CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY
RESPIRATORY AND CARDIOVASCULAR SYSTEM

Fourth Conference

ORGANIZED BY

SOCIETY OF TOXICOLOGIC PATHOLOGY - INDIA (STP-I)

NOVEMBER 1-3, 2012

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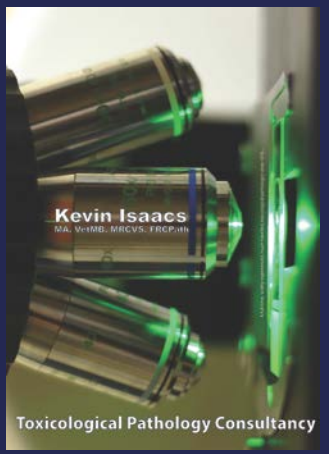
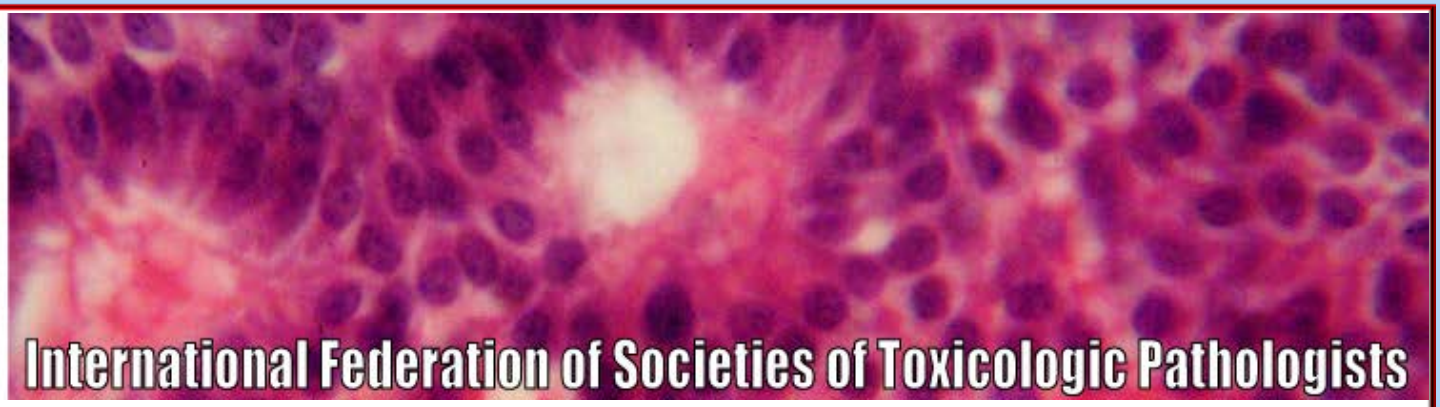
SPONTANEOUS CARDIOVASCULAR PATHOLOGY (MOSTLY)





The International Federation of Societies of Toxicologic Pathologists is pleased to sponsor the lectures given by:

Kevin ISAACS

during the 4th STPI conference
1-3 November 2012, in Bangalore

http://www.ifstp.com

THE HEART

OUTLINE OF CHANGES SEEN

- Developmental lesions
- Nothing (Sudden death)
- Myocardial lesions
- Endocardial lesions
- Epicardial & Pericardial lesions
- Conducting system
- Vascular changes
- Tumours

WORDS OF CAUTION

Spontaneous disease is not uncommon

Differentiating treated lesions from spontaneous disease

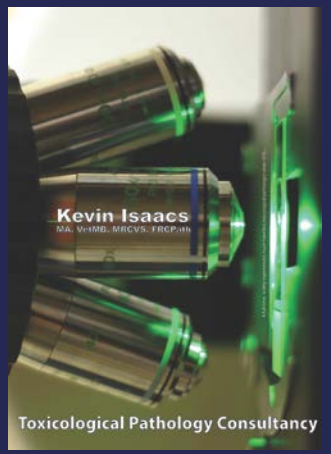
- Pathognomonic changes are relatively infrequent
- Weight of evidence
  - Patterns of effect may be important
  - Small changes can be significant

The sampling regime is central to diagnosis

- Accurate and careful recording of findings may be crucial

Some illustrations are effects of treatment for comparison

MYOCARDIAL LESIONS



CONGENITAL LESIONS

Septal defects

- Rarely reported
- Seen at necropsy

Situs inversus

- Rare
- May be missed

Heterotopic tissue

- Thyroid tissue in dogs

‘SUDDEN CARDIAC DEATH’

