# Non-proliferative and Proliferative Lesions of the Cardiovascular System of the Rat and Mouse

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The INHAND Project (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) is a joint initiative of the Societies of Toxicologic Pathology from Japan (JSTP), Europe (ESTP), Great Britain (BSTP) and North America (STP) to develop an internationally-accepted nomenclature for proliferative and non-proliferative lesions in laboratory animals. The primary purpose of this publication is to provide a standardized nomenclature for characterizing lesions observed in the cardiovascular (CV) system of rats and mice commonly used in drug or chemical safety assessment. The standardized nomenclature presented in this document is also available electronically for society members on the internet (http://goreni.org). Accurate and precise morphologic descriptions of changes in the CV system are important for understanding the mechanisms and pathogenesis of those changes, differentiation of natural and induced injuries and their ultimate functional consequence. Challenges in nomenclature are associated with lesions or pathologic processes that may present as a temporal or pathogenic spectrum or when natural and induced injuries share indistinguishable features. Specific nomenclature recommendations are offered to provide a consistent approach. (DOI: 10.1293/tox.2016-I001; J Toxicol Pathol 2016; 29: 1S–47S)

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## Introduction

The INHAND Project (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) is a joint initiative of the Societies of Toxicologic Pathology from Europe (ESTP), Great Britain (BSTP), Japan (JSTP), and North America (STP) to develop an internationally-accepted nomenclature for proliferative and non-proliferative lesions in laboratory animals. The purpose of this publication is to provide a standardized nomenclature for classifying lesions observed in the cardiovascular system of rats and mice (Mann *et al.* 2012). The standardized nomenclature of the cardiovascular system presented in this document is also available electronically on the internet (www.goreni.org). A widely accepted and applied nomenclature for cardiovascular lesions in rodents will decrease confusion among regulatory and scientific research organizations and provide a common language to increase and enrich international exchanges of information among toxicologists and pathologists.

Terms of systemic non-proliferative lesions that occur across organ systems and are not specific to the cardiovascular system are not included in this monograph. Similarly, systemic neoplasms such as lymphoma or histiocytic sarcoma are described in separate documents under the hematopoietic system and not discussed in this document. Proliferative lesions that have their histogenic origin in the cardiovascular system (e.g.

hemangiosarcoma) are included here but may also be included in other organ systems in which they may occur with frequency enough for a reader to consult that publication. Every effort is made to harmonize descriptions among the different sources.

The nomenclature recommended here is generally intended to be specific and descriptive rather than diagnostic. We do recognize the occasional need to use terms that are more collective (e.g. cardiomyopathy, valvulopathy) for lesions that are morphologically variable but represent a temporal continuum (e.g. the collective incidence of necrosis, inflammatory cell infiltrate, and fibrosis that can be associated with rodent progressive cardiomyopathy in older animals). In these cases, the term should be descriptively qualified in the accompanying textual report. The diagnostic criteria used for the terms in this monograph are generally those that can be seen with standard hematoxylin and eosin-stained (H&E) paraffin sections. A few (e.g. amyloid, lipid) can be suspected with H&E-stained sections but confirmed with special staining techniques (e.g. Congo red for amyloid, Oil Red O for lipid). Histochemical or immunohistochemical techniques may be mentioned in the comments section of individual terms as diagnostic aids.

## ANATOMY AND PHYSIOLOGY

Accurate and precise morphologic descriptions of xenobiotic-induced changes in the cardiovascular system are important for understanding the mechanisms and pathogenesis of those changes as well as the ultimate impact of those changes on the function of the system. Accordingly, a working understanding of the relationship between structure and function is important.

The four-chambered heart is contiguous with a closed-loop network of arteries, capillaries, and veins that circulate blood as a substrate for essential nutrients, waste products and xenobiotics. Valves between atria and ventricles and between ventricles and outflow tracts (i.e. aorta, pulmonary artery) ensure unidirectional blood flow. Structural injury to components of the cardiovascular system can alter function or induce exceptional work demands that can have long term implications for the system itself as well as the organism as a whole.

Functionally, the cardiovascular system is remarkably adaptable with consistent basal function throughout a range of physiologic and pathophysiologic conditions. The rate (chronotropy) and force (inotropy) of contraction of the heart quickly respond to changes in physiologic need. Likewise, return to basal levels is also rapid when the need diminishes. The pressure against which the heart is pumping can be altered by relaxation or constriction of muscular arteries to maintain a stable and positive pressure within the closed loop. The rate and rhythmicity of the heart as well as the tone of arterial vessels are maintained and modified by a variety of endogenous neural (autonomic nervous system), hormonal (e.g. catecholamines), and hemodynamic (e.g. plasma volume) factors. These regulatory signals are integrated and "plastic" in health but potentially altered in disease.

Rhythmic contraction of individual cardiomyocytes is initiated by waves of action potentials that originate in nodal centers of electrical stimulation in the wall of the right atrium (sinoatrial node) and the base of the interventricular septum (atrioventricular node). Communication of the excitatory signals from the AV node to the ventricular myocardium is via the interventricular bundle of His and Purkinje fibers. At the individual cardiomyocyte cell level, action potential propagation is closely linked to calcium transients in and outside the cell as well as within intracellular compartments (e.g. sarcoplasmic reticulum). Changing intracellular calcium concentrations alter binding affinity of regulatory troponin proteins for actin and myosin myofilaments. Unbinding of inhibitory troponin I allows "sliding filament" contraction (Bers 2002).

The cellular components of the cardiac myocardium (not including coronary arteries covered in the 'blood vessels' section) include cardiomyocytes and an extensive supporting network of interstitial capillaries and fibrocytes. By volume, cardiomyocytes comprise approximately 80% of the myocardium but only 25% of the cellular content by number (Porter and Turner 2009). A capillary-to-myofiber ratio of approximately 1:1 supports the profound energy substrate (e.g. $O_2$, free fatty acids, glucose) needs of the contracting heart. Cardiomyocytes are arranged in tightly packed parallel bundles or fascicles with

varying orientation across the myocardium (e.g. vertical orientation along the long axis of the heart at the subendocardium, horizontal orientation at the mid-myocardium). Atrial cardiomyocytes are generally smaller and more loosely arranged. Subcellularly, myofibrils (~50%) and mitochondria (~40%) largely constitute the intracellular content of bi-nucleated adult rat cardiomyocytes. A unique and extensive network of sarcolemmal invaginations (T-tubules) provide intimate dissemination of action potentials throughout individual cardiomyocytes.

Heart valves in the rodent heart are of two types- larger atrioventricular valve leaflets whose free borders are attached to papillary projections of the myocardium by thin chordae tendinae and smaller semilunar valve cusps at the origins of the aorta and pulmonary artery. Individual valve leaflets or cusps are composed of mesenchymal stromal or interstitial cells embedded within a loose myxomatous matrix and covered by a single and flattened layer of endothelial cells.

Within the vascular system, the arterial segments are the most common target of xenobiotic injury. Structurally, they can be subdivided by size and mural architecture with larger elastic arteries (e.g. aorta) distinguished from tissue-penetrating muscular arteries/arterioles (e.g. coronary arteries, mesenteric arteries) by their greater distensibility and elastin content. Arteries are characteristically composed of a thin, endothelium-lined tunica intima, a predominating tunica media of smooth muscle cells, and a loose tunica adventitia that is contiguous with the perivascular connective tissue. Each of these 'tunics' may be specific targets of drug-induced injury with the tunica media most commonly affected. Drug-induced injuries often extend throughout the vessel wall (i.e. become transmural) and may even involve the perivascular tissues.

## MANIFESTATIONS OF CARDIOVASCULAR TOXICITY

The components of the cardiovascular system are susceptible to a wide spectrum of injuries from natural disease and xenobiotics. These injuries may present as a change in function with or without a correlative change in structure or morphologic injury that may or may not be accompanied by a detectable change in function. For example, xenobiotics with vasopressor effects (i.e. vasoconstrictors) will raise systemic blood pressure and potentially restrict blood flow to tissues. Though the most immediate effect is increased blood pressure and ischemia, persistence of the effect can result in intimal proliferation, medial hypertrophy and even cardiac hypertrophy.

Classes of chemicals and drugs for which cardiovascular injury have been described include drugs intentionally targeted at the cardiovascular system and those that are not (Dawson and Moffatt 2012; Leung *et al.* 2012; Slørdal and Spigset 2006). Some xenobiotics may have little direct effect on the cardiovascular system but may elicit "toxicity" by complicating or exacerbating pre-existing cardiovascular disease (Feenstra *et al.* 1999; Golomb *et al.* 2007; Golomb *et al.* 2009).

Responses to injury by the cardiovascular system are similar to those of other organs with the exception that cardiomyocyte regenerative capacity is largely recognized to be minimal and insufficient to replace significant loss of cardiac mass. Accordingly, compensatory hypertrophy of remaining cardiomyocytes is more the norm. Necrosis of cardiomyocytes elicits a mixed inflammatory response that becomes more mononuclear in character with macrophages ultimately phagocytizing cell debris (Clements *et al.* 2010). Loss of individual or small numbers of cells results in hypertrophy of adjacent cells and myocardial contraction that obscures evidence of that cell loss. Alternatively, large areas of necrosis are often replaced by contracted areas of fibrosis with altered contractile capability relative to native myocardium (Vracko *et al.* 1989).

Global changes in heart wall stress can result in changes in cardiac mass reflected in quantifiable changes in heart weight. Increases in wall stress induced by increased preload (e.g. secondary to plasma volume expansion or valvular insufficiency) or increased afterload (e.g. outflow obstruction, systemic vasoconstriction) can be accompanied by increased mass distributed regionally (right vs. left side) reflecting the source of the stress.

## Cardiovascular Risk Characterization

*In vivo* cardiovascular risk characterization in contemporary drug development or environmental hazard identification is traditionally characterized in two distinct assessments- a single dose functional assessment conducted in instrumented animals (most often non-rodents but increasingly including rodent models as well) and a morphologic evaluation in repeat-dose general toxicity studies. The morphologic assessment of xenobiotic-induced injury generally includes macroscopic assessments as well as light microscopic evaluation of hematoxylin and eosin-stained sections of formalin-fixed tissues collected from rodent and non-rodent animal models. The microscopic terminology, descriptions, and recommendations included in this monograph are defined accordingly.

### Morphologic Evaluation of the Cardiovascular System

A complete morphologic assessment of the cardiovascular system begins with a thorough external examination to determine the presence of localized edema or discoloration (e.g. hyperemia, congestion, hemorrhage) that might be evidence of cardiovascular dysfunction or vascular injury. Internal organs and body cavities are evaluated for evidence of congestion, hemorrhage, or fluid accumulation. Likewise, the heart and major vessels are examined *in situ* for changes in size, color, or shape (e.g. a rounded heart may be evidence of ventricular hypertrophy; dilation of a major vessel may be evidence of aneurysm). Heart weights are generally measured. The frequent absence of cardiovascular functional assessment in repeat-dose rodent toxicology studies increases the importance of a thorough macroscopic examination.

**Fixation.** Immersion fixation of cardiovascular tissues in 10% neutral buffered formalin (NBF) is routine for histopathologic examination in nonclinical safety studies (Elangbam 2005; Morawietz *et al.* 2004). An interest in non-routine assessments may require different handling of fresh tissues. Transcriptional/genomic analysis requires snap freezing of appropriate samples or use of an appropriate nucleic acid preservative (e.g. RNAlater, Qiagen, Valencia, CA). Although adequate ultrastructural examination can be performed on expeditiously formalin-fixed tissues, fixation in glutaraldehyde or some other relevant electron microscopy fixative is preferred. Plans for post-microtomy immunolabeling (i.e. immunohistochemistry) might require limiting immersion time in formalin, fixation in paraformaldehyde or collecting snap frozen samples for frozen sectioning.

**Trimming methods.** Two trimming methods have been used most commonly for routine examination of the rodent heart. A longitudinal bisection of the heart perpendicular to the plane of the interventricular septum allows visualization of many major components of the heart including three of the four valves (left and right atrioventricular, aortic) (Elangbam 2005; Morawietz *et al.* 2004; Nyska *et al.* 2005) (Fig. 1). Serial sections of the trimmed heart would be required to also examine the pulmonic valve which will be deep to the plane of section described for longitudinal trimming.

Alternatively, the Isaac's method that includes a mid-ventricular cross-section with longitudinal bisections of the base and apex on either side of that sample allows proportional assessment of ventricular free wall and septal thicknesses (Isaacs 1998; Nyska *et al.* 2005) (Fig. 2). In the view of these authors, the whole heart longitudinal section is best for routine examinations while the Isaac's method might be best for characterizing regional changes in cardiac mass (e.g. cardiomegaly).

**Routine staining.** Hematoxylin and eosin (H&E)-stained, 4-5 μm thick sections of paraffin-embedded tissue samples are routinely evaluated in nonclinical safety/risk assessment studies. Standard tissue lists generally include the heart as a potential target organ of toxicity and may also include a cross-section of aorta. Additional blood vessels are commonly examined in the context of the tissues in which they are set. Many of the morphologic changes and injuries captured in this monograph are easily detected with routine H&E staining. Special stains or preparations may be useful for further characterization of a morphologic change or to facilitate recognition of subtle changes.

**Electron microscopy.** Transmission electron microscopy (TEM) is probably the most common ancillary evaluation done to detect subcellular injuries or further characterize changes seen at the light microscopic level. Small samples of tissue (approximately 1x1x1 mm) fixed with glutaraldehyde-containing fixative and osmium tetroxide are processed for plastic embedment (e.g. PolyBed 812, Polysciences, Inc., Warrington, PA) and sectioned at 800-1000 angstroms for ultrastructural examination. Uranyl acetate and/or lead citrate 'stains' are often used to provide needed contrast to resolve subcellular detail. TEM examination allows critical evaluation of cellular organelles (e.g. mitochondria, sarcoplasmic reticulum, contractile myofilaments) and identification of intracellular and extracellular tissue elements that might have been noted with light microscopic examination (e.g. lipid accumulation within cardiomyocytes; amyloid accumulation between cardiomyocytes). Alternatively, ultrastructural examination may reveal organelle changes not visible with routine light microscopy (Chu *et al.* 2007; French *et al.* 2010; Nyska *et al.* 2009).

**Special stains.** Special histochemical stains are sometimes useful to further characterize intra- or extracellular material identified with H&E or to highlight specific tissue components for better or more quantitative assessment. For example, amyloid, an amorphous pink with H&E, polarizes apple green with Congo red staining; clear vacuoles can be confirmed as cardiomyocyte lipid accumulation in frozen sections with Oil Red O staining; granular pigment deposits can be identified as lipofuscin with PAS staining (Sheehan and Hrapchak 1980) (Fig. 3, 4). Alternatively, interstitial collagen accumulation in the myocardium that may be inconspicuous with HE staining is more easily visualized with either picrosirius red or trichrome staining (e.g. Masson's, Gomori's) (Fig. 5). Picrosirius red has

also been used to facilitate automated methods for quantitating myocardial fibrosis (Azevedo et al. 2010; Berry et al. 2011). Phosphotungstic acid hematoxylin (PTAH) staining that highlights the striated morphology of cardiomyocytes has been used to visualize sub-cellular changes of early cell injury in the heart (Clements et al. 2010) (Fig. 6). PTAH can also be used to highlight fibrin content of thrombi or the "fibrinoid" change of vascular inflammation and necrosis (Sheehan and Hrapchak 1980).

**Immunohistochemistry.** Immunohistochemical labeling of cellular or extracellular proteins is another technique for characterizing pathophysiology in the heart. Increased intracellular concentrations of cleaved caspase 3 visualized with immunolabeling is one method to identify cells in the apoptotic pathway that can be very difficult to detect with routine H&E staining (Mikaelian et al. 2010). Immunolabeling with Lamp2 allows recognition of lysosomal accumulation characteristic of phospholipidosis that might be an alternative to an ultrastructural examination (Obert et al.. 2007) (Fig. 7, 8). Loss of troponin immunolabeling has been used to identify cardiomyocytes in the early stages of necrosis and to link those changes to increased circulating concentrations of cardiac troponin (Clements et al. 2010) (Fig. 9, 10).

Many routine immunohistochemical labeling techniques can be applied to formalin-fixed, paraffin-embedded tissue sections though fixation time prior to processing may be limiting (i.e. 'over-fixation' can decrease the immunolabeling potential of some proteins). Alternatively, some antigen targets may require unfixed tissues.

**Plastic embedding.** Though 4-5 μm thick paraffin sections are adequate for routine characterization of morphologic changes in the heart, more critical assessment of sub-cellular changes may require thinner sections (1-2 μm) of tissue samples embedded in a matrix that makes those thin sections possible. Doxorubicin cardiotoxicity in rodents is characterized by a progressive vacuolar degeneration of cardiomyocytes. Histologic characterization of that change in experimental models is often done in 2 micron, toluidine-blue stained sections of methylmethacrylate embedded heart samples (Herman et al. 2001) (Fig. 11).

**Artifacts.** As a dynamically contracting muscle in life, the heart and muscular arteries are susceptible to a number of postmortem morphologic artifacts that can be confused with xenobiotic-induced injuries. A cardiomyocyte "fixed" in contraction will be hypereosinophilic and may exhibit accentuation and deformity of cytoplasmic striations closely resembling the early morphologic changes of hyaline or contraction band degeneration (Fig. 12, 13). Also, the vacuolation of t-tubule dilation or swollen mitochondria may occur either as post-mortem change or real evidence of injury. Likewise, hyper-contracted muscular arteries that might be seen in a number of tissues resemble the medial hypertrophy of chronic hypertension. Smooth muscle cells of the tunica media may also be vacuolated suggesting early degeneration or lipid accumulation.

Artifactual changes in the cardiovascular system are minimized much like they are for any organ system with rapid postmortem excision, minimal handling (e.g. trimming), and rapid fixation.

### Biomarkers

Biomarkers are important tools for detecting and monitoring cardiovascular injury. Serum proteins that report myocellular necrosis (cardiac troponins, creatine kinase, myoglobin, fatty acid binding protein) or myocardial wall stress (natriuretic peptides) can be applied non-clinically or clinically (Clements et al. 2010; Walker 2006). Circulating nucleic acids are a focus of current interest departing from traditional protein-focused assays (Mikaelian et al. 2013). Electrocardiograms (ECGs) are commonly used to detect electrical conductance abnormalities, and a variety of imaging modalities are available for assessing changes in cardiac structure or contractile function (Casartelli et al. 2011; French et al. 2010; Guth et al. 2019). Non-invasive measures of blood pressure are a staple in the clinical setting and reflect a balance of vascular tone and cardiac contractile force. The relevance of these biomarkers for a particular injury is determined by their cellular or whole organ source and their relationship to the pathogenesis of the lesion being "biomarked".

## General Principles of Nomenclature

Morphologic descriptions of pathologic changes in the cardiovascular system should accurately reflect a predominating process but should also reflect as best as possible the target of that process. For example, injuries that specifically affect cardiomyocytes- either individually or in aggregate- should be qualified with "cardiomyocyte". In general, these lesions will be discrete and focal to multifocal. An example might be multifocal cardiomyocyte necrosis induced by catecholamine overdose. Contrastingly, lesions that are more regional and involve both cardiomyocytes and interstitial components should be described as "myocardium". An example of this might be myocardial infarcts that result from coronary artery occlusion producing regional and fulminant necrosis. Accurate morphologic descriptors can give insight to pathogenesis. Localized (focal) "cardiomyocyte hypertrophy" is likely to have a different pathogenesis than a more generalized cardiomegaly though both involve increases in size of individual cardiomyocytes.

Morphologic descriptors should also be chosen with care to avoid the potential for confusion with similar terms used by related medical disciplines. For example, "myocarditis" in human clinical medicine is a primary inflammatory process most often associated with viral infection, drug-induced hypersensitivity reactions, or heart transplant rejection. Immune-mediated mechanisms are also often associated with vasculitides (i.e. vasculitis) in clinical medicine (Silver et al. 2001). These processes are not modeled in rodents in preclinical safety studies. Inflammation in the myocardium of rodents or the walls of blood vessels is most often a response to injury for which the terms "myocarditis" or "vasculitis", respectively, should not be used so as not to suggest a mechanistic link that does not exist.

Many animal models are recognized to have age-related background diseases or lesions for which distinction from xenobiotic-induced injury may be challenging (Greaves 2007). These distinctions become even more challenging when one considers that a possible manifestation of toxicity is exacerbation of pre-existing disease not unlike that which can occur in human patients. Nevertheless, background pathology findings are often reliably distinguished from toxic injury by differences in incidence, severity, distribution and morphology (Clemo et al. 2003). Making that distinction requires a precise approach to recording a lesion when it's present, using consistent terminology and applying defensible interpretive criteria.

There are a number of terms that have been used widely in the toxicologic pathology community that won't be recommended here for reasons articulated above. These include cardiomyopathy, rodent progressive cardiomyopathy, arteritis, and vasculitis. Rationales for their exclusion will be included with the appropriate and recommended alternatives. There are also terms not represented here because they don't demonstrate any uniqueness in the cardiovascular system or are not widely recognized in rodents. Examples include hemorrhage, atrophy, and fatty infiltration.

## Nomenclature, Diagnostic Criteria, and Differential Diagnoses

### I. Non-Proliferative Lesions

### Heart

The cellular and interstitial components of the myocardium are susceptible to a variety of morphologic changes. The following terms are the most common morphologies and processes encountered in routine toxicity studies.

### Cardiomegaly (Note: this is a macroscopic morphology that could be observed in a routine histologic tissue section)

*Other Terms*
- Cardiac enlargement
- Cardiac hypertrophy

*Pathogenesis*
Diffuse enlargement of the heart may result from expansion of interstitial tissue elements (e.g amyloid deposition), cellular infiltration (e.g. lymphoma) or increases in cardiomyocyte size. Since cardiomyocytes can increase in length, width or both depending on the stimulus, an increase in whole organ size may not be reflected in an observable change in cardiomyocyte cross-sectional area at the histologic level. Increases in cardiomyocyte size may result from increased hemodynamic load (e.g. valvular dysfunction, plasma volume expansion) or as a response to anabolic signaling at the molecular level (e.g. myostatin inhibition, anabolic steroids, triiodothyronine, $\beta_2$ adrenergic agonism)

*Diagnostic Features*
- Grossly observable increase in heart size that can be symmetrical and involve the whole organ or asymmetrical involving predominately one side of the heart (Fig. 14, 15)
- ± Quantifiable increase in individual cardiomyocyte cross-sectional area
- ± Change in ventricular volumes
  - o Concentric hypertrophy = decreased ventricular volume
  - o Eccentric hypertrophy = increased ventricular volume
- ± Grossly observable increase in ventricular wall thickness

*Differential Diagnoses*
- Focal cardiomyocyte hypertrophy that is regionally restricted or borders an area of injury (e.g. as compensatory hypertrophy)

*Comments*
Cardiomegaly is defined as an increase in the mass of the heart beyond the limits of normal for age, sex, and body weight (Berridge et al. 2013). Chemically-induced cardio-

megaly in preclinical safety studies is most often manifest as a grossly observable increase in heart size and/or weight. Quantitative or qualitative assessment of individual cardiac chambers (i.e. right vs. left, atrium vs. ventricle) may reveal a disproportionate contribution by one or more chambers or the entire heart may be proportionately affected (Greaves 2007). Increases in cardiac mass visible at the macroscopic level may not be reflected in an easily detectable change in individual cardiomyocyte size or morphology. In fact, individual cardiomyocyte size may be increased by lengthening (i.e. addition of sarcomeres; eccentric hypertrophy secondary to volume overload) rather than increasing cross-sectional area (e.g. concentric hypertrophy secondary to pressure overload) complicating detection with even quantitative or morphometric measures.

Cardiomegaly may occur as an adaptive or maladaptive response to increased wall stress. The distinction and outcome of these two different pathogeneses are important but beyond the scope of this manuscript. The reader is referred to other literature to learn more about contemporary concepts (Hill et al. 2008; Opie et al. 2006; Selvetella and Lembo 2005).

Cardiomegaly as a response to trophic or mechanical stimuli that affect either the heart as a whole or substantive sub-regions (i.e. left ventricle) should be distinguished from focal cardiomyocyte hypertrophy that occurs in individual cardiomyocytes in response to localized myocardial injury and is the subject of a separate term.

### Nomenclature recommendation
Substantive changes in heart size are generally reflected in increases in heart weight. If quantitative heart weight data is available, then this assessment should be deferred to that data. This terminology should be used when heart weight is not available but gross enlargement of the heart is obvious.

## Hypertrophy, cardiomyocyte
### Other Terms
• Myocardial hypertrophy

### Pathogenesis
Cardiomyocytes may add contractile elements and increase cell area in response to trophic signals (e.g. growth hormone, anabolic steroids) or increased work stress. Increase in cross-sectional area of individual cardiomyocytes may occur diffusely (more difficult to recognize) or as a regional change. Histologically, cardiomyocyte hypertrophy is most easily recognized when it is localized and adjacent to an area of cardiac injury/insult (e.g. locally-extensive myocardial fibrosis, endocardial fibrosis, or increased ventricular volume) where it represents compensatory hypertrophy (Greaves 2007) (Figs. 16, 17). Quantifiable changes in heart mass may not be associated with observable hypertrophy of individual cardiomyocytes. Also, morpho-

logic features of cardiomyocyte hypertrophy can be seen in untreated mice or rats (more common in mice) without obvious injury.

### Diagnostic Features
• Increase in size of individual or small groups of cardiomyocytes that may border a region of myocardial injury
• Cytoplasm may be hypereosinophilic but with intact striations
• Nuclei are often enlarged, irregular in contour, and hyperchromatic
• Cell alignment may be distorted by the injury (e.g. fibrosis) that the cells border

### Differential Diagnoses
• Hypercontraction artifact where the cells will be hypereosinophilic and enlarged but striations will be prominent, irregular and spaced closely together.

