

Non-proliferative Lesions of the Heart and Vasculature in Rats

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INTRODUCTION

Spontaneous non-proliferative changes of the heart and vasculature in laboratory rats are common (3, 5, 13, 20). The vast majority of these are degenerative and inflammatory changes. Knowing the morphology and background incidence of these changes (e.g., murine progressive cardiomyopathy, myocardial mineralization, arteritis) is important because xenobiotics may induce similar lesions or increase their severity/incidence in toxicologic evaluation studies. This document outlines a simplified nomenclature for spontaneous non-proliferative pathologic findings occurring in the heart and vasculature of laboratory rats and is based primarily on histopathologic findings in hematoxylin and eosin (H&E)-stained tissue sections from Sprague-Dawley and F-344 rats.

MORPHOLOGY

HEART

Murine Progressive Cardiomyopathy (Figures 1-4)

The terms cardiomyopathy and degenerative cardiomyopathy have also been used for this spectrum of spontaneously occurring degenerative changes in the heart. The modifier "murine" may be included in the diagnostic terminology to distinguish this condition from the unique types of cardiomyopathy that occur in humans. Murine progressive cardiomyopathy occurs at higher prevalence in older male rats (1, 3, 6, 11). Female rats have less extensive changes than males of the same age (3, 6). In males morphologic changes characteristic of murine progressive cardiomyopathy may occur as early as 3-4 months of age. Sites of predilection include the myocardium of the apex, below the fibrous rings, papillary muscles and free wall of the left ventricle. The subendocardial myocardium is a commonly affected layer. In some instances, changes can be seen adjacent to intramural coronary arteries. The early stages of cardiomyopathy consist of minute foci of mononuclear

inflammatory cells, edema and degenerated cardiac myofibers.

The changes in the later stages may be focal or diffuse in distribution and consist of fibrosis, degeneration/necrosis of cardiac myofibers, atrophy, mineralization, and cartilaginous/osseous metaplasia. Cartilaginous metaplasia should be distinguished from the normal cartilage which may be present in a section through the trigone area.

Mineralization (Figure 5)

Mineralization occurs in aged rats, especially in those with chronic renal disease/parathyroid gland hyperplasia. It is more severe in males than in females (8, 20). The left ventricle is more affected than the atria, interventricular septum, or right ventricle. Mineralization can be detected by H&E stain and is characterized by irregularly-shaped small or large basophilic deposits in the cytoplasm of single or groups of cardiac myofibers. Mineralized cells are well demarcated from surrounding normal cells. Early mineralization is characterized by rows of minute basophilic spherules, which are calcified mitochondria. Mineralization may be accompanied with myofiber degeneration, mononuclear inflammatory cell reaction and/or fibrosis. Focal mineralization of the cartilage in the trigone area may be present in older rats.

Fatty Infiltration

Fatty infiltration consists of subepicardial adipose cells in the myocardial stroma displacing adjacent cardiac myofibers. It is most prominent in older obese animals (19, 21).

Fatty Change

Fatty change is characterized by an accumulation of numerous, variable size spherical fat droplets in the sarcoplasm of cardiac myofibers. Fat stain may be required to confirm the diagnosis.

Lipofuscinosis

Lipofuscinosis may occur at any age but is most common in aged rats (3, 21). It is characterized by brownish-yellow granules at the poles of the nuclei of affected cardiac myofibers. Pigment granules are yellow-orange by autofluorescence. Lipofuscin stains such as Schmorl's or carbol fuchsin may be used for demonstration of the lipochromic pigment.

Thrombosis (Figure 6)

Thrombosis occurs mostly in aged rats and more often in males than in females (4). Thrombi occur much more frequently in the atria than in ventricles, and the left atrium is much more affected than the right atrium.

Atrial Thrombosis: The thrombus often fills the atrial lumen and on occasion projects into the mitral orifice. The

thrombi are usually well organized. Platelets, leukocytes (particularly neutrophils) and fibrin may be present. Atrial thrombosis may be present with atrial myocarditis and/or severe cardiomyopathy.

Ventricular Thrombosis: Ventricular mural thrombi may occur in cases of severe cardiomyopathy (22). Acute endocarditis and/or myocarditis may occur adjacent to the thrombus.

