CELL AND TISSUE DEGENERATION

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- **Cell injury** occurs if a cell cannot adapt quickly to a hostile environment.
- Cell injury maybe **sublethal** and **reversible** (= **cell degeneration**)
- Alternatively, the injury can be irreversible and lethal (= **cell death** via **oncotic necrosis** or **apoptosis**)

DEGENERATION OF CELLS

- Sublethal injury -> morphological changes in cells that are useful indicators of such injury and of disturbed cell function.
- Typically, with light microscopy, the **cytoplasm of affected cells appears abnormal** in routine haematoxylin-and-eosin (H&E) stained sections, due to the accumulation of cytoplasmic water, lipid or other substances.

HYDROPIC DEGENERATION

Hydropic degeneration = acute cell swelling

- The **most common expression of cell injury**
- Essentially represents **intracellular oedema.**
- Affected cells can no longer maintain homeostasis and regulate entry and exit of water and ions, and their cytoplasm swells with water.

- In health, homeostatic control of intracellular water and ion concentrations is largely a function of the **membrane Na^+-K^+-atpase pump.**
- For each molecule of ATP consumed, the pump moves three Na^+ ions out of the cell in exchange for two K^+ ions

The **two major causes** of hydropic degeneration of cells are:

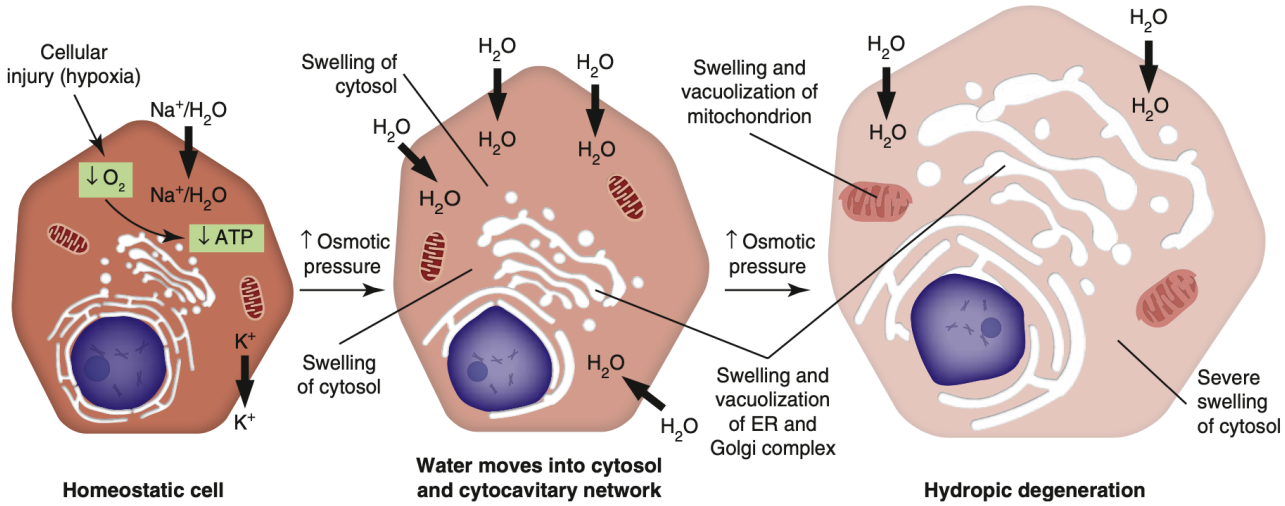
- **Physical damage to plasma membranes and/or organelle membranes**
  - By reactive oxygen species (free radicals)
  - By covalent binding of toxic chemicals
  - By bacterial toxins (e.g. Phospholipases)
  - By cytotoxic lymphocytes
  - By complement activation
  - The damaged membranes are leaky, allowing entry of water and Na^+ and Ca^{++} ions, and loss of intracellular K^+ and Mg^{++} ions

- **Failure of cell energy production**
  - In **hypoxia**, depletion of cell oxygen stores cessation of aerobic oxidative phosphorylation within mitochondria -> depletion of ATP stores
  - In most cells (NOT neurons), decreased ATP -> increased inorganic phosphate -> stimulation of phosphofructokinase -> metabolic switch to **anaerobic metabolism.**
    -> glycogenolysis -> depletion of cell glycogen stores, generation of osmotically active molecules such as lactate, and a decrease in cell pH

- Anaerobic metabolism may allow short term cell survival, but it is an inefficient way to generate ATP.
- Eventually, inadequate ATP stores -> failure of the Na^+-K^+-atpase pump-> Movement of Na^+ and water into the cell

- the process of hydropic degeneration is summarised in **Figure 1**

Figure 1 – Hydropic Degeneration



Reference: "Pathologic Basis of Veterinary Disease" - J.F. Zachary, 6th edition, Elsevier, St Louis, Missouri (2022)

Gross Appearance

- Affected tissues may be heavy, pale and swollen (turgid), and may bulge and ooze fluid when incised.
- Affected tissues may be softer and more friable than normal.

