our system, aggregations of smooth muscle cells seem to be a necessary prerequisite for lipid accumulation. Further

work on the development of cushions and the role of blood flow in vascular morphogenesis is needed.

The role of smooth muscle cells as the major cell type involved in the development of atherosclerotic lesions in a variety of animals, including cows,[32] swine,[31] rabbit,[33] baboons,[34] humans,[35] chickens[36] and pigeons,[9] has been well documented. Lesions expand by a proliferative response of normal smooth muscle cells adjacent to regions of necrosis in a manner similar to that seen in the healing of arterial wounds.[37] This study and others[9,31–36,38] suggest that modified smooth muscle cells found in early atheromatous lesions and even those found in fibrous caps of advanced lesions are very active metabolically. Such an interpretation is consistent with the presence of increased granular endoplasmic reticulum, many polysomal rosettes, a prominent Golgi apparatus, and numerous mitochondria in these cells. As Zemplenyi[30] has emphasized, the smooth muscle cell seems to dominate the metabolism of the normal arterial wall and of lesions at all stages of atherosclerotic involvement.

This study demonstrates that atherosclerosis in the celiac bifurcation is a predictable phenomenon in terms of histopathology, topography, age and breed differences. It should be possible to isolate metabolic changes associated with the spontaneous disease process in this model system. A better understanding of the role of energy production, altered enzyme patterns and acid mucopolysaccharide metabolism is needed. Unequivocal answers to these questions and others concerning the role of metabolism in atherogenesis will be obtained only through the use of such a defined model system.

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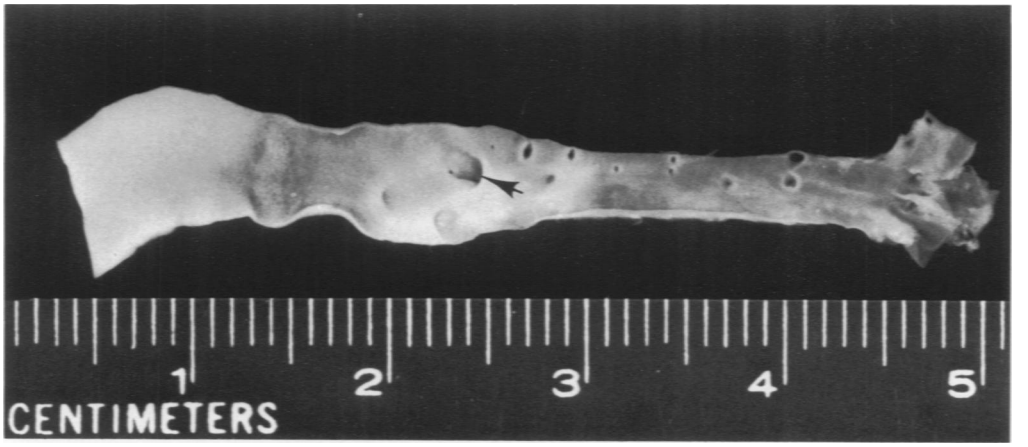

Fig 1—Noninvolved aorta with ridge-like thickenings extending diagonally proximal from the celiac orifice (*arrow*). White Carneau male, 3-weeks-old (unstained whole mount, × 2.6).

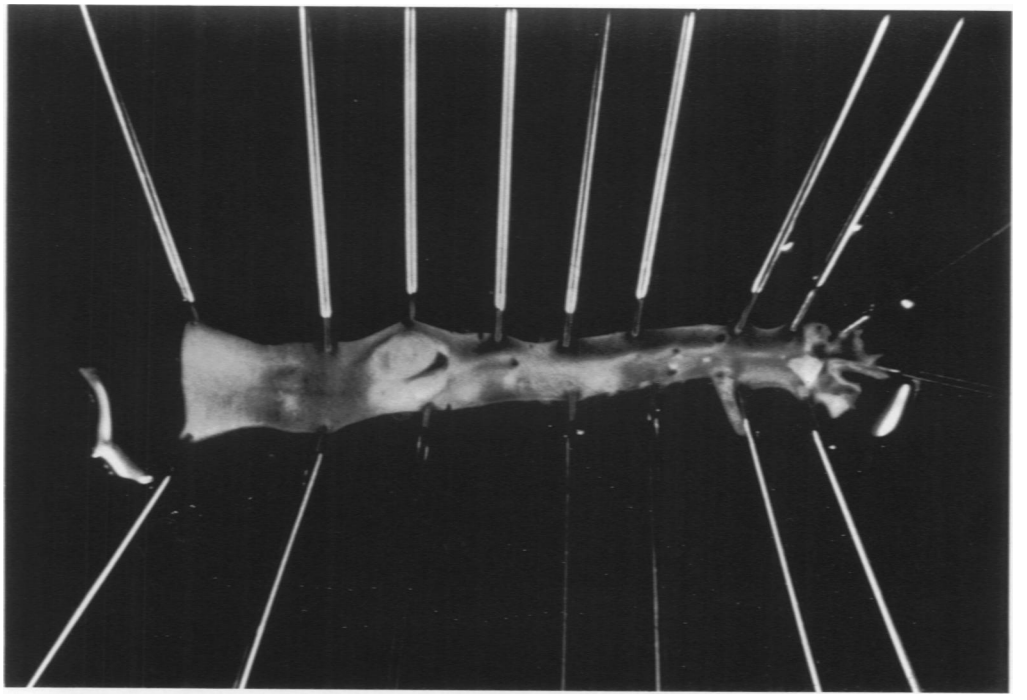

Fig 2—Two large plaques on either side of the celiac orifice. Smaller plaques are also present at the renal branches and in the trifurcation. White Carneau female, 5-years-old (unstained whole mount, × 2.1).