Endocardial Myxomatous Change (Figure 7)

Endocardial myxomatous change usually occurs more in older rats than in younger rats (2, 12). The change consists of focal or diffuse thickening due to presence of loose myxomatous tissue in the subendocardium. Hypertrophy or slight hyperplasia of the endocardium may also occur. There may be focal areas of hemorrhage and hemosiderin deposits. In severe cases of valvular involvement, hyalinization, unorganized or organized thrombi, accumulation of inflammatory and mast cells, and polypoid vegetation may be found.

Inflammation

Inflammation of the pericardium, epicardium, myocardium, and endocardium may occur with varying degrees of severity. The inflammation is usually acute to chronic and more frequent in older than in younger rats (7, 17).

Congenital Malformations

Although congenital malformations are rare, the most common is interventricular septal defect (19). Less common is atrial septal defect which usually arises from an aberration in formation of the membranes of the upper portion of the septum.

BLOOD VASCULATURE

Medial Degeneration (Figure 8)

Medial degeneration occurs in several arteries. The coronary arteries are commonly involved, most frequent among males and older animals (9, 10, 22). Affected vessels have a decreased number of muscle cell nuclei. Remaining nuclei are enlarged, pleomorphic, irregularly arranged and surrounded by increased deposition of a matrix substance which is weakly PAS positive (most likely mucopolysaccharides). Although uncommon, there may be replacement with collagen, reticulin and elastic fibers.

Medial Hypertrophy

Medial hypertrophy is rare (14), and occurs primarily in medium-size pulmonary arteries.

The tunica media is thickened due to enlarged muscle cells. Hyperplasia of muscle cells may also occur. Critical judgment should be exercised to exclude apparent thick tunica media due to oblique sections of blood vessels which have external oblique muscles.

Arteritis (Panarteritis Nodosa, Periarteritis, Polyarteritis) (Figures 9-12)

Arteritis occurs most frequently in older male rats (5, 15, 18, 23). It is a degenerative, inflammatory and necrotizing multifocal lesion of arteries and arterioles involving all vascular layers. The lesions are often segmented, nodular or tortuous, and may result in vascular dilatation, aneurysm, thrombosis or occlusion. The mesenteric arteries are most frequently affected. Vessels in several other organs, including the pancreas and testes, may also be involved.

Microscopically, the early stages are characterized by focal fibrinoid necrosis of the arterial wall and intense inflammatory infiltrates of neutrophils, lymphocytes and eosinophils. This is followed by medial and adventitial fibroplasia and chronic perivascular inflammation. The later stages are characterized by narrowing and obliteration of the lumen, focal hypertrophy of the tunica media and/or aneurysmal formation. Medial fibrinoid necrosis is more predominant in testicular than in mesenteric arteries (4). Arterial fibrinoid necrosis and thrombosis have been associated with infarcts in various organs, particularly in the kidney and intestines (4).

Arterial Thrombosis (other than arteritis)

Arterial thrombosis, not associated with arteritis, may occasionally occur in the lung, kidney, liver, adrenal, or other organs. These thrombi may be metastatic emboli from atrial thrombosis. Arterial thrombosis occurs most frequently in older rats. In the kidney it has been associated with renal infarcts (3, 22).

Capillary Thrombosis

Capillary thrombosis is rare (22). It consists of fibrinous thrombi in pulmonary alveolar capillaries of old rats. Thrombosis in the pulmonary parenchyma is usually accompanied with focal interstitial tissue reaction. Thrombosis in subpleural capillaries is usually associated with minute infarct and pleural necrosis. Glomerulonephritis is associated with capillary thrombosis in the lung (13).

Atherosclerosis (Figure 13)

Atherosclerosis is rare. When it occurs, the aorta or coronary arteries are involved and the extent of the changes is limited. Atherosclerosis consists of small focal areas of foam cells in the tunica intima or subintima/tunica media. Atheromatous plaques may occur.

Mineralization (Figures 14, 15)

Mineralization of the tunica media and/or intima is commonly observed in older rats (14, 22), especially in males with chronic renal disease and/or parathyroid gland hyperplasia (22). Veins in addition to arteries may be mineralized. Vascular mineralization occurs in various tissues and organs. Intimal and/or medial sclerosis with extensive

mineralization of the elastic fibers occur in the aorta. The earliest change of mineralization is fine basophilic granules in the internal elastica lamina. Vascular mineralization may also occur in areas where tissues undergo necrosis.