### Comments
Cardiomyocytes that are increased in size due to an increase in subcellular accumulation of other than contractile elements (e.g. vacuolation, glycogen accumulation, phospholipidosis) should use terminology that reflects the morphology of that content.

### Nomenclature recommendation
'Hypertrophy, cardiomyocyte' should be used when there is a clearly observable increase in cardiomyocyte width relative to other regions of myocardium within the section (e.g. when the change is focally or regionally distributed) or the accompanying untreated control animals. Diffuse cardiomyocyte hypertrophy is difficult to demonstrate unless profound and may need to be accompanied by quantitative cell measures.

## Degeneration, cardiomyocyte
### Other Terms
• Degeneration/Necrosis, cardiomyocyte

### Pathogenesis
Individually or collectively, cardiomyocytes can be injured in a number of ways with an ultimate common pathway that involves disruption of cellular homeostasis that may or may not result in cell death. Those ways may be directly acting on the cell (e.g. mitochondrial injury) or involve some extrinsic change like increased workload or decreased energy substrate supply (e.g. oxygen, fatty acids, glucose)

### Qualifiers (secondary descriptors)
• Hyaline – 'glassy' hypereosinophilia
• Vacuolar – discrete but frequently pleomorphic clear spaces in the cytoplasm
• Fatty – variably sized and discrete clear round spaces within which neutral lipid can and should be demon-

strated with electron microscopy or special histochemistry (Oil Red O, osmium tetroxide staining)

*Diagnostic Features*

Sub-lethal injury of individual cardiomyocyte characterized by one or more of the following:
- hyaline cytoplasmic eosinophilia (Fig. 18)
- cytoplasmic vacuolation (Fig. 19, 20, 21, 22)
- intracellular lipid accumulation
- contraction bands

*Differential Diagnoses*

Cardiomyocyte necrosis – distinguished by the presence of recognizable necrotic cell debris as nuclear karyolysis and/or eosinophilic granular material not confined within an intact cell membrane. Straightforward 'degeneration' shouldn't have the inflammatory cell component often associated with cardiomyocellular necrosis.

*Comments*

'Degeneration' is a relatively imprecise term used and abused commonly in descriptions of pathologic changes. Dorland's defines 'degeneration' as "deterioration; change from a higher to a lower form, especially change of tissue to a lower or less functionally active form" (Dorland 2012). Accordingly, the term describes more a process than a specific morphology. In the heart as well as other target organs, it is frequently applied to describe cellular or tissue changes for which a more discrete and predominating process like necrosis, fibrosis, or inflammation cannot be defined. Cytoplasmic vacuolation characterizes pre-lethal cellular changes of doxorubicin cardiotoxicity (Berridge et al. 2013; Greaves 2007). Hyaline eosinophilia and hypercontraction characterize the early stages of cellular injury in models of catecholamine overdose (Clements et al. 2010). The term has also been used interchangeably with myocytolysis or cardiomyocyte fragmentation (Greaves and Faccini 1984).

*Nomenclature recommendation*

The term 'degeneration' should be accompanied by a morphologic qualifier like 'hyaline', 'vacuolar', or 'fatty'.

### Degeneration/necrosis, cardiomyocyte

*Other Terms*
- Degeneration, hyaline, cardiomyocyte

*Pathogenesis*

Sub-lethal cellular injury with persistence or repeated insult can and often does progress to cell death or necrosis.

*Diagnostic features*
- Coincidence of morphologic changes in individual cardiomyocytes with a spectrum that bridges those of sublethal degeneration and necrosis
- One or more features of hyalinization, vacuolation or

hypercontraction
- Should include individual cell fragmentation

*Comment*

Coincidence of morphologic changes consistent with sublethal and lethal injury generally represent an early timepoint in a progressive process where necrosis and inflammatory cell infiltration will become more prominent or a very mild and transient injury that involves small numbers of cells.

*Nomenclature recommendation*

This conjugated terminology should be applied when there is clear evidence of sublethal injury and cell death (necrosis) but inflammatory cell infiltrates are not prominent. Very early or equivocal lesions may also be described as hyaline degeneration (Fig. 18).

### Necrosis, cardiomyocyte

*Other Terms*
- Myocytolysis
- Cardiomyophagy

*Pathogenesis*

Cardiomyocyte injury that results in cell death and fragmentation.

*Qualifiers (secondary descriptors)*

Coagulative, lytic, contraction band (Zenker's)

*Diagnostic Features*
- Discrete region of cardiomyocytes that are hypereosinophilic and lack nuclear detail (coagulative necrosis)
- Fragmentation of individual or groups of cardiomyocytes (lytic necrosis)
- Cytoplasmic hypereosinophilia with irregular and hypercontracted Z-bands. Intracellular dissolution of myofilaments resulting in heterogenous areas of cytoplasmic rarefaction (Zenker's or contraction band necrosis) (Fig. 23)
- Early stages may be accompanied by interstitial edema
- An accompanying cellular infiltrate that may include lymphocytes, plasma cells, neutrophils, eosinophils, and macrophages; macrophages often predominate in subacute and chronic lesions serving to phagocytize cellular debris
- Small, discrete foci of necrosis may resolve without fibrosis
- Larger regions of necrosis often resolved by fibrosis

*Differential Diagnoses*
- Hypercontraction artifact – morphologically resembles contraction band necrosis and may be difficult to distinguish from contraction band necrosis in very acute studies
- Hyaline degeneration – distinguished by cellular frag-

mentation and the presence of an inflammatory cell response.

### Comments

Cardiomyocytes are terminally differentiated and therefore do not proliferate to replace dead cells. Accordingly, cardiomyocyte necrosis represents permanent loss of contractile capability with the clinical impact largely determined by the magnitude of loss and the subsequent occurrence of additional cell loss.

The pattern or distribution of necrosis in the myocardium may offer clues to pathogenesis. Large discrete regions of coagulative necrosis typical of myocardial infarcts seen in human patients with atherosclerotic coronary artery disease are most consistent with coronary artery occlusion with ischemic necrosis of the region at risk (i.e. that region supplied by the affected coronary artery). Likewise, multifocal myocardial necrosis with a predilection for the left ventricle, subendocardial regions, and the apex of the heart (e.g. catecholamine cardiotoxicity) is generally thought to have a "work-energy mismatch" origin. More broadly distributed necrosis without a regional predilection and often accompanied by sublethal cardiomyocyte degeneration is more likely direct cardiomyocellular cytotoxicity.

Cardiomyocyte necrosis often instigates a predictable cascade of morphologic events that can give insights into the 'age' of individual lesions (and thereby their relationship to xenobiotic exposure). Necrotic cell fragmentation will be quickly followed by an inflammatory cell infiltrate (18-24 hours) whose magnitude is determined by the number of cells affected. Macrophages within that infiltrate will phagocytize cell debris. Repair will be by fibrosis if the cell loss is substantive (i.e. more than a few cells). Small foci of necrosis may resolve without much connective tissue response.

### Nomenclature recommendation

Precise morphologic terminology can communicate the temporal stage of a myocardial lesion. 'Necrosis' should be applied to those lesions where cardiomyocyte fragmentation is present but the inflammatory cell response is minimal. 'Necrosis/inflammatory cell infiltrate' should reflect those lesions where the infiltrate is prominent and evidence of accompanying cardiomyocyte necrosis is present (i.e. to reflect that the inflammatory cell infiltrate is a response to the primary event of necrosis). 'Mononuclear cell infiltrate/fibrosis' is used for those lesions where fibrosis repair has begun. 'Fibrosis' would be reserved for those lesions where inflammation has largely resolved.

## Necrosis/inflammatory cell infiltrate, cardiomyocyte

### Other Terms
• Necrosis, cardiomyocyte

### Pathogenesis

Non-apoptotic cardiomyocyte cell death initiates an infiltration by mixed inflammatory cells that may be the most prominent morphologic evidence of that cell death in histologic sections. These infiltrates are predominated by mononuclear cells but may also include fewer numbers of neutrophils or eosinophils. Macrophages within the infiltrate phagocytize cell debris.

### Diagnostic features
• Focal to multifocal lesions
• Cardiomyocyte eosinophilia and/or fragmentation
• Localized mixed inflammatory cell infiltration including lymphocytes, plasma cells, macrophages with fewer neutrophils or eosinophils (Fig. 24)
• Fibrosis as a repair process is generally not prominent

### Differential Diagnosis
• Degeneration, cardiomyocyte – would not be accompanied by evidence of cellular fragmentation or inflammatory cell infiltrate
• Degeneration/necrosis, cardiomyocyte – may include very minimal inflammatory cell infiltration
• Inflammatory cell infiltrate/fibrosis, myocardium – connective tissue proliferation much more prominent component of the morphology

### Comments

Focal infiltrates of inflammatory cells are common in the hearts of rodents and may be associated with naturally-occurring (e.g. rodent progressive cardiomyopathy) or xenobiotic-induced disease. Focal cardiomyocyte necrosis is a common initiating event. Cardiomyocyte necrosis frequently instigates a predictable cascade of events whose morphology can give insights into the 'age' of individual lesions (and thereby their relationship to xenobiotic exposure). Necrotic cell fragmentation is quickly followed by an inflammatory cell infiltrate (18–24 hours) whose magnitude is determined by the number of cells affected (Brady et al. 2010; Clements et al. 2010; Clements 2008). Macrophages within that infiltrate will phagocytize cell debris. Repair will be by fibrosis if the cell loss is substantive (i.e. more than a few cells). Small foci of necrosis may resolve without much connective tissue response.

Multifocal cardiomyocyte necrosis with inflammatory cell infiltration is a morphologic manifestation of rodent or murine progressive cardiomyopathy in young rodents (≤ 3 months) (Chanut et al. 2013). Rodent progressive cardiomyopathy (PCM) is a spontaneous, chronic and progressive myocardial disease that occurs in many strains of rats and mice with some variability in incidence and severity. The lesions of PCM are generally considered to be more prominent in male rodents than females. Fibrosis can be a more prominent feature in older animals. The typical distribution has supported a hypothetical pathogenesis related

to ischemia and vascular disease. Dietary restriction has been demonstrated to ameliorate the severity of PCM and chronic renal disease (another common spontaneous disease of laboratory rodents) is also believed to have some pathogenic influence (Greaves 2007; Jokinen et al. 2005; Kemi et al. 2000). The pattern and morphology of the lesions could also support a hypothesis of endogenous catecholamine release.

Terminology for the lesions that characterize PCM has been highly variable leading to mis-communication or disagreement among the reviewers of pharmaceutical risk assessment packages. Though the disease is widely recognized by experienced toxicologic pathologists, morphologic similarities with some forms of xenobiotic-induced cardiomyocyte injury sometimes complicate differentiation- particularly in short duration toxicity studies. Differentiation is significantly enabled by precise descriptive terminology, consistent reporting practices and robust historical control data.

### Nomenclature recommendations

Precise morphologic terminology can communicate the temporal stage of a myocardial lesion. 'Necrosis/inflammatory cell infiltrate' should reflect those lesions where the infiltrate is prominent and evidence of preceding cardiomyocyte necrosis is present (i.e. to reflect that the inflammatory cell infiltrate is a response to the primary event of necrosis). 'Necrosis' should be applied to those lesions where cardiomyocyte fragmentation is present but the inflammatory cell response is minimal. 'Mononuclear cell infiltrate/fibrosis' is used for those lesions where fibrosis repair has begun. 'Fibrosis' would be reserved for those lesions where inflammation has largely resolved.

In young animals, 'necrosis/inflammatory cell infiltrate' is a common manifestation of the lesions of PCM and the term should therefore be applied accordingly. This harmonized practice was recently adopted by one global pharmaceutical organization to enable more accurate differentiation of xenobiotic-induced cardiac lesions from those of this spontaneous background disease (Chanut et al. 2013).

### Mononuclear cell infiltrate/fibrosis, myocardium

#### Other Terms
• Cardiomyopathy

#### Pathogenesis
Localized areas of cardiomyocyte necrosis are often associated with a predictable and temporally progressive process of mixed inflammatory cell infiltration and reparative fibrosis. The full spectrum of these changes can be seen with repeated injuries as might occur with chronic dosing of a cardio-toxic compound or rodent progressive cardiomyopathy.

#### Diagnostic Features
• Focal, multifocal or regionally-extensive lesions
• Inflammatory cell infiltration ± evidence of preceding cardiomyocyte necrosis
• Interstitial proliferation of fibroblasts and connective tissue (fibrosis)
• Individual lesions may have one or more of the features above but the spectrum of changes should be represented across lesions
• Chronic lesions of mononuclear cell infiltration and fibrosis generally predominate

#### Differential Diagnoses
• Necrosis/inflammatory cell infiltrate, myocardium-smaller or less chronic lesions may lack fibrosis and display a more mixed inflammatory cell infiltrate

#### Comment
"Cardiomyopathy" is a term often used in sub-chronic and chronic studies (e.g. ≥ 3 months duration) to capture the spectrum of morphologic changes that include necrosis, inflammation and fibrosis (Jokinen et al. 2011). A collective term is useful for concise communication but this term is often associated synonymously with rodent progressive cardiomyopathy in the toxicologic pathology community or human heart diseases that are accompanied by clinical dysfunction (Boudina and Abel 2010; Frey et al. 2012; Gavazzi et al.. 2000; Silver et al. 2001).

#### Nomenclature recommendations
"Mononuclear cell infiltrate/fibrosis, myocardium' should be used when chronic lesions predominate but a full spectrum of morphologic changes may be seen. This is most likely to occur in sub-chronic and chronic studies where the changes may represent those of rodent progressive cardiomyopathy or xenobiotic-induced injury. This term is preferred to 'cardiomyopathy' which is considered a synonym. 'Myocardium' is applied as a locator to represent the involvement of interstitial connective tissue. Coincident presence of more than very minimal cardiomyocyte necrosis should prompt an accompanying 'necrosis, cardiomyocyte'.

### Vacuolation, cardiomyocyte

#### Other Terms
• Degeneration, vacuolar, cardiomyocyte

#### Pathogenesis
Clear cytoplasmic vacuolation of otherwise normal appearing cardiomyocytes can be induced by lipid accumulation, T-tubule or sarcoplasmic reticulum dilation, or mitochondrial swelling.

#### Diagnostic features
• Clear, discrete vacuoles within the cytoplasm of cardiomyocytes of otherwise normal morphology (Fig. 25)

- Vacuoles are generally 'microvesicular' and do not distort the cell size or shape
- Not associated with degeneration or necrosis
- Ultrastructural examination or special stains generally required to characterize the composition of the vacuoles (e.g. lipid, phospholipid, swollen mitochondria, etc.)

*Differential Diagnoses*
- Vacuolar degeneration – distinguished by some evidence of cellular dysfunction or injury either by distortion of cellular morphology (change in size or shape), increases in serum cTn, or coincidence of more obvious degeneration and necrosis

*Comments*

Vacuolation of cardiomyocytes can present a diagnostic challenge. Light microscopic vacuolar changes are most often associated with an abnormal accumulation of energy substrates (e.g. lipid), altered processing of phospholipid membranes (e.g. phospholipidosis), or enlargement/dilation of cellular organelles (e.g. mitochondria, sarcoplasmic reticulum). Though these changes often involve some alteration of cellular physiology, they may be difficult to associate with a detectable change in electrophysiology or contractile function. Additionally, vacuolation at the light microscopic level can be very non-specific requiring additional assessments (e.g. special stains, electron microscopy) to characterize the source.

*Nomenclature recommendation*

Use of the term 'vacuolation, cardiomyocyte' at the light microscopic level should be reserved for those instances where cytoplasmic vacuolation is not accompanied by demonstrable evidence of cell injury or necrosis. Vacuolation should be further characterized with ancillary assessments to understand the content of the vacuoles which could inform an understanding of pathogenesis and possibly even risk assessment.

### Apoptosis, cardiomyocyte

*Pathogenesis*

Activation of known apoptotic molecular pathways results in initiation of a 'programmed cell death' with characteristic morphologic features.

*Diagnostic Features*
- Condensed or spherical membrane bound cell fragments
- Hypereosinophilic cytoplasm
- Pyknosis or karyorrhexis of nuclei
- Positive TUNEL and/or cleaved caspase 3 immunolabeling
- Absence of a 'responding' inflammatory cell infiltrate

*Differential Diagnosis*
- Lytic cardiomyocyte necrosis – characterized by loss of membrane integrity and associated with a responding inflammatory cell infiltrate
- Coagulative cardiomyocyte necrosis – intact cardiomyocyte 'ghosts' with a 'faded' or pyknotic nucleus; often associated with vascular occlusion/injury

*Comment*

Co-administration of ephedrine and caffeine induced cardiomyocyte apoptosis (Dunnick et al. 2007). The morphological characteristics were as follows: in the hearts of animals found dead or sacrificed in distress 4–5 h after the first dosing of ephedrine and caffeine, changes were observed chiefly in the interventricular septum and, to a lesser extent, the left and right ventricular walls. Massive interstitial hemorrhage occurred at the subendocardial myocardium of the left ventricle and interventricular septa. The hemorrhage was associated with degeneration of the surrounding myofibers that appeared hyalinized, vacuolated, and with loss of striations. In some of these cells the nuclei were apoptotic (pyknotic) or had disappeared (Fig. 26). Other changes consisted of a multifocal, generalized loss of myofibers associated with macrophage infiltration and the presence of basophilic fragments of nuclei. The macrophages appeared to be digesting lysed fibers and fragments of nuclear chromatin. Application of the histochemical Barbeito-Lopez Trichrome Stain (BLTS) for diagnosis of myocardial degeneration and/or necrosis indicated the presence of generalized patchy yellow myofibers, consistent with cytoplasmic homogenization and loss of striation (Milei and Bolomo 1983; Milei and Storino 1986). Normal-appearing fibers in control and treated animals stained green to light blue.

The fragmented nuclei were strongly positive for anti-phospho-H2A.X, a histone variant that becomes phosphorylated during apoptosis (Talasz et al. 2002). Caspase-3 immunolabeling revealed the presence of multifocal, intracytoplasmic, positive myofibers present most frequently in the interventricular septa. Infrequently these positively labeled myofibers demonstrated morphological degenerative alterations only, indicating the usefulness of this method in detecting an activated apoptotic process at an early stage.

It is important to note that pyknosis and karyorrhexis are not exclusive to apoptosis and can be a part of the spectrum of cytomorphological changes that occurs with necrosis (Kumar and Abbas 2010). The presence of nuclear fragmentation (i.e. karyorrhexis) suggests that the process is irreversible, leading to death of the myofiber.

Apoptotic cell death is frequently implicated in xenobiotic-induced and even natural diseases but difficult to recognize in cardiomyocytes without the aid of immunolabeling for relevant 'markers' (e.g. TUNEL, activated caspase 3). Accordingly, recognition of this process and use of this term may require the application of special labeling techniques (e.g. TUNEL, cleaved Caspase 3 immunolabeling).

### Infarct, myocardium

*Other Terms*
- Necrosis, myocardium (as a pathogenically less specific term)
- Necrosis, cardiomyocyte

*Pathogenesis*

Occlusion of a coronary artery results in regional ischemic/coagulative necrosis of cardiomyocytes supplied by that vessel. The extent of the infarct is determined by the level at which the vessel is occluded, the volume of myocardium supplied by that vessel, and the collateral circulation to that region of the heart.

*Diagnostic Features*
- Discrete region of myocardial necrosis that represents the field of supply for an occluded vessel
- Cytoplasmic hypereosinophilia
- Depending on age of the lesion, may include interstitial edema and infiltrates of inflammatory cells that originate from the margins of the lesion
- Resolution/repair is generally by fibrosis
- Microthrombi and vascular changes may be seen in the region

*Differential Diagnosis*
- Locally extensive cardiomyocyte necrosis not associated with vascular occlusion is more often lytic, morphologically variable and associated with mixed inflammatory cell infiltration.

*Comment*

Xenobiotic-related cardiac infarction is very rarely reported in rats or mice. In the 2-butoxyethanol-induced rat model of hemolysis with disseminated thrombosis, thrombosis in the heart occurred in small-caliber arteries and was associated with coagulative myocardial necrosis and inflammation (Ezov et al. 2002). Neither thrombosis nor infarction seemed to have a predilection for any particular anatomical sites of the heart, and both were located in atria and ventricles.

*Nomenclature recommendation*

'Infarct' should only be applied when there is evidence for vascular occlusion causing a discrete region of necrosis since it infers a specific pathogenesis. 'Necrosis, myocardium' would be most appropriate otherwise. The myocardial necrosis of infarction is associated with intense neutrophilic response in the first few days. Depending on the time passed from the initial thrombotic event, the infarct may be replaced by scar tissue (Ezov et al. 2002).

### Fibrosis, myocardium

*Other Terms*
- Scar, myocardium

*Pathogenesis*

Necrosis of the myocardium involving more than a few cells in a localized region generally repairs ('heals') by replacement with collagenous connective tissue (Fig. 27). The resulting 'scar' lacks the contractile properties of normal myocardium and may be bordered by hypertrophied cardiomyocytes compensating for loss of contractile tissue.

*Diagnostic Features*
- Localized replacement of cardiomyocytes with collagenous connective tissue
- Large areas may have discrete borders that interdigitate with adjacent cardiomyocytes
- Smaller areas may be less discrete and dissect the adjacent intact myocardium

*Differential Diagnosis*
- Fibrosis, subendocardium – more discrete subendocardial location lacking evidence of preceding necrosis or inflammation
- Amyloid, myocardium – distinguished by special staining (Congo red)

*Comment*

Fibrosis is a normal 'healing' response to myocardial necrosis (Jourdan-LeSaux et al. 2010; Kumar and Abbas 2010; Vracko et al. 1989). The connective tissue infrastructure of the normal myocardium is generally widely distributed and largely invisible with routine H&E staining. Localized accumulation of collagen within the myocardium is often evidence of previous myocardial necrosis. Extensive regions of fibrosis lacking the contractile compliance of normal myocardium may lead to cardiac dysfunction. Care must be taken to differentiate fibrosis of 'healing' from normal concentrations of connective tissue in the heart at the insertion of chordae tendinae or at the heart base.

### Inflammatory cell infiltrate, myocardium

*Other Terms*
- Inflammation, myocardium
- Necrosis/inflammatory cell infiltrate, cardiomyocyte

*Pathogenesis*

Inflammatory cell infiltration of the heart is most commonly a response to cardiomyocellular injury and necrosis but infiltrates without evidence of precedent or associated necrosis may also be seen. These infiltrates are often composed of mixed mononuclear cells with fewer neutrophils or eosinophils.

*Diagnostic Features*
- Infiltration of the myocardium by one or several types of inflammatory cells: neutrophils, macrophages/histiocytes, lymphocytes, plasma cells, eosinophils
- Chronic or recurring lesions may also be associated with fibrosis

*Differential Diagnoses*
- Neoplasia (lymphomas, leukemias) – more monomorphic populations of cells not focused at sites of cardiomyocellular injury
- Primary cardiomyocyte necrosis – accompanied by evidence of cardiomyocyte fragmentation

*Comments*

Inflammatory cell infiltrates or inflammation may not be associated with foci of necrosis and/or fibrosis in rats and mice (Elwell and Mahler 1999; Greaves 2007). But, inflammatory cell infiltration responding to cardiomyocellular injury is much more common in rodents than primary myocardial inflammation as described for viral infections or transplant rejection in human patients. (Silver et al. 2001).

*Nomenclature recommendations*

Inflammatory cell infiltration with evidence of precedent or accompanying cardiomyocyte necrosis should be termed 'necrosis/inflammatory cell infiltrate' to most accurately reflect the primary pathogenesis. 'Inflammatory cell infiltrate, myocardium' may be used in those instances where necrosis is absent.

### Edema, myocardium

*Pathogenesis*

Acute myocardial injuries that result in increased vascular permeability or endothelial injury can cause excessive accumulation of low protein fluid in the myocardial interstitium. Edema may also result from increased capillary hydrostatic pressure or decreased lymphatic drainage.

*Diagnostic Features*
- Expansion of the interstitial space
- Pale basophilic amorphous material (Fig. 28)
- Interstitial vacuolation

*Differential Diagnoses*
- Artifact of tissue processing – artificial separation of cardiac myofibers during microtomy
- Autolysis – postmortem autolysis may result in increased interstitial fluid
- Myxomatous degeneration of the myocardium or heart valves – special histochemical stains reveal compositional differences

*Comments*

Localized edema may be due to lymphatic blockage or increased vascular permeability (early inflammation) (Cheville 1994).

Clements, et al. described interstitial edema as an early change in the progression of myocardial injury that occurred with high dose isoproterenol cardiotoxicity (Clements et al. 2010).

### Thrombus, atrium

*Pathogenesis*

Pathogenesis of this spontaneous condition is unknown but likely involves endothelial injury and/or altered atrial contractile function. Xenobiotic-induced atrial thrombosis likely involves endothelial injury or altered atrial contractile function.

*Diagnostic Features*
- Atrial luminal distension with mass of laminated fibrin admixed with leukocytes and red blood cells, often segmentally adherent to the adjacent atrial endocardium (Fig. 29, 30)
- More chronic lesions may be re-canalized by endothelium-lined channels; covered by endothelium continuous with the endothelium of the non-occluded atrium, or include dense fibrous connective or metaplastic cartilage/bone
- Left atrial involvement is most common

*Differential Diagnosis*

Primary neoplasms of the heart, such as atrio-caval mesothelioma and hemangiosarcoma may also involve the left atrium but are distinguished by hypercellular infiltrations of the atrial wall that may extend to the endocardial surface.