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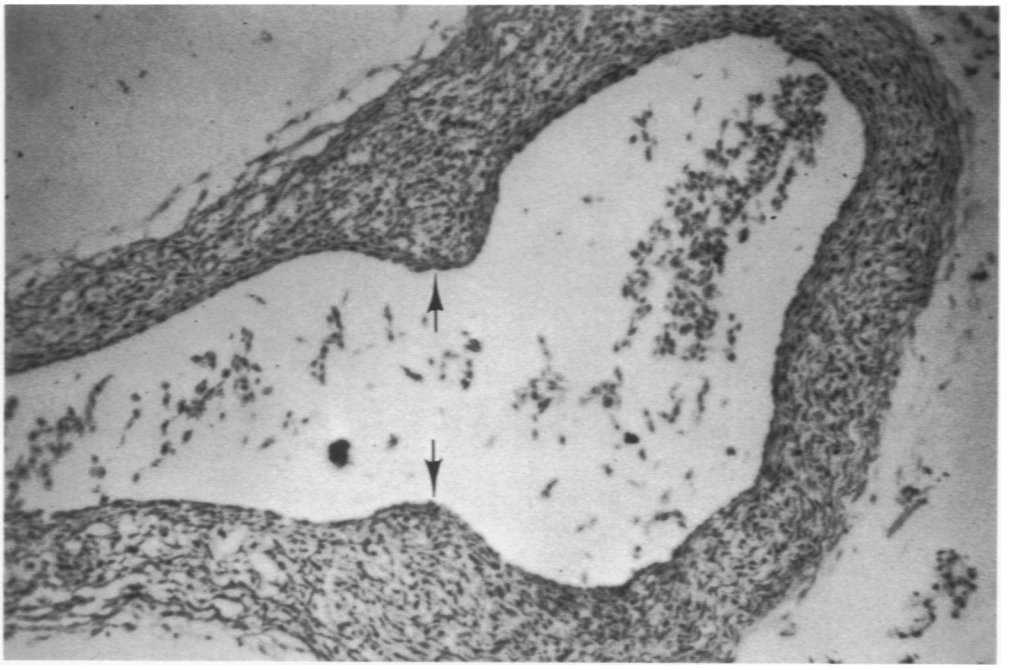

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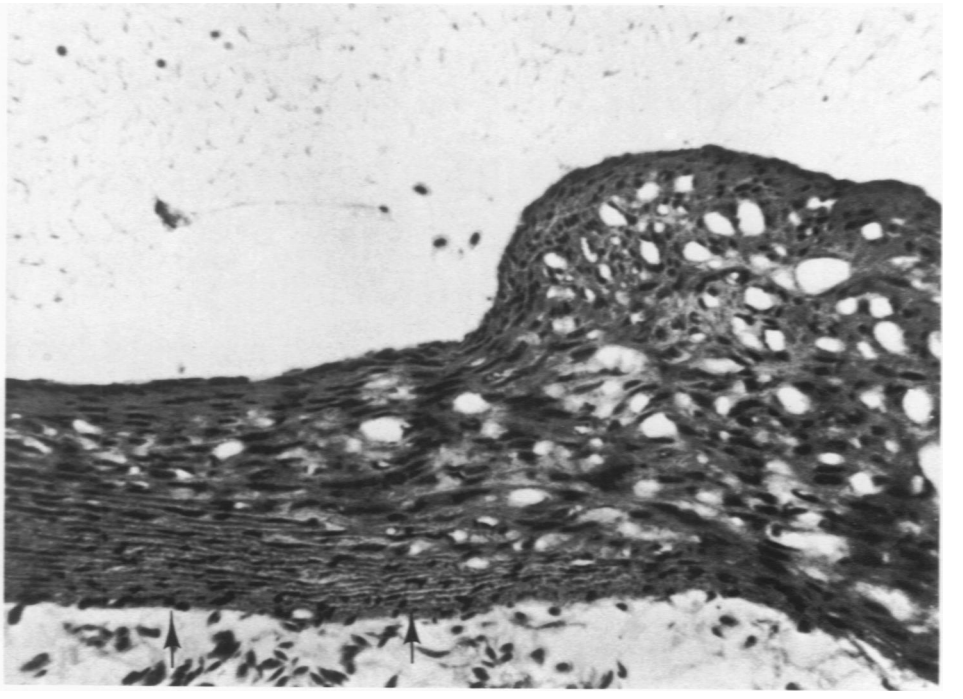
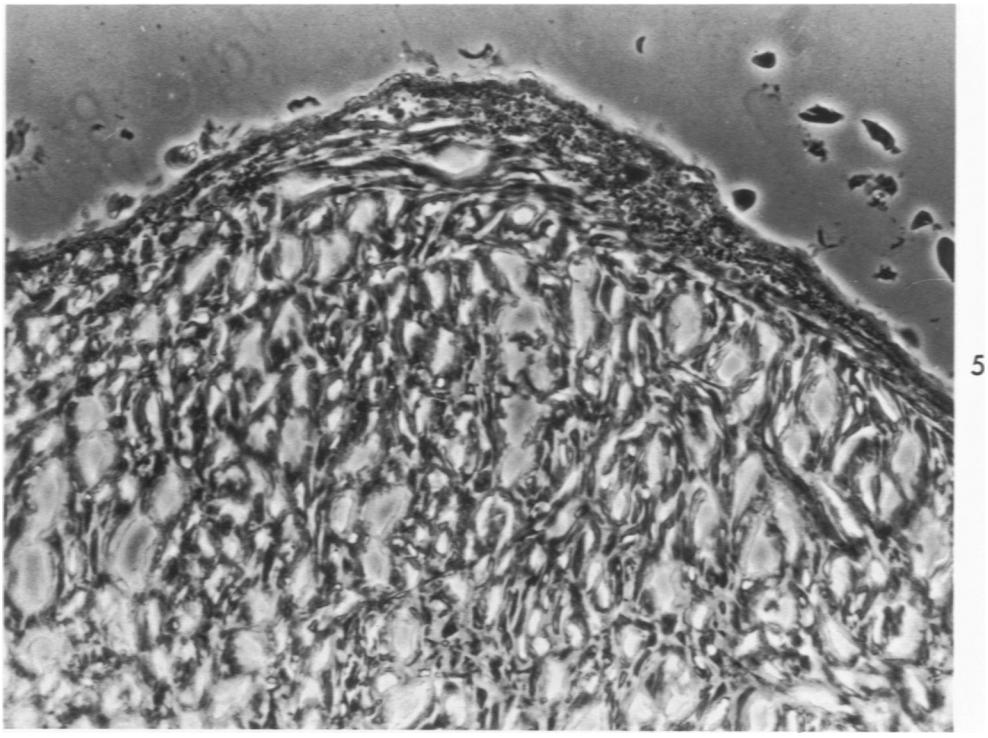