Animals can die with no obvious cause of death

- Long term rodent studies mainly
  - Found dead
- Very difficult for a pathologist
  - No less serious than necrosis!
- Could be arrhythmias
  - Heart chambers are often dilated at necropsy

Major cause of death in man

- Probably underdiagnosed in lab animals

Could be ionic/metabolic changes

- Potassium, Sodium, Magnesium, Calcium
- Hypoglycaemia

Conductivity changes

- Some treatment-related deaths
  - Local anaesthetics i.v.
  - Prolonged QT intervals

HYPERTROPHY, MYOCARDIAL

Thickening of chamber walls

- Judged visually at histopathology or necropsy
- Can be measured quite simply

Increase in myocyte size

- With nuclear hypertrophy
  - Increased ploidy

Compensatory

- Response to increased workload
  - Concentric
- Vasoactive peptides and growth factors

Maladaptive

- May involve increased apoptosis
  - Asymmetric
- Fibroplasia and EC matrix deposition
  - Not related to apoptosis
- Heart failure
  - Lesions in other organs e.g. lung, liver

Usually secondary change in rodents

- Degeneration of valves
- Cardiomyopathy
- Amyloidosis
- Atrial thrombus

VACUOLATION, MYOCARDIAL

Diffuse or focal

- Can be difficult to assess in H&E sections

Neutral lipid

- Fasting
- Cardiomyopathy in rats
- Hypothyroidism
- Also toxicity
  - Allylamine

Phospholipids

- Treatment related
  - EM

SER swelling

- Anthracyclines

Mitochondrial swelling

- More likely to be a toxic effect
- Reversible

NECROSIS, MYOCARDIAL

Irreversible damage

- Therefore, cannot be described as “degeneration”
- Inflammation is variable

Histological appearance of necrosis

- Coagulative (Zenker’s) necrosis
  - Hypereosinophilic appearance and loss of striations
  - Contraction bands – may be seen as an early change
  - Both can be easily confused with artefact
    - Loss of TnI or myoglobin staining or autofluorescence may help
- Inflammatory cells
  - Mainly macrophages
- Myocyte loss as a sequel
- May also see mineralisation in acute changes

SPONTANEOUS INCIDENCE OF NECROSIS

Part of cardiomyopathy syndrome in rats

- Incidence in old rats may be high
  - Many animals may have some at 2 years
  - Severity varies widely with strain, location, diet, pathologist etc.
- Seen as focal change in young rats
  - Recorded as separate lesions
    - Necrosis fibrosis/inflammation

Also seen with renal failure in rats

- Widespread degenerative changes in myocardium
  - Can be accompanied by mineralisation
  - End stage CPN with hyperparathyroidism
    - Mineralisation of many blood vessels & BMs
  - Severe obstructive nephropathy

Occasional focal change in NHP & dogs

- Cynomolgus monkeys
  - Confounding factor in toxicity studies
  - See below

INFLAMMATION, MYOCARDIAL

Mainly associated with necrosis, fibrosis or myocyte loss

Occurs spontaneously in most species

- Young Beagles 5% males, 2% females
- Rats – varies with strain and laboratory

Macrophages

- Most common cell type with myocyte necrosis

Polymorphs

- In some acute lesions

Lymphocytes

- Scattered foci seen commonly in NHP
  - 13% (Chamanza et al)
  - No identified cause
  - Not associated with necrosis
  - No site of predilection

FIBROSIS, MYOCARDIAL

Sequel to necrosis & inflammation

- Focal change, usually
- Major feature of cardiomyopathy in rats

Sequel to necrosis and haemorrhage in NHP

- Cynomolgus (especially Mauritian origin)

May be associated with hypertrophy

- Diffuse, interstitial change in man
- Focal change more frequent

Idiopathic change in wild marmosets

- Interstitial

Post-viral disease in dogs

- Parvovirus
  - Could confound toxicity studies

RAT CARDIOMYOPATHY

Common

- Progressive
- How early does it start?

Random distribution of lesions at low severity

- Vacuolation
- Necrosis
- Fibrosis
- Inflammation

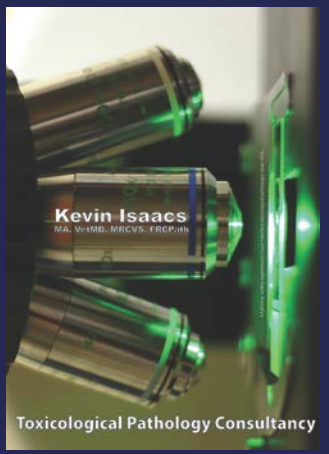
A pattern can emerge in more advanced lesions

- Heart base
- Left ventricle
  - Papillary muscles
- Not usually atria
- Some compensatory hypertrophy

What is not part of the syndrome?