*Comment*

Spontaneous rates of atrial thrombosis were determined for control Fischer 344 rats and B6C3F1 mice: 0% in rats and mice in 90-day studies and 0.7% in both genders of mice, 4% in male rats, and 1% in female rats in 2-year studies (Yoshizawa et al. 2005). Some genetic mouse models of hyper-coagulation, abnormal myocardial contraction and dietary disorders are associated with atrial thrombosis. Examples include At[m/m] mice showing a mutation in the antithrombin gene, ja/ja (jaundiced) mice with a deficiency in erythroid β-sectrin; β-tropomyosin-overexpression mice exhibiting abnormal cardiomyocytic contraction and relaxation resulting in altered blood flow and thrombus formation; and dietary fat associated with heart thrombosis in C3H/OUJ mice developing dystrophic cardiac calcinosis (Dewerchin et al. 2003; Everitt et al. 1998; Kaysser et al. 1997; Muthuchamy et al. 1998).

Xenobiotic-induced atrial thrombosis can be associated with myocardial injury, endothelial injury, circulatory stasis, hypercoagulability, and impaired atrial mechanical activity (e.g. atrial fibrillation) resulting in stasis of blood within the left atrial appendage (Yoshizawa et al. 2005).

A retrospective study of the NTP database suggested that atrial thrombosis was usually associated with death of these animals (Yoshizawa et al. 2005).

### Mineralization, cardiomyocyte or myocardium

*Other Terms*
- Calcinosis
- Calcification

*Pathogenesis*

Deposition of mineral within individual cardiomyocytes at a site of cellular necrosis or within the interstitial connective tissue.

*Diagnostic Features*
- Basophilic granular material or larger amorphous to crystalline aggregates in myocardium or epicardium; visible in H&E sections; confirmed with special stains

*Differential Diagnoses*
- Formalin pigment – artifact of tissue fixation with haphazardly distributed granular basophilic material.
- Osseous metaplasia – will include features of skeletal bone that are distinct from normal cardiac histo-architecture

*Comments*

Myocardial mineralization is found sporadically in most strains of aged rats and mice, particularly following myocardial injury (Greaves 2007). In the Fischer rat, mineralization of the cardiac trigone begins to occur by six months of age (MacKenzie and Alison 1990). Myocardial mineralization may occur with rodent progressive cardiomyopathy or secondary to advanced renal disease (Fig. 31). This finding has been reported under conditions of systemic calcium-phosphorus imbalance (Greaves 2007).

In certain CD-1 mice, cardiac mineralization is uncommon. The lesion is found in the epicardium and adjacent myocardium over the right ventricle and the epicardium is sometimes fibrotic (Faccini et al. 1990). Genetic susceptibility exists for epicardial or myocardial mineralization in some inbred strains of mice, where frequency and severity depend on age, sex, diet and number of pregnancies (Fig. 32). Mineralization can be seen in the heart of animals as young as 6-7 weeks of age (Hagiwara et al. 1996).

### Pigment, cardiomyocyte or myocardium

*Pathogenesis*

Lipofuscin deposition – May be associated with advancing age (i.e., a fourfold increase was documented in cardiomyocytes of rats between 6 and 30 months of age) or oxidative stress from mitochondrial damage or production of reactive oxygen species (Schmucker et al. 2002; Terman et al. 2004).

Hemosiderin deposition – May develop following drug induced myocardial necrosis and hemorrhage, like in the case of rat treatment with a vasodilating antihypertensive drug (Greaves 2007). Iron loading, like in the case of hypotransferrinaemic (hpx/hpx) mice, a model for genetic (primary) haemochromatosis, was associated with the presence of hemosiderin-loaded macrophages (Simpson et al. 1993).

*Diagnostic Features*
- Lipofuscin deposition: Presence of intracytoplasmic and intra-lysosomal golden-brown pigment. Ultrastructurally characterized by the presence of prominent intracytoplasmic dense bodies with areas of electron lucidity in their matrix (Schmucker et al. 2002). Deposits fluoresce brown in UV light and stain with fat-soluble dyes and the PAS reaction (Sheehan and Hrapchak 1980).
- Hemosiderin deposition: Interstitial yellowish or reddish-brown, Perl's prussian blue-positive pigmented granule accumulation located within macrophages or free in the interstitium (Fig. 33, 34). The accumulation of iron may be associated with myocardial hemorrhage, degeneration, necrosis and fibrosis (Carthew et al. 1993; Carthew et al. 1994; Whittaker et al. 1996). In the hypotransferrinaemic (hpx/hpx) mouse, the presence of iron-loaded macrophages was not associated any other damage in the heart (Simpson et al. 1993). With EM, iron-filled lysosomes (siderosomes) were found in macrophages in the heart muscle cells (Iancu et al. 1987).

*Differential Diagnosis*
- Artifactual formalin pigment deposits which are granular and birefringent
- Stain precipitates are often less specifically located and outside the plane of section (i.e. sitting on top of the tissue rather than within it)
- Melanin pigment that may be present in heart valves and other tissues of pigmented mice.

*Nomenclature recommendation*

The level of detail used in the terminology for pigment deposits in cardiomyocytes should be determined by the effort applied to identify the content of the pigment. For example, pigment not characterized with special stains or ultrastructure should bear a more general term like 'pigment, cardiomyocyte'.

### Amyloid, myocardium

*Other Terms*
- Amyloid deposit
- Amyloidosis

*Pathogenesis*

Deposits of immunoglobin light chains or acute phase proteins in a β-pleated sheet conformation can accumulate in the interstitium of the myocardium, arterial walls and other organs. These deposits are generally not associated with a cellular reaction.

*Diagnostic Features*
- Hyaline amorphous matrix infiltrating and expanding the cardiac interstitium or walls of blood vessels.
- Positive staining with Congo red or Thioflavine T

*Differential Diagnoses*
- Fibrinoid necrosis of small blood vessels – frequently associated with medial hemorrhage and/or cell necrosis
- Fibrin deposition – frequently associated with hemorrhage

*Comments*

Amyloid is frequently seen in mice and hamsters, but is very rare in rats (Fig. 35, 36). It occurs both spontaneously and as an induced lesion in mice, where it can be caused by repeated injections of pro-inflammatory substances (eg. casein). There are two types of amyloid in mice; AA and AapoAII, with AapoAII being more commonly seen in the heart (Percy and Barthold 2007).

Amyloid may occur as intramural deposits in the small blood vessels of numerous organs including the jejunum, pancreas and testes (Elwell and Mahler 1999). Amyloid can be stained with Congo Red, Oil Red O, Alcian Blue and Thioflavine T, but staining intensity may vary considerably. Amyloid is birefringent under polarised light (Sheehan and Hrapchak 1980).

**Heart Valves**

Valve injury can involve any or all of the cellular and non-cellular components of the valves. Thrombi can form along disrupted endothelial surfaces. Activated valve interstitial cells can adopt a myofibroblast phenotype with expression of smooth muscle actin, proliferate and increase production of extracellular matrix material. Physical disfigurement of valve leaflets may lead to disruption of laminar blood flow and increased hemodynamic stresses worsening injuries to the valve. Disfigurement may also lead to backflow of blood resulting in myocardial hemodynamic stresses (e.g. increased preload) as well (Donnelly 2008). Non-inflammatory degenerative processes present in the heart valves must be distinguished from spontaneous conditions that occur with aging in laboratory animals (Elangbam et al. 2002b).

Drug-induced valve lesions have not been described frequently enough in rodents for the terminology to be well-established. Accordingly, a descriptive approach is recommended with the potential need to contextualize the descriptions in a textual report. It's possible to have morphologic changes that span the terms offered below. A term that most accurately reflects the predominant change should be applied.

### Degeneration, myxomatous, valve

*Other Terms*
- Degeneration, myxomatous, endocardial
- Valvulopathy
- Endocardiosis, valvular

*Pathogenesis*

The valve stroma is expanded by hypocellular amorphous matrix material composed predominately of glycosaminoglycans, a normal product of valve stromal cells. The pathogenesis of degenerative valvular disease of rodents (as with other species) is unknown. It likely involves activation of valvular interstitial cells leading to either increased production or decreased remodeling of interstitial matrix.

*Diagnostic features*
- Diffuse or nodular thickening of valve leaflets that may extend along chordae tendinae
- Expansion of the spongiosa of the valve leaflet with hypocellular extracellular matrix composed predominately of glycosaminoglycans (Fig. 37, 38)
- Incidence increases with age
- Hypercellular variants with increased numbers of haphazardly arranged valvular interstitial cells may resemble drug-induced valvulopathy of human patients

*Differential Diagnoses*
- Stromal proliferation, valve – not definitively recognized in nonclinical animal models but would be expected to be more cellular
- Inflammation, valve – would be distinguished by greater cellularity with infiltrates of inflammatory cells as well as frequently including hemorrhage and cellular necrosis

*Comment*

Mitral valve prolapse in human patients is the result of an age-related myxomatous degeneration of atrioventricular valves (Freed et al. 1999). The valvular disfigurement that can result may allow systolic regurgitant blood flow (valvular insufficiency) and myocardial disease from increased hemodynamic preload. Though not characterized functionally, morphologically similar changes have been described as an age-related spontaneous condition in atrioventricular and semilunar valves of rodents (Donnelly 2008; Elangbam et al. 2002b; Ruben et al. 2000).

A significant interpretive challenge can present in nonclinical studies with ergotamine, serotonergic, anorexigenic, or amphetamine compounds given the similarities between what has been described in human patients as drug-induced valvulopathy and spontaneous disease in rodents- particularly hyper-cellular variants of the spontaneous disease (Connonlly et al. 1997; Elangbam et al. 2006; Gustafsson et al. 2005; Rothman et al. 2009). But, the ability to reliably model these drug-induced valvular injuries is not firmly established.

*Nomenclature recommendations*

The rodent lesion has been more commonly termed "endocardial myxomatous change" in the historic and even

contemporary literature (Elangbam et al. 2002b). In the interest of translational communication (i.e. nonclinical to clinical) and more 'descriptive' terminology, 'degeneration, myxomatous' is probably more useful.

This term should be reserved for the spontaneous valvular degeneration of aging rats. As with other forms of spontaneous disease in rodents (progressive cardiomyopathy, progressive nephropathy), xenobiotic treatment could exacerbate the condition resulting in a xenobiotic-associated change in incidence or severity.

### Stromal proliferation, valve

*Other Terms*
- Valvulopathy

*Pathogenesis*
Valve stromal cells can be activated by mechanical (i.e. hemodynamic) and xenobiotic stimuli. Stimulation induces hypertrophy, proliferation and the production of extracellular matrix that may be an admixture of glycosaminoglycans and collagen.

*Diagnostic features*
- Increased numbers of hypertrophied stromal cells ± mitotic figures ± expansion of the extracellular matrix
- 'Activated' stromal cells acquire a myofibroblast phenotype and immunolabel with smooth muscle actin
- Valve leaflet may become expanded and disfigured.
- Overlying endothelium may become hypertrophied if exposed to altered hemodynamic forces with associated valve dysfunction.

*Differential diagnoses*
- Myxomatous degeneration – distinguished by hypocellularity and matrix expansion of the valve body. Also a lesion predominately seen in older rodents.
- Valvular endocarditis – typically more chronic vegetative lesions that occur with low incidence. Often include evidence of bacteremia.

*Comment*
The best known drug-induced valvulopathy is that reported in human patients taking the anorexigenic combination phentermine-fenfluramine. The lesions were characterized as fibrous plaques along the surface of valve leaflets (so called "onlays") composed of stromal cells in a dense matrix of collagen and glycosaminoglycans that distorted the architecture of the affected valve leading to dysfunction and secondary cardiac insufficiency (patients presented with murmurs and congestive heart failure) (Connolly et al. 1997). The pathogenesis of phen-fen-induced valve lesions is strongly linked to agonism of the 5HT2B serotonin receptor and believed related to valvulopathies also described in patients taking the anti-Parkinsonian drugs pergolide and cabergoline (ergot-derived drugs with po-

tent 5HT2B receptor activity) or with serotonin-secreting carcinoid tumors (Bratter et al. 1999; Cosyns et al. 2013; Droogmans et al. 2007; Elangbam et al. 2008; Gustafsson et al. 2005; Roth 2007). Though modeling these clinical valvulopathies in animals has been difficult, Fielden, et al. reported a rapidly developing proliferative valvulopathy in rats given a proprietary compound with in vitro 5HT2B receptor activity (Fielden et al. 2010).

*Nomenclature recommendations*
'Stromal proliferation' should be reserved for those lesions that are clearly not inflammatory but involve proliferation of valve stromal cells with an accompanying increase in amorphous and fibrous matrix. This 'proliferation' term is included here rather than with other proliferative terms because it is almost never associated with a continuum that could include dysplastic or neoplastic proliferation and it is useful to put it into the context of the other valve terms.

### Inflammation, valve

*Other Terms*
- Endocarditis, valvular

*Pathogenesis*
Septic emboli adhere to the surface of valve leaflets/cusps initiating a cascade of inflammation, hemorrhage, thrombus formation and reactive hyperplasia of endothelial and/or stromal cells. The inflammatory process may extend into the stroma of the valve. Alternatively, xenobiotics with TGFβ activity can induce a spectrum of inflammatory changes within the valve matrix.

*Diagnostic Features*
- Vegetative plaque composed of mixed inflammatory cells including neutrophils in a matrix of fibrin that may also contain micro-colonies of coccoid bacteria on the surface of one or more valve leaflets (Fig. 39, 40)
- Expansion of the valve stroma by edema, cellular necrosis, hemorrhage and inflammatory cell infiltrates
- Reactive hyperplasia may be present in the adjacent endothelium or underlying stromal cells

*Differential diagnoses*
- Stromal proliferation, valve – primarily involve the stroma and lack the infiltration of inflammatory cells

*Comment*
Septic valvular endocarditis (inflammation of the valve and endocardium) is most often seen in laboratory rodents as an incidental finding but may occur more frequently in animals that are immune-compromised or become septic secondary to the stress or insult of drug toxicity (Anderson et al. 2006). Large vegetative lesions may compromise valve function (i.e. ensuring unidirectional flow of blood) leading to secondary myocardial changes (e.g. hypertrophy due to increased pre-load) and cardiac dysfunction.

Alternatively, Anderton et al. more recently reported valve lesions in rats induced by ALK5 inhibitors (Anderton et al. 2011). These lesions were rapidly developing and shared morphologic features with septic valvular inflammation (e.g. inflammatory or degenerative changes that included hemorrhage, necrosis, fibrin deposition and inflammatory cell infiltrate) but more consistently across treated animals and without evidence of bacteria. Unpublished personal experiences/communications indicate that the Anderton experience has been shared by others targeting ALK5 or other intermediates of the TGFβ pathway.

*Nomenclature recommendation*

This term should be reserved for the incidental and vegetative lesions often presumed to be secondary to bacteremia. We acknowledge the potential confusion with the inflammatory lesions of ALK5 inhibitors but those lesions generally also include stromal proliferation and have been obviously drug-associated indicated by dose-relationship and incidence.

### Angiectasis, valve

*Other Terms*
- Hemocyst
- Hematocyst
- Blood cyst

*Pathogenesis*

Dilation of thin-walled blood vessels in atrioventricular valves.

*Diagnostic Features*
- Thin walled cystic structure lined by a continuous single layer of endothelium and filled with blood. (Fig. 41)

*Differential Diagnoses*
- Hematoma or hemorrhage – red cells are extravasated

*Comments*

Valvular angiectasis is identified as single or multiple cystic, blood-filled lesions on valves. The lesions are observed predominantly on the septal cusp of the right atrioventricular valve and occasionally found on the parietal cusp of the right atrioventricular valve or on the left atrioventricular valve. There is no direct relationship with inflammatory cell infiltration. Thrombosis, degeneration of blood cells or of the vessel itself and mineralization have not been reported in rats and mice. Fang *et al.* demonstrated with serial sections of the heart of rats that the lesion including multiple blood-filled cysts was interconnected with vessels (Fang *et al.* 2007).

The cause is unknown. There is no evidence that development of valvular angiectasis is induced by trauma or associated with necropsy procedures. There is no evidence that the incidence increases with advancing age as well.

*Nomenclature recommendation*

Some confusing terms have been used to describe cystic lesions on the valves in animals. For example, a term of "hematoma" or "congenital hematoma" is used for expressing similar valvular lesions. "Hematoma" is an abnormal and localized collection of blood, in which the blood is clotted, and is generally the result of a break in the wall of a blood vessel. Therefore, 'hematoma' is an inaccurate term to describe valvular angiectasis in rats and mice.

**Blood Vessels**

Mechanistically, the pathogenesis of xenobiotic-induced vascular injury may be due to biomechanical, immunologic, or direct cellular cytotoxicity (Berridge et al. 2013) (Kerns et al. 2005). Of these, biomechanical and direct cytotoxicity predominate in rodent species used in nonclinical pharmaceutical safety assessment. Alternatively, immune-mediated vasculitides are more common in human patients either as natural disease or a drug-related adverse event when it occurs. Since '-itis' terms (e.g. vasculitis/arteritis) are entrenched in clinical medicine and recognizing that they refer to injuries with pathogeneses distinct from those most commonly described in nonclinical species, '-itis' terms are not recommended.

Spontaneous or non-drug-induced vascular lesions (Spontaneous Polyarteritis of Rodents) do occur in rodents and may provide an interpretive challenge. A necrotizing arteriopathy of small and medium-sized arteries resembling polyarteritis nodosa in human patients is well-described in rodents and may have a predilection for vascular beds where drug-induced injury may also occur (e.g. mesenteric arteries) (Greaves 2007; Jokinen et al.. 2005; Ruben et al. 2000). Also, multifocal arterial necrosis can occur in uremic animals with renal failure or with severe hypertension. These lesions may be morphologically indistinguishable from drug-induced vascular injury and require differentiation by considering incidence, dose response, and historical experience with spontaneous disease in the strain of animals being used.

The terms listed below represent common presentations of drug-induced vascular injury in rodents. These changes often present as a continuum and may be influenced by the magnitude and persistence of the insult as well as the age of the lesion. Conjugated terms (e.g. degeneration/necrosis, necrosis/inflammation, inflammation/fibrosis) may be preferred to represent that spectrum. Morphologic descriptors for the changes of vascular injury should capture either the primary event (e.g. necrosis) or the most prominent morphology represented in the sample examined (which can be variable depending on the age of the lesion). The injuries these terms describe are most commonly seen in small to medium-sized arteries.

### Hypertrophy, endothelial

*Other Terms*
  "Plump" endothelial cells

*Pathogenesis*

"Activation" of endothelial cells results in cellular hypertrophy and increased production of vasoactive and pro-inflammatory mediators.

*Diagnostic features*

- Endothelial cells with large, prominent nuclei that protrude into the vascular lumen
- Apparent increase in interstitial cellularity of a tissue due to increased visibility of endothelial cells and small vessels (Fig. 42)

*Differential Diagnoses*

- Endothelial hyperplasia – increased numbers of endothelial cells in small interstitial vessels, mitotic figures

*Comments*

Endothelial hypertrophy has been observed in the capillaries of the parotid glands of rats treated with isoproterenol and in the pancreatic arteries of rats infused with fenoldopam (Hand and Ho 1985; Ikegami 2002). The endothelium of fenoldopam-treated pancreatic arteries was immunohistochemically observed to have increased vWF and Factor VIII content compared to saline controls; this was interpreted to be the result of increased protein synthesis. Endothelial hypertrophy was induced in small pulmonary arteries in rats by infusing large volumes of saline over a 30-day period (Morton et al. 1997).

Endothelial cells may be stimulated (rapid, reversible responses that are independent of protein synthesis) or activated (longer term responses involving gene expression and protein synthesis) as a result of exposure to various stimuli (Mitchell and Schoen Frederick 2010). Whether endothelial cell hypertrophy is a morphological representation of either of these conditions cannot be determined by light microscopy.

### Hypertrophy, medial or mural, artery

*Other Terms*

- Medial thickening
- Hypertrophy/hyperplasia, medial

*Pathogenesis*

Increased hemodynamic pressure within the arterial system results in hypertrophy of smooth muscle cells of the tunica media.

*Diagnostic Features*

- Increase in vascular wall, mainly tunica media, thickness due to hypertrophy of vascular smooth muscle cells (Fig. 43).

*Differential Diagnoses*

- Amyloid, artery – thickening of vascular wall due to accumulation of extracellular amyloid rather than increase

in size of smooth muscle cells
- Rat pulmonary vessels appearing irregularly thickened at branch points
- Artificial hypercontraction of a coronary artery as a postmortem change

*Comments*

Thickening of the media of muscular wall most often as a result of hypertrophy of smooth muscle fibers is commonly considered evidence of hypertension and is believed to be an adaptive response of the vessel wall to increased intramural stresses (Greaves 2007). This change is observed as a typical feature in the spontaneous hypertensive rat (SHR) or the experimental hypertension model of dogs (Cimprich 1986; Limas et al. 1980). Smooth muscle cell hyperplasia in the thickened vascular wall may be seen together with smooth muscle cell hypertrophy.

The medial hypertrophy of arteries and veins without evidence of systemic hypertension can be seen in rats receiving an inotropic vasodilator, and it may be a result of an adaptive response to marked vessel wall tension being achieved by excessive vasodilatation (Westwood 1990).

Another form of medial thickening associated with an increase in extracellular matrix within the media may be seen in the experimental diabetes rat (Vranes et al. 1999).

As in the systemic circulation, medial thickening of pulmonary arteries is characteristic of pulmonary hypertension. There is no good animal model for human pulmonary hypertension, but experimental hyperoxia or hypoxia has been shown to produce medial thickening of muscular pulmonary artery in animals. Medial thickening of pulmonary arteries was described in rats given infusions of saline for 30 days (Morton et al. 1997).

Note regarding a unique feature of rat pulmonary vasculature- a vessel appearing to have an irregularly thickened muscle can be seen at the level of the terminal bronchiole, which is an external oblique muscle surrounding a right angle branch artery.

### Hemorrhage, medial or mural, artery

*Pathogenesis*

Mechanical vasoactivity (constriction or dilation) alters the 'tone' of small to medium-sized arteries leading to increased luminal sheer stresses and mechanical injury to medial smooth muscle cells. Hemorrhage is one consequence of those injuries. Direct medial smooth muscle cytotoxicity may also occur.

*Diagnostic Features*

- Segmental or circumferential infusion of erythrocytes into the tunica media of small to medium-sized arteries (Fig. 44, 45)
- Often accompanied by segmental to circumferential medial necrosis

- May be a component of medial necrosis

*Differential Diagnoses*
- Necrosis, medial or mural
- Inflammation, medial or mural

*Comment*

Medial arterial hemorrhage may be seen as an acute change with either potent vasodilators or vasoconstrictors. It also often accompanies medial necrosis and may be a component of medial inflammation. Arterial medial necrosis and hemorrhage is best exemplified by the arterial lesions seen in rats given intravenous infusions of the dopaminergic vasodilator fenoldopam (Ikegami 2002; Yuhas et al. 1985).

*Nomenclature recommendation*

'Hemorrhage, medial/mural' should be reserved for those minimal lesions not complicated by obvious necrosis or inflammation.

### Degeneration/necrosis, medial or mural, artery

*Other Terms*
- Arteritis, necrotizing
- Vasculitis, arterial
- Necrosis/inflammation, medial or mural, artery

*Pathogenesis*

Mechanical vasoactivity (constriction or dilation) alters the 'tone' of small to medium-sized arteries leading to increased luminal sheer stresses and mechanical smooth muscle stresses that could result in smooth cell injury and death. Direct smooth muscle cytotoxicity may also occur.

*Diagnostic Features*
- Loss or fragmentation of smooth muscle cell nuclei
- Fragmentation of smooth muscle cells
- Expansion of the tunica media by hyaline eosinophilic amorphous material (fibrinoid change)
- May be accompanied by hemorrhage and inflammatory cell infiltrate

*Differential Diagnoses*
- Hemorrhage, medial/mural – distinguished by the presence of extra-vasated erythrocytes in the arterial wall without evidence of cell injury.
- Inflammation, medial/mural – distinguished by mural infiltration with inflammatory cells without evidence of cell injury.

*Comment*

Medial necrosis of small and medium arteries is a common feature of vascular injury in the rodent (Fig. 46). It is often seen as a xenobiotic-related event with molecules that are recognized to be vasoactive (Greaves 2007; Greaves 2000; Louden *et al.* 2006; Zhang *et al.* 2006). It is also a feature of the spontaneous necrotizing arteriopathy of rodents

(Greaves 2007; Mitsumori 1990).

*Nomenclature recommendation*

Since spontaneous arteriopathy cannot be differentiated from xenobiotic-induced injury based on morphology alone, similar morphologic descriptors should be used (e.g. necrosis, medial/mural) with a distinction made in a textual interpretation that considers incidence, location of the lesions, dose-response, and historical data for the strain of rodent used.

'Degeneration/necrosis', 'Necrosis/inflammation', and 'Inflammation' are likely morphologies along the continuum of temporal progression or severity for arterial wall injuries. The term that most reflects the morphology should be applied recognizing that there's not likely a distinction in considerations of mechanisms, pathogenesis, or risk assessment.

### Necrosis/inflammation, medial or mural, artery

*Other Terms*
- Arteritis
- Vasculitis, artery
- Necrosis, medial or mural, artery

*Pathogenesis*

Inflammation as a response to primary necrosis can occur within arterial walls as it does in the myocardium. Hemorrhage and edema are more likely to accompany necrosis in arterial walls.

*Diagnostic Features*
- Combined features of necrosis and inflammation (Fig. 47, 48)
- Karyolytic nuclear debris
- Amorphous eosinophilic fibrinoid matrix
- Mixed inflammatory cell infiltration with a prominent neutrophilic component
- Hemorrhage and edema possible

*Differential Diagnoses*
- Degeneration/necrosis, medial or mural – may be early or a mild form of the pathogenesis of arterial wall injury lacking a significant component of inflammation
- Inflammation, medial or mural – necrosis not prominent or obscured by inflammatory cells

*Nomenclature recommendation*

'Degeneration/necrosis', 'Necrosis/inflammation', and 'Inflammation' are likely morphologies along the continuum of temporal progression or severity for arterial wall injuries. The term that most reflects the morphology should be applied recognizing that there's not likely a distinction in considerations of mechanisms, pathogenesis, or risk assessment.