Fig 3—Cushions of smooth muscle cells protruding into the aortic lumen at the celiac bifurcation (*arrows*). White Carneau 10-day embryo (H&E, × 150). **Fig 4**—Normal cushion with circumferentially oriented smooth muscle cells. Laminar organization of elastic laminae at left (*arrows*) of figure is disrupted in the region of the cushion. Show Racer female, 4-weeks-old (H&E, × 330).



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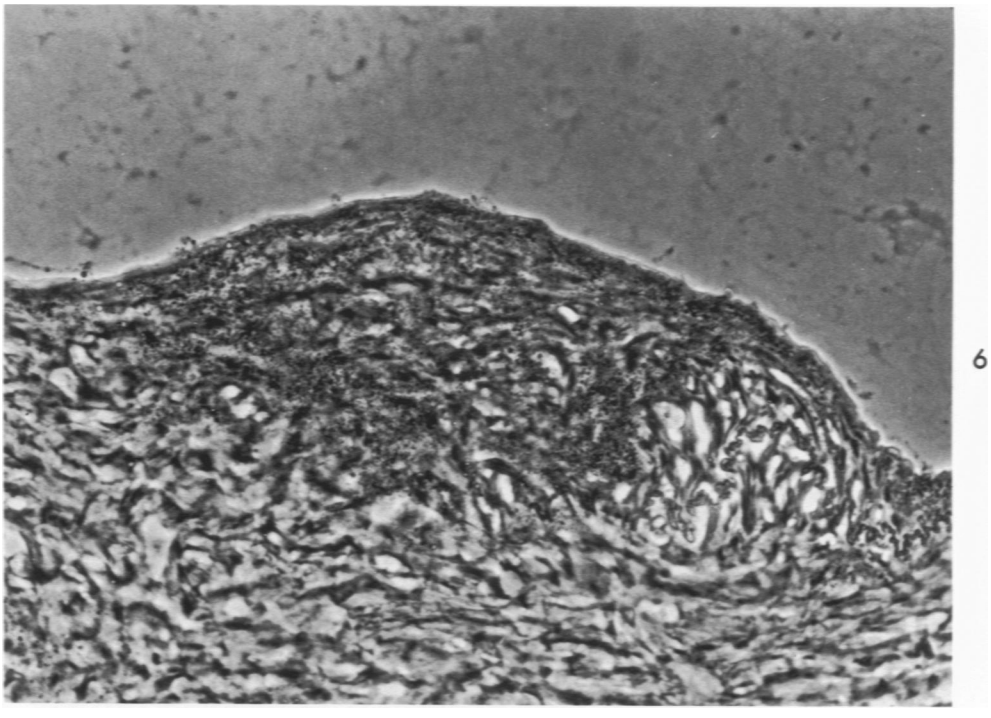

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Fig 5—Endothelial and subendothelial lipid deposition in an otherwise normal celiac cushion. White Carneau female, 3-months-old (hematoxylin–oil red 0, phase contrast, × 420).
Fig 6—Grade 1 lesion. Many fine lipid droplets are present, some in deeper regions. Slight proliferation is visible. Show Racer male, 6-years-old (hematoxylin–oil red 0, phase contrast, × 420).