- Vascular lesions
- Valvular lesions
- Atrial myocardial hypertrophy

A TREATED LESION FOR COMPARISON



CYNOMOLGUS MONKEY ‘CARDIOMYOPATHY’

Spontaneous Lesions of the Cardiovascular System in Purpose-Bred Laboratory Nonhuman Primates

RONNIE CHAMANZA, NICOLA M. A. PARRY, PETRINA ROGERSON, JEN R. NICOL AND ALYS E. BRADLEY

Toxicologic Pathology, 34:357–363, 2006

Spontaneous Findings in the Heart of Mauritian-Origin Cynomolgus Macaques (Macaca fascicularis)

JUSTIN D. VIDAL, LITA S. DROBATZ, DENISE F. HOLLIDAY, LEE E. GEIGER, AND HEATH C. THOMAS

Toxicologic Pathology, 38: 297-302, 2010

Spontaneous Cardiomyopathy in Cynomolgus Monkeys (Macaca Fascicularis)

TANJA S. ZABKA, MICHAEL IRWIN, AND MUDHER A. ALBASSAM

Toxicologic Pathology, 37: 814-818, 2009

CYNOMOLGUS MONKEY 'CARDIOMYOPATHY'

Confounding factor in toxicity studies

Mauritian supplier (Vidal et al, 2010)

- 29 males, 42 females
- Subendocardial haemorrhage 30%
- Haemosiderin 17%
- Myocardial degeneration/necrosis 13%
- Coronary arterial degeneration/haemorrhage 6%
- Fibrosis, papillary muscle 4%
- Lesions can be large
  - Ventricles

Various suppliers (Chamanza et al, 2006)

- 1025 males, 1025 females
- Myocarditis, focal 5%
- Mineralisation 0.5%
- Endocarditis 0.4%
- Myocardial fibrosis 0.1%

Unidentified supplier (Zabka et al 2009)

- 4 animals affected
- Fibrosis, myocardial disarray, vacuolation
- Ventricles and septum

CYNOMOLGUS MONKEY 'CARDIOMYOPATHY'

Pathogenesis

- Attributed to catecholamine release
  - 'Capture stress'
  - Inductive reasoning – beware!
- Resembles descriptions of catecholamine-related changes
  - Mainly from literature reports
- No reports of concurrent adrenal changes

Unreported lesion (my observation)

- Large cynomolgus monkeys
- Heavily restrained
- Haemorrhage and necrosis
  - Mainly ventricular

Beware 'writing off' cardiac lesions in NHPs

- Case is still unproven, in my view

MYOCARDIAL HAEMORRHAGE

Rarely affects myocardium alone

- Endocardial and epicardial too

Clotting defects

- Thrombocytopaenia
  - Dog
  - Pig
- Vit K deficiency
  - Dietary insufficiency in rodents
  - Haemorrhage & inflammation
  - Confounding factor in toxicity studies

Capture stress in NHP

- With myocardial necrosis

MINERALISATION, MYOCARDIAL

Ca^{++} ions present in abundance in myocardium

Focal changes (usually dystrophic)

- Accompaniment to acute necrosis
- Sequel to haemorrhage

Diffuse changes (metastatic)

- Renal insufficiency
  - Rats with advanced CPN
  - Acute obstructive nephropathy
- Occasionally with tumours
  - E.g. lymphoma

PIGMENT DEPOSITION

Not common as a spontaneous lesion

- Occasionally in cardiomyopathy

Haemosiderin

- Sequel to haemorrhage
- Perl's Prussian blue positive

Lipofuscin

- Old dogs
- Not commonly reported in toxicity studies
- Schmorl's or EM

AMYLOID DEPOSITION

Mice

- Aged animals
- Some strains have more than others

Interstitial

- Can be easily missed

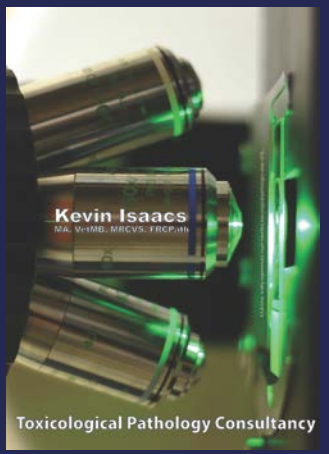
Deposits in other tissues

- Kidneys
- Spleen
- Liver

Pale, amorphous eosinophilic material

- Low levels difficult to see
- Sirius red or Congo red + birefringence
- Thioflavine T + UV