### Inflammation, medial or mural, artery

*Other Terms*
- Arteritis
- Vasculitis, artery
- Necrosis/inflammation, medial or mural, artery

*Pathogenesis*

Acute medial arterial necrosis may progress to a spectrum of inflammatory changes that includes hemorrhage, edema, and mixed inflammatory cell infiltrates. Severe lesions may extend into perivascular tissues.

*Diagnostic Features*
- Medial expansion or disruption by admixture of hemorrhage, edema, and mixed inflammatory cells
- Often accompanies medial necrosis
- May extend into the perivascular space

*Differential Diagnoses*
- Necrosis, medial/mural

*Comment*

The inflammation that accompanies mural necrosis and may extend into the perivascular space with severe injuries are distinguished from the more bland perivascular inflammatory cell "cuffs" that may not be associated with substantive mural injury.

*Nomenclature recommendation*

'Degeneration/necrosis', 'Necrosis/inflammation', and 'Inflammation' are likely morphologies along the continuum of temporal progression or severity for arterial wall injuries. The term that most reflects the morphology should be applied recognizing that there's not likely a distinction in considerations of mechanisms, pathogenesis, or risk assessment.

### Vacuolation, medial or adventitial, artery

*Other Terms*
- Vacuolar degeneration, artery
- Fatty infiltration; fatty change; fatty degeneration; steatosis, artery

*Pathogenesis*

Intracellular accumulation of fluid or lipid causes discrete clear spaces in the cytoplasm of arterial smooth muscle cells.

*Diagnostic Features*
- Vacuoles of various sizes within the cell cytoplasm may be distinct, individual, and round (fat), or indistinct and irregular (water); special stains such as Oil Red O and Sudan IV (lipid-soluble dyes) on frozen tissue samples differentiate vacuoles containing lipid from those containing water.

*Differential Diagnoses*
- Glycogen accumulation – smooth muscle cell cytoplasm would be lacy and lack discrete vacuoles.

*Comments*

Vacuolation is a morphologic presentation of subcellular injury. Ultrastructurally, one may observe the accumulation of water in fragmented endoplasmic reticulum or in mitochondria in affected cells (Cheville 1983a).

Fat accumulation (other terms: fatty change, fatty degeneration, steatosis) is a degenerative change that consists of the accumulation of globules of neutral lipids (triglycerides) in non-lipogenic cells. Any cellular injury that causes an imbalance among supply, use, synthesis, or release of lipids may induce this change. Ultrastructurally, lipid globules are free within the cytoplasm (Cheville 1983b).

The administration of peroxisome proliferators-activated receptor-γ (PPAR-γ) agonists has been shown to induce an increase in the size and number of vacuoles within the tunica media and/or adventitia of small- to medium-sized arteries and arterioles of brown adipose tissue. Some vacuoles may contain thin, peripherally located basophilic nuclei. Oil Red O and osmium staining are positive, indicating the presence of lipid. Ultrastructural features are consistent with differentiated adipocytes.

This change has been observed in short-, medium-, and long-term oral (gavage) toxicity studies conducted in Han Wistar rats (Elangbam et al. 2002a). The vessels of brown adipose tissue were selectively affected by compound administration. Males were more sensitive to the change than were females. The incidence and severity of the vacuolation decreased with increasing study duration, suggesting an adaptive effect.

PPAR-γ is a central regulator of fat formation (Tang et al. 2008). Using a PPAR-γ-reporter strain of mouse (PPARγ-R26R), Tang et al. discovered that adipocyte progenitors reside in the mural cell compartment of adipose vasculature, but not in the vasculature of other tissues. These cells express PPAR-γ; they divide, are mobilized from, and repopulate the adipose stromal-vascular fraction of adipose tissue. Thus, the lipid accumulation that occurs with the administration of PPAR-γ proliferators may represent differentiation of lipogenic precursor cells into adipocytes, as opposed to an accumulation of lipid in a non-lipogenic cell.

### Inflammatory cell infiltrate, perivascular

*Other Terms*
- Perivasculitis
- Inflammation, perivascular

*Pathogenesis*

Perivascular inflammation or inflammatory cell infiltration frequently accompanies vascular injuries of varying

etiology. This is particularly true when the primary injury is subacute to chronic where the inflammation may be accompanied by fibrosis (cf. fibrosis, perivascular).

*Diagnostic Features*
- "Inflammation" characterized by infiltrates of inflammatory cells in a perivascular stroma expanded by edema, hemorrhage, congestion, and/or proliferating fibroblasts (fibroplasia)
- May be bland perivascular hypercellularity composed of mixed inflammatory cells (neutrophils, mononuclear inflammatory cells) or predominately mixed mononuclear (lymphocytes, macrophages) inflammatory cells
- Chronicity often accompanied by fibroplasia

*Differential Diagnoses*
- Inflammation, medial/mural that spills into the perivascular space

*Comment*
'Inflammatory cell infiltrate, perivascular' should be reserved for those presentations where adjacent medial/mural injury and inflammation are not present as the primary site of injury (Fig. 49). One should consider whether a lesion primarily characterized by perivascular inflammation is an extension of an injury that involves the arterial wall somewhere outside the plane of section being examined. Examination of samples from multiple animals with a spectrum of change or step-sections through the lesion might be instructive with respect to pathogenesis. Perivascular inflammation can also be a feature of some viral diseases so care should be made in a toxicity study to ensure the lesion is not the result of background disease. In the lung, it is a common feature of allergen-induced pulmonary disease (Sur et al. 1999; Tschernig et al. 2008). Eosinophilic inflammatory cell infiltrates occurred around pulmonary arteries in rats given infusions of saline for 30 days (Morton et al. 1997).

### Fibrosis, perivascular

*Pathogenesis*
Chronic hemodynamic stress on a small to medium-sized artery can induce circumferential proliferation of fibrous connective tissue with a characteristic 'onion skin' appearance. Alternatively, 'healed' vascular and perivascular inflammation may have perivascular fibrous connective tissue as an outcome.

*Diagnostic Features*
- Dense, circumferential "collar" of mature fibrous connective tissue or fibroblasts surrounding small to medium arteries (Fig. 50)
- 'Healed' inflammatory lesions may include an admixture of mixed mononuclear inflammatory cells
- Perivascular fibrosis of chronic hemodynamic stress may be accompanied by mural hypertrophy of the as-

sociated artery

*Differential Diagnoses*
- None

*Comment*
The two most common presentations for perivascular fibrosis in rodents are the circumferential fibrosis of chronic hypertension and as a sequelae to vascular wall inflammation that extends into the periadventitial space.

### Intimal thickening, acellular, artery

*Pathogenesis*
Unknown

*Diagnostic Features*
- An increase in matrix, without an increase in cell numbers, between the endothelium and the internal elastic lamina

*Differential Diagnoses*
- Intimal proliferation – distinguished by increased subintimal cellularity.

*Comments*
Limas et al. (1980) observed gradual thickening of the intima with ground substance, elastin and collagen fibers for up to 20 weeks in the aorta of spontaneously hypertensive rats (Limas et al. 1980). Smooth muscle cells and fibroblasts subsequently appeared in the subendothelial space, signaling the transition of the lesion from intimal thickening to intimal hyperplasia.

Acellular intimal thickening has been found in the dermis and subcutis of untreated skin from the dorsum of 19-20 week old Sprague-Dawley rats (Fig. 51). The composition of the matrix (e.g. endothelial cell cytoplasm, extracellular matrix) is not presently known (Wells et al. 2010).

### Mineralization, medial or mural, artery

*Other Terms*
- Calcinosis
- Calcification

*Pathogenesis*
Deposition of mineral in the walls of blood vessels (arteries or veins) following injury (dystrophic mineralization) or as a primary process of disordered systemic calcium-phosphorus balance (malignant calcification).

*Diagnostic Features*
- Basophilic granular material or larger amorphous to crystalline aggregates in the walls (tunica media) of arteries or veins; visible in H&E sections; confirmed with stains for minerals (Fig. 52, 53)

*Differential Diagnoses*
- Artifact of tissue fixation – basophilic particulate material more haphazardly distributed
- Osseous metaplasia – include histologic features of bone

*Comments*

In aged Sprague-Dawley rats, arterial mineralization can be seen as a sequela to chronic renal disease. Associated with parathyroid hyperplasia and osteodystrophia fibrosa, it can be especially prominent in the aorta but can involve the coronary arteries and other sites. Initially, mineral appears on the elastic fibers of the media, but the whole wall of the vessel can later become affected (Lewis 1992). Mineralization of the thoracic and abdominal aorta and the intima of small pulmonary vessels can be found with chronic renal disease in Fischer rats (Mitsumori 1990; Plendl et al. 1996). Subintimal mineral deposits can be seen in medium to large arteries of almost all lungs in rats over the age of 6-9 months; these are frequently seen at or near points of bifurcation (Lewis 1992).

Mineralization in the absence of severe chronic nephropathy can be seen in arteries of multiple organs. Calcium salts are first deposited on the elastic lamina, the elastic fibers of the media or the basal lamina of the endothelium. Lesions may progress to extensive mineralization of the media and intima, generally without an associated inflammatory infiltrate (Dungworth et al. 1992).

In mice, small vessels in the myocardium as well as the aorta may be affected by mineralization (Elwell and Mahler 1999). In aged mice, the incidence of this finding depends on strain and sex and can occur with pre-existing arteriosclerosis or atherosclerosis (Plendl et al. 1996). The elastic fibers of the muscle wall are affected in vessels in many organs and are commonly seen in the small vessels of the thalamus (Elwell and Mahler 1999).

### Aneurysm, artery or aortic

*Pathogenesis*

Weakness due to injury or remodeling of a segment of an artery allows blood pressure to dilate the vessels to the extent the adventitial tissues allow.

*Qualifiers*

Dilated; dissecting; aortic

*Diagnostic Features*
- Dilated types of aneurysms- an increase in vascular diameter forming a prominent out-pouching or irregular dilated area. It may occur concentrically, or along one side of the vessel only. An increase in vascular diameter is almost universally accompanied by thinning of the vascular wall, primarily of the media, at the site of the aneurysm.
- Dissecting types of aneurysms- a blood filled space within the vascular wall produced by circulating blood entering through an intimal tear. The lesion expands outward together with the formation of cystic spaces filled with blood and necrotic debris.

*Differential Diagnoses*
- Hematoma – extravasated red blood cells
- Angiectasis – likely to be less segmental and involve smaller blood vessels (e.g. capillaries)

*Comments*

An aneurysm is a persistent, localized abnormal dilatation of any vessel (Elwell and Mahler 1999; Mitsumori 1990; Stehbens 2001). It may occur not only in arteries but also in veins, microcirculation, lymphatics, and heart. However, in standard toxicology studies, the term 'aneurysm' is generally applied to a lesion of the aorta or a large elastic artery (Boor and Conklin 2008). For the lesions of vessels other than the large elastic arteries, 'angiectasis', 'lymphangiectasis' or other terms would be better to express each lesion. Aortic aneurysms produce serious clinical disease and often cause death by rupture.

Aneurysms rarely occur in the common strains of laboratory rodents, but aortic aneurysms can be seen in rats as a lesion secondary to degenerative changes in the media (Mitsumori 1990). There are also several genetically disposed or experimentally produced animal models (Daugherty et al. 2006).

A false aneurysm or pseudo-aneurysm results from partial or complete mural disruption of the vessel wall, usually traumatic (Stehbens 2001). This is essentially 'hematoma' that communicates with a vascular lumen and the sac wall is formed by the surrounding fibrous tissue and the fibrin coagulum by extra-vasated blood. It may also occur following rupture of a true aneurysm.

### Angiectasis

*Other Terms*

telangiectasia (in the liver)

*Pathogenesis*

May occur with congestion, increased hemodynamic pressures or weakening of small capillary walls.

*Diagnostic features*
- Clear spaces circumscribed by thin capillary walls in exsanguinated animals.
- Spaces lined by flattened endothelial cells not increased in number.
- Few erythrocytes may be present in the luminal spaces.
- Affected regions not often discretely circumscribed.

*Differential diagnosis*
- Hemangioma – generally circumscribed area of dilated vascular spaces that may be expansile and compress adjacent parenchyma.

- Endothelial hyperplasia – vascular spaces lined by increased numbers of hypertrophied endothelial cells. Mitotic figures may also be present.

*Comment*

Angiectasis can be seen in any organ and may be an artifact of the exsanguination procedure. As a pathologic feature, it is best described in the liver where it has also been termed 'telangiectasia' (Thoolen et al. 2010).

### Thrombus

*Pathogenesis*

Fibrin thrombus forming within a vascular space resulting from endothelial injury or dysregulation of the clotting cascade.

*Diagnostic Features*

- Brightly to mildly eosinophilic concentric lamellar structures most often occurring in veins.
- Varying numbers of degenerating white blood cells may be present, usually neutrophils.
- Often attached or integrated into wall of blood vessel.
- Occasionally seen as a spontaneous finding in the lung. (Fig. 54)

*Differential Diagnoses*

- Postmortem blood clotting – typically less organized and not attached to blood vessel wall

*Comment*

Thrombi rarely occur as a toxicologically induced lesion but capillary thrombi in the pulmonary vasculature have been associated with monocrotaline administration in stump-tailed monkeys and rats (Chesney and Allen 1973; Gopinath et al. 1987; Lalich et al. 1977). Thrombi may occur secondary to damage of the vessel wall caused by spontaneous or induced vasculitis. In Fisher 344 rats, thrombosis of large vessels such as the pulmonary or hepatic vein is sometimes observed. The finding is usually secondary to degenerative changes in the media, with thrombi becoming organized and consisting of dense fibrous connective tissue with foci of dystrophic mineralization (Mitsumori 1990). With toxic vascular injury, usually affecting arteries, occlusive thrombi are frequent in both acute and healing stages (Berridge et al. 2013).

Thrombi are often seen in infusion studies, associated with presence of intravenous catheters. In early stages they may appear as a fibrinoid 'sleeve' around the catheter tip. In intravenous studies of longer duration, or if the compound being infused is irritant, thrombi often become very large, partially occluding the vessel. They can become organized and integrated within the vessel wall with fibrosis and neo-vascularization sometimes becoming re-canalized.

### Embolus

*Other Terms*

- Embolism

*Pathogenesis*

May occur with infiltration of blood vessels by invasive tumors or artifactual introduction of external tissues (skin, hair) during intravascular injection or infusion.

*Diagnostic Features*

- Tissue or foreign material not generally seen within a blood vessel present in the vascular lumen
- Examples include rafts of neoplastic cells, hair shafts, talc crystals, plastic fragments from catheters
- May include fibrin deposits

*Differential Diagnoses*

- Thrombus – fibrillar eosinophilic fibrin with or without inflammatory cells
- "Floaters" from the water bath during the preparation of histologic sections
- Tissue compression artifact – local tissues pushed into vascular spaces by mechanical compression (e.g. liver, adrenal).

*Comments*

Most often associated with intravenous procedures and may originate from dissemination of local thrombi (thromboemboli) in infusion studies. May also be foreign material or rafts of neoplastic cells (Fig. 55). Thromboemboli appear as amorphous eosinophilic or purplish acellular masses within a vein, often attached to the intima. Foreign body emboli may contain hair shafts, fragments of skin or adipose tissue or foreign material. In studies where lipid is administered, fat emboli can be seen in the vasculature of various organs. Pulmonary thromboembolism in rats has been associated with intra-peritoneal administration of PAP, due to its toxic effect on mesenteric tissues and vasculature, resulting in thrombosis (Carthew et al. 1995).

### Amyloid, medial/mural, artery

*Other Terms*

- Amyloid deposition
- Amyloidosis

*Pathogenesis*

Increased production of immunoglobin light chains or acute phase proteins by the liver can result in deposition of those proteins in a variety of organs- including the tunica media of arteries. These proteins generally have a β-pleated sheets configuration that resists degradation.

*Diagnostic Features*

- Hyaline amorphous material within the muscular wall of arteries (Fig. 56)
- Confirmed with Congo red special histochemistry or

Thioflavine T

*Differential Diagnoses*
- Fibrinoid necrosis, artery – often accompanied by karyolitic debris
- Non-amyloid hyaline deposits – differentiated with special stains

*Comments*

Amyloid is seen frequently in mice and hamsters, but is very rare in rats. It occurs both spontaneously and as an induced lesion in mice, where it can be caused by repeated injections of inflammatory stimuli e.g. casein. There are two types of amyloid in mice; AA and AapoAII, with AapoAII being more commonly seen in the heart (Percy and Barthold 2007).

Amyloid may occur as intramural deposits in the small blood vessels of numerous organs including the jejunum, pancreas and testes (Elwell and Mahler 1999). Amyloid can be stained with Congo Red, Oil Red O, Alcian Blue and Thioflavine T, but staining intensity may vary considerably. Amyloid is birefringent under polarised light.

### Intramural plaque, artery

*Other Terms*
- Arterial plaque

*Pathogenesis*

Unknown

*Diagnostic Features*
- Granular material (often PAS-positive) and collagenous fibers with interspersed spindle cells in the tunica intima of pulmonary vessels; covered by vascular endothelium;
- Focal protrusion of matrix into the vascular lumen.
- Variable mineralization of the matrix (Fig. 57, 58)

*Differential Diagnoses*
- Organizing thrombus – distinguished by presence within the lumina of vessels and a composition predominated by amorphous or laminated thrombin.
- Intimal thickening, acellular – generally a circumferential lesion in small vessels with more collagen.

*Comments*

The lesion appears to be limited to the mouse lung. Reported previously in aging Han:NMRI, CBA, and C57BL mice (Ernst et al. 1996; Rehm et al. 1985), it has also been observed in Swiss albino mice from short term (28-day) preclinical safety studies (personal communication) and untreated heterozygous MnSOD (SOD2) mice (personal communication). Inflammation is generally absent. Multiple vessels may be affected within a single lung lobe (Rehm et al. 1985). The lesion has been traditionally described in arteries but in the young mice that were affected, it was not possible to definitively identify the affected vessels as arteries.

The pathogenesis of the lesion is unknown; it may represent various stages of organizing thrombus. Larger lesions may have a pedunculated appearance. References describe the lesion as subintimal, or as arising in the tunica media. However, because there is no indication that special stains identifying the internal elastic lamina were performed, one cannot exclude the possibility that the lesion occurs in the intimal space.

Intramural plaque is distinguishable from an atherosclerotic plaque by the absence of lipid in the lesion.

## II. Proliferative Lesions

The heart is not a common site of proliferative lesions in rodents. A number of the processes described represent continuums where discrete terminology may not be backed by a thorough understanding of biological behavior. For example, Schwann cell proliferations occur spontaneously in laboratory rats with incidences generally <1.0%. Subendocardial hyperplasia, endocardial schwannoma, and intramural schwannoma may all be variants of the same proliferative process. Likewise, hemangioendothelial hyperplasia, hemangioma, and hemangiosarcoma may represent a similar continuum in mice. The translational significance of an increase in incidence or a bias to one morphology over another in the context of xenobiotic treatment is unknown. Neoplastic lesions are designated benign (B) or malignant (M) based on morphologic features and general biologic behavior.

### A. Non-neoplastic proliferative changes

### Heart

### Hyperplasia, Schwann cell, subendocardium

*Other Terms*
- Hyperplasia, Schwann cell
- Neurofibromatosis, endocardial
- Schwannomatosis

*Pathogenesis*

Non-neoplastic subendocardial proliferation of mesenchymal cells of Schwann cell origin.

*Diagnostic Features*
- The lesion is composed of a thin (<20 cells), hypercellular layer of mesenchymal ovoid cells beneath an intact endocardium and distinct from the underlying myocardium; myocardial infiltration is minimal (Fig. 59).
- Elongate cells have faintly eosinophilic cytoplasm, indistinct cellular borders and round, ovoid or slightly elongated nuclei (Fig. 60).
- In basal layers (adjacent to myocardium), cells may be more spindle-shaped and fibrocyte-like, have orientation

parallel to the endocardium, and have elongated hyperchromatic nuclei.
- The left ventricular endocardium is most often affected though the change may rarely be present in atria and valve leaflets.

*Differential Diagnoses*
- Subendocardial fibrosis – Cells with histological features of fibroblasts/fibrocytes with pale nuclei are more haphazardly arranged and localized. Nuclear density less than that seen with endocardial hyperplasia with an accompanying increase in extracellular matrix.
- Schwannoma, endocardial – Schwannomas may infiltrate into the adjacent myocardium or form a discrete mass that protrudes into the ventricular lumen (generally left).

*Comment*
Novilla et al. described a low incidence (< 1%) of spontaneous endocardial hyperplasia in a review of Fischer, Wistar, Sprague-Dawley, and Long Evans rats used in chronic toxicity and oncogenicity studies (Novilla et al. 1991a). S-100 immunoreactivity and ultrastructural features of Schwann cells were common. These lesions are most often seen in older rats (Elwell and Mahler 1999; Ruben et al. 2000). They may represent a proliferative continuum with subendocardial schwannoma.

Naylor et al. compared a series of lesions (termed 'spontaneous endomyocardial disease') in aged Sprague-Dawley rats to what is described as 'endomyocardial fibrosis' in man. The rat lesions were morphologically very similar to those described by Novilla but lacked S-100 immunoreactivity and ultrastructural features of Schwann cells in the few lesions that were examined (Naylor et al. 1986). Despite that, the lesions described by Naylor and Novilla are likely to be the same and distinct from the human condition. Likewise for lesions described as 'subendocardial fibrosis' by Lewis (Lewis 1980).

### Hyperplasia, mesothelial, epicardium or pericardium
*Other Terms*
- Pericardial hyperplasia
- Epicardial hyperplasia
- Villous hyperplasia

*Pathogenesis*
Frequently a reactive proliferation of mesothelial cells lining body cavities or the epicardial surfaces of the atria.

*Diagnostic Features*
- Cuboidal cells arranged in multiple layers along mesothelial surfaces without a significant amount of supporting stroma and no expansile protrusion into the body cavity
- Cells with moderately abundant basophilic cytoplasm

have large, prominent, dark-staining basophilic nuclei
- Papillary projections of the surface may be observed (Fig. 61)

*Differential Diagnoses*
- Mesothelioma, pericardial – more pleomorphic proliferation of epithelial and stromal components
- Mesothelioma, atriocaval – more pleomorphic proliferation of epithelial and stromal components uniquely located at right atrial-vena caval junction. Local invasion and metastasis are possible (i.e. malignant behavior).
- Mesothelial inflammation – not accompanied by proliferation of mesothelial epithelium.

*Comment*
Mesothelial hyperplasia is often observed in association with inflammatory effusions of the body cavities where the hyperplastic cells may have a focal, multifocal or diffuse distribution. Thus, a focus of mesothelial hyperplasia may be infiltrated by inflammatory cells. Gavage dosing errors or trauma may be a source of the inflammation that ultimately initiates the reactive mesothelial proliferation.

## Blood Vessels

### Proliferation, intimal, artery or vein
*Other Terms*
- Intimal hyperplasia
- intimal plaque formation
- intimal thickening

*Pathogenesis*
Expansion of the vascular intima with smooth muscle cells.

*Diagnostic Features*
- Expansion of the tunica intima
- Proliferation of cells (smooth muscle cells or, less commonly, fibroblasts) between endothelial cells and the internal elastic lamina

*Differential Diagnoses*
- Intimal cushions (when part of the circumference of the vessel is affected) – normal structures.
- Intramural plaque – less cellular, not generally circumferential and most commonly seen in the lung of mice.
- Arteriosclerosis/Atherosclerosis – expansion of the intima in medium to large arteries with a heterogenous matrix of lipid, smooth muscle cells, and mononuclear inflammatory cells that can be 'foamy'

*Comments*
Intimal proliferation can affect part of or the entire circumference of the intima. It can be symmetrical or asymmetrical. Endothelial cells in affected vessels may be discontinuous, sparse, and plump (hypertrophic). This is a nonspecific change that may be observed in aged rats

(Greaves and Faccini 1984) and only occasionally observed in mice (Faccini et al. 1990). Administration of allylamine or phosphodiesterase inhibitors can induce this lesion in rats (Berridge et a*l*. 2013). The change may also be seen in infusion studies with chronically indwelling catheters.

### Nomenclature recommendations
This lesion is not generally thought to be a precursor to neoplasia. Accordingly, 'proliferation' is preferred to 'hyperplasia' to communicate the distinction from those processes that do have more pre-neoplastic potential.

## Hyperplasia, hemangioendothelial

### Other Terms
- Hyperplasia, endothelial
- Hyperplasia, hemangioendothelial cell
- Hyperplasia, capillary

### Pathogenesis
Proliferation of small vessel (capillaries) endothelial cells with luminal dilation/ectasia.

### Diagnostic Features
- Poorly circumscribed proliferation of normal or hypertrophied endothelial cells lining normal or increased numbers of variably dilated capillary spaces. (Fig. 62, 63)
- This lesion may occur primarily in the heart or be seen in other organ systems (e.g. the subcapsular sinuses of lymph nodes).
- Generally, not associated with compression of the surrounding tissues.
- The vascular spaces may contain a proteinaceous fluid and variable numbers of lymphoid cells (should consider lymphaniectasia) or erythrocytes.
- Fibrous connective tissue generally not a prominent feature.

### Differential Diagnoses
- Angiectasis – Vessels are not increased in number, have normal structure, and well differentiated endothelial cells.
- Hemangioma or Lymphangioma – These tumors usually cause compression of the surrounding tissues, have a greater size, and slight cytological abnormalities of the endothelial cells.
- Endothelial hyperplasia – described in the liver IN-HAND monograph as a "proliferation of normally present sinusoidal lining endothelial cells without sinusoidal dilation."

