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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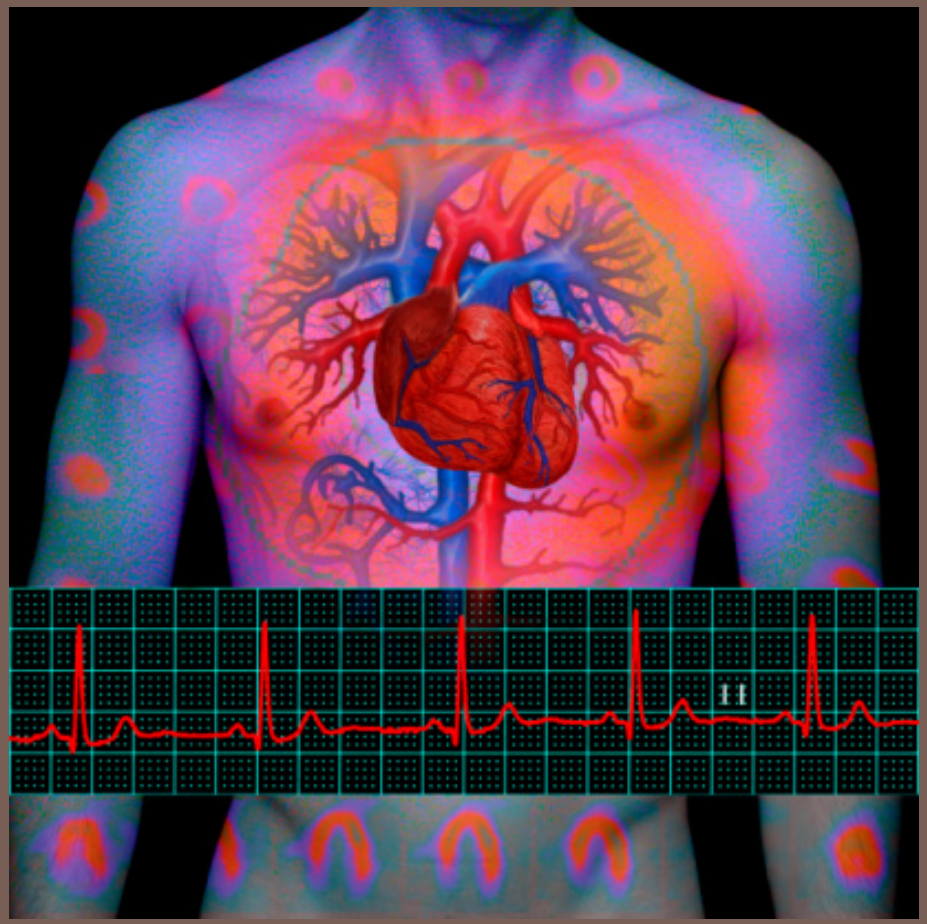
Heart: Practical Aspects of Assessment in Toxicological Pathology



International Federation of Societies of Toxicologic Pathologists

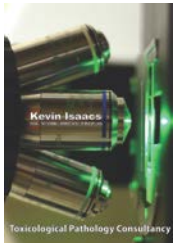
The International Federation of Societies of Toxicologic Pathologists is pleased to sponsor the lectures given by:

Kevin ISAACS

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

http://www.ifstp.com

Introduction



Complex structure

- Heterogeneous structure
  - Right & Left sides
    - Different pressures
  - Valves
  - Conducting system
  - Blood vessels
  - Nerves
  - Heart base
  - Muscle fascicles are arranged to pump blood efficiently

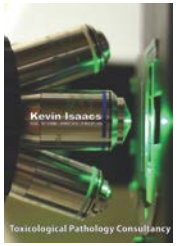
Complex physiology

- Electrical activity & contraction coupling
- Hormones
- High energy demand
- Low detoxification potential
  - Vulnerable to injury
  - Myocyte regeneration negligible (possibly 1%/year)

We concentrate on certain aspects

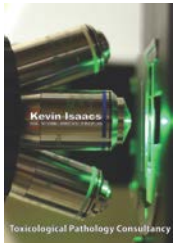
- Some things are ignored
  - Until something goes wrong

Excitation-contraction coupling



- The action potential travels down T-tubules and triggers calcium channels during the plateau phase of the cardiac action potential, causing a net flux of calcium ions into the cardiac myocyte.
- The increase in intracellular calcium ions is detected in the membrane of the sarcoplasmic reticulum which transport calcium out into the cytosol
- The cytoplasmic calcium binds to Troponin C, moving the troponin complex off the actin binding site allowing the myosin head to bind to the actin filament.
- Using ATP hydrolysis the myosin head pulls the actin filament to the centre of the sarcomere.
- Intracellular calcium is taken up by the sarcoplasmic reticulum ATPase pump into the sarcoplasm, ejected from the cell by sodium-calcium exchange or plasma membrane calcium ATPase or taken up by the mitochondria
- Intracellular calcium concentration drops and the troponin complex returns over the active site of the actin filament, ending contraction.

Objectives of morphological evaluation



Assess effects of treatment on the heart

In-life

- Study design
- Clinical signs
- Heart rate/ECG
- BP
- Clinical chemistry
- Haematology

Necropsy

- Standardised procedures
- Thorough examination and recording of findings
- Preservation of macroscopic abnormalities
- Special preparation techniques (if required)

Histology

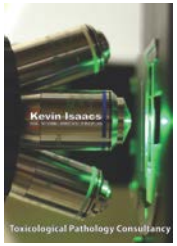
- Full survey of all relevant structures
- Standardised sections
- All lesions
- Additional observations
- Special stains (if necessary)

Histopathology

- Full and thorough examination
- Record all relevant findings
- Present results appropriately
- Correlate all relevant data
- Make an interpretation for study
- (Risk assessment)

If the practical part is not performed properly, the results may be of limited value