Microscopic Appearance

- Affected cells appear swollen, with pale, cloudy or wispy to finely vacuolated cytoplasm.
- The mildest form is often referred to as **cloudy swelling.**
- Extreme hydropic degeneration is referred to as **ballooning degeneration**, with marked cell enlargement and voluminous clear cytoplasm due to water accumulation and degradation of cytoplasmic proteins (e.g. Virus-infected epithelial cells)
- Presence of intra-cellular water can be confirmed by the failure of the cells to stain with special histochemical stains for fat or glycogen.

Fate of Affected Cells

- Cells that have undergone hydropic degeneration are **dysfunctional.**
- However, the injury is still **potentially reversible.**
- If the membrane damage can be repaired or the oxygen supply restored before the "point of no return" is reached, affected cells can return to normal appearance and function.

FATTY CHANGE

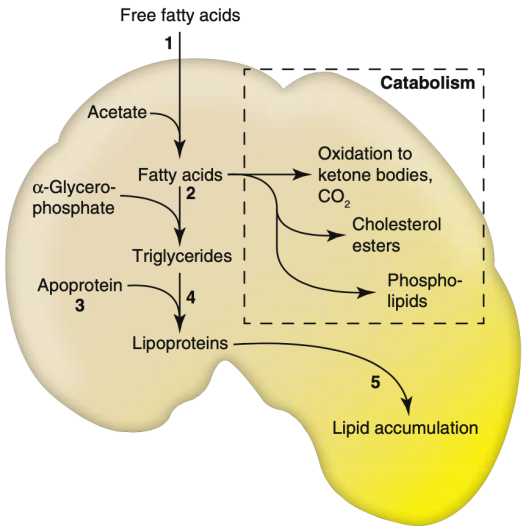
Fatty change = intracellular accumulation of excess lipid

- Also called **lipidosis**, **steatosis** or **fatty degeneration**
- A **common manifestation of sublethal cell injury**
- Develops most often in cells that normally metabolise a lot of lipid (**especially hepatocytes** but also **renal tubular epithelial cells** and **myocardial fibres**)
- The lipid that accumulates is predominantly in the form of **triglycerides**(triacylglycerols)

Hepatic Lipidosis

- Lipidosis is a common finding in the liver because of the vital role that this organ normally plays in lipid metabolism (see **Figure 2**)
- Some of the fatty acids that enter the liver (e.g. Chylomicrons derived from the diet, very low-density lipoproteins (VLDL) in circulation, low density lipoproteins (LDL)mobilised from body fat depots etc.) Are oxidised as an energy source in the mitochondria of hepatocytes.
- Others are used by the hepatocytes to synthesise cholesterol esters or phospholipids or are oxidised to form ketone bodies.
- However, most incoming fatty acids are esterified by the hepatocytes to form **triglycerides.**
- The triglycerides are then packaged by the hepatocytes with **apoproteins** to form **VLDL**-> Exported into the circulation as a readily available energy source for other tissues.

Figure 2 – Lipid metabolism in the liver and possible mechanisms resulting in lipid accumulation



Reference: "Pathologic Basis of Veterinary Disease" - J.F. Zachary, 6th edition, Elsevier, St Louis, Missouri (2022)

- Hepatic lipidosis may develop if any of the steps in normal hepatic lipid metabolism shown above is compromised.
- The steps that are often compromised are the synthesis of apoproteins and the packaging of apoproteins and triglycerides into VLDL for export, as these steps require considerable energy consumption by hepatocytes.
- Esterification of fatty acids to triglycerides is less energy-dependent and may continue, even in an injured hepatocyte.

- **Causes of hepatic lipidosis** include:
  - **Entry of excess fatty acids into the liver**, exceeding its capacity for rapid processing
    - E.g. High lipid diets (including milk in suckling animals)

- E.g. Increased mobilisation of fat stores - e.g. Late pregnancy, peak lactation, anorexia, diabetes mellitus

- **Inadequate supply of proteins or cofactors to permit synthesis of apoproteins.**
  - E.g. Chronic protein malnutrition
  - E.g. Cobalt or vitamin B_{12} deficiency

- **Sublethal hypoxia**
  - > diminished hepatocyte ATP (energy) stores available for synthesis of apoproteins and packaging of VLDL for export

- **Sublethal toxic injury**
  - E.g. Carbon tetrachloride (ccl4), aflatoxin and white phosphorus poisoning - > damage to rough endoplasmic reticulum of hepatocytes -> decreased apoprotein synthesis

- Some of these mechanisms are **physiological** rather than pathological.
- Therefore, **fatty change is NOT always indicative of cell injury or of cell dysfunction.**