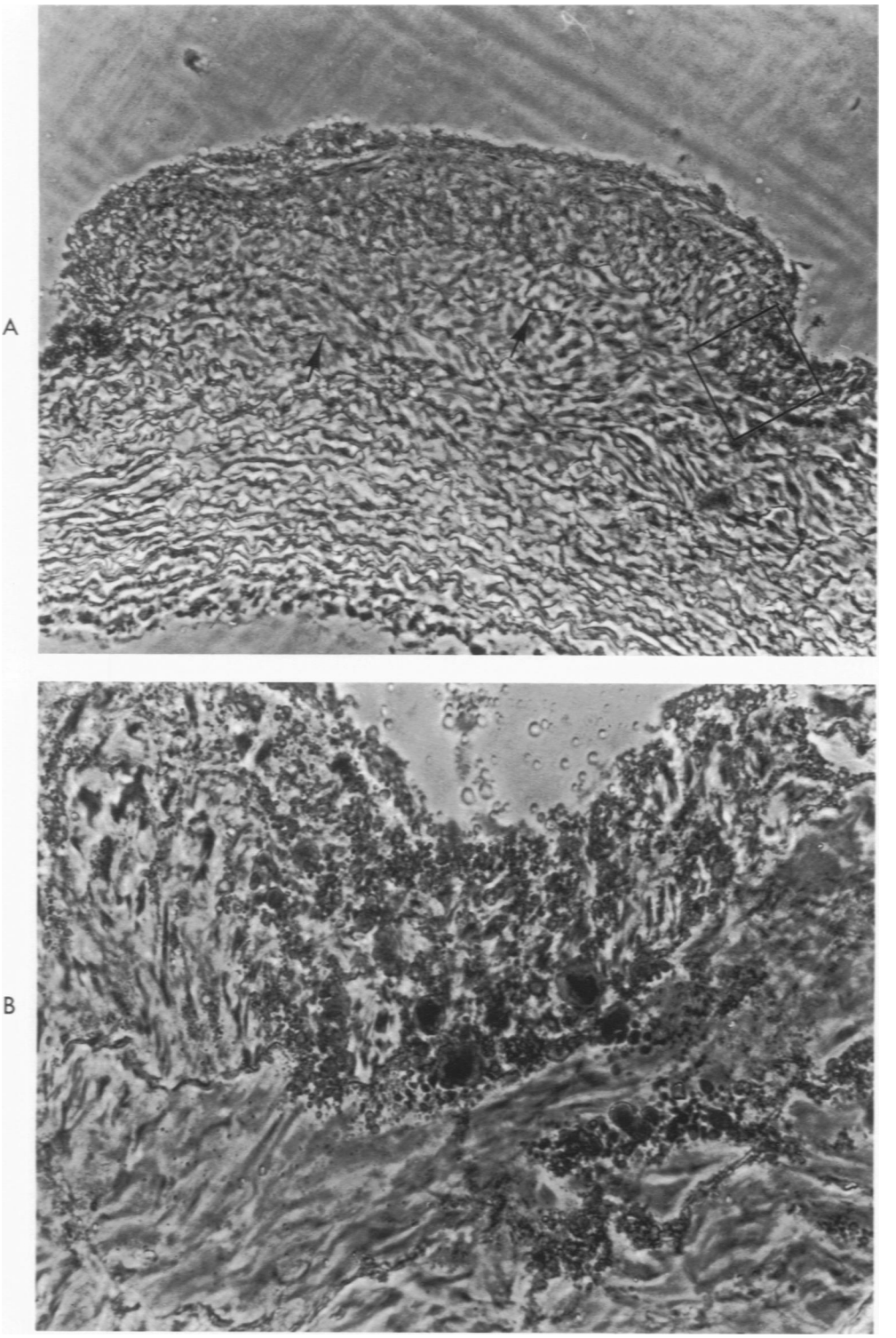


Fig 7—Grade 2 lesion. Show Racer female, 1-year-old. **A**—Marked proliferation has enlarged this cushion. Fragmented elastica is evident (*arrows*) (hematoxylin–oil red 0, phase contrast, × 165). **B**—Higher magnification of area outlined in 7A. Lipid in large pools as well as fine droplets can be seen (× 660).