Study Design



Choice of species

- Anatomy
  Physiology
  Size
- Pharmacology
- Relevance to man

Study type

- Investigative (non-GLP) or Regulatory (GLP)
- Acute, subacute or chronic administration
- Route of administration

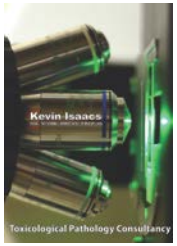
In-life monitoring

- ECG (various), Heart rate, Telemetry
- Ultrasound
  MRI
- Clinical chemistry
  - CK-MB
  - LDH
  - Troponin I
  - Natriuretic peptides

Terminal procedures

- Necropsy
- Histology
- Special techniques
  - Stereology
  - EM
  - Histochemistry
  - Immunohistochemistry
  - Genomics

Necropsy



Examine

- Externally

Remove

- Open larger animals

Examine internally

- Carefully

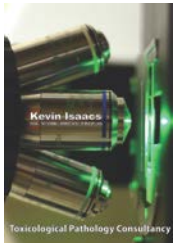
Preserve

- NBF

Special techniques

- Perfusion
  - Whole animal (rat)
  - Heart only (dog)
- EM
- Frozen

Histology



Trimming

- Standardised
- Present the same structures in every animal

Rodents

- Three pieces in rat (my preference)
- Others use one piece
- One in mouse

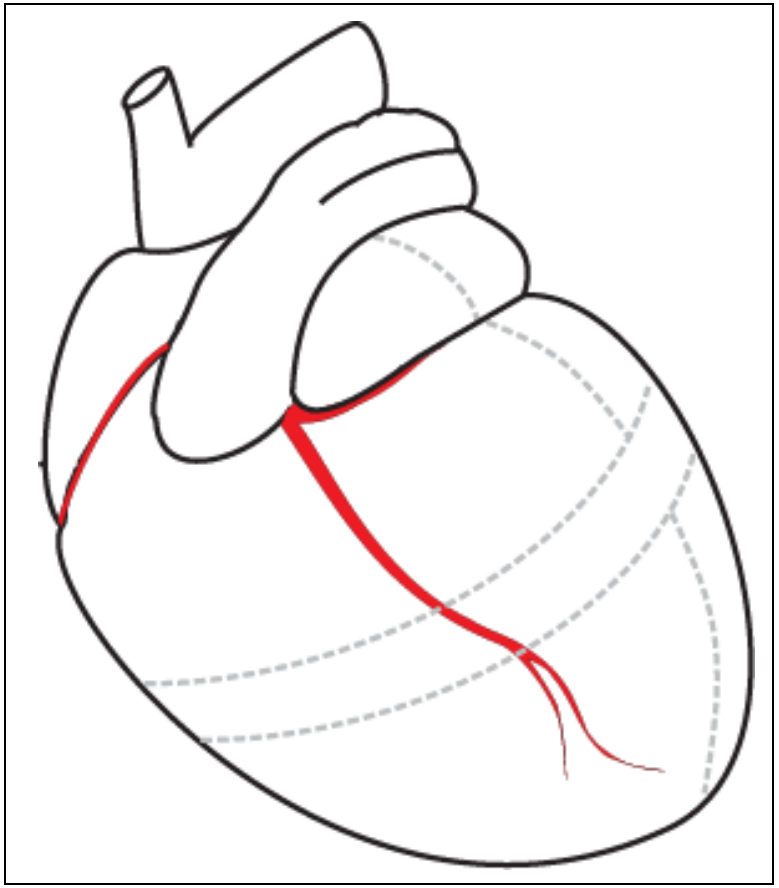
Larger species