ENDOCARDIAL & VALVE CHANGES



ENDOCARDIAL CHANGES

Can affect heart chambers &/or valves

Haematocysts

- Vascular ectasia
- Incidental and usually harmless

'Myxoid' degeneration, valves

- Prominent GAGs give "myxoid" or "mucoid" appearance
- Older rats and dogs
- Secondary changes seen due to valve incompetence
- Underdiagnosed in routine studies
  - Good sampling required

Amyloid deposits, valves

- Old mice

Inflammation

- Occasionally in old rats
- Apex of heart
- Also seen with valvular degeneration

ENDOCARDIAL CHANGES

Haemorrhage

- Can be an agonal event, occasionally, in valves
- Can be difficult to see

Thrombus

- Distinguish from post mortem clotting
- Usually atrial
  - Atrial fibrillation
- Seen with amyloidosis in mice and hamsters

Fibrosis

- Uncommon
- May be seen in valves of NHP

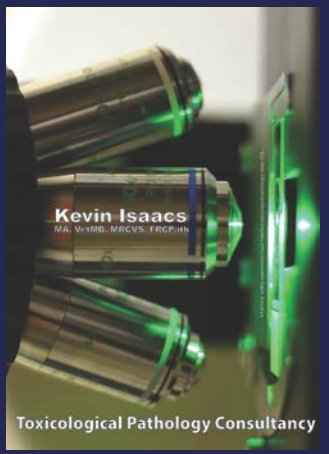
Osseous metaplasia

- Old rats
  - Occasionally

Pigment

- Haemosiderin
  - Secondary to haemorrhage
  - Rare
- Melanin
  - Valves of pigmented mice

EPICARDIAL/PERICARDIAL CHANGES



EPICARDIAL CHANGES

Pericardium and epicardium often overlooked at necropsy

- Not easy to see in rodent
- Minor changes in larger animals often overlooked

Mesothelial hyperplasia

- Atrial fronds in dogs
- Probably related to motility
- Also seen with inflammation

Inflammation

- Sporadically in dogs, rodents, NHP
  - Often atrial, focal lesions
- May be related to gavage injury in rodents
  - Can see mediastinal abscess
- May involve epicardial fat

EPICARDIAL CHANGES

Fibrosis

- Usually a sequel to inflammation
- Dogs, NHP, rodents

Mineralisation

- Has been seen spontaneously in mouse
- Not common

Haemorrhage

- Occasionally in NHP
- May be related to handling or restraint

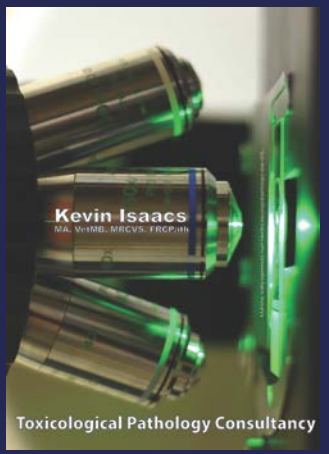
Squamous plaques

- NHP

Metaplasia, osseous

- Old rats
- Associated with chronic cardiomyopathy

VASCULAR SPONTANEOUS LESIONS



VASCULAR LESIONS

Range of reaction in vessel walls is limited

- Makes attribution to treatment difficult
  - Small differences may be important