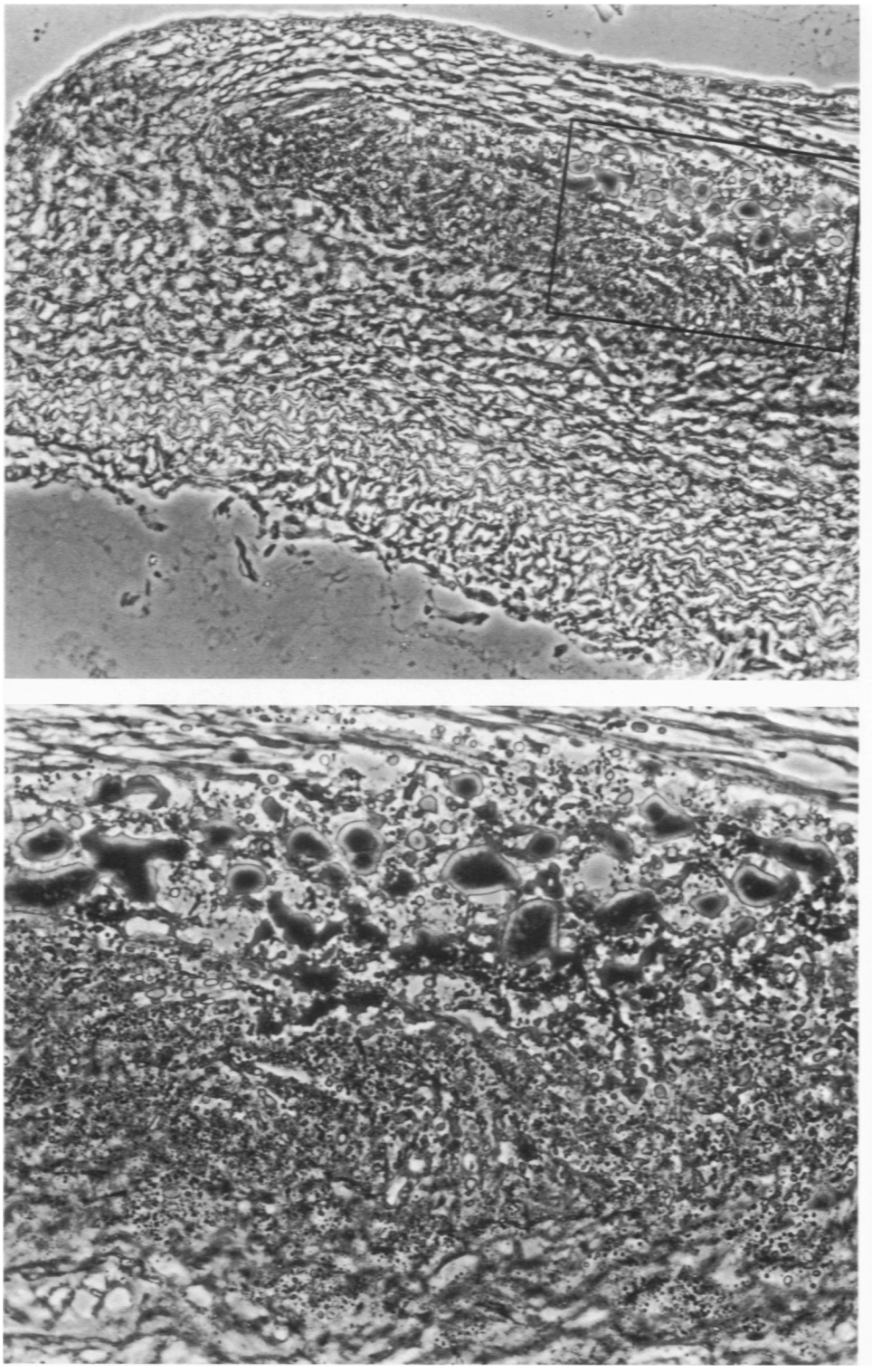
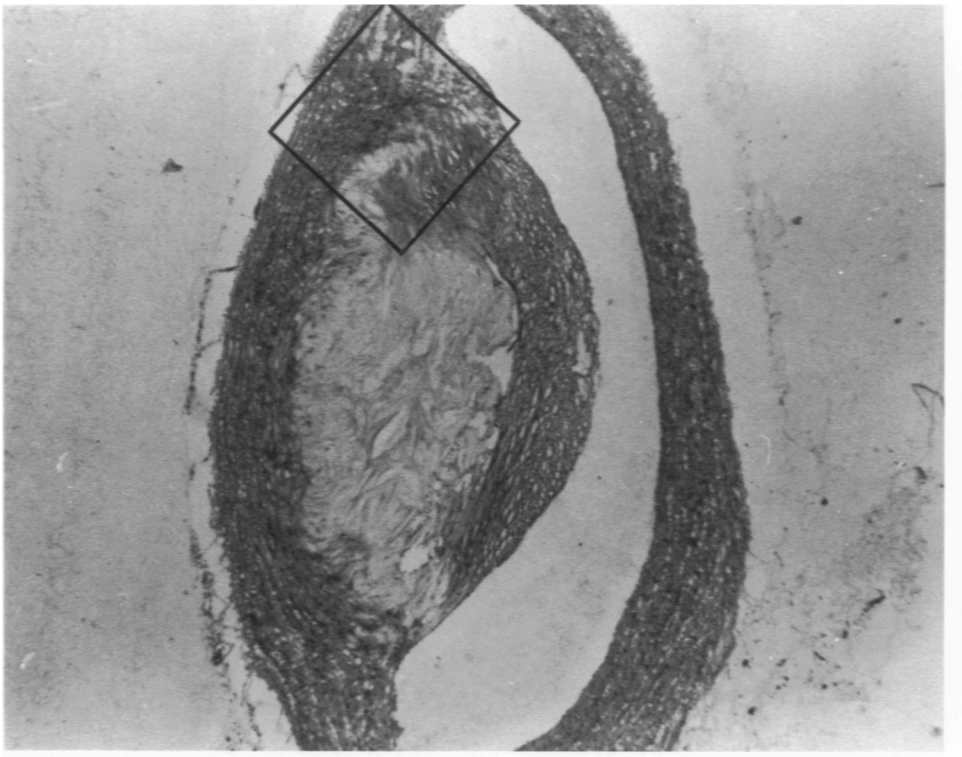


Fig 8—Grade 3 lesion. White Carneau female, 6-years-old. **A**—Lipid-filled plaque exhibiting considerable luminal protrusion, central necrosis, and involvement of surrounding media (hematoxylin–oil red 0, phase contrast, × 165). **B**—Higher magnification of area outlined in a. Necrosis and large amorphous lipid pools are evident. × 420.

Fig 9—Grade 4 lesion. White Carneau male, 6-years-old. **A**—A large fatty lesion with prominent fibrous cap and extensive central necrosis (H&E, × 60). **B**—Higher magnification of an adjacent serial section depicting cholesterol clefts (*arrows*) in the area outlined in 9A (hematoxylin–oil red 0, phase contrast, × 165).



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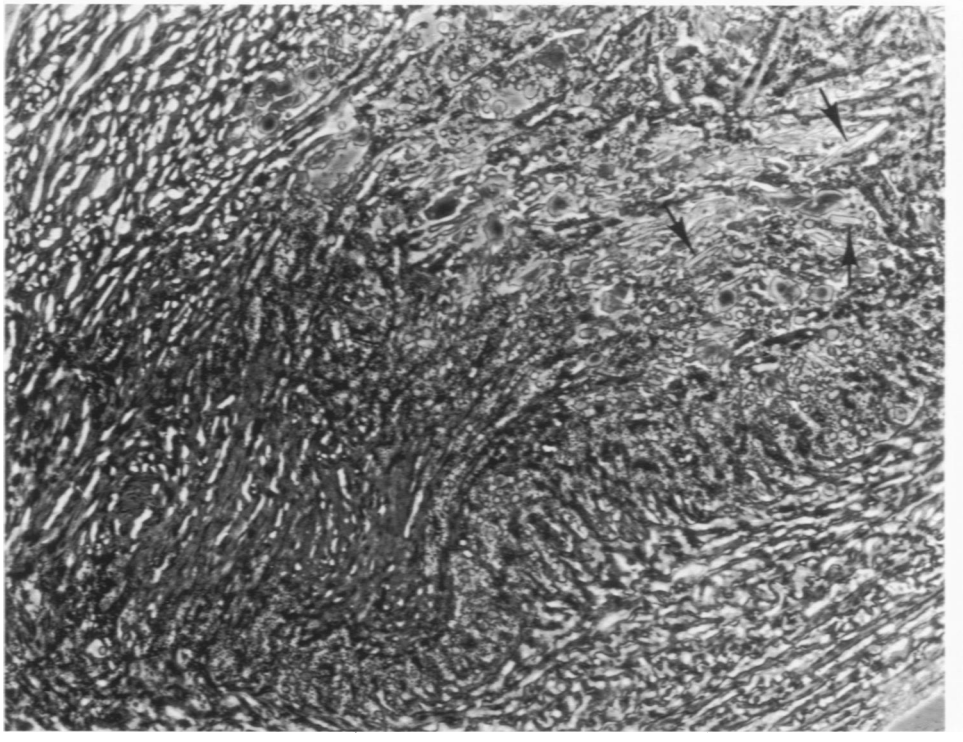