Telangiectasia

Telangiectasia is defined by large blood-filled cavities lined by endothelium. This change occurs mostly in the liver, where it is a cavernous dilatation of groups of sinusoids.

Congenital Malformations

Congenital malformations of blood vessels are rare (22). Hamartomas, patent ductus arteriosus, right-sided aortic arch and absence of azygos vein may occur.

LYMPHATIC VASCULATURE

Lymphangiectasia

Lymphangiectasia occurs in lymph nodes, among which the mesenteric lymph nodes are primarily affected. This change is characterized by dilated lymphatic capillaries.

DISCUSSION

Spontaneous non-proliferative pathologic changes in the heart and vasculature of laboratory rats are common, particularly in old males (3, 5, 13, 21). The vast majority are degenerative and inflammatory. Xenobiotics may increase the incidence/severity of many of these changes (7, 16, 19). For example, exacerbation of murine progressive cardiomyopathy, arteritis or mineralization are quite common. In such cases, comparison with control animals and with known spontaneous lesions is important for determination of toxicity. Drugs and chemicals may also induce morphologic changes in the heart and vasculature which are outside the range of spontaneous changes. Medial hemorrhage and vascular leakage are examples of such effects (9, 10).

NOMENCLATURE AND DIAGNOSTIC CRITERIA

HEART

Murine Progressive Cardiomyopathy

1. Fibrosis, infiltration with mononuclear inflammatory cells and/or myofiber degeneration/necrosis.
2. Focal or multifocal distribution.
3. Sites of predilection are the apex, below the

fibrous rings, papillary muscles, and free wall of the left ventricle.

4. Early stages usually consist of small foci of mononuclear inflammatory cells, edema and degenerated cardiac myofibers, primarily around intramural coronary arteries.
5. Late stages are more extensive and consist of foci with fibrosis, degeneration/necrosis, atrophy and occasional mineralization of cardiac myofibers, and cartilaginous/osseous metaplasia.

Mineralization

1. Small or large, regularly shaped, intracytoplasmic basophilic deposits.
2. Individual groups of cardiac myofibers are affected.
3. May be accompanied by infiltration with inflammatory mononuclear cells, fibrosis, myofiber degeneration and atrophy.
4. At early stages, rows of minute basophilic spherules (mineralized swollen mitochondria) can be distinguished in the cytoplasm of affected myofibers.

Fatty Infiltration

1. Infiltration of subepicardial adipose cells into the myocardial interstitium, displacing cardiac myofibers.

Fatty Changes

1. Intracytoplasmic fat droplets, of variable size, within cardiac myofibers.
2. Fat stain (e.g., Oil Red O, Sudan Black) may be required to confirm the diagnosis.

Lipofuscinosis

1. Intracytoplasmic brownish-yellow granules around nuclei of affected cardiac myofibers.
2. Granules are yellow-orange by autofluorescence.
3. Stains for lipofuscin (e.g., Schmorl's, carbol fuchsin) may be used to confirm the diagnosis.

Thrombosis

Atrial:

1. Usually are well organized.
2. Platelets, leukocytes (primarily neutrophils) and fibrin may occur.

Ventricular:

1. Usually are acute to subacute. Acute: neutrophils, fibrin and hemorrhage predominate Subacute: mixed inflammatory cellular infiltrates, of which mononuclear cells are in considerable amount.
2. Adjacent endocarditis and/or myocarditis may occur.

Endocardial Myxomatous Changes

1. Usually valvular.
2. Focal or diffuse thickening due to subendocardial

loose myxomatous tissue.

3. Hypertrophy or slight hyperplasia of the endocardium may occur.
4. In severe cases, hyalinization, unorganized or organized thrombi, accumulation of inflammatory and mast cells, and polypoid vegetation may occur.

Inflammation

1. Acute—infiltrates in the epicardium, myocardium and/or endocardium composed primarily of polymorphonuclear leukocytes and/or fibrin and edema.
2. Chronic—infiltrates in the epicardium, myocardium, and/or endocardium composed primarily of mononuclear inflammatory cells.
3. Chronic/active—a mixture of inflammatory cells in the epicardium, myocardium, and/or endocardium; fibrosis, myofiber degeneration/necrosis and abscess formation may be components of this type of inflammatory lesion.