### Comment
Proliferation of normal or hypertrophied endothelial cells lining capillaries has been described most commonly in mice both spontaneously and with chronic exposure to some xenobiotics. A low incidence of 'hemangioendothe-lial cell hyperplasia' has been described by Iwata, et al. as a spontaneous lesion coincident with mammary adeno-carcinomas in B6C3F1 female mice (Iwata et al. 1994). 'Endothelial hyperplasia' was described in the continuum to hemangiomas and hemangiosarcomas in NTP bioassays for 1,3-butadiene (Melnick et al. 1990; Solleveld et a*l*. 1988). When seen coincidently with more obviously neoplastic vascular neoplasms in chronic studies, these lesions likely represent a pre-neoplastic continuum.

### Nomenclature recommendations
As noted below for neoplastic vascular processes, nomenclature is a challenge given our limited understanding of the pathobiology of these processes. Though a variety of terms have been used for this proliferation of endothelial cells and small vessels, 'hemangioendothelial hyperplasia' seems to capture the potential for an increase in both endothelial cells and vascular space/volume.

## Hyperplasia, angiomatous

### Other Terms
- Hyperplasia, hemangiomatous
- Hyperplasia, angiomatous

### Pathogenesis
Localized proliferation of thin-walled vascular spaces lined by normal endothelial cells lacking nuclear atypia or mitoses. May be a pre-cursor for vascular neoplasia (e.g. hemangioma or hemangiosarcoma).

### Diagnostic features
- Localized areas of increased capillaries and other vascular structures
- Vascular spaces often blood-filled and uniform or variable in size
- Varying increase in adjacent extracellular matrix
- Lined by flattened endothelium without mitotic figures or nuclear atypia
- Generally don't distort adjacent normal tissue
- May be seen coincidentally with vascular neoplasia

### Differential Diagnoses
- Angiectasis – dilated vascular spaces not increased in number.
- Hemangioendothelial hyperplasia – endothelial cell prominence and proliferation distinguishes.
- Hemangioma – more discrete and expansile distorting adjacent tissue
- Hemangiosarcoma – increased heterogeneity of vascular and stromal morphology with prominent endothelial atypia and mitoses. More infiltrative.

### Comment
A Pathology Working Group (PWG) deliberating over a range of common neoplasms seen in chronic peroxisome proliferator-activated receptor (PPAR) studies (Hardisty

et al. 2007) described a spectrum of proliferative vascular lesions with typical hemangiomas and hemangiosarcomas among them. A lesion in the subcutis more discrete than the endothelial hyperplasias described above and more akin to a variant of hemangioma (Hardisty et al. 2007) was described as 'angiomatous hyperplasia'. Additionally, this same group described a subcutaneous proliferative vascular lesion that they termed 'angiolipoma' to recognize the coincidence of mature adipocytes and small blood vessels. These processes were considered to be unique to PPARs (Hardisty et al. 2007).

The female reproductive system INHAND monograph also describes angiomatous hyperplasia in the uterus of rodents as a "focal, well-demarcated lesion within the endometrium or myometrium consisting of an increased number of closely packed vascular structures, not distorting the surrounding tissue".

An interesting "vascular anomaly" is also described in rasH2 mice where it can be seen in a variety of tissues but is most commonly seen along the serosal surface of the uterus and urinary bladder. These proliferative lesions are characterized as thick walled, blood-containing vessels lined by well-differentiated endothelial cells. They have occasionally been seen in the presence of hemangiosarcoma suggesting a biological continuum (Paranjpe et al. 2013b).

### Nomenclature recommendation

Differentiating hemangioendothelial hyperplasia, angiomatous hyperplasia, and even neoplastic hemangiomas and hemangiosarcomas can be a challenge. The prominence of endothelial cell hypertrophy and proliferation should distinguish hemangioendothelial hyperplasia from angiomatous hyperplasia. Also, 'angiomatous hyperplasia' has been established for some unique presentations (e.g. chronic PPAR treatment, uterus) where consistent terminology should be applied. The term 'vascular anomaly' as applied to the non-neoplastic vascular proliferations in rasH2 mice is not morphologically descriptive and could be termed 'angiomatous hyperplasia' in the future. Hemangiomas are distinguished by their discreteness and expansile growth while hemangiosarcomas would be expected to be more invasive, possibly multicentric and include prominent nuclear atypia and mitoses.

## B. Neoplastic Lesions

## Heart

### *Schwannoma, endocardial (M)*
#### Other Terms
- Neurofibroma/sarcoma
- Neurosarcoma
- Sarcoma, anitschkow cell

### Pathogenesis
Neoplastic proliferation of subendocardial Schwann cells that may infiltrate the adjacent myocardium as well as protrude into the ventricular lumen.

### Diagnostic Features
- Expansile, spindle cell mass generally originating within the ventricular subendocardium that may protrude into the ventricular lumen as well as infiltrate the underlying myocardium (Fig 64)
- Pleomorphic polygonal or fusiform cells (> 20 layers) with vesicular nuclei, prominent nucleoli, and varying numbers of mitotic figures (Fig 65).
- Fusiform cells immediately subjacent to the endocardium may have more elongate and hyperchromatic nuclei.
- Ovoid to spindle-shaped cells are intermingled and may form separate parallel-arranged zones.
- Some regions may have nuclear palisading (Antoni type A tissue) and some regions have loose network of cytoplasmic processes and increased ground substance (Antoni type B tissue).
- Neoplasm may have deep infiltrations of basophilic spindle-shaped cells into the adjacent myocardium.

### Differential Diagnoses
- Endocardial fibrosis – more monomorphic population of fibrocytes that do not invade the adjacent myocardium
- Subendocardial hyperplasia – less pleomorphic and more hypercellular population of mesenchymal cells that do not invade adjacent myocardium

### Comment
Schwannomas of the heart are recognized to be spontaneous neoplasms of a number of rat strains but are not described in mice (Brix et al. 2005; Haseman et al. 1998; Novilla et al. 1991b; Ruben et al. 2000). Endocardial and intramural variants are recognized. S-100 immunoreactivity is common in both variants (Novilla et al. 1991b). Endocardial schwannomas tend to have more discrete boundaries with expansile growth but infiltration of subjacent myocardium is common as well. These neoplasms vary in their histomorphology and distinctions have occasionally been made between benign and malignant phenotypes. Generally, they exhibit a malignant biological behavior with local invasion more common than distant metastases (Elmore et al. 2013).

### *Schwannoma, intramural (M)*
#### Other Terms
- Neurosarcoma
- Sarcoma, anitschkow cell

### Pathogenesis
Neoplastic proliferation of intramural Schwann cells that often invade and interdigitate with adjacent myocardium.

*Diagnostic Features*
- Poorly circumscribed spindle cell mass within the ventricular myocardium that is generally more infiltrative than expansile (Fig. 66, 67)
- Pleomorphic polygonal or fusiform cells with vesicular nuclei, prominent nucleoli, and varying numbers of mitotic figures.
- Ovoid to spindle-shaped cells are intermingled and may form separate parallel-arranged zones.
- Some regions may have nuclear palisading (Antoni type A tissue) and some regions have loose network of cytoplasmic processes and increased ground substance (Antoni type B tissue).

*Differential Diagnoses*
- Myocardial fibrosis of rodent progressive cardiomyopathy – generally not as locally extensive, more interdigitation with adjacent cardiomyocytes and with rare mitoses. Remnants of mixed mononuclear inflammation may also be a feature.
- Chronic myocardial infarction – likely to have more discrete borders, represent the field of perfusion for an occluded vessel at the artery or arteriole level, and include more eosinophilic collagenous matrix.

*Comment*
Schwannomas of the heart are recognized to be spontaneous neoplasms of a number of rat strains but are not described in mice (Brix et al. 2005; Haseman et al. 1998; Novilla et al. 1991b; Ruben et al. 2000). Endocardial and intramural variants are recognized. Intramural schwannomas tend to have less discrete boundaries with infiltrative growth into adjacent myocardium. These neoplasms vary in their histomorphology and distinctions have occasionally been made between benign and malignant phenotypes. S-100 immunoreactivity may be demonstrated. Generally, they exhibit a malignant biological behavior with local invasion more common than distant metastases (Elmore et al. 2013).

### Mesothelioma, epicardium or pericardium (M)

*Pathogenesis*
Neoplastic proliferation of mesothelial cells lining body cavities.

*Diagnostic Features*
- may have epithelioid and mesenchymal histomorphologic elements that may bias to one morphology
- epithelioid components may be papillary, tubular, or solid
- mesenchymal components include spindle-shaped cells and collagen
- may be exophytic or plaque-like

*Other qualifiers/modifiers*
Epithelioid type – predominated by epithelial components with papillary or glandular structures common.

Sarcomatoid type – predominated by spindle cells arranged in interlacing bundles or whorls.

Biphasic type- mixed populations of epithelial and spindle cell components.

*Differential Diagnoses*
Mesothelial hyperplasia – bland histomorphology without invasion into subjacent tissues

Atriocaval mesothelioma – distinguished by localization at the junction of the right atrium and vena cava; tend to be more invasive

*Comment*
Mesotheliomas are uncommon spontaneous neoplasms that can occur in any body cavity (e.g. pleural, abdominal, etc.) and have been described in the pericardium (Ruben et al. 2000). A single pericardial mesothelioma was identified in nearly 80,000 F344 rats used in chronic carcinogenicity studies at the NTP (Alison et al. 1987). Pericardial mesotheliomas may be bland or exuberant in morphology. Pericardial mesotheliomas are distinguished from the more predictable and more aggressive looking atriocaval mesotheliomas. These neoplasms are likely too rare to make definitive judgements of typical biological behavior but cellular atypia, mitotic activity and invasive growth seen in some sites support malignancy. Additional characterization of this neoplasm can be found in the Soft Tissue INHAND monograph (Greaves et al. 2013).

### Mesothelioma, atriocaval (M)

*Other Terms*
- tumor, atriocaval node

*Pathogenesis*
Neoplastic proliferation of mesothelial-like cells at the right atrial-vena caval junction

*Diagnostic features*
- expansile and infiltrative epithelioid mass at the junction of the right atrium and cranial vena cava
- varying proportions of epithelial cells in tubular and alveolar structures with a fibrous stroma (Fig. 68)
- alveolar and tubular structures are lined by a single or multiple layers of squamous to low cuboidal cells (Fig. 69)
- epithelial-like cells form solid masses, small clumps, nests and single polygonal cells within the stroma with nuclear pleomorphism and mitotic activity
- larger neoplasms sometimes invade adjacent atrial structures, have emboli of neoplastic cells in lymph vessels of the mediastinum, and metastasize to distant sites such as

the lungs, thoracic lymph nodes and liver

*Differential diagnoses*
- Mesothelioma, pericardial – more bland histomorphology and less aggressive biologic behavior

*Comment*
As described for pericardial mesotheliomas, these spontaneous neoplasms are generally rare and most often reported in rats when they do occur. An exception to a generally very low incidence is a report by Goodall et al. of a high incidence (approximately 20%) of these neoplasms in a single inbred strain of albino rats, NZR/Gd (Goodall et al. 1975). Contrastingly, Alison et al. reported 8 in nearly 80,000 F344 rats included in cardinogenecity studies at the NTP but Haseman et al. didn't report any in the same rat strain in a later report (Alison et al. 1987; Haseman et al. 1998). A review of tumor incidence in a smaller selection of female Harlan SD rats didn't report any as well (Brix et al. 2005). As noted, the location and morphology of these neoplasms is distinctive enough to warrant a differentiation from pericardial mesotheliomas that more commonly resemble mesotheliomas of other mesothelium-lined body cavities.

### Paraganglioma (B)

*Other Terms*
- Aortic body tumor

*Pathogenesis*
Locally invasive neoplasm of the aortic body paraganglion located in the interatrial septum.

*Diagnostic features*
- Nests of round cells with granular, faintly basophilic cytoplasm and vesicular, lightly stippled nuclei (Fig. 70)
- Nests are surrounded by a delicate reticulin network
- Mitotic figures rare
- May locally invade through to epicardial surface (Fig. 71)

*Differential diagnoses*
- Atriocaval mesothelioma – location and bias toward the mesothelial surface should differentiate

*Comment*
Aortic body paragangliomas are rare but have been described as spontaneous neoplasms in rats. Their morphology and location are distinctive features (Alison et al. 1987; Li et al. 2013). These neoplasms are reportedly immunoreactive for synaptophysin and Chromogranin A (Li et al. 2013).

### Blood Vessels

Neoplastic vascular lesions can be a diagnostic challenge given the spectrum of potential presentations (e.g. angiec-

tasis to hemangiosarcoma), lack of robust understanding about the biology of these processes in our animal models, and the considerable variability in the human pathology literature. Of particular challenge (particularly for shorter duration studies) is recognizing morphologic changes likely to progress to overt neoplasia with continued dosing. Accordingly, a simplified approach to the nomenclature is likely most prudent until a better understanding allows us to justifiably distinguish morphologically similar processes.

### Hemangioma (B)

*Other Terms*
- Hemangioendothelioma, benign

*Pathogenesis*
Non-invasive but expansile proliferation of capillaries.

*Diagnostic features*
- Variably-sized vascular spaces (capillary to cavernous) lined by a monolayer of endothelial cells with hyperchromatic nuclei (Fig. 72, 73)
- Mitotic figures are rarely present
- Rarely encapsulated
- Hypocellular collagenous intervascular stroma
- Moderate compression of surrounding tissues is usually seen
- Thrombosis of vascular spaces and interstitial hemorrhage may be present

*Modifiers*
Capillary- generally small vascular spaces

Cavernous- large vascular spaces

*Differential diagnoses*
- Angiectasis – no increased number of vessels; no compression of surrounding tissues
- Hemangiosarcoma – much more endothelial pleomorphism; local to metastatic invasion
- Angiomatous hyperplasia – less circumscribed and expansile than hemangioma but less pleomorphism and atypia than hemangiosarcoma

*Comment*
As with hemangiosarcomas, hemangiomas are more common in mice than rats (Brix et al. 2005; Haseman et al. 1998; Poteracki and Walsh 1998). They can be multicentric and probably occur more commonly in the liver, uterus, skin, spleen, and bone marrow.

Hemangiomas and hemangiosarcomas have been described in rasH2 transgenic mice with hemangiosarcomas far more common. Though they can occur in a variety of tissues, they are most commonly seen in the spleen (Nambiar et al. 2012; Paranjpe et al. 2013a).

### Hemangiosarcoma (M)

*Other Terms*
- Hemangioendothelioma, malignant
- Angiosarcoma

*Pathogenesis*

Neoplastic proliferation of pluripotential mesenchymal stem cells or endothelial cells of blood vessels.

*Diagnostic features*
- Atypical endothelial cells form vascular channels (capillary to cavernous) and solid cellular masses supported by variably developed fibrovascular stroma (Fig. 74, 75)
- Tumor cells can be round, polygonal or extremely irregular in form; commonly they are spindle-shaped
- Tumor cells have single or multiple irregular shaped and sized nuclei that are often large and lobulated with large masses of chromatin
- Mitotic figures can be common and bizarre
- Local invasion and metastases are often present
- Hemorrhage, congestion, and thrombi within vascular spaces are common
- Immunolabeling for Factor VIII-related antigen can be a useful immunomarker for identifying well-differentiated tumors

*Differential diagnoses*
- Hemangioma – more regular pattern of discrete vascular spaces with no or only a few mitotic figures in single layer of lining endothelial cells; no invasiveness or metastases
- Hemangiopericytoma – spindle cell mass with cytologic features of malignancy encircling less prominent thin-walled vascular channels
- Granulation tissue – newly-formed blood vessels are arranged perpendicular to fibroblasts, collagen bundles, and surfaces with no cytological and histological features of neoplasia
- Fibrosarcoma – lacks distinct vascular pattern and prominent endothelial cells

*Comment*

Hemangiosarcomas are fairly common in laboratory rodents. A number of surveys of 2-year bioassay data for control animals revealed that hemangiosarcomas occur more commonly than hemangiomas, that these tumors are more common in mice than rats and that the liver and spleen are the most common anatomic sites of origin (Brix et al. 2005; Haseman et al. 1998; Poteracki and Walsh 1998). But, primary hemangiosarcomas of the heart have also been seen sporadically in untreated rats and mice. Cardiac hemangiosarcomas were prominent in mice treated with 1,3-butadiene (Hong Tox Path 2000). They have also been seen in PPAR carcinogenicity studies (Hardisty Tox Path 2007).

Hemangiomas and hemangiosarcomas have been described in rasH2 transgenic mice with hemangiosarcomas the second most common spontaneous neoplasm (behind pulmonary bronchoalveolar adenomas) described in this strain. Though they can occur in a variety of tissues, they are most commonly seen in the spleen (Nambiar et al. 2012; Paranjpe et al. 2013a).

An interesting "vascular anomaly" is also described in rasH2 mice where it can be seen in a variety of tissues but is most commonly seen along the serosal surface of the uterus. These proliferative lesions are characterized as thick walled, blood-containing vessels lined by well-differentiated endothelial cells. They have occasionally been seen in the presence of hemangiosarcoma suggesting a biological continuum (Paranjpe et al. 2013b).
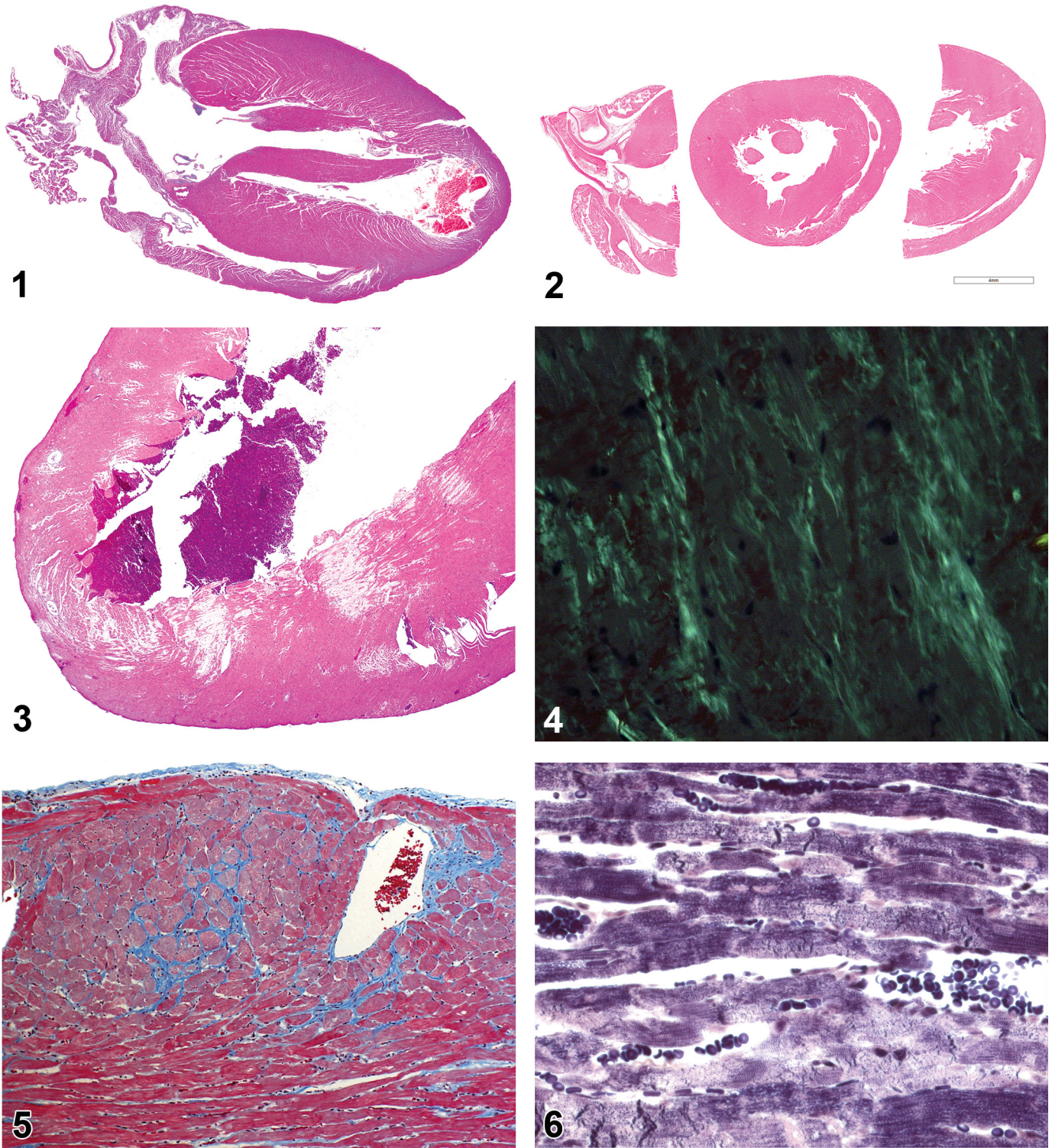
FIGURE 1. —— Longitudinal section of rat heart generally allowing assessment of 4 chambers and 3 valves.

FIGURE 2. —— Isaac's method of rodent heart trimming allowing proportional assessments of ventricular wall thicknesses.

FIGURE 3. —— Multifocal amyloid deposition in the heart of a mouse.

FIGURE 4. —— Congo red staining of amyloid is apple green with polarization.

FIGURE 5. —— Interstitial collagen of myocardial fibrosis stains blue with a Masson's trichrome.

FIGURE 6. —— Phosphotunstic acid hematoxylin (PTAH) staining of acutely injured cardiomyocytes reveals disruption and fragmentation of myofibril striations. Rat isoproterenol cardiotoxicity.
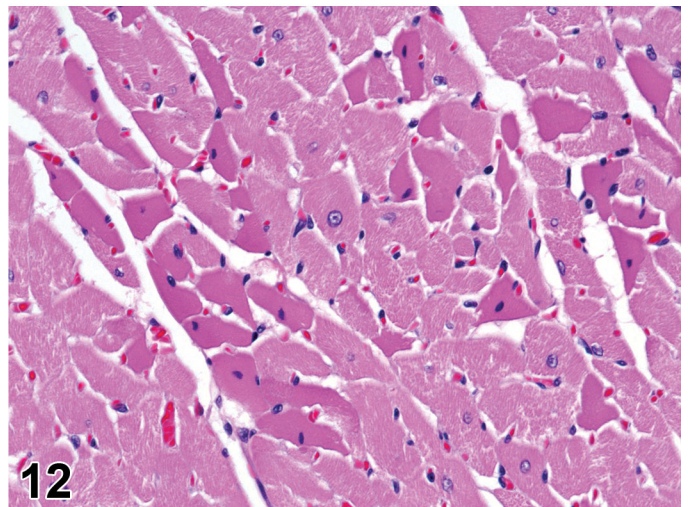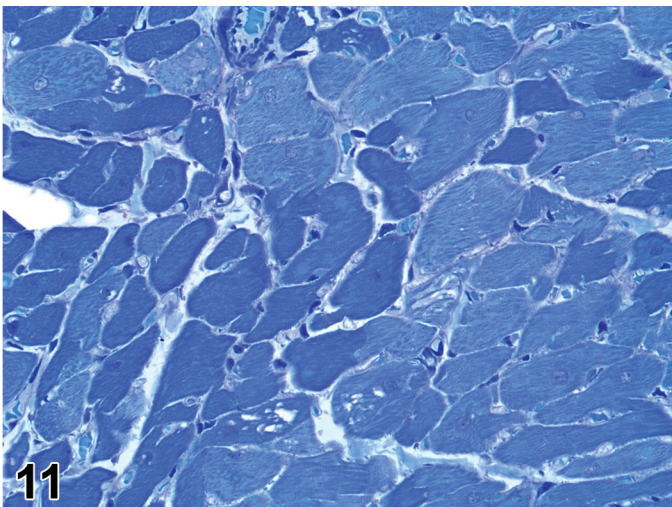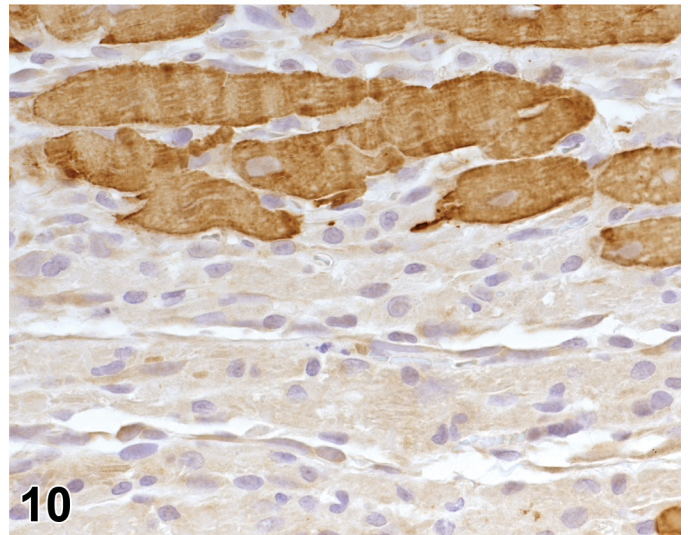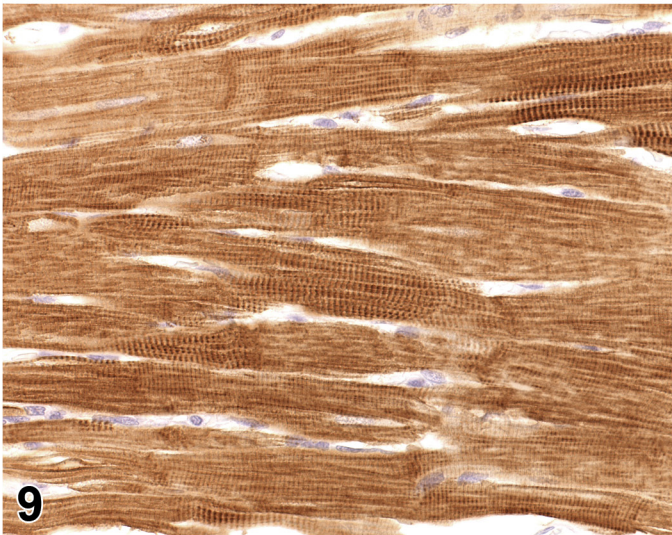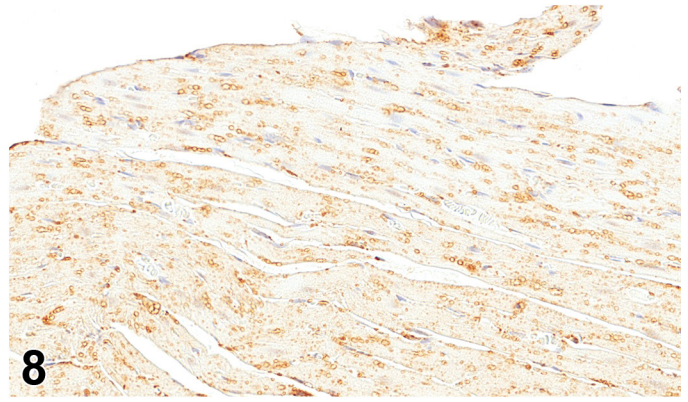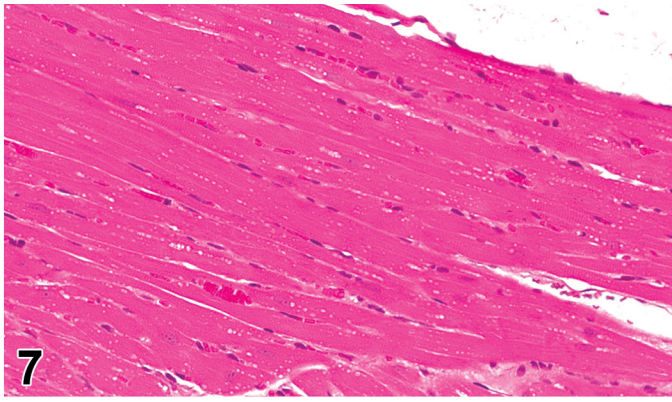
FIGURE 7. —— Cardiomyocyte vacuolation as a fine cytoplasmic microvesiculation with hematoxylin and eosin (HE) staining.