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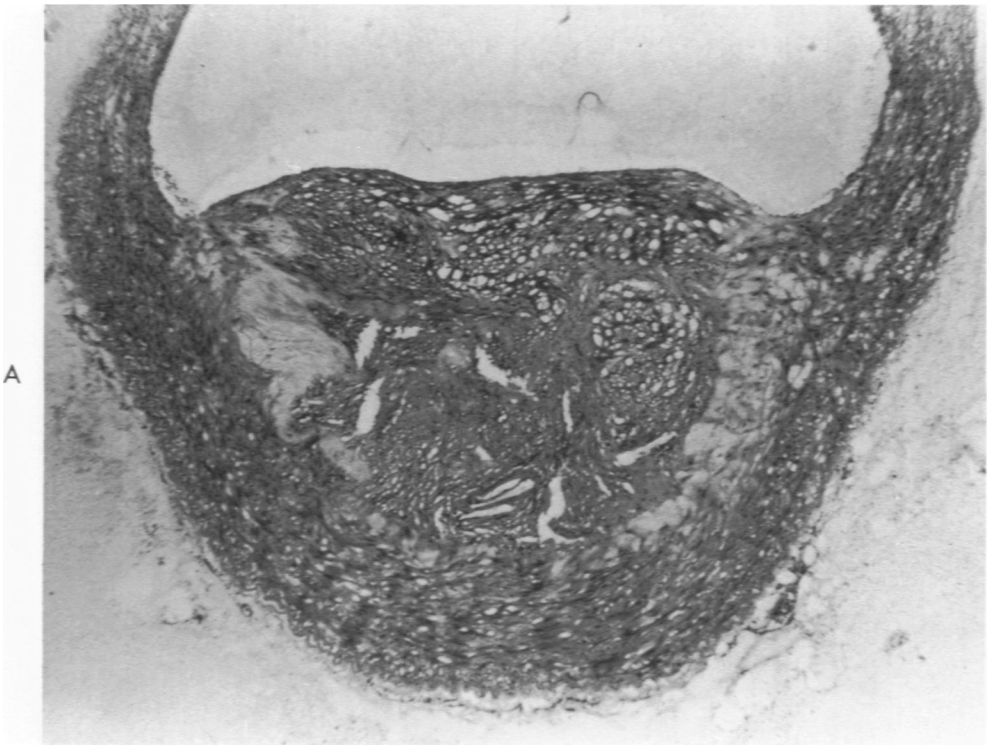
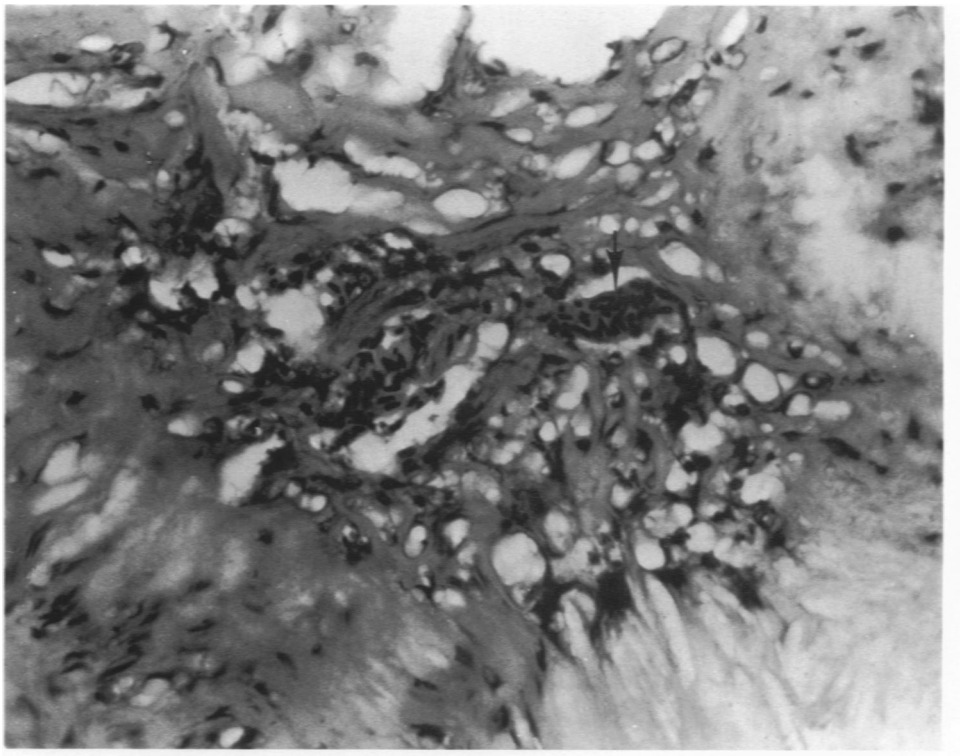


Fig 10—Grade 5 lesion. White Carneau male, 6-years-old. **A**—Massive fatty lesion occluding more than half the lumen, of this vessel (H&E, × 60). **B**—Higher magnification of an adjacent serial section showing clusters of red blood cells (*arrow*) in the center of the plaque (H&E, × 420). **C**—Higher magnification of an adjacent serial section showing bone formation (*arrow*) and what appears to be formation of cartilage (*dart*) (H&E, × 420).