- Up to 9+ pieces
- Major anatomical features
- Abnormalities

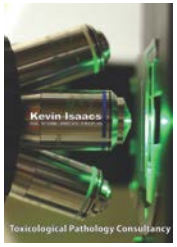
Staining

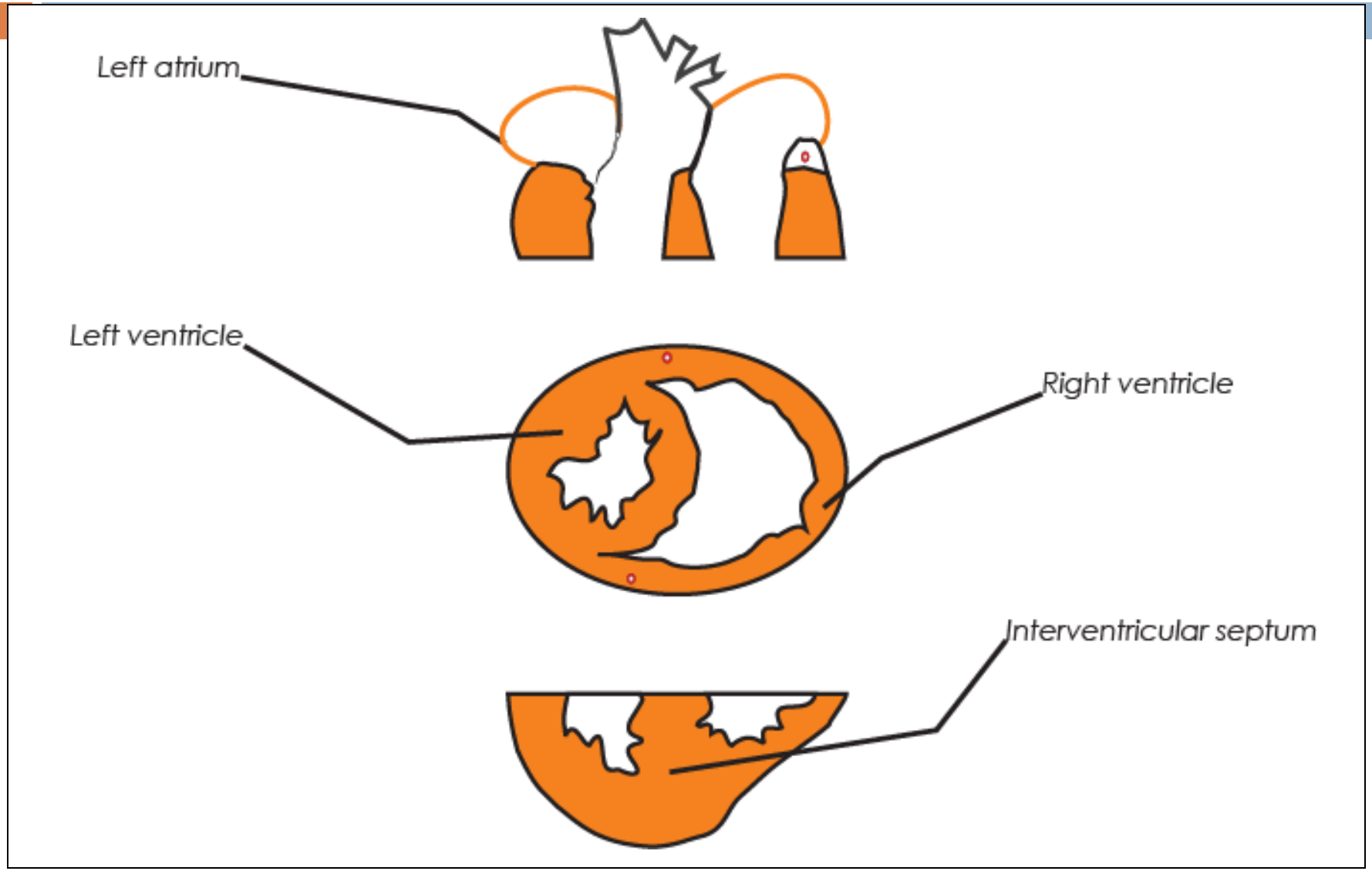
- Standardised
- Special stains

Sectioning rat heart

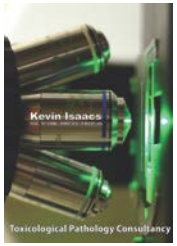


Sections of rat heart





Larger species



Dog, Pig, Monkey

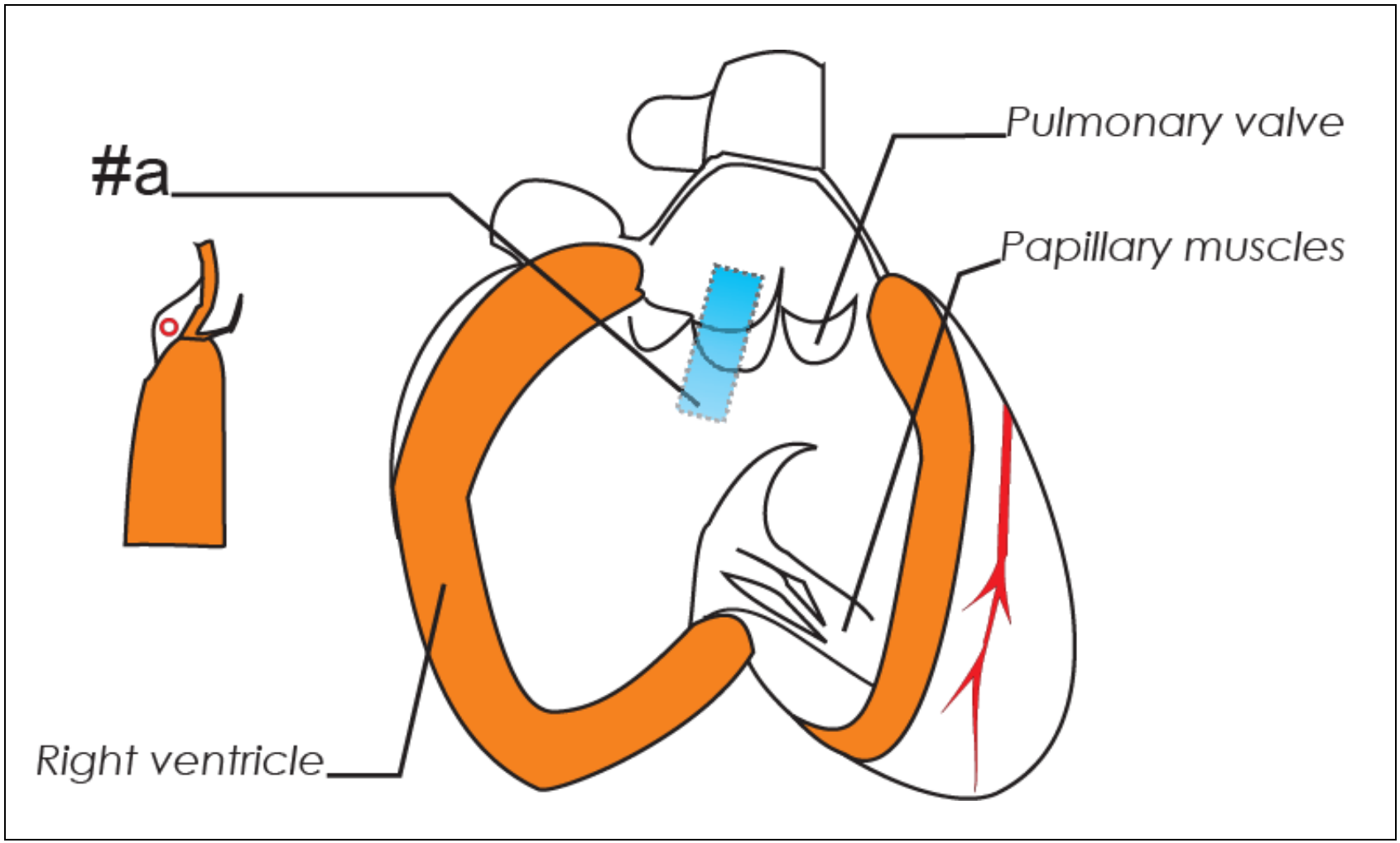
Open the heart along blood flow

- Examine internal surfaces
- Photograph abnormalities

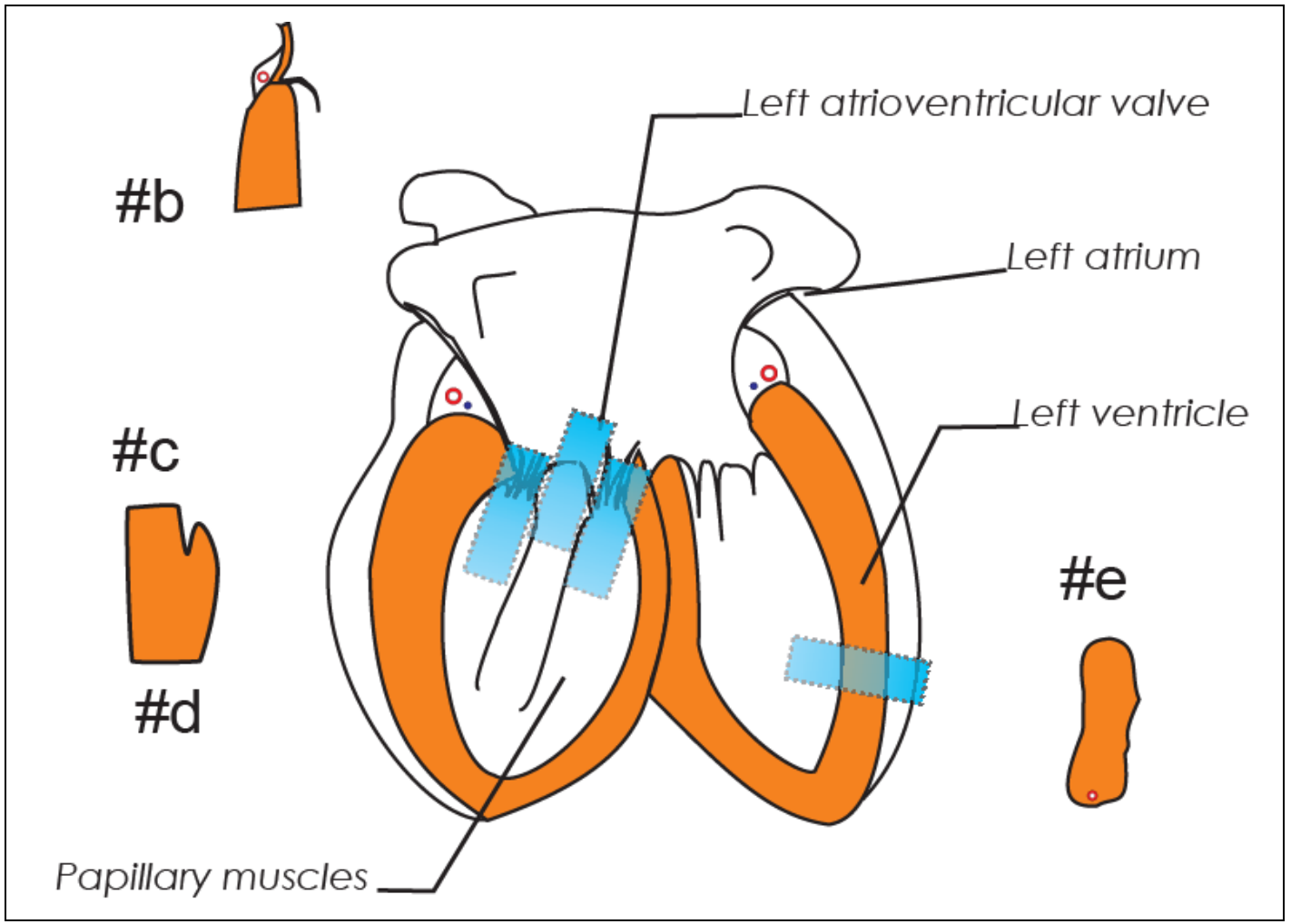
Fix before trimming (or not)