FIGURE 8. —— Lamp2 IHC allows characterization of the microvesiculation as increased lysosomes (due to phospholipidosis)

FIGURE 9. —— Normal cardiomyocytes exhibit homogeneous cytoplasmic labeling with cTn IHC.

FIGURE 10. — Acutely injured cardiomyocytes lose cTn IHC immunolabeling. Rat isoproterenol cardiotoxicity.

FIGURE 11. — Toluidine blue staining of methylmethacrylate embedded heart samples allows easier recognition of the macrovesicular cytoplasmic change of doxorubicin cardiotoxicity (lower center of image).

FIGURE 12. — Artifactual hypercontracted and hypereosinophilic myofibers in cross-section.
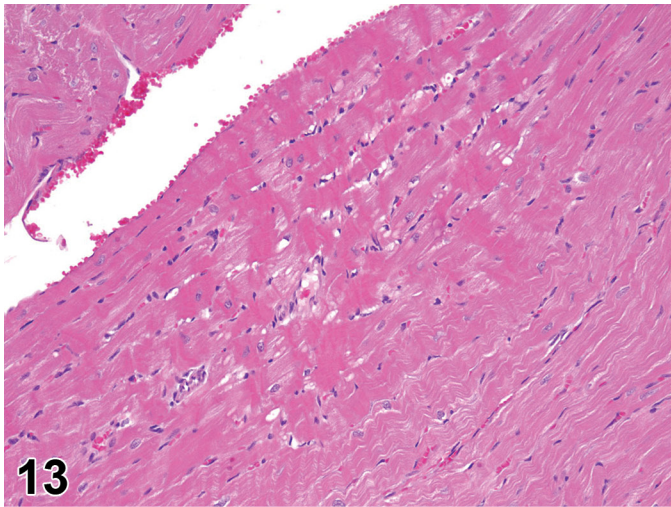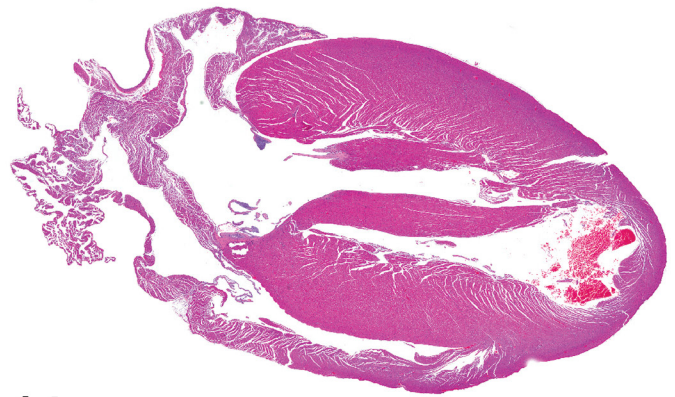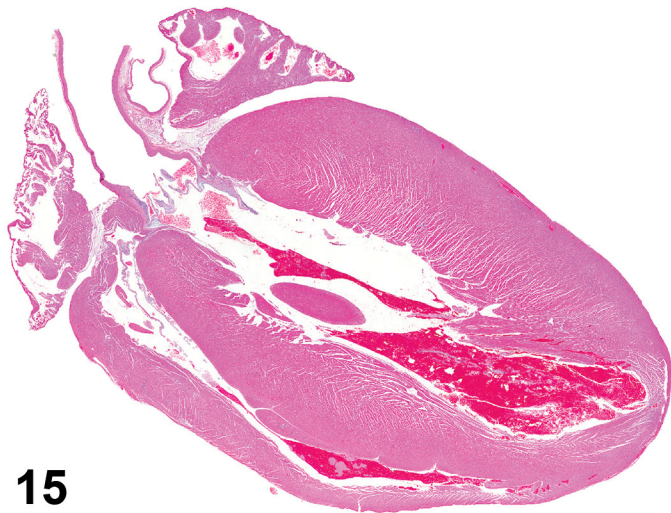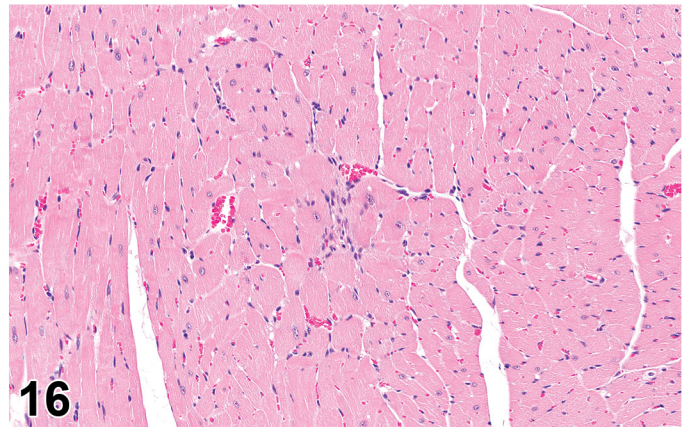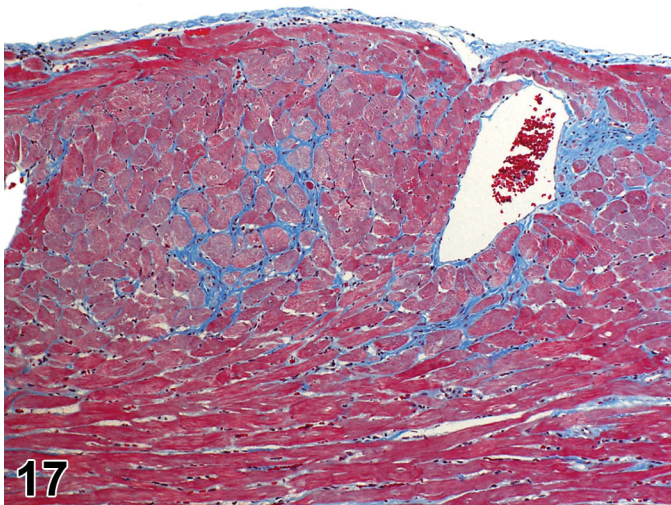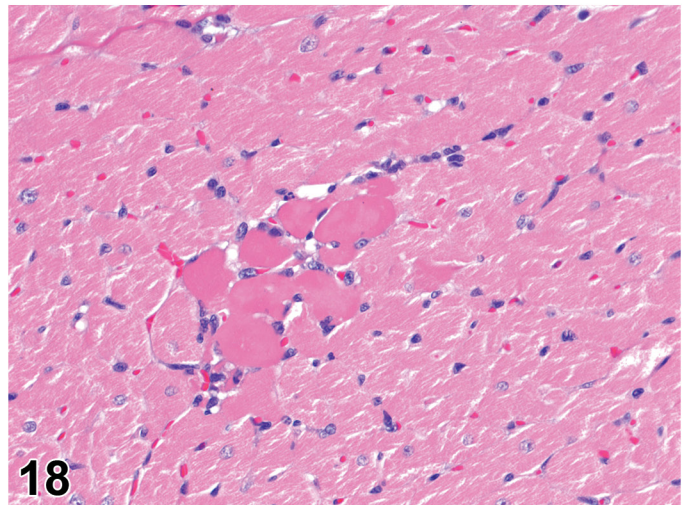
FIGURE 13. — Hypercontracted and hypereosinophilic myofibers in longitudinal section. This change could be confused with peracute myofiber injury and hyaline degeneration.

FIGURE 14. — Normal rat heart.

FIGURE 15. — Age-matched heart from a rat treated with a compound that induced symmetrical heart enlargement and increased heart weight.

FIGURE 16. — Focal cardiomyocyte hypertrophy with minimal accompanying hypercellularity. Presumed remnant of a focus of myocyte necrosis.

FIGURE 17. — Enlarged cardiomyocytes in an area of the myocardium dissected by interstitial fibrosis. Masson's Trichrome stain.

FIGURE 18. — Degeneration, hyaline. Cardiomyocyte hypereosinophilia of peracute injury.
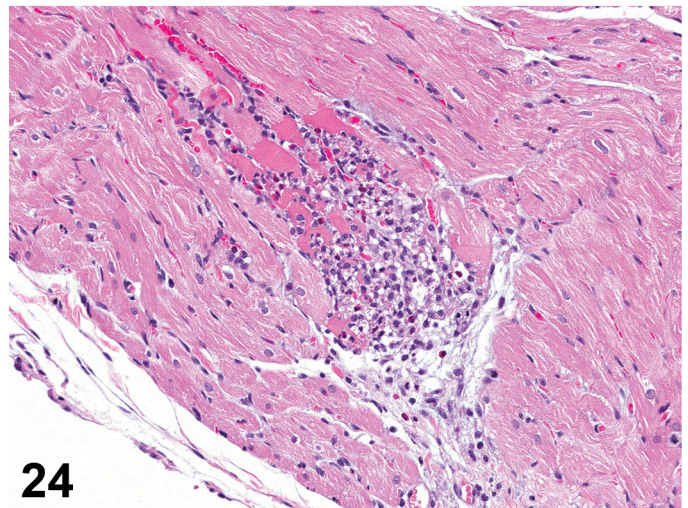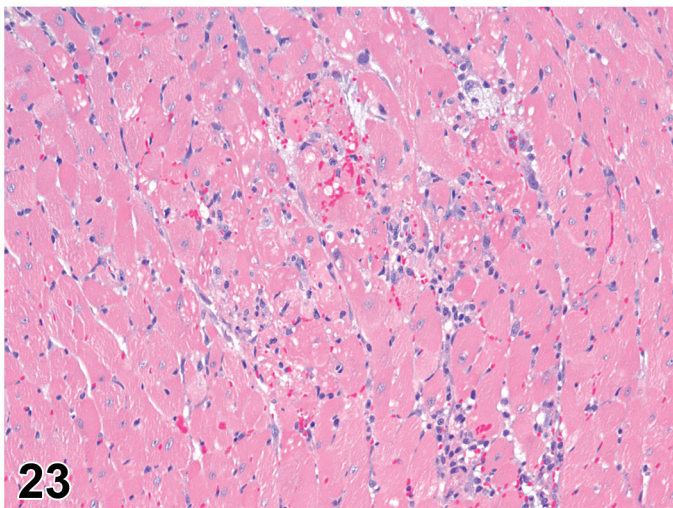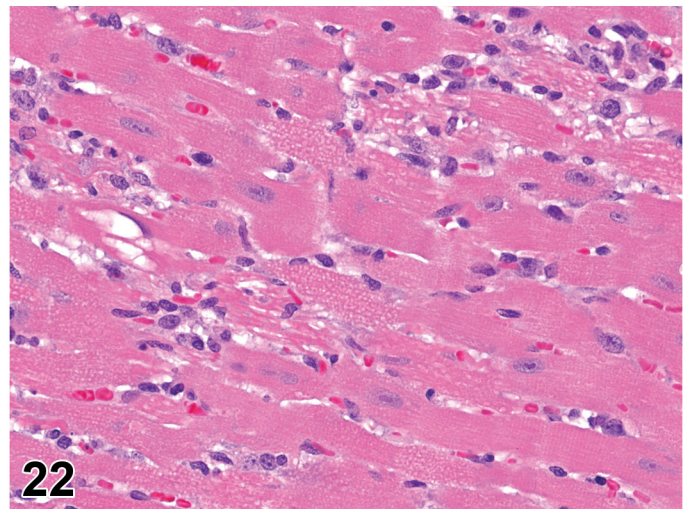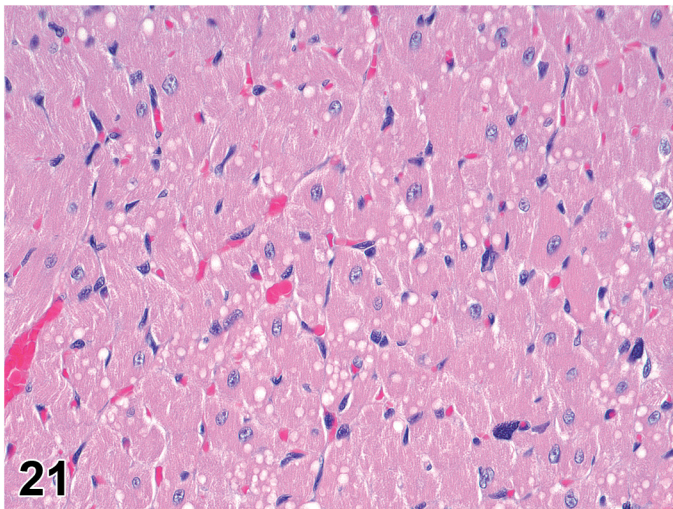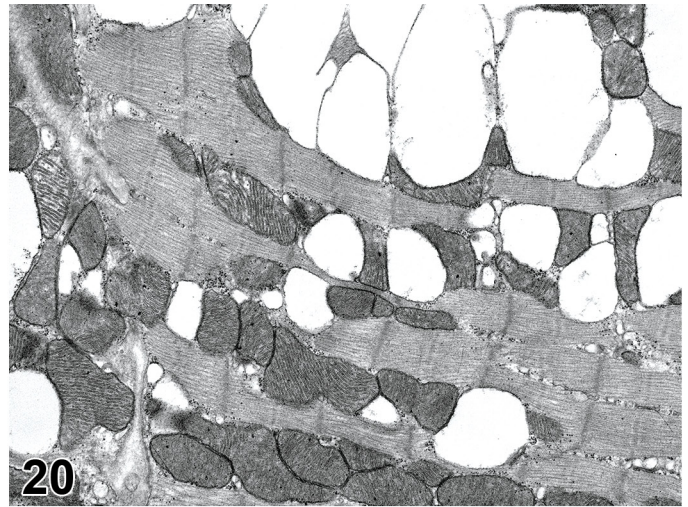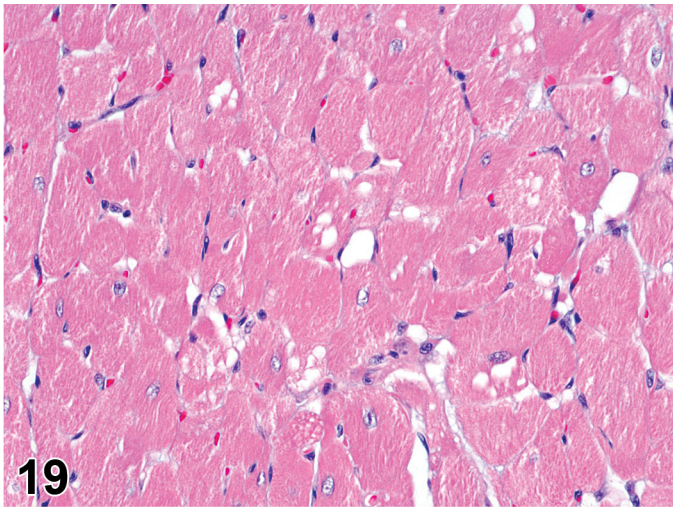
FIGURE 19. — Degeneration, vacuolar. Macrovesicular degeneration of doxorubicin cardiotoxicity.

FIGURE 20. — Degeneration, vacuolar. TEM image characterizing macrovesiculation as dilation of sarcoplasmic reticulum.

FIGURE 21. — Degeneration, vacuolar. Discrete clear vacuoles of varying size scattered within cardiomyocytes. Multifocal mitochondrial swelling.

FIGURE 22. — Degeneration, vacuolar. Fine and diffuse microvesiculation of cardiomyocytes. Diffuse mitochondrial swelling.

FIGURE 23. — Necrosis, cardiomyocyte. Cardiomyocyte necrosis is characterized by hyaline condensation and fragmentation of the cytoplasm, minimal hemorrhage and early inflammatory cell infiltration.

FIGURE 24. — Necrosis/inflammatory cell infiltrate. Temporal progression of cardiomyocyte necrosis reveals a growing prominence of the responding inflammatory cell infiltrate but the inciting process was still necrosis.
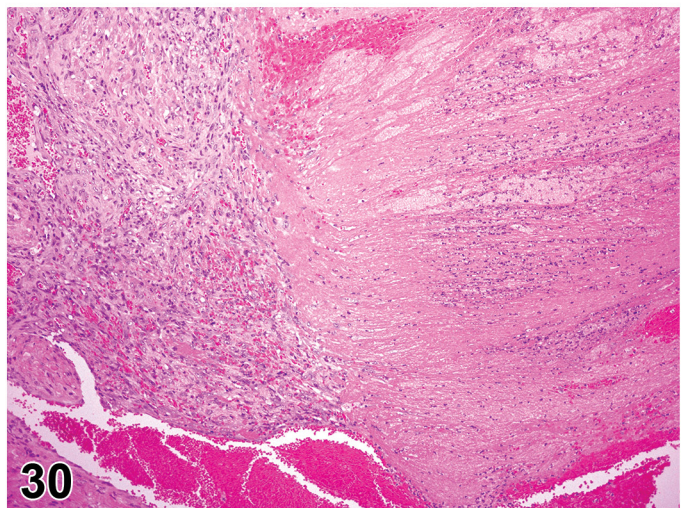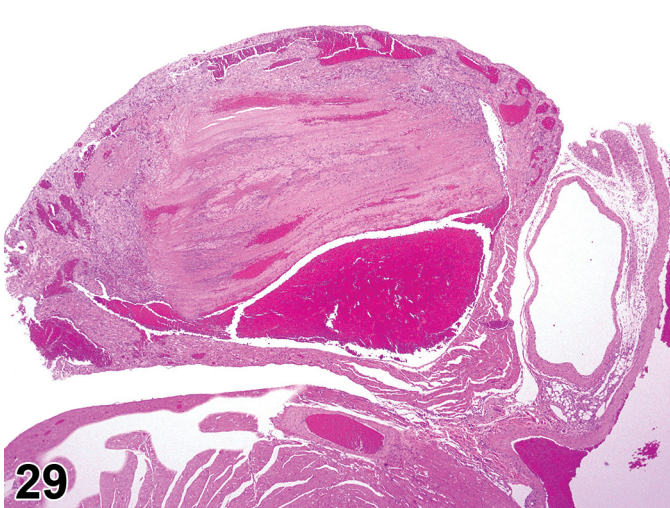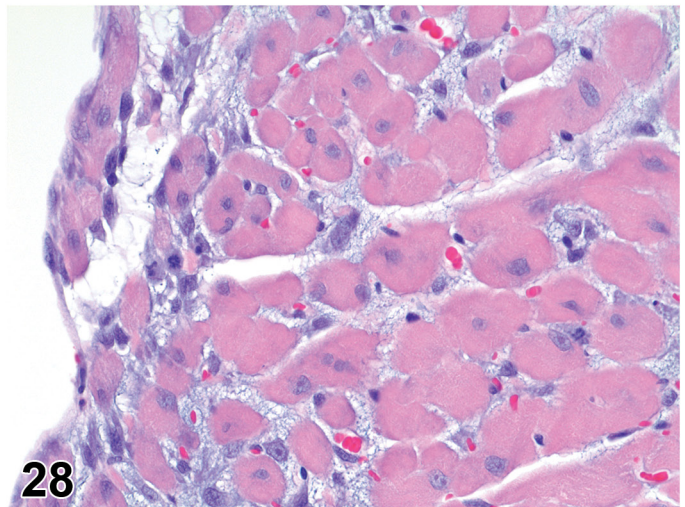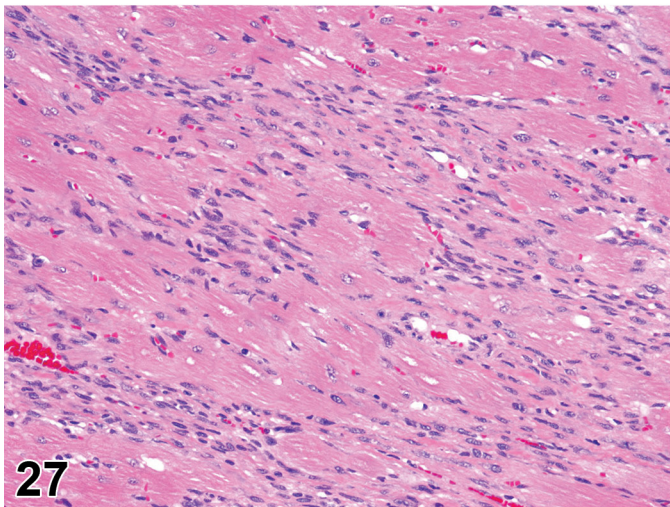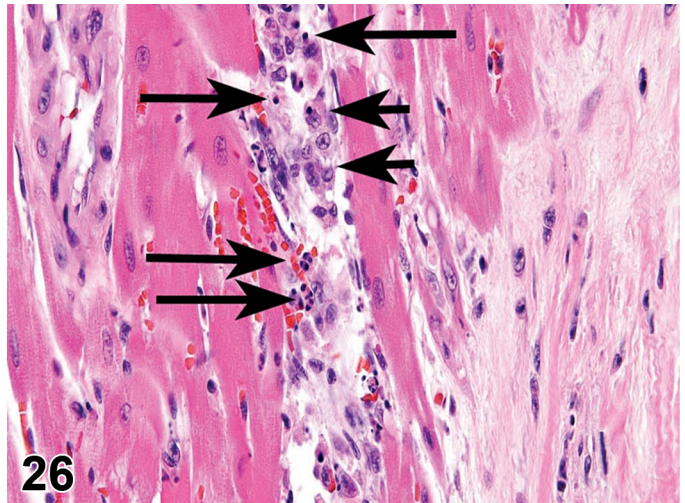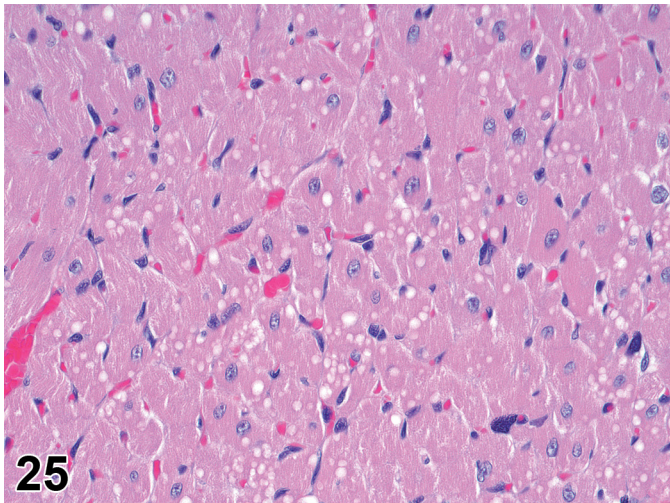
FIGURE 25. — Vacuolation, cardimyocyte. Normal appearing cardiomyocytes have clear cytoplasmic vacuoles. Further characterization of the vacuoles with special stains or TEM might reveal organellar injury that could be characterized as 'degeneration'. Other terms = degeneration, vacuolar (see Figure 21).

FIGURE 26. — Apoptosis, cardimyocyte. Apoptotic cells indicated by arrows. Ephedrine and caffeine treatment, rat (Image courtesy of the National Toxicology Program).

FIGURE 27. — Fibrosis, myocardium.

FIGURE 28. — Edema, myocardium. Myocardial interstitium is expanded by pale blue amorphous material.

FIGURE 29. — Thrombus, atrium. Atrial lumen occluded by an adherent mass of laminated fibrin.

