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**

<table>
<thead>
<tr>
<th>Thickening of chamber walls</th>
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<tbody>
<tr>
<td>• Judged visually at histopathology or necropsy</td>
</tr>
<tr>
<td>• Can be measured quite simply</td>
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<table>
<thead>
<tr>
<th>Increase in myocyte size</th>
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<tbody>
<tr>
<td>• With nuclear hypertrophy</td>
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<tr>
<td>• Increased ploidy</td>
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<table>
<thead>
<tr>
<th>Compensatory</th>
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<tbody>
<tr>
<td>• Response to increased workload</td>
</tr>
<tr>
<td>• Concentric</td>
</tr>
<tr>
<td>• Vasoactive peptides and growth factors</td>
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<table>
<thead>
<tr>
<th>Maladaptive</th>
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<tbody>
<tr>
<td>• May involve increased apoptosis</td>
</tr>
<tr>
<td>• Asymmetric</td>
</tr>
<tr>
<td>• Fibroplasia and EC matrix deposition</td>
</tr>
<tr>
<td>• Not related to apoptosis</td>
</tr>
<tr>
<td>• Heart failure</td>
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<tr>
<td>• Lesions in other organs e.g. lung, liver</td>
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<table>
<thead>
<tr>
<th>Usually secondary change in rodents</th>
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<tbody>
<tr>
<td>• Degeneration of valves</td>
</tr>
<tr>
<td>• Cardiomyopathy</td>
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<tr>
<td>• Amyloidosis</td>
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<tr>
<td>• Atrial thrombus</td>
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</table>
Can be difficult to assess in H&E sections

Diffuse or focal

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
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  
<table>
<thead>
<tr>
<th></th>
<th>• Major feature of cardiomyopathy in rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequel to necrosis and haemorrhage in NHP</td>
<td>• Cynomolgus (especially Mauritian origin)</td>
</tr>
</tbody>
</table>
| 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
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
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
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
- Thrombocytopenia
  - Dog
  - Pig
- Vit K deficiency
  - Dietary insufficiency in rodents
  - Haemorrhage & inflammation
  - Confounding factor in toxicity studies

Capture stress in NHP
- With myocardial necrosis
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
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**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Details</th>
</tr>
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<tbody>
<tr>
<td>Can affect heart chambers &amp;/or valves</td>
<td></td>
</tr>
<tr>
<td>Haematocysts</td>
<td>• Vascular ectasia</td>
</tr>
<tr>
<td></td>
<td>• Incidental and usually harmless</td>
</tr>
<tr>
<td>‘Myxoid’ degeneration, valves</td>
<td>• Prominent GAGs give “myxoid” or “mucoid” appearance</td>
</tr>
<tr>
<td></td>
<td>• Older rats and dogs</td>
</tr>
<tr>
<td></td>
<td>• Secondary changes seen due to valve incompetence</td>
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<tr>
<td></td>
<td>• Underdiagnosed in routine studies</td>
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<td></td>
<td>• Good sampling required</td>
</tr>
<tr>
<td>Amyloid deposits, valves</td>
<td>• Old mice</td>
</tr>
<tr>
<td>Inflammation</td>
<td>• Occasionally in old rats</td>
</tr>
<tr>
<td></td>
<td>• Apex of heart</td>
</tr>
<tr>
<td></td>
<td>• Also seen with valvular degeneration</td>
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### ENDOCARDIAL CHANGES

<table>
<thead>
<tr>
<th>Condition</th>
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</table>
| **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

- Inflammation
- Haemorrhage
- Necrosis
- Intimal thickening
- Splitting & duplication of IEL
- Thrombi
- Medial hypertrophy
- Adventitial fibrosis
- Angiectasis
- Neovascularisation
- Endothelial hyperplasia
- Aneurysm
- Amyloid
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.”
Some terms, however, are best avoided:
- Panarteritis nodosa
- Polyarteritis nodosa

Often diagnosed as ‘arteritis’

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
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
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

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

Haemangiosarcoma

Lymphangioma
• Mesenteric lymph nodes (rat)

Lymphangiosarcoma
• Very rare

Haemangiopericytoma
• Usually has been classified as fibrosarcoma