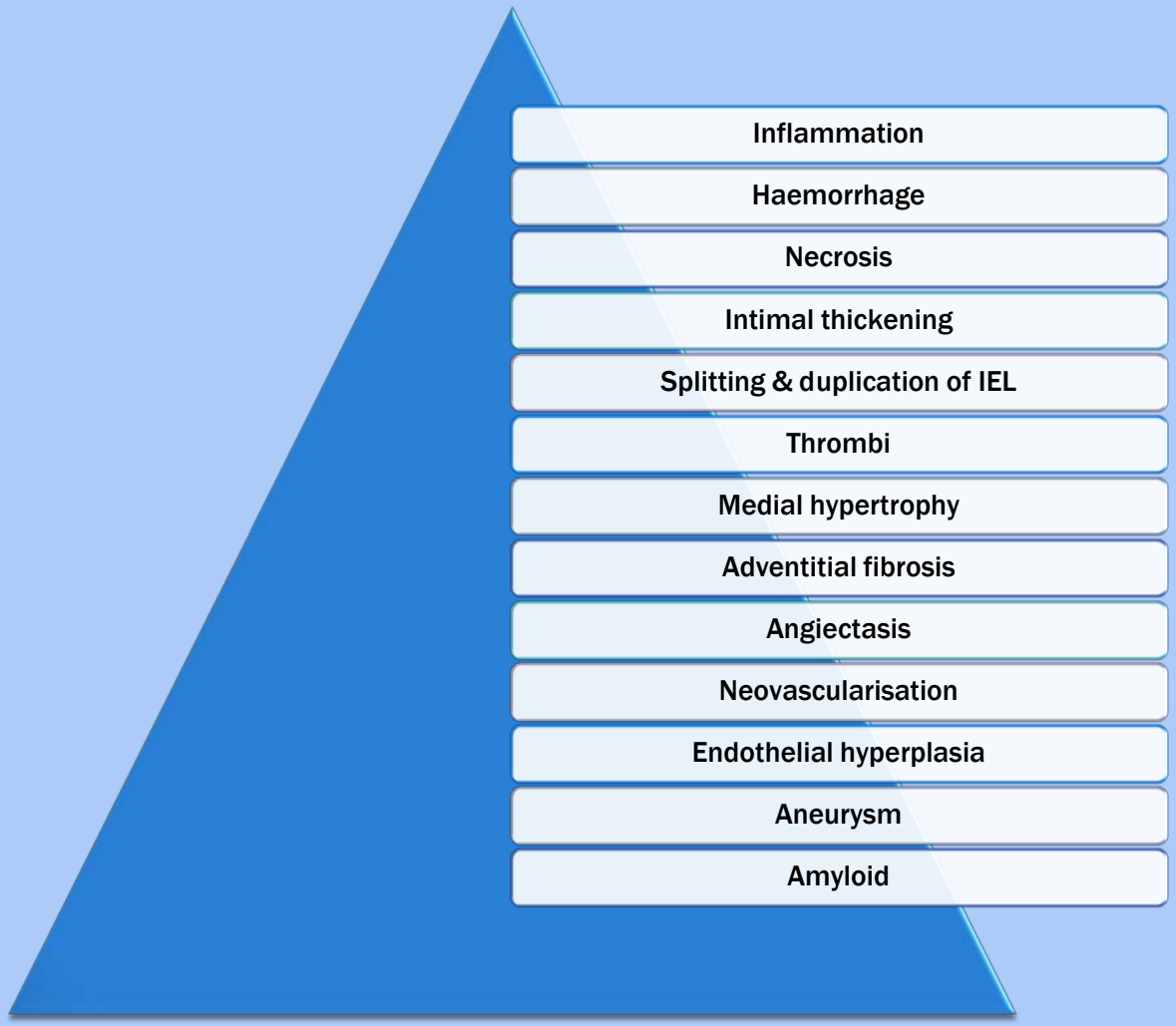
Causes of changes

- Infectious agents
  - Relatively uncommon in rodent studies
- Immune system involvement
  - INAS in dogs
  - Some glomerular lesions
- Haemodynamic changes
- Humoral factors
  - Renin-angiotensin
- Dietary factors
  - Restriction reduces spontaneous incidence and severity

Biomarkers

- No simple, reliable markers

VASCULAR SPONTANEOUS LESIONS



VASCULAR INFLAMMATION

Inflammation

- Most spontaneous lesions are segmental
- Can affect all layers of vessels
- Mixed inflammatory cell infiltration
  - Varies with duration of lesion and causative agent
  - Usually associated with other changes in vessel wall
- Mature lesions mainly in intima and adventitia
  - Media repairs effectively
- Medial necrosis
- Fibrinoid material

Terminology

- Arteritis, phlebitis, vasculitis - commonly used
  - Do not ‘travel’ well
- I prefer “Inflammation, arterial/venous etc.”