Trim when tissues have hardened

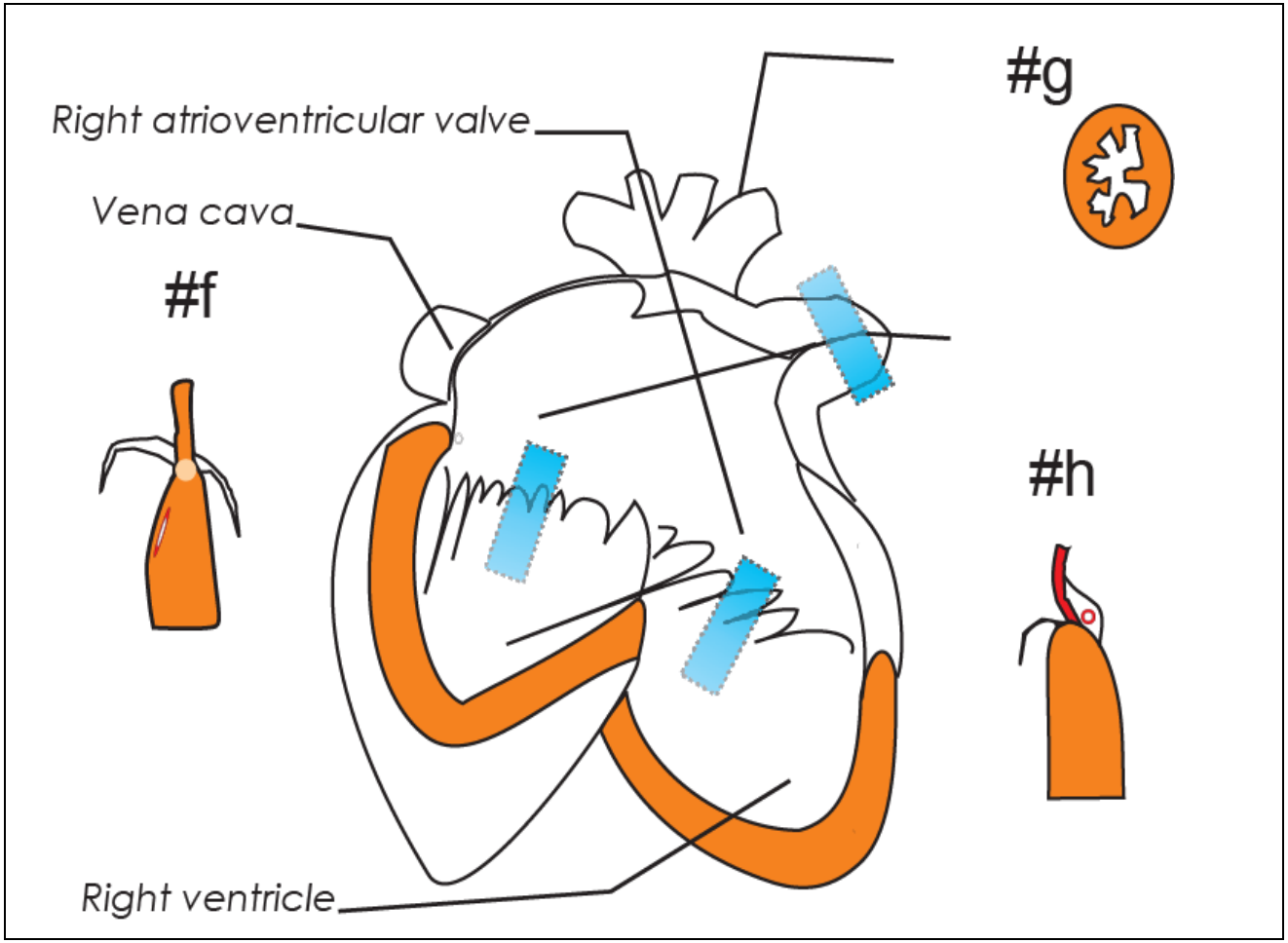
Dog heart trimming 1



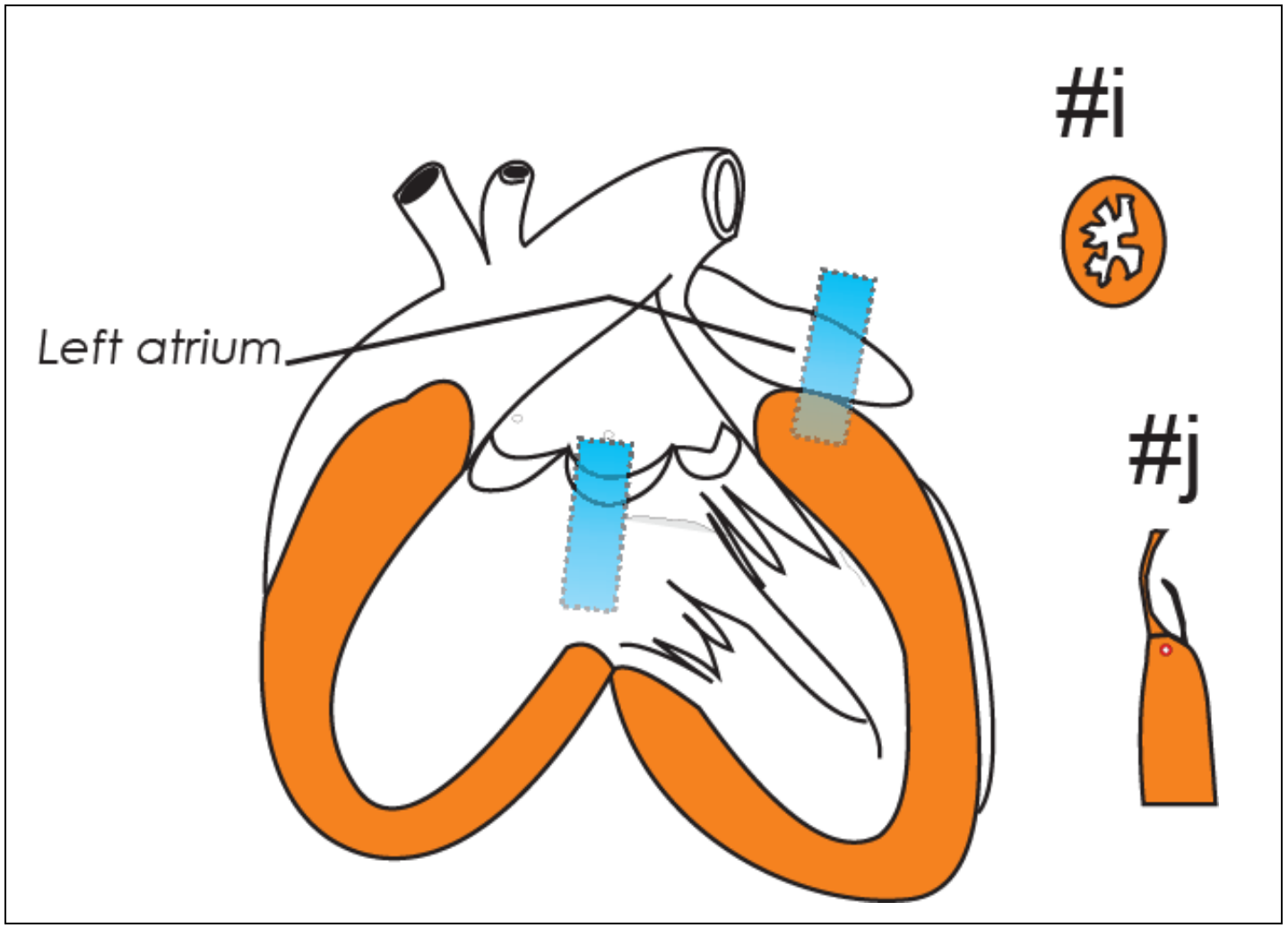
Dog heart trimming 2



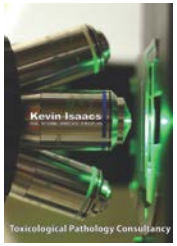
Dog heart trimming 3



Dog heart trimming 4



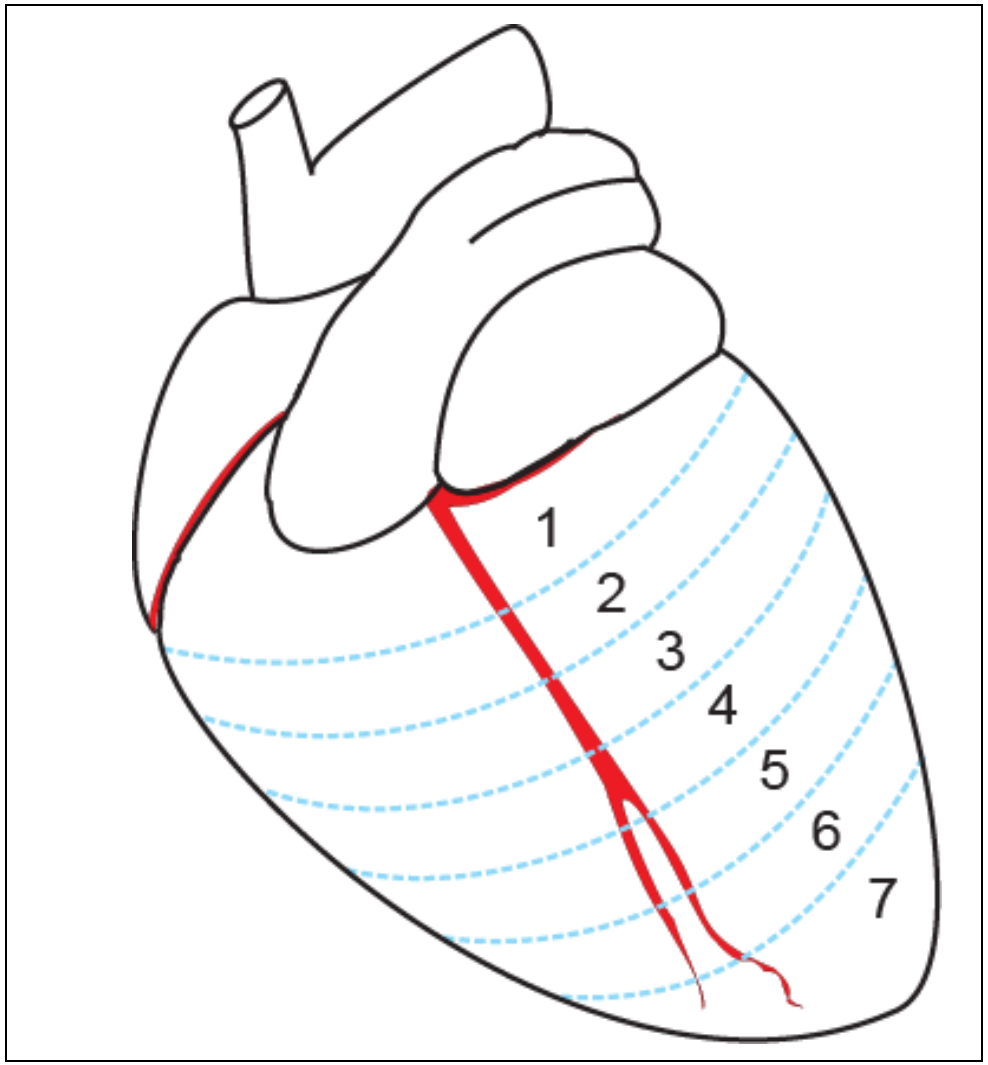
Dog heart trimming 5



This is what the sections will look like in the blocks

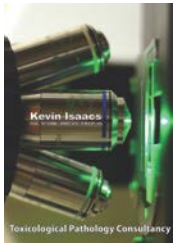
Block	Structures sampled	
#a	Right ventricle, pulmonary artery, right circumflex artery, pulmonary valve	
#b	Left ventricle, left atrium, left circumflex artery, left ventricular artery, left atrioventricular valve	
#c #d	Left papillary muscle	
#e	Interventricular septum, left interventricular artery	
#f	Interventricular septum, left septal artery, left atrioventricular valve, right atrioventricular valve, atrioventricular node	
#g	Right atrium, right atrial artery	
#h	Right ventricle, right atrium, right circumflex artery, right ventricular artery, right atrioventricular valve	
#i	Left atrium, left atrial artery	
#j	Left ventricle, aortic valve, left circumflex artery	