BLOOD VASCULATURE

Medial Degeneration

1. Occurs most commonly in coronary arteries.
2. Decreased number of nuclei.
3. Remaining nuclei are enlarged, pleomorphic, irregularly arranged, and surrounded by abundant deposit of a matrix substance which is weakly PAS positive.
4. Collagen, reticulum and elastic fibers in the matrix are not common.

Medial Hypertrophy

1. Medium-size pulmonary arteries are most commonly affected.
2. Muscle cells are enlarged.
3. Muscle cell hyperplasia may occur.
4. Exclude apparent thick tunica media due to oblique sections of blood vessels which have external oblique muscles.

Arteritis

1. At early stages, focal fibrinoid necrosis of the tunica media and an inflammatory infiltrate with neutrophils, lymphocytes and eosinophils.
2. At mid stages, medial and adventitial fibroplasia and perivascular mononuclear inflammatory cell infiltration.
3. At early stages, narrowing and obliteration of the lumen, focal hypertrophy of the tunica media, considerable fibrosis, and aneurysmal formation.

Arterial Thrombosis (other than arteritis)

1. Thrombi composed of fibrin and mixed inflammatory cells in lumen of arteries of various

organs, particularly lung, kidney, liver and adrenal gland.

Capillary Thrombosis

1. Fibrin thrombi in pulmonary capillaries, usually accompanied with interstitial tissue reaction of mixed inflammatory cells.
2. Pleural capillari thrombi composed of fibrin and associated with minute infarcts and pleural necrosis.

Atherosclerosis

1. Subintimal/medial foam cells in coronary arteries or aorta.
2. Small area of foam cells in tunica intima and subintima/tunica media.

Mineralization

1. Tunica media and/or intima are affected.
2. Veins, in addition to arteries, may be affected.
3. Earliest change is seen as fine basophilic granules in the internal elastic lamina.

Telangiectasia

1. Large blood-filled cavities lined by endothelium.
2. In the liver, cavernous dilatation of groups of sinusoids.

LYMPHATIC VASCULATURE

Lymphangiectasia

1. Dilated lymphatic capillaries.

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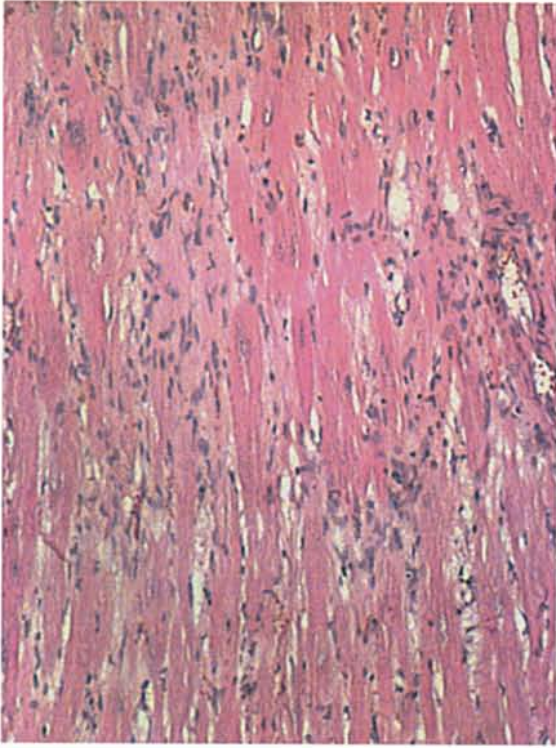


Fig. 1 - Murine progressive cardiomyopathy: interstitial fibrosis (H&E).