ARTERIAL INFLAMMATION

Often diagnosed as 'arteritis'

- Some terms, however, are best avoided:
  - Panarteritis nodosa
  - Polyarteritis nodosa

Coronary arteries

- See below

Splanchnic arteries

- Rats
  - Mesenteric arteries
  - Pancreatic arteries
- Low incidence, now
- Unrestricted diets led to high incidence in past

Testes

- Rats

Kidneys

- Old rats
  - Sometimes associated with CPN

CORONARY ARTERIAL INFLAMMATION

Dog (Hartman lesion)

- Non-suppurative
- Low frequency (1 - 5%)
- Relatively low severity
- Sub-clinical

Dog - INAS

- Idiopathic Necrotising Arteritis Syndrome (Beagle Pain Syndrome)
- Rare in controls
- Coronary, mediastinum, meninges, epididymides, urinary bladder
- Acute, suppurative lesion
  - Can be severe
- Chronic lesions are non-suppurative
- Clinical signs in young dogs
- Often in treated animals but no dose relationship

Rat (heart base)

- Low incidence & severity, usually

INFLAMMATION, PERIVASCULAR

Terminology

- 'Periarteritis' often conflated with 'arteritis'
- Confusing and often inaccurate

Commonly seen

- Part of widespread inflammatory changes
  - May be residual lesion
- Vessel wall usually undamaged
- May be sporadic and unexplained
- Lungs
  - Infectious agents – virus, bacteria, mycoplasma
- Kidneys
  - Not uncommon in rats with or without CPN
- Liver
  - Common in rats
- Brain
  - Occasionally in rats
- Thyroid gland
  - Dogs

MINERALISATION

Dystrophic

- Focal lesions
- Endothelium of pulmonary arteries of rat lung
  - Very common
- Lingual artery of F344 rats
  - Increases with age

Generalised

- Renal failure
  - End-stage CPN
    - hyperparathyroidism
  - Obstructive nephropathy

AMYLOID

Aged mice

- Aged hamsters

Affects many blood vessels

- Liver: central and portal veins
- Kidney: glomeruli
- Thyroid, gut, heart, lungs

SMALL VESSELS

Angiectasis

- Age-related
- Adrenals, ovaries, pituitary
  - Mainly rats
- Bone marrow
  - Mice
- Liver
  - Mice and rats
- Dilatation of thin-walled vessels
  - Normal endothelial cells
  - May be apparently incomplete lining
- Secondary thrombi
- Complex lesions can be confused with tumours
  - Endothelial hyperplasia
  - Neovascularisation
- Lymphatics
  - In lymph nodes

ATHEROMA AND ARTERIOSCLEROSIS

Atheroma

- Rare in most species
- Occasionally in old rats
- Specific models
  - Rabbits

Arteriosclerosis

- Old rodents in some sites
- Old dogs

MISCELLANEOUS CHANGES

IEL duplication

- Motility
  - Right atrium
  - Left papillary
- Post-inflammatory

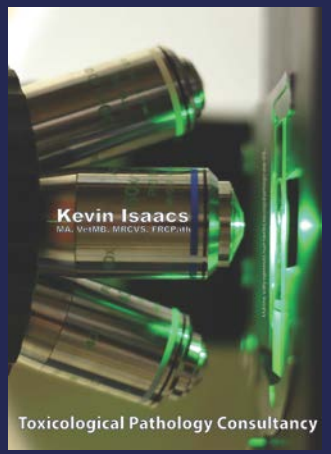
Oedema, perivascular, lungs

- Rat
- Altered permeability
  - CO_2 euthanasia

Hypertrophy, media

- Vasoconstriction
- Hypertension
- No apparent cause

PROLIFERATIVE LESIONS



PROLIFERATIVE LESIONS – HEART, RODENTS

- **Schwannoma, benign**
  - Endocardial
  - Intramural
- **Schwannoma, malignant**
  - Endocardial
  - Intramural
- **Haemangioma**
- **Haemangiosarcoma**
- **Mesothelioma, malignant**
  - Atriocaval
  - Pericardial
- **Rhabdomyoma**
- **Rhabdomyosarcoma**
- **Fibroma**
- **Fibrosarcoma**
- **Paraganglioma**
- **Metastases**
  - Lymphoma, malignant
  - Histiocytic sarcoma
  - Mammary adenocarcinoma
  - Bronchiolo-alveolar carcinoma

PROLIFERATIVE LESIONS – VASCULAR, RODENTS

Haemangioma

Haemangiosarcoma

- Many potential sites
  - Liver
  - Spleen
  - Subcutis
  - Mesenteric lymph nodes (rat)

Lymphangioma

- Mesenteric lymph nodes (rat)

Lymphangiosarcoma

- Very rare

Haemangiopericytoma

- Usually has been classified as fibrosarcoma