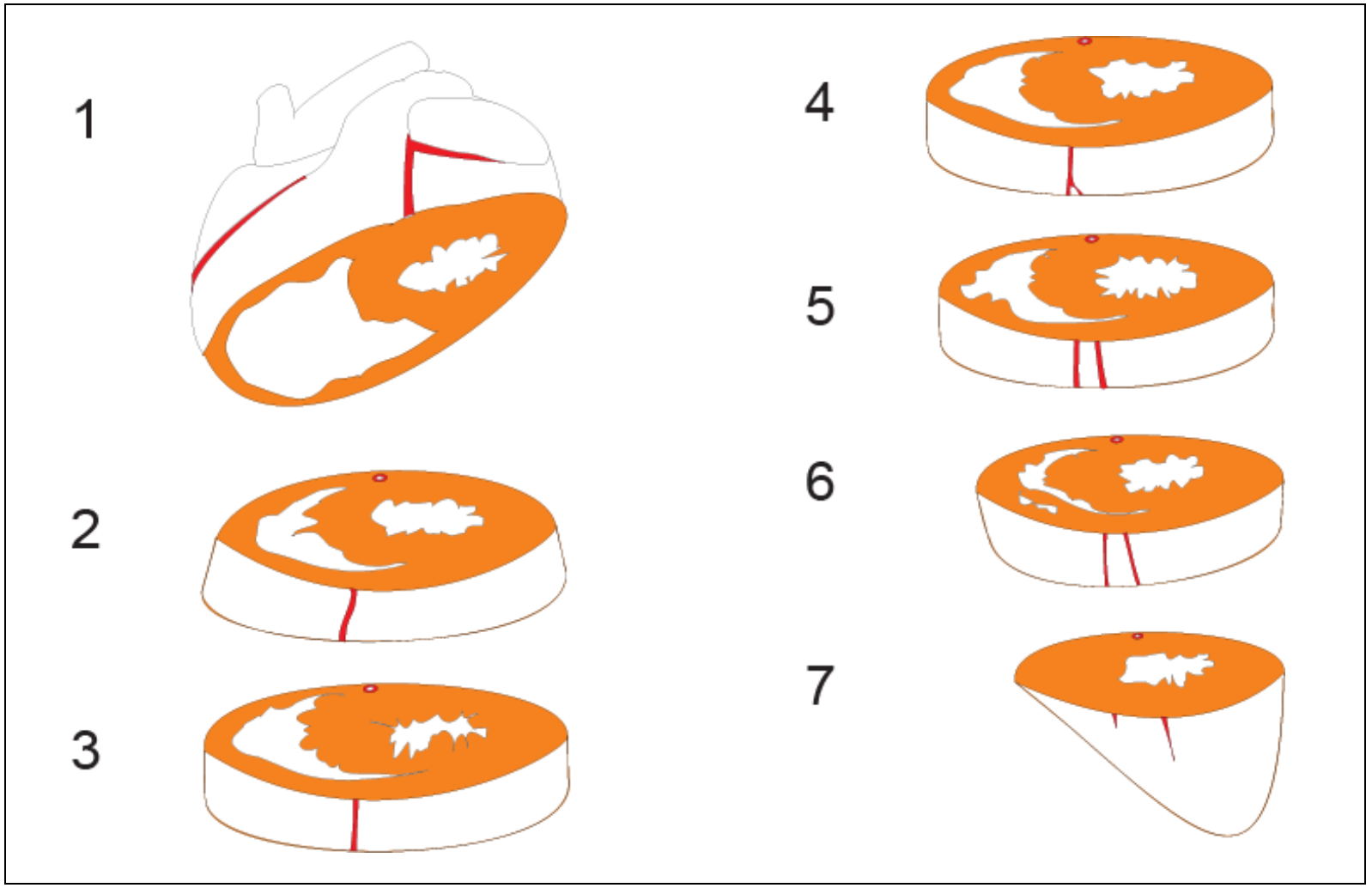
Breadloaf method - trimming



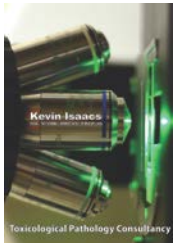
Useful for stereology

Breadloaf method - sections





Coronary vessels, dog



Perfuse fix heart

- Beer pump
  - Low flow rate
  - Saline, then NBF
  - Very easy

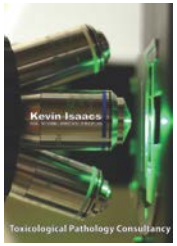
Dissect out coronary tree

- Time consuming
- Gives good map
- No two dogs are quite the same

Monastral blue

- 20 minutes before necropsy
- Identifies many lesions

Histology – staining



H&E (with variants)
- Good for most things

Elastic van Gieson
- IEL, elastin

Trichromes; MSB
- Fibrosis

PTAH
- Fibrin, cross striations

Von Kossa/Alizarin Red
- Phosphates/Calcium

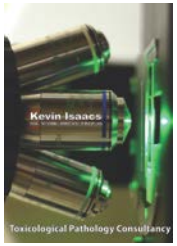
ORO/Sudan Black

Perl's
- Ferric iron

Immunohistochemistry
- Peptides

Plastic sections
- GMA, HEMA for fine detail

Histology – specific stains



Vacuolation

- H&E
- Plastic
- ORO

Fibrosis

- Picrosirius red + polarisation
- Trichrome stains

Early necrosis

- Autofluorescence (450 – 490 nm)
- IHC: TnI, Myoglobin, Fibronectin, Actin, C9
- Azan trichrome

Mineral

- Alizarin red
- von Kossa

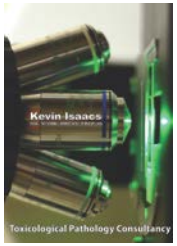
Pigment

- Schmorl's
- Perl's
- Masson-Fontana

Neural markers

- PGP 9.5
- NF-160

Normal histology



Some normal anatomical features are ignored

- Sampling regime inadequate
- Not required by SOP

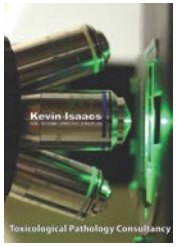
Some cannot be found easily

- Specific blood vessels
- Neuro-endocrine tissue
- Conducting tissues
  - Sino atrial node
  - AV node
  - Nerves & ganglia

Some are mistaken for lesions

- Sino atrial node
- Chordae tendineae insertions
- Duplication of IEL in papillary muscles

Histopathology objectives



Assess and diagnose

- Diagnostic criteria
- Terminology

Interpretation

- Species and strain
- Pharmacology
- Chemical structure
- Published data

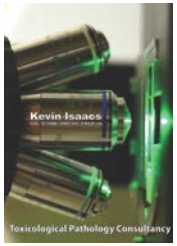
Grading

- Comparison – controls
- Consistent sampling
- Consistent recording
- Topographical detail

Results

- Tabulation
- Incidence
- Severity
- Dose response
- Statistical analysis
- Historical data