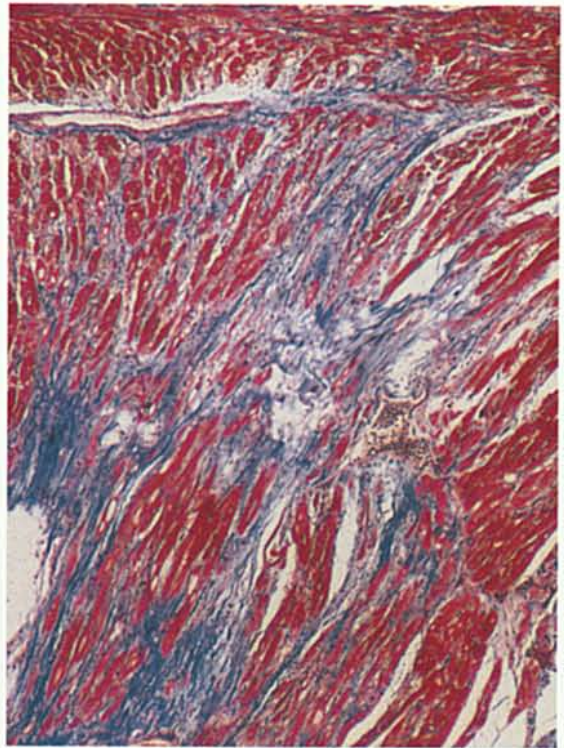


Fig. 2 - Murine progressive cardiomyopathy: interstitial fibrosis, (trichrome stain).

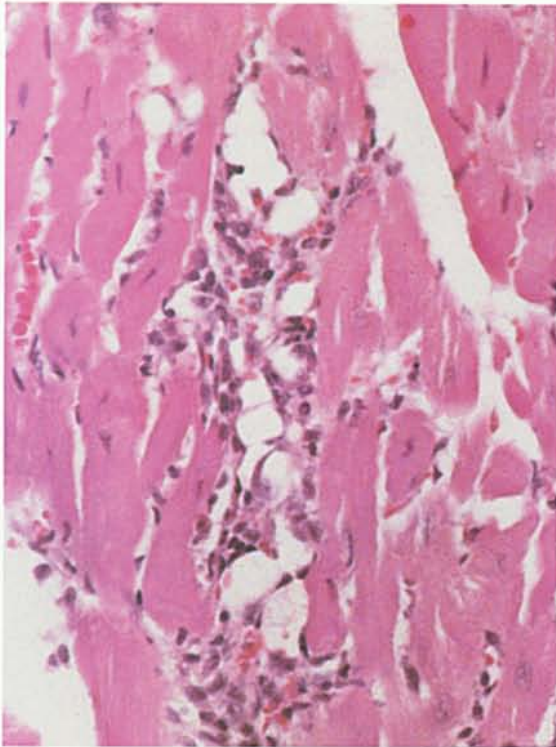


Fig. 3 - Murine progressive cardiomyopathy: accumulation of mononuclear inflammatory cells (H&E).



Fig. 4 - Murine progressive cardiomyopathy: osseous metaplasia (H&E).

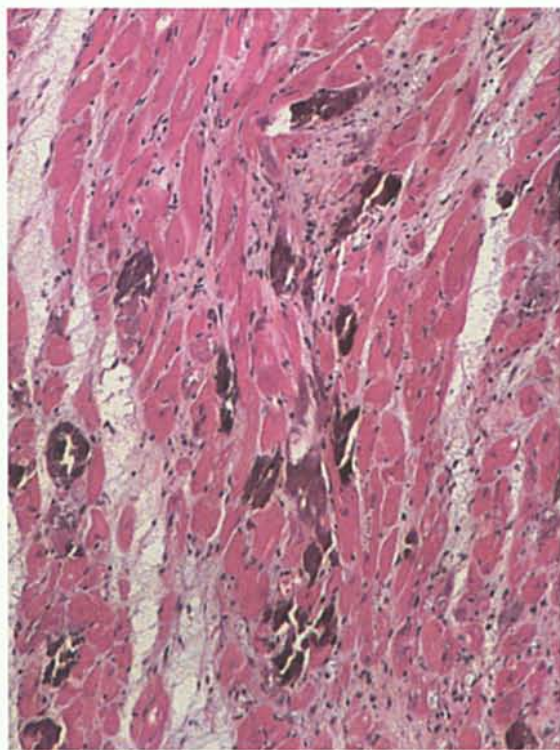


Fig. 5 - Mineralization, myocardium (H&E).