FIGURE 30. — Thrombus, atrium. Higher magnification of Figure 29.
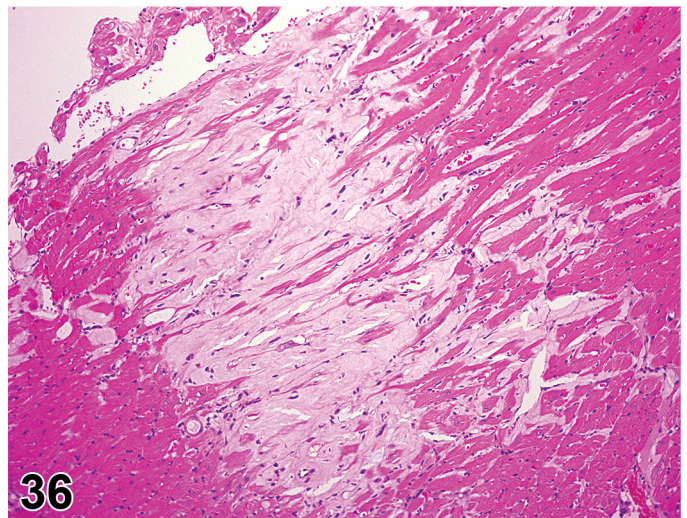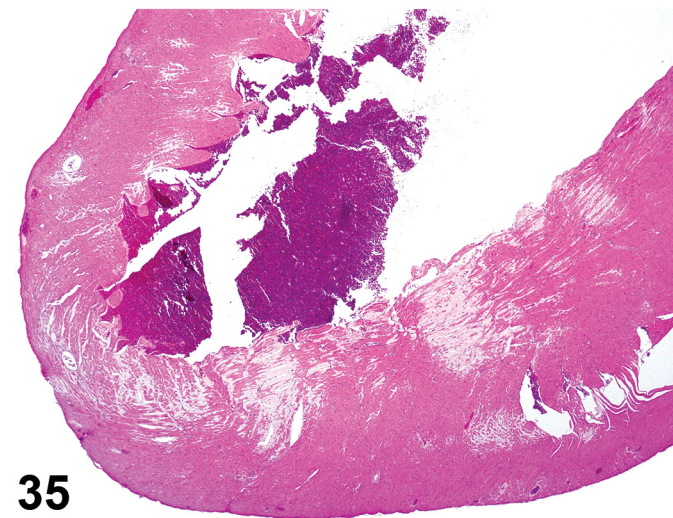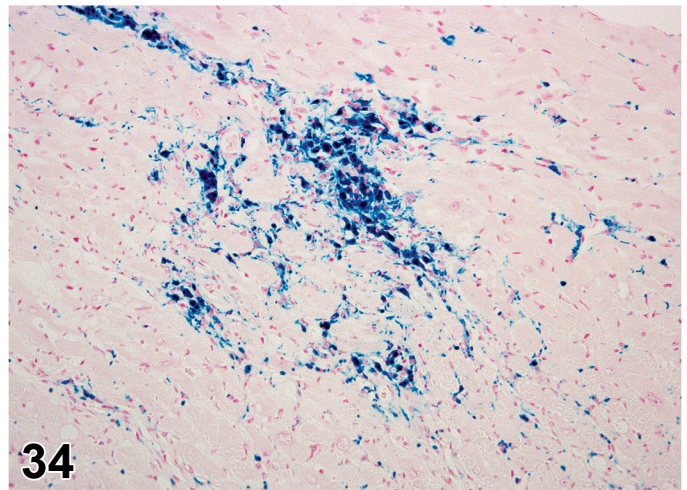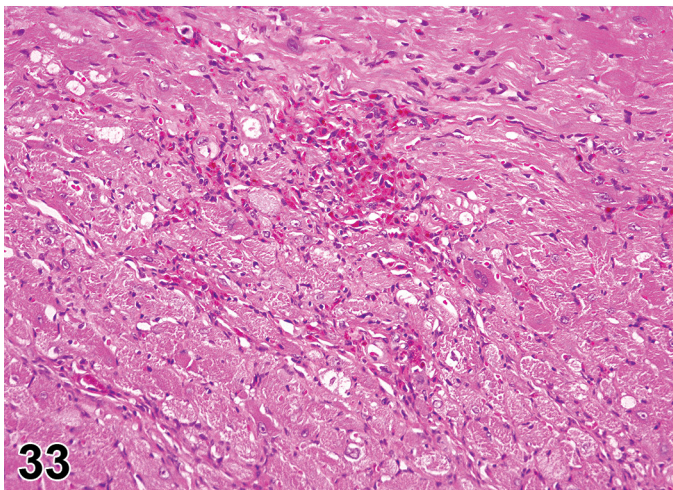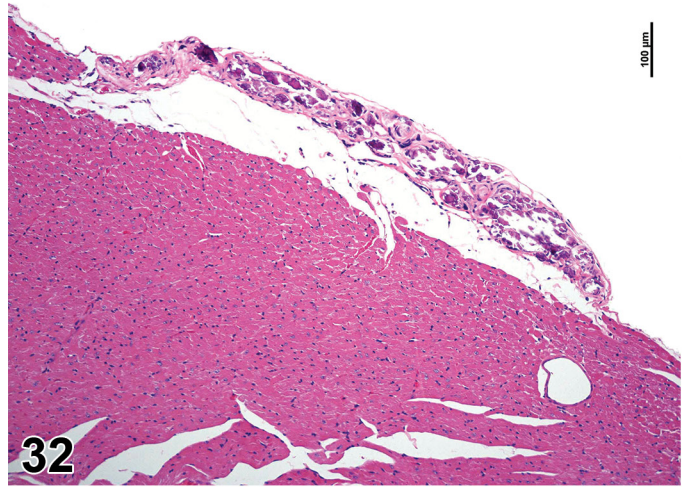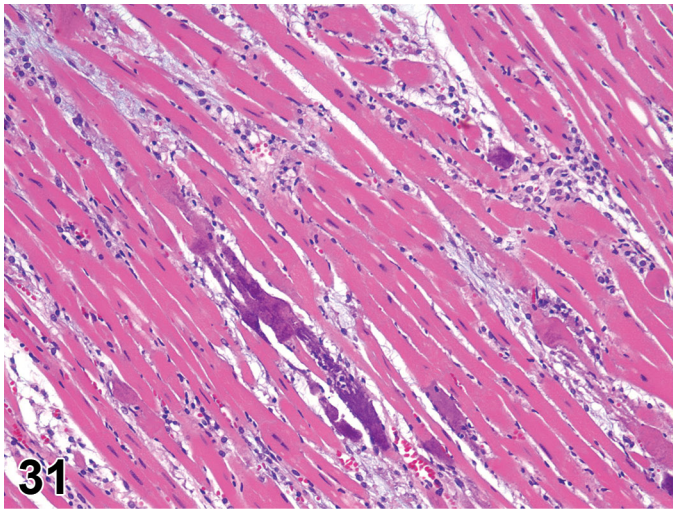
Figure 31. — Mineralization, cardiomyocyte or myocardium. Single fiber mineralization in the heart of a rat with severe nephrotoxicity.
Figure 32. — Mineralization, myocardium or epicardium. Mouse.
Figure 33. — Pigmentation, myocardium. Hemosiderin deposition in an area of resolving hemorrhage.
Figure 34. — Pigmentation, myocardium. Perl's prussian blue stain reveals iron content in deposit of hemosiderin.
Figure 35. — Amyloid, myocardium.
Figure 36. — Amyloid, myocardium. Higher magnification of Figure 35.
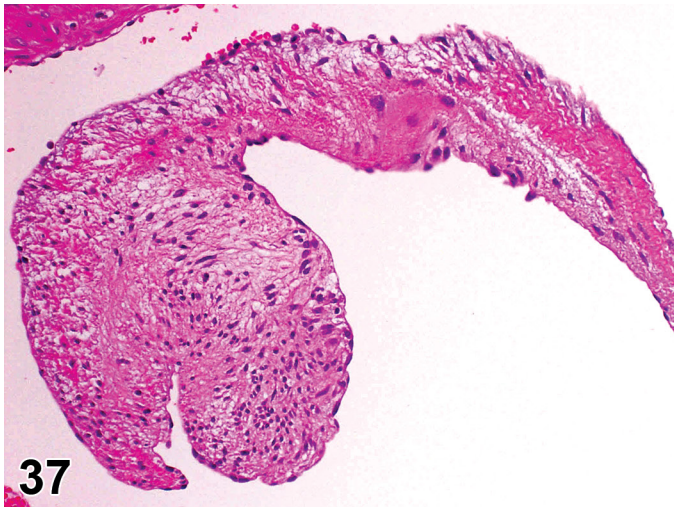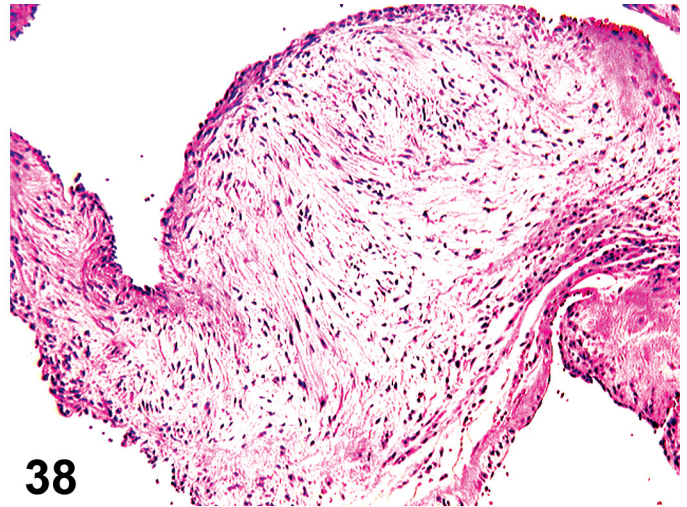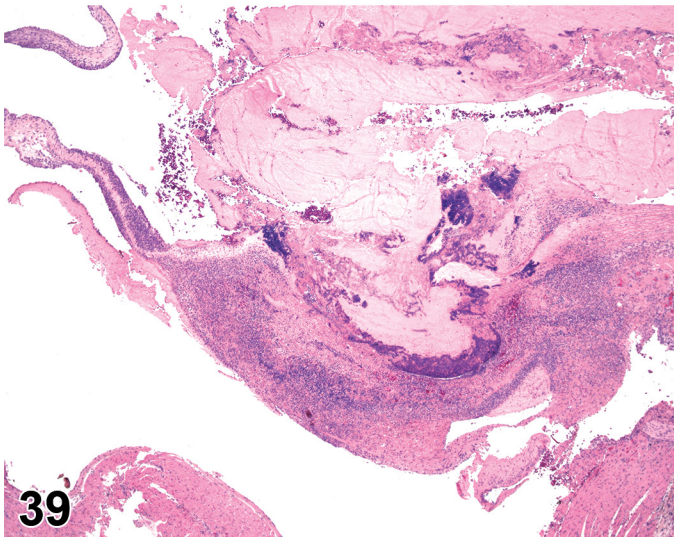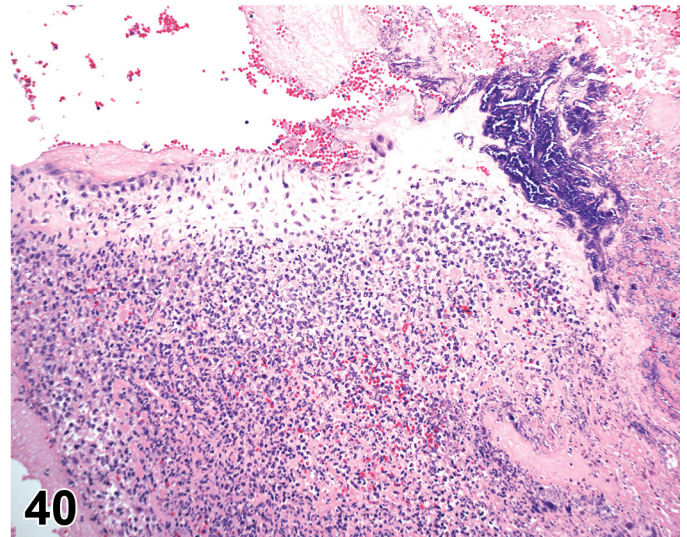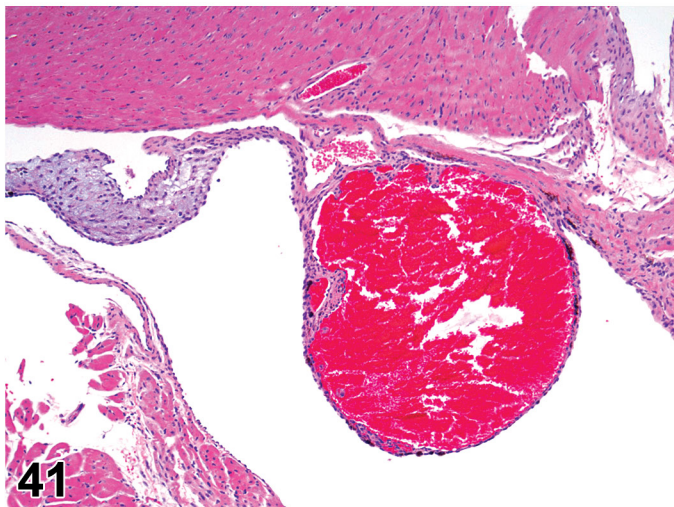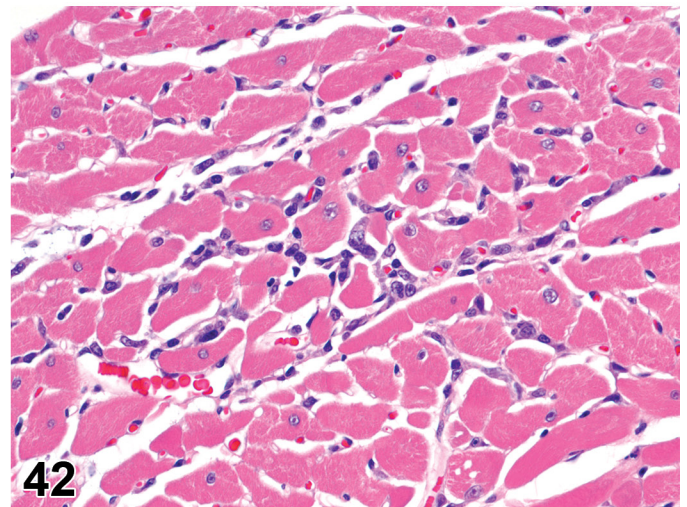
FIGURE 37. — Degeneration, myxomatous, valve.

FIGURE 38. — Degeneration, myxomatous, valve (compliments of Chandi Elangbam).

FIGURE 39. — Inflammation, valve. Hypercellular valve leaflet with an adherent thrombus.

FIGURE 40. — Inflammation, valve. Higher magnification of Figure 39 with dark blue microcolonies of bacteria (upper right of image).

FIGURE 41. — Angiectasis, valve.

FIGURE 42. — Hypertrophy, endothelial.
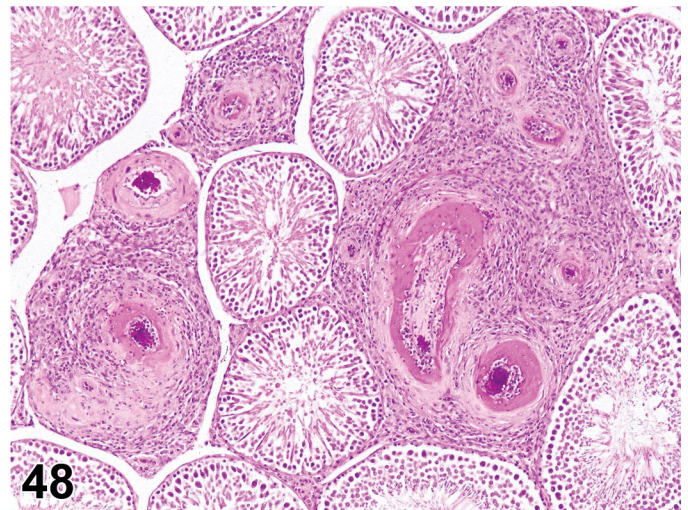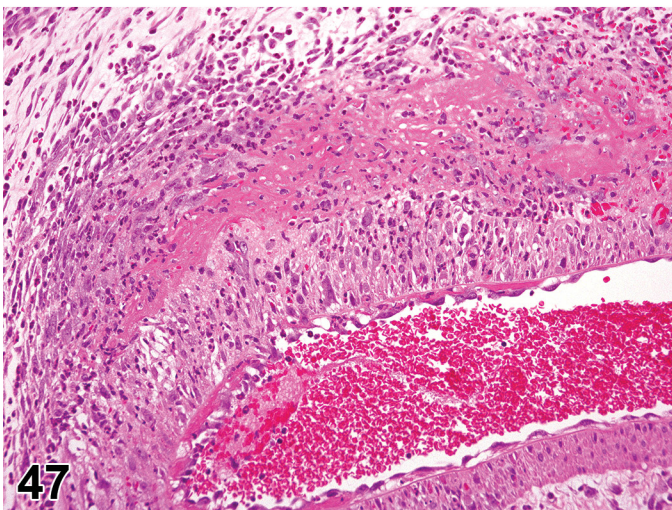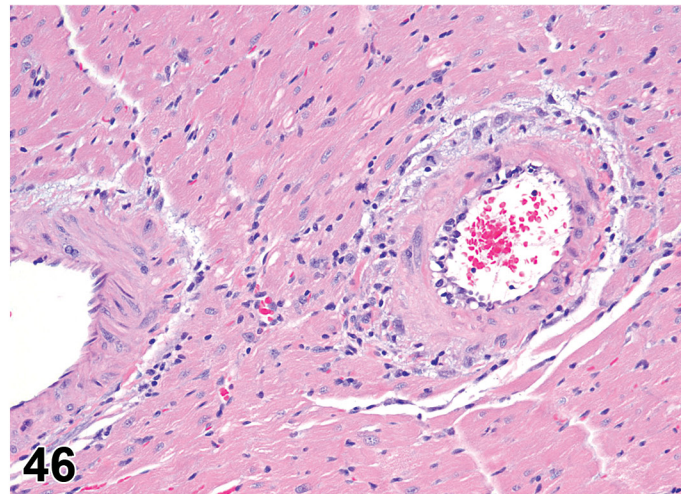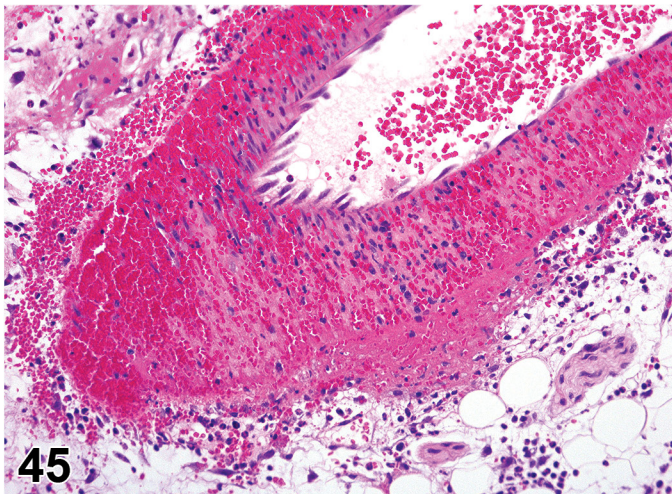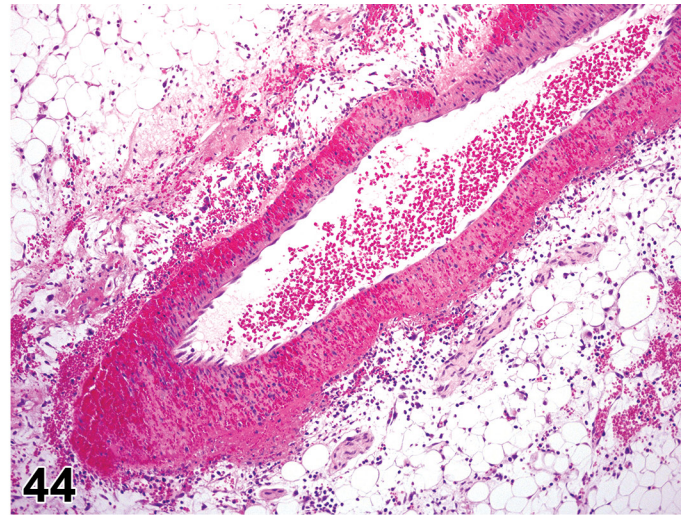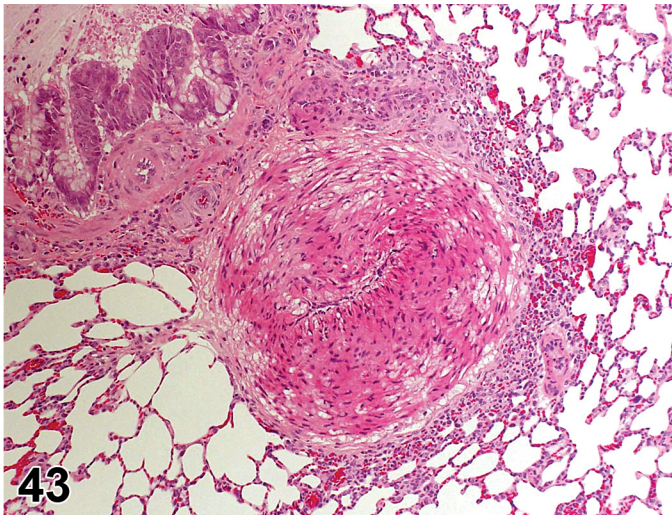
FIGURE 43. — Hypertrophy, medial or mural, artery.

FIGURE 44. — Hemorrhage, medial or mural, artery.

FIGURE 45. — Hemorrhage, medial or mural, artery. Higher magnification of Figure 44.

FIGURE 46. — Degeneration/necrosis, medial or mural, artery. Characterized by loss of nuclear detail, nuclear pyknosis and medial fragmentation and vacuolation. Accompanied by perivascular edema in this lesion.

FIGURE 47. — Necrosis/inflammatory cell infiltrate, medial or mural, artery.

FIGURE 48. — Necrosis/inflammatory cell infiltrate, medial or mural, artery. More chronic lesion than Figure 47 accompanied by perivascular fibrosis.
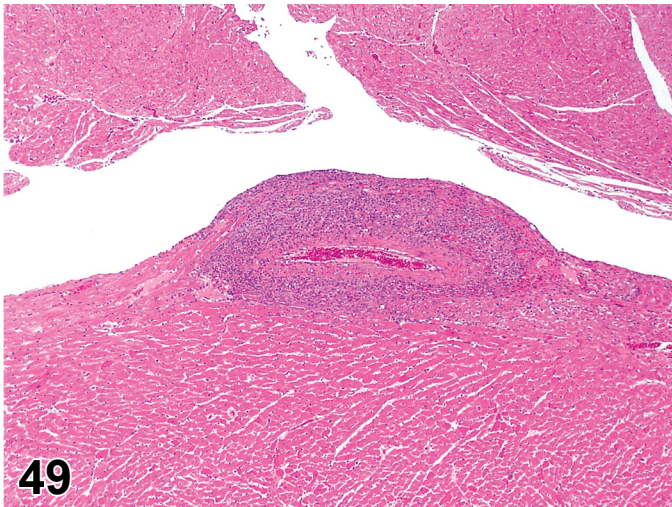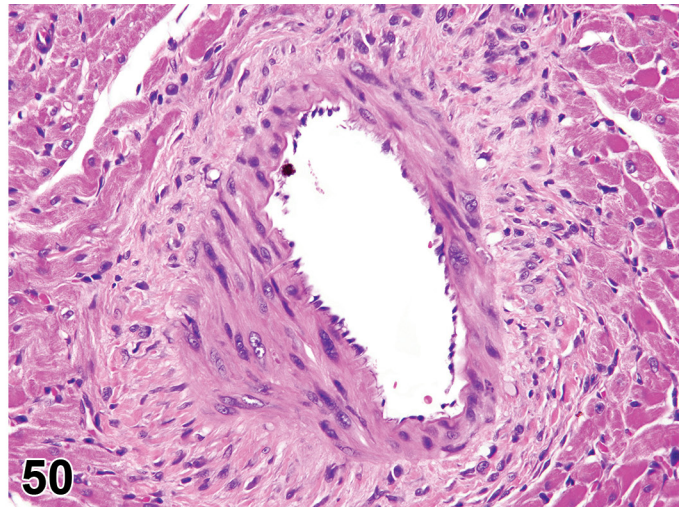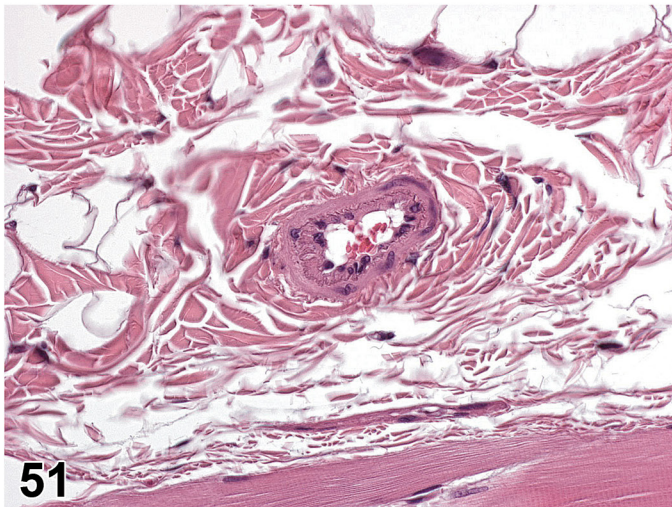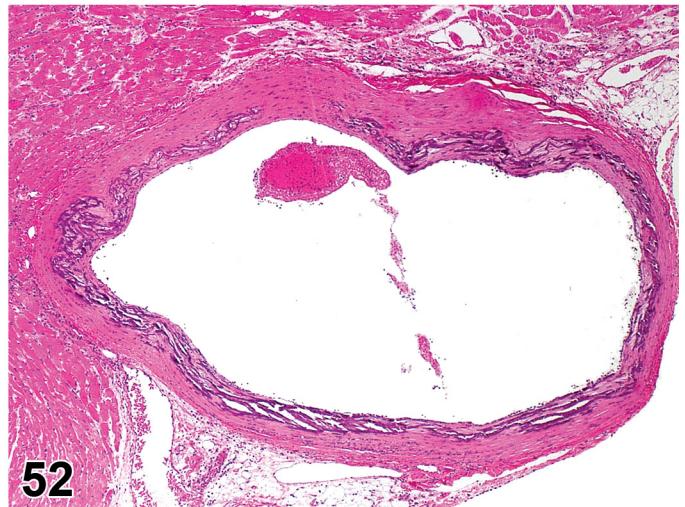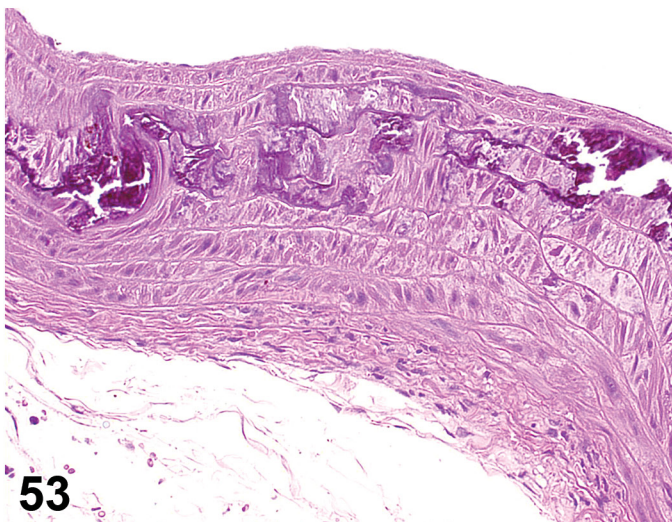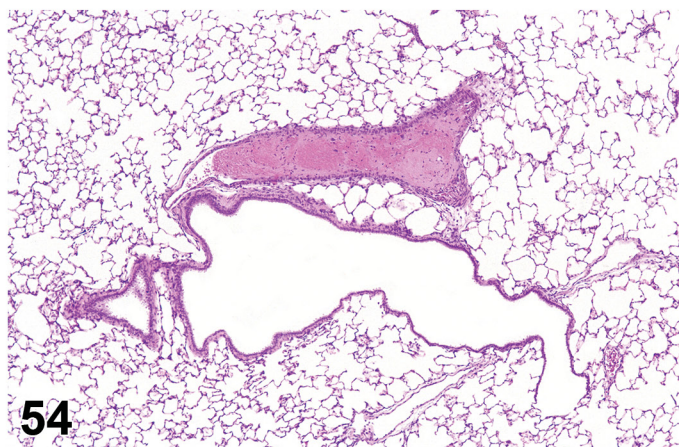
FIGURE 49. — Infiltrate, inflammatory cell, perivascular.