Heart – the locations



Developmental abnormalities

- Rare and depend on excellent necropsy to detect them
- Persistent foramina
- Situs inversus

Myocardial lesions

- Common - spontaneous and induced

Endocardial lesions

- Common in aged animals
- Can be easily induced in dogs with turbulent blood flow

Epicardial/Pericardial lesions

- Not uncommon as spontaneous
- Pericardium is often neglected

Conducting system changes

- Extremely rare, except with widespread damage

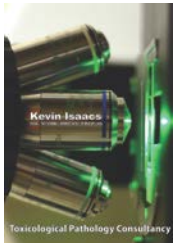
Vascular changes

- Not uncommon – spontaneous and induced, especially dogs

Tumours

- Occasionally in rodents
- Schwann cell, endothelial and mesothelial tumours as primary tumours
- Metastases, occasionally

Possible lesions



Basic lesions can be similar irrespective of cause

- Necrosis
- Inflammation
- Fibrosis
- Haemorrhage
- Hypertrophy
- Atrophy
- Pigment deposition
- Mineralisation

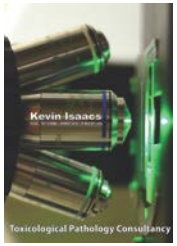
Distribution of lesions may be different

- Subendocardial
- Subepicardial
- Apical
- Atrial
- Diffuse
  - Random
  - Pattern of distribution

Combinations of lesions may be distinctive

- e.g. Necrosis +/-
  - Inflammation
  - Vacuolation
  - Fibrosis
  - Haemorrhage
  - Mineralisation

Confounding factors



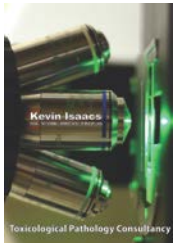
Spontaneous disease

- Can mimic treated lesions
- Can have a high background incidence
  - Cardiomyopathy in rats
  - Lymphocyte aggregations in NHP
  - Valvular degeneration in rats and dogs
  - Coronary arterial disease in dogs
  - Necrosis and fibrosis in myocardium of NHP

Incomplete information

- Poor sampling regime
- Poor recording at histopathology

Reporting



Simple computer-generated tabulations

- May lack detail
- Grade details necessary

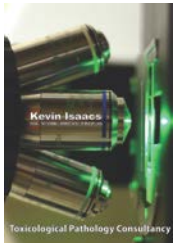
Topographical breakdown

- Better than complex diagnoses
- Helps with pathogenesis
- Logical presentation

Syndromes

- 'jet' lesions
- Cardiomyopathy
  - Spontaneous
  - Treatment-related

Interpretation of findings 1



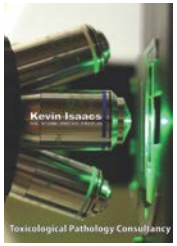
Correlation with all other findings

- Clinical chemistry, haematology, in-life signs
- Extra measurements: ECG, BP, HR
- Other biomarkers BNP, ANP, Troponins
- Exposure levels

Exaggerated pharmacology

- Inotropy
  - ⇧contractility, turbulent flow
- Ischaemia/hypoxia
  - ⇧O_2 demand, ⇩blood flow, ⇩ O_2 supply
- Chronotropy
  - ⇩BP (reflex tachycardia)
- Vascular flow changes
  - arteriopathy
- Membrane changes
  - local anaesthetics

Interpretation of findings 2



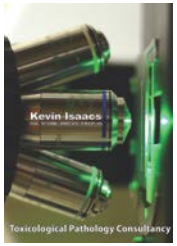
Altered physiology

- Electrolyte changes
  - K^{+}, Mg^{++}, Ca^{++}

Direct toxicity

- Cytotoxicity
  - Doxorubicin
  - Allylamine
- Organelles
  - Mitochondria
  - Myofibres
- Generalised muscular toxicity
  - Ionophores
  - Vit E defy

Interpretation of findings 3



Recovery/Repair

- Recovery for many changes is not common
  - Fibrosis persists
  - Remodelling
- Mitotic rate is very low in adult life (essentially zero)
- Myocardial hypertrophy may resolve
  - Can leave fibrosis

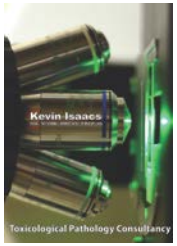
Functional changes

- ECG (QT interval) – no pathology
- Hypertrophy (PPAR) – ⇧blood volume?
- Atrophy (ACE inhibitors)

Species differences

- Sick human patient rather than a normal animal at a high dose
  - Restoring normal values
- Anatomy
  - Vascular supply
- Physiology
- BMR (rodents)
- Pharmacology
- Receptor distribution (e.g. endothelins)
- Kinetics/Metabolism
- Distribution, bioavailability

Investigation 1



Morphometry

- Preservation & sampling
- Simple assessments
  - Sufficient animals
- Manual or computer-based
- IHC if necessary

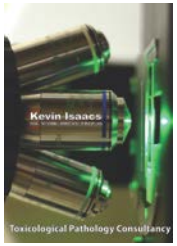
Perfusion

- GMA, EM, blood vessels, measurements

Dissection

- Muscles, valves (weigh or measure)

Investigation 2



Plastic sections

- More detail, more effort

EM

- Organelle changes, measurements

IHC

- Structural proteins
  - Troponins
  - Myoglobin
- Peptides
- Vacuolation

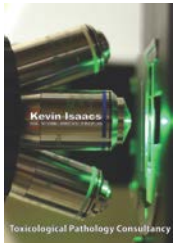
Frozen

- Histochemistry (enzymes, lipid)

Function

- ECG/Echo
- Telemetry
- Ultrasound
- MRI
- Pharmacology (antagonism studies)
- Genomics

Conclusion



Basic principles

- Same for all organ systems
- Clear strategy, clear thinking
- Good science

Do the basic, routine things well

- Set the study up carefully
- Attention to detail at all stages
- Good technique
- Ensure consistency