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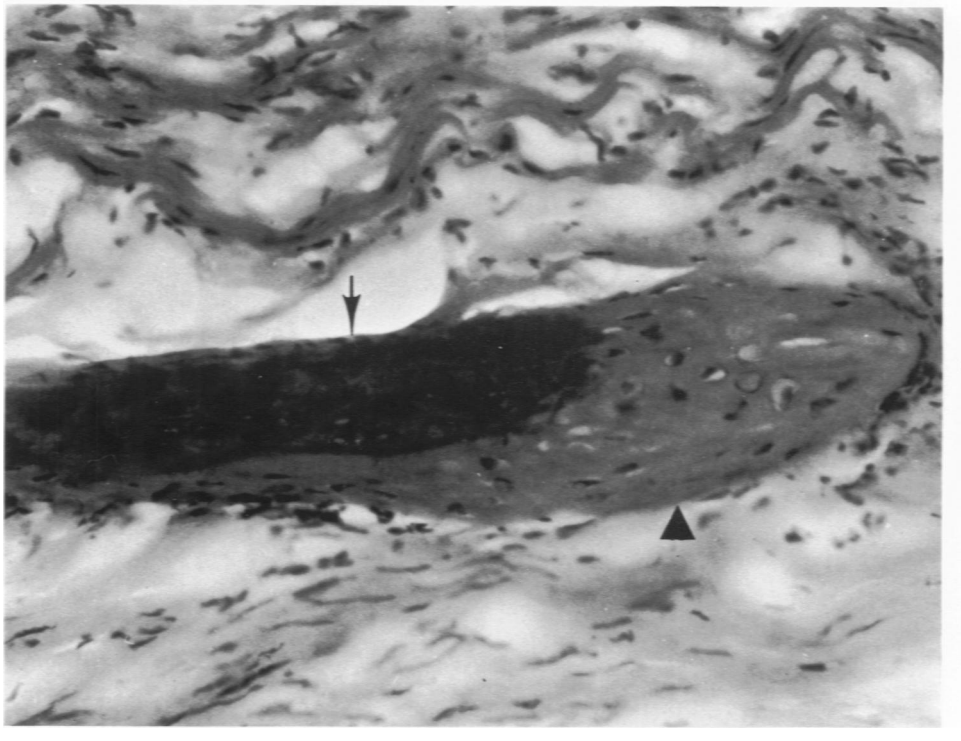

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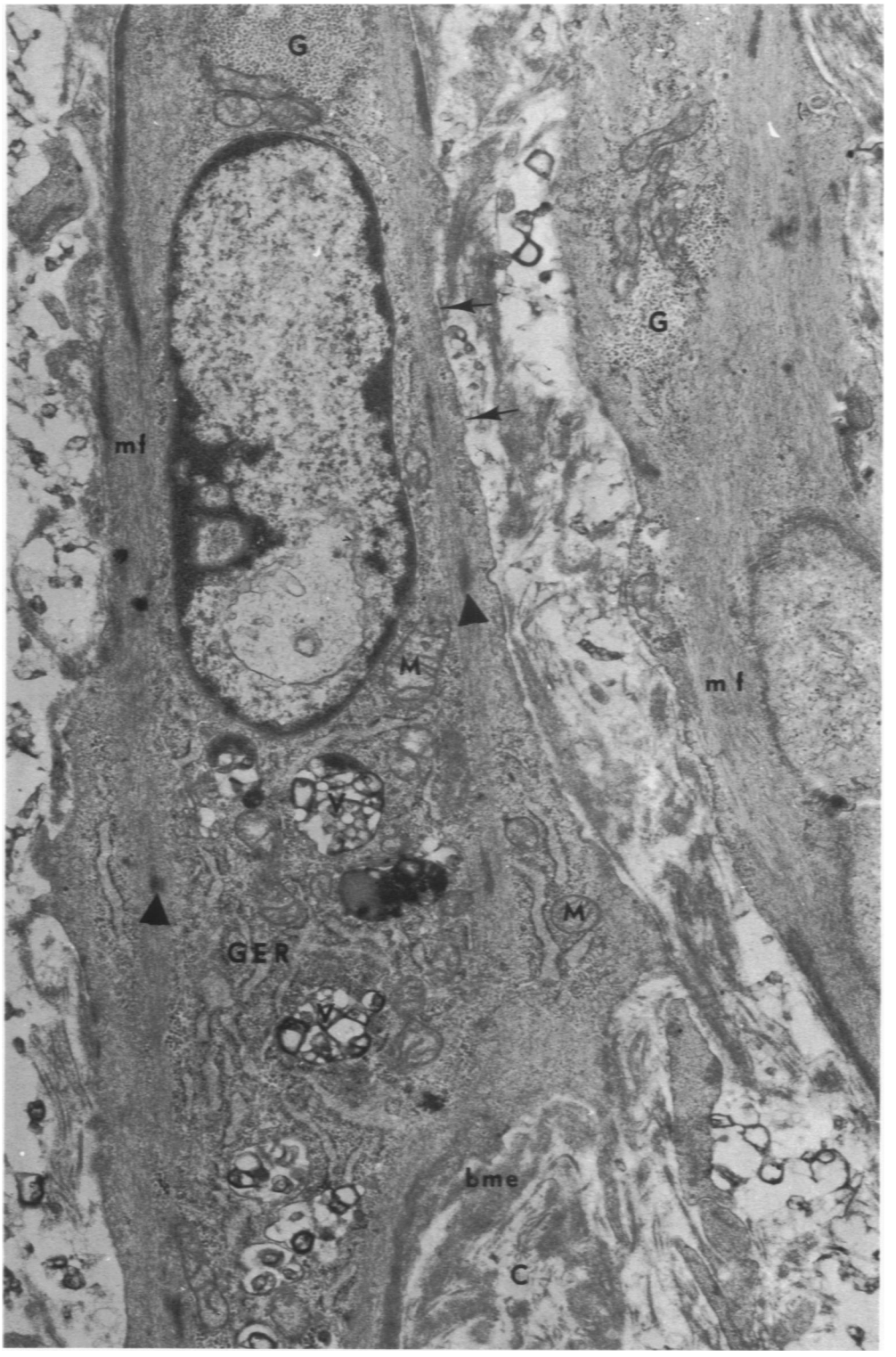