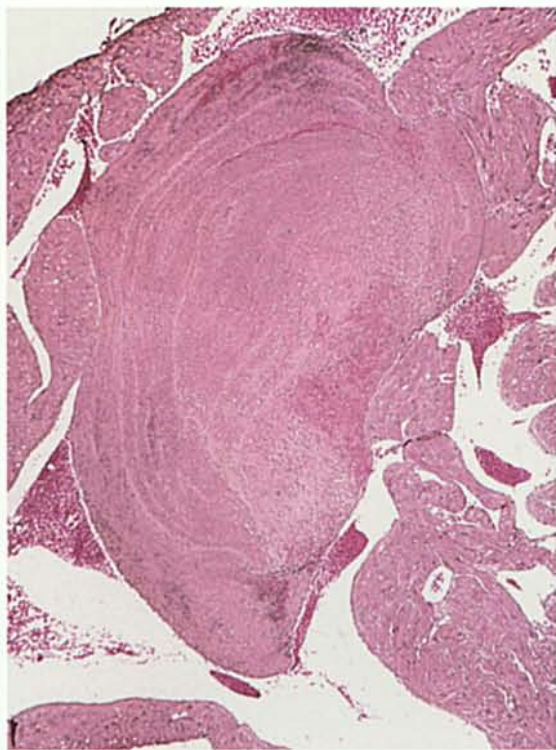


Fig. 6 - Thrombosis, left atrium (H&E).

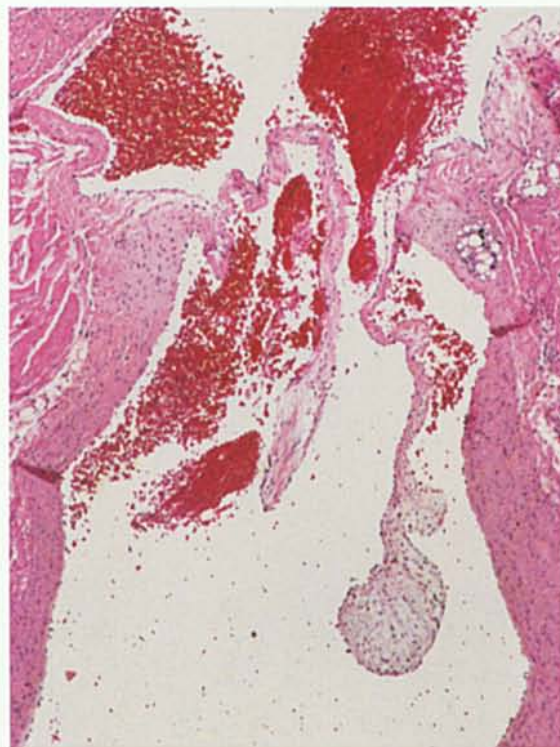


Fig. 7 - Endocardial myxomatous change (H&E).

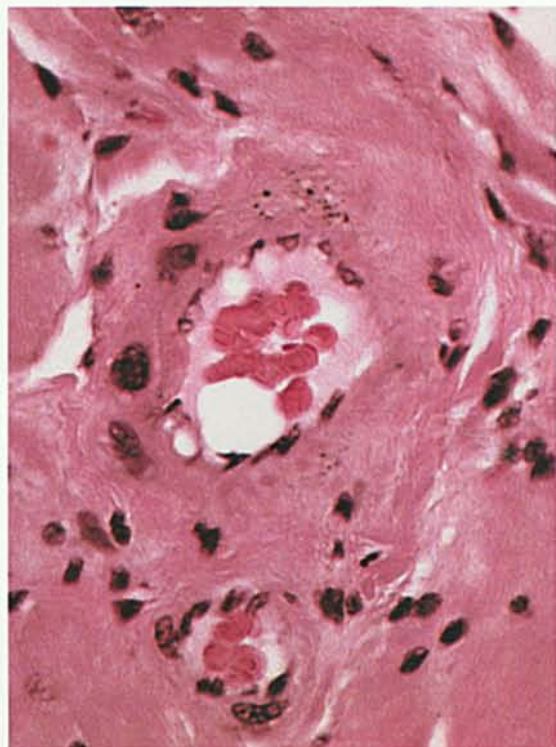


Fig. 8 - Medial degeneration, intramural coronary artery (H&E).



Fig. 9 - Arteritis: mesenteric artery (low magnification) (H&E).