FIGURE 50. — Fibrosis, perivascular. Angiotensin II treatment, rat (Image courtesy of the National Toxicology Program).

FIGURE 51. — Intimal thickening, acellular, artery. Subcutis of rat.

FIGURE 52. — Mineralization, medial or mural, artery.

FIGURE 53. — Mineralization, medial or mural, artery.

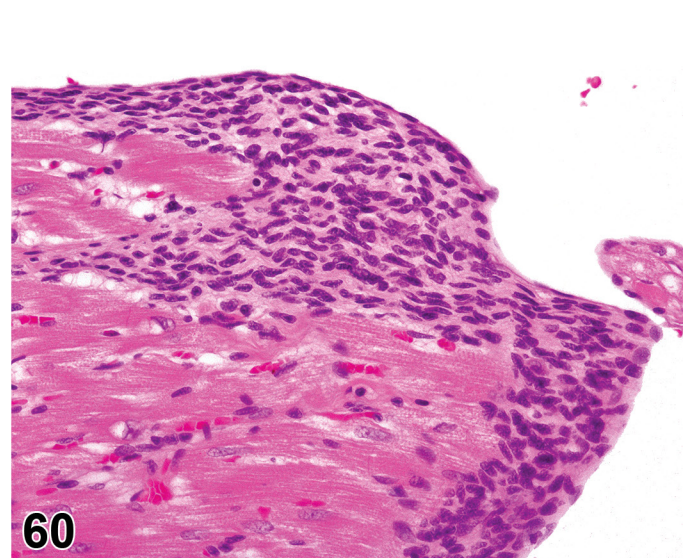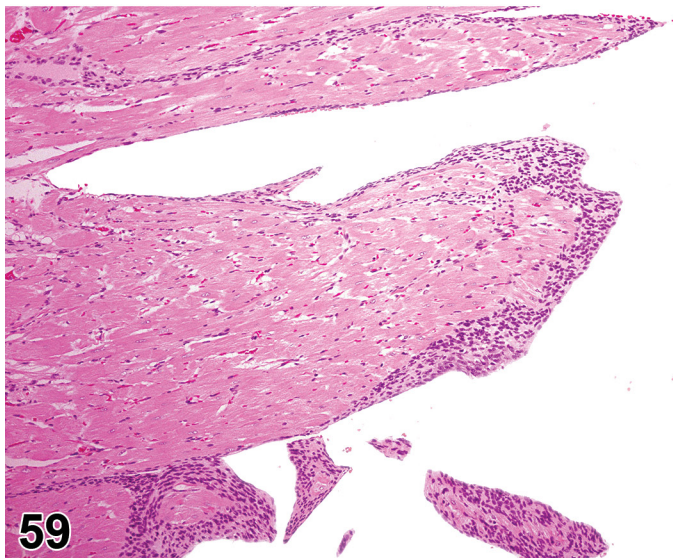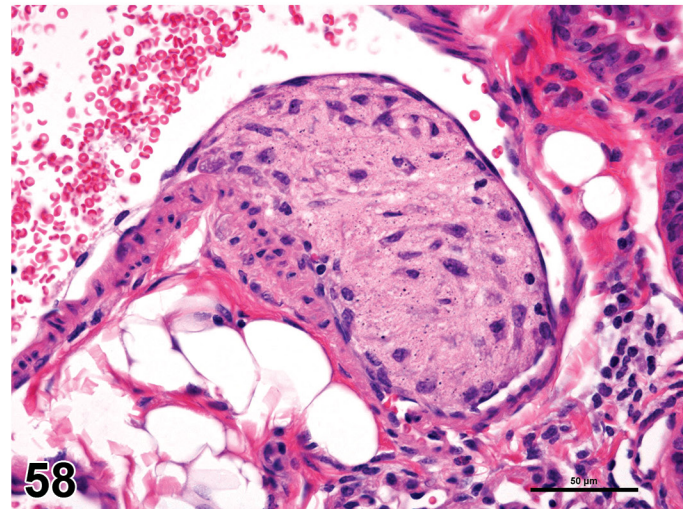FIGURE 54. — Thrombus. Pulmonary artery partially occluded by a poorly organized thrombus. (Image courtesy of the National Toxicology Program).
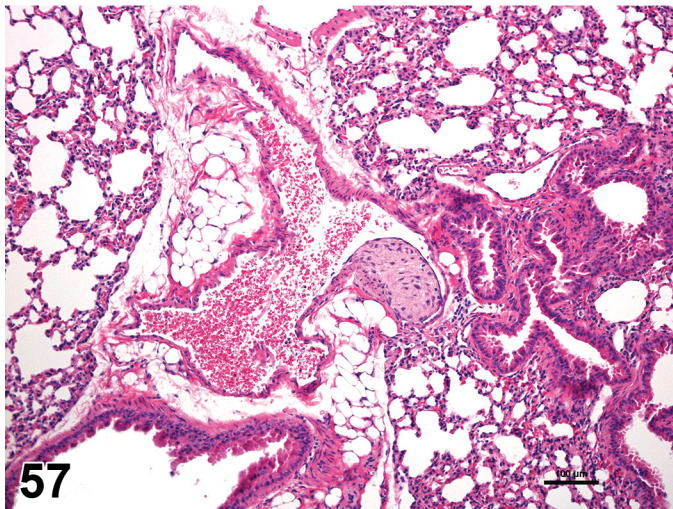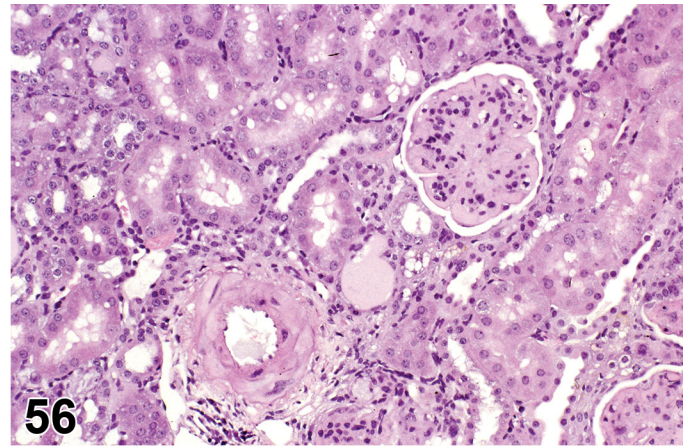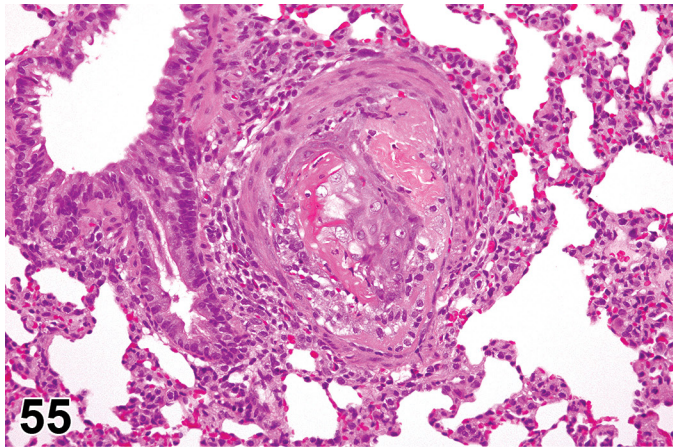
FIGURE 55. — Embolus. Artery lumen is occluded by an irregular mass of non-local cells and fibrin.

FIGURE 56. — Amyloid, medial or mural, artery. Tunica media of a renal artery is effaced by pale eosinophilic amorphous material. Similar material expands glomerular capillary loops and mesangia (Image courtesy of the National Toxicology Program).

FIGURE 57. — Intramural plaque, artery.

FIGURE 58. — Intramural plaque, artery.

FIGURE 59. — Hyperplasia, Schwann cell, subendocardium.

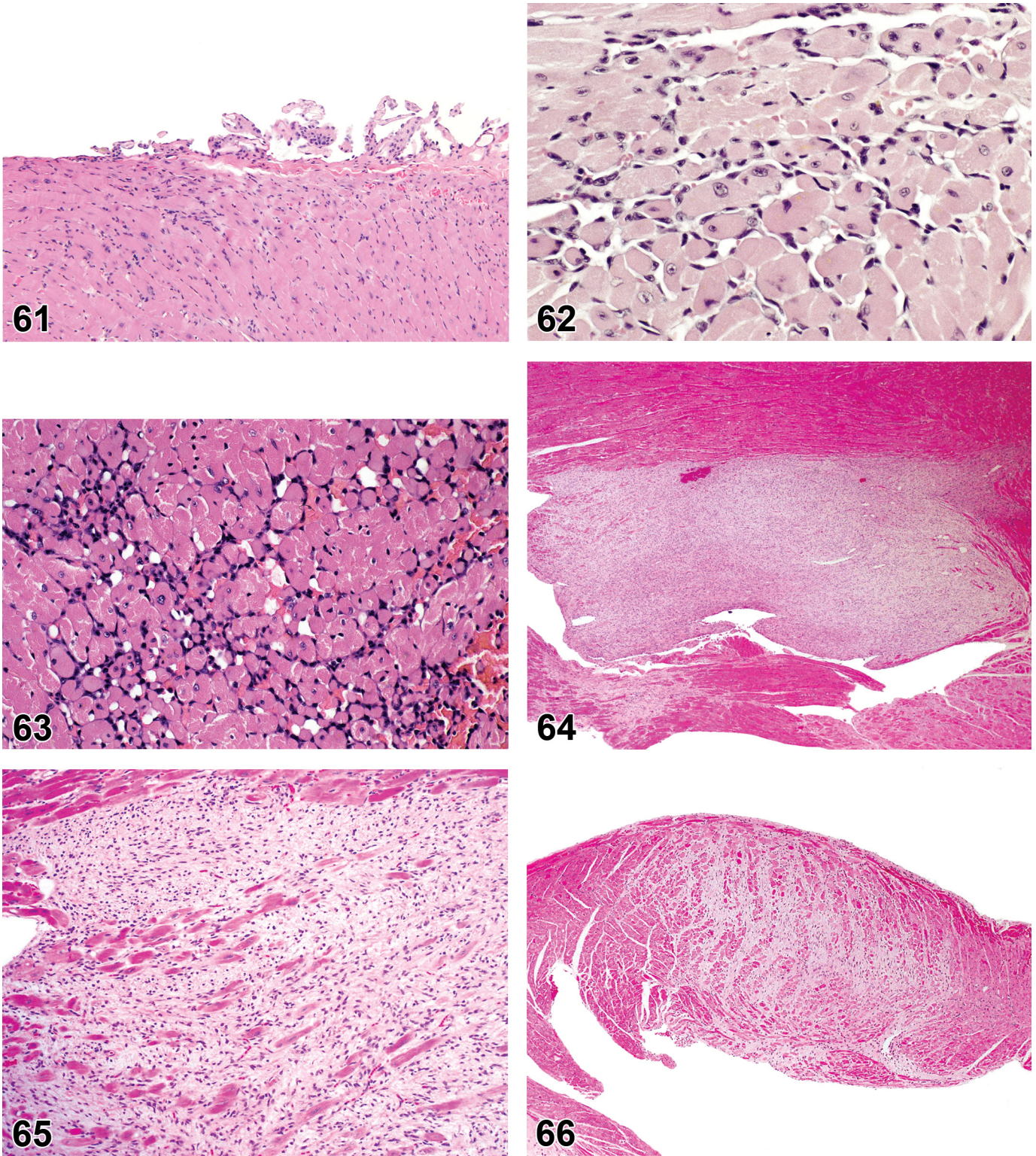FIGURE 60. — Hyperplasia, Schwann cell, subendocardium. Higher magnification of Figure 29.

FIGURE 61. — Hyperplasia, mesothelial, epicardium (Image courtesy of the National Toxicology Program).

FIGURE 62. — Hyperplasia, hemangioendothelial. 1,3-Butadiene, mouse (Image courtesy of the National Toxicology Program).

FIGURE 63. — Hyperplasia, hemangioendothelial. Benzophenone, mouse (Image courtesy of the National Toxicology Program).

FIGURE 64. — Schwannoma, subendocardial.

FIGURE 65. — Schwannoma, subendocardial. Higher magnification of Figure 64.
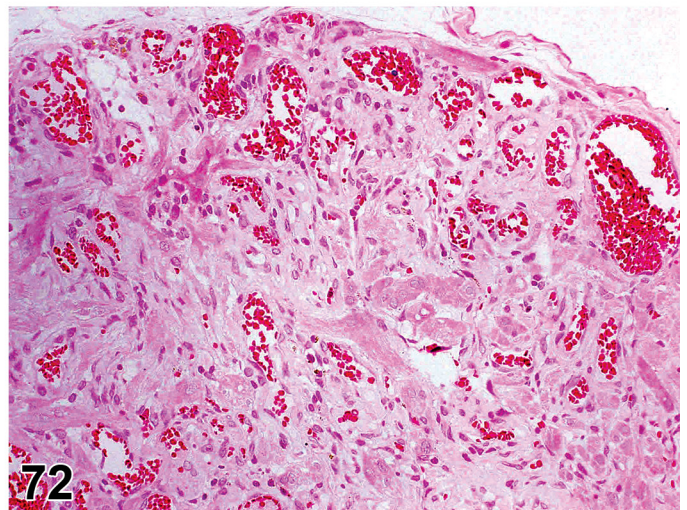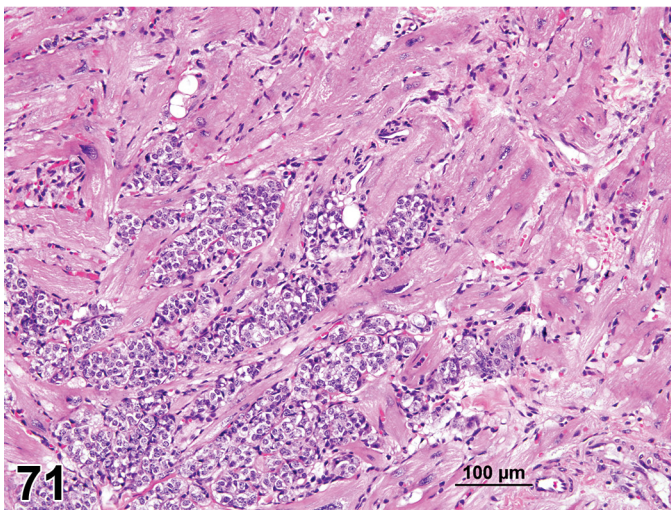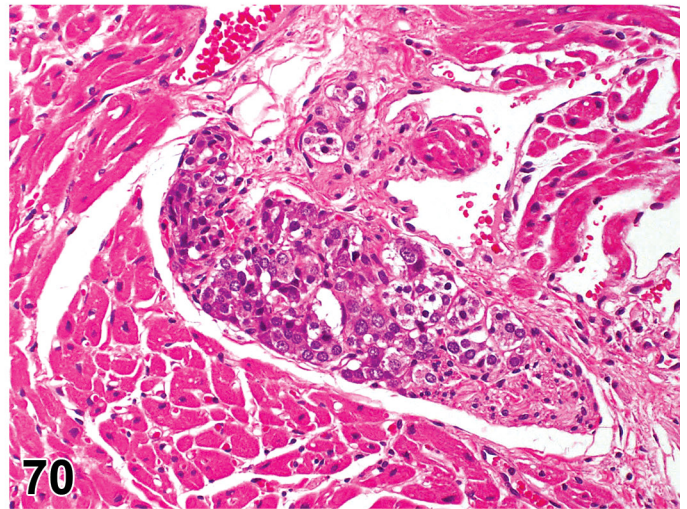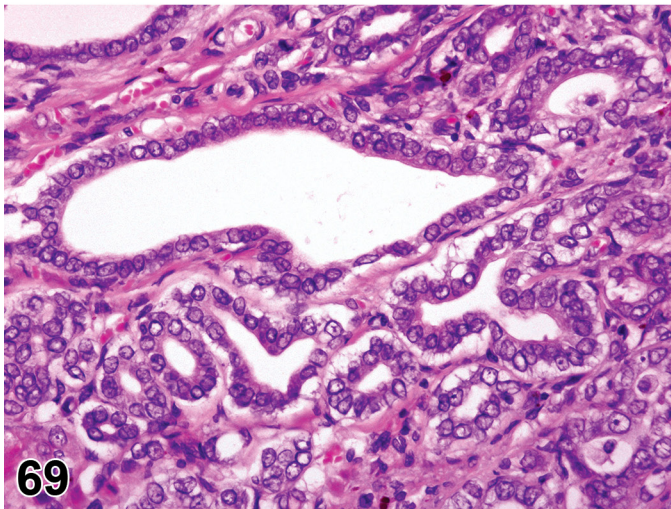
FIGURE 66. — Schwannoma, intramural.
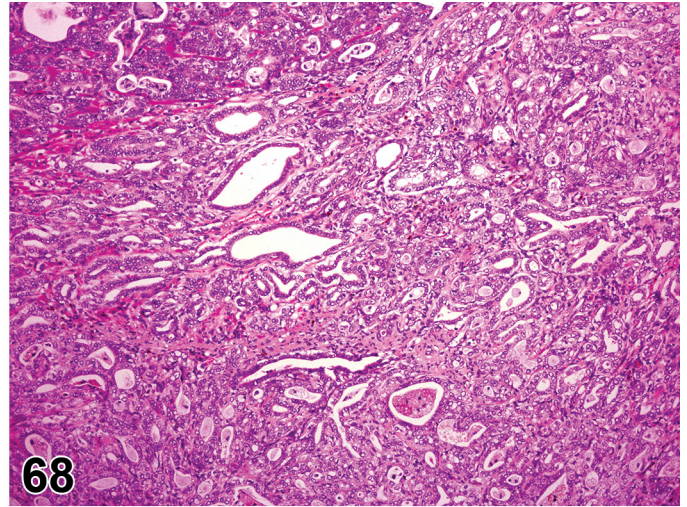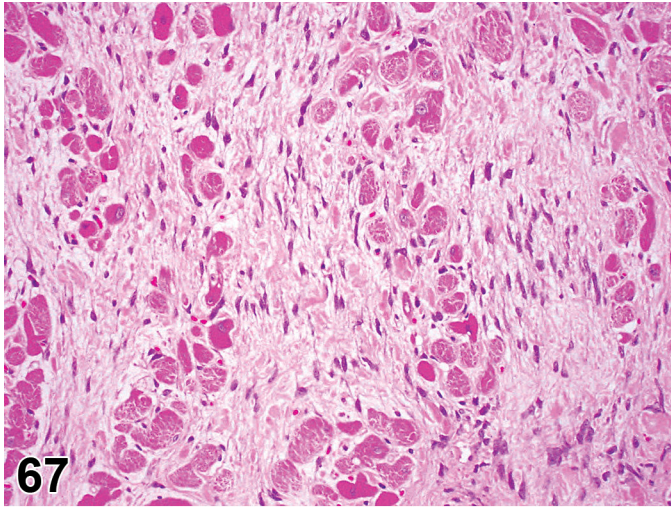
FIGURE 67. — Schwannoma, intramural. Higher magnification of Figure 66.

FIGURE 68. — Mesothelioma, atriocaval.

FIGURE 69. — Mesothelioma, atriocaval. Higher magnification of Figure 68.

FIGURE 70. — Paraganglioma.

FIGURE 71. — Paraganglioma (Image courtesy of the National Toxicology Program).

FIGURE 72. — Hemangioma.
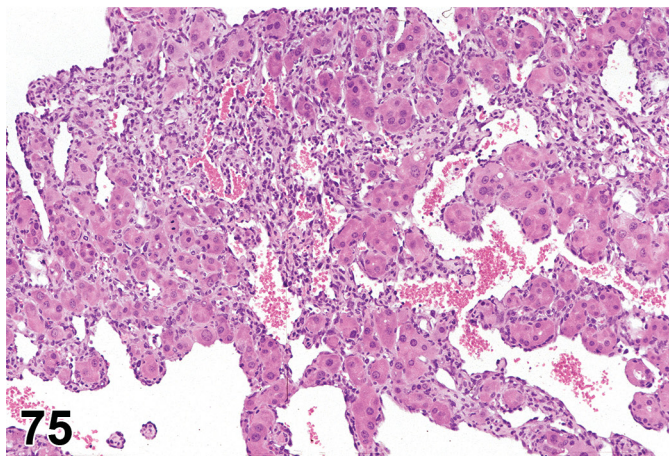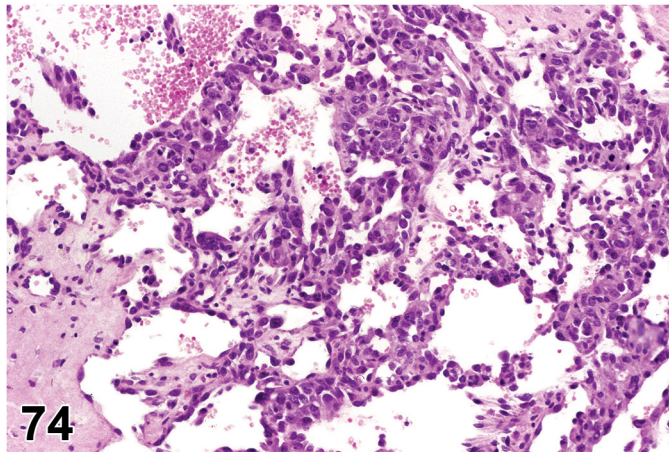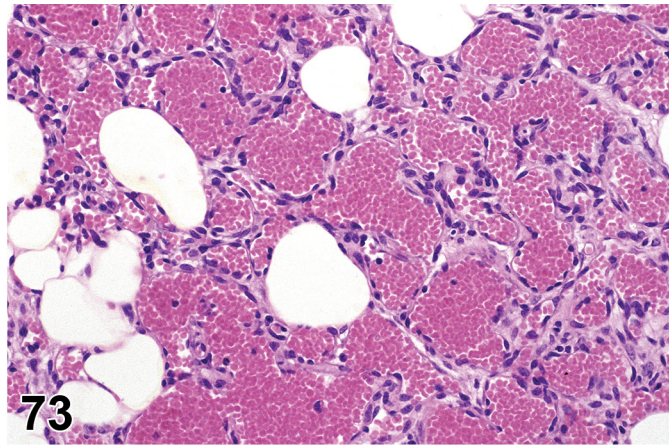
FIGURE 73. — Hemangioma. 1,3-Butadiene, mouse, subcutaneous (Image courtesy of the National Toxicology Program).

FIGURE 74. — Hemangiosarcoma. 1,3-Butadiene, mouse (Image courtesy of the National Toxicology Program).

FIGURE 75. — Hemangiosarcoma. P-Nitroaniline, liver (Image courtesy of the National Toxicology Program).

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