Fig 11—Modified smooth muscle cell in the upper region of the fibrous cap from an advanced lesion in a 7-year-old White Carneau female containing myofilaments (*mf*) with dense bodies (*darts*), much granular endoplasmic reticulum (*GER*), many mitochondria (*M*) and glycogen (*G*). Pinocytotic vesicles are prominent (*arrows*). The extracellular space contains fragmented basement membrane envelope (*bme*) and some collagen (*C*). Numerous vacuoles (*V*) containing material resembling that found in extracellular spaces are present (× 18,900).

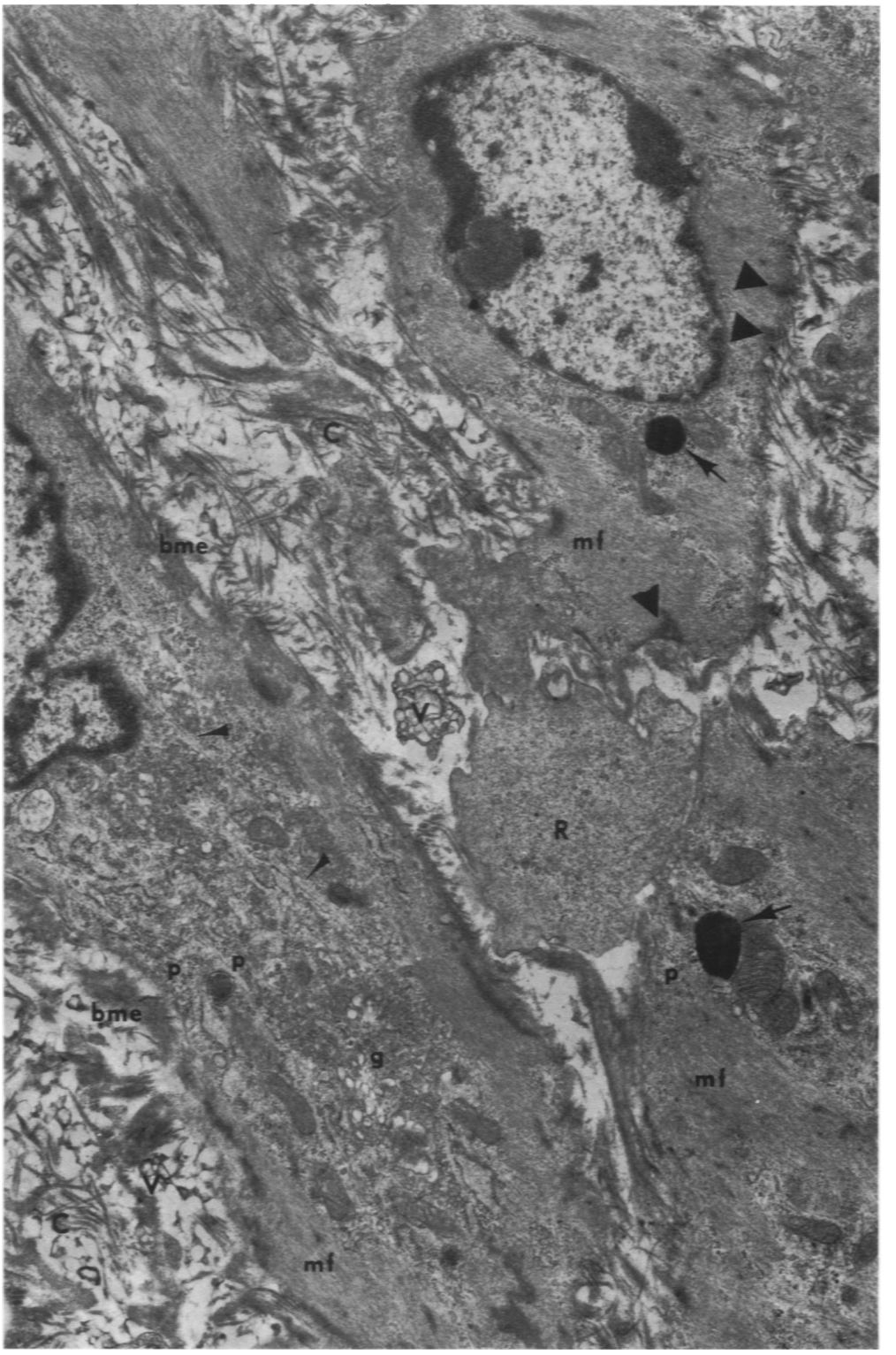

Fig 12—Deeper-lying region of the fibrous cap shown in Fig 11. Modified smooth muscle cells in this region contain a similar array of organelles including a prominent Golgi apparatus (*g*), many free ribosomes (*R*) and polysomes (*p*), and microtubules (*small darts*). These cells have many more myofilaments (*mf*) with dense bodies (*large darts*) and a distinct basement membrane envelope (*bme*). Darkly stained inclusions (*arrows*) are present, and extracellular vesiculated material (*V*) is abundant. Collagen (*C*) is more prominent here (× 16,500).

[*End of Article*]