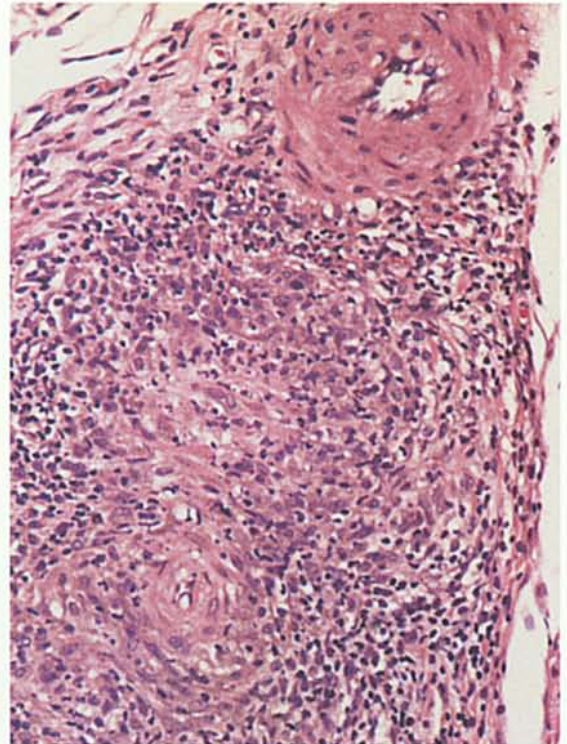


Fig. 10 - Arteritis: mesenteric artery (high magnification) (H&E).

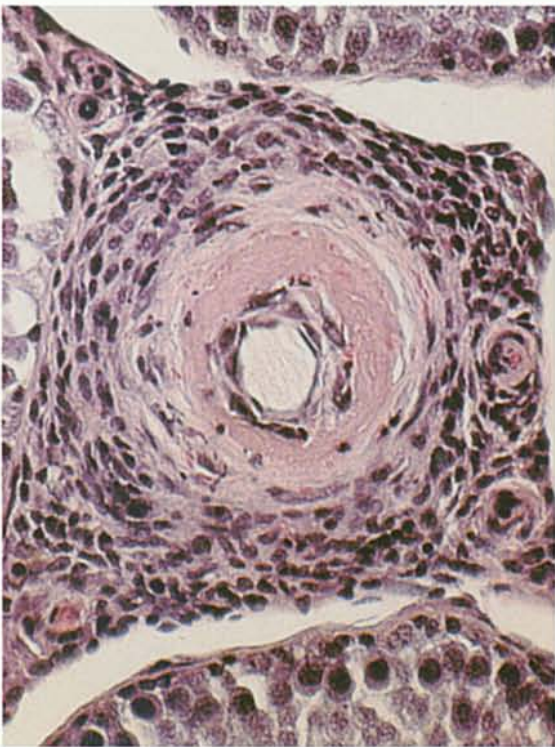


Fig. 11 - Arteritis: testis (H&E).



Fig. 12 - Arteritis: thrombus in duodenal artery (H&E).



Fig. 13 - Atherosclerosis, intramural coronary artery (H&E).

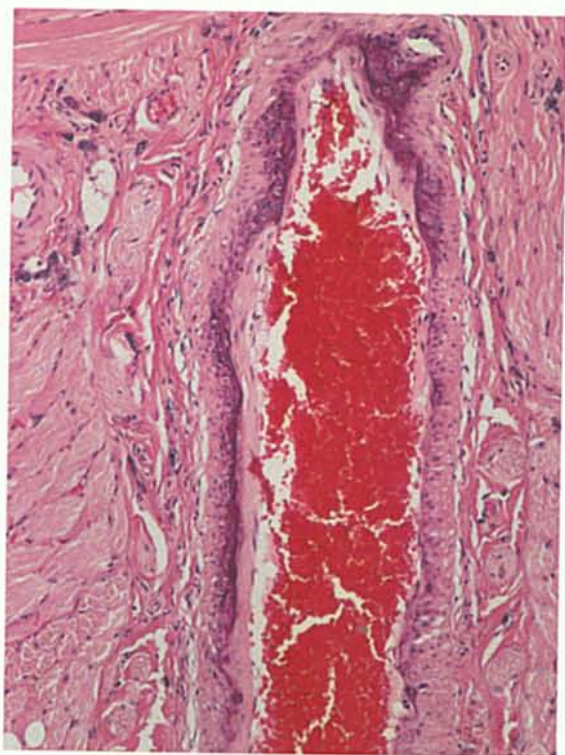


Fig. 14 - Mineralization: artery, tongue (H&E).



Fig. 15 - Mineralization: intramural coronary artery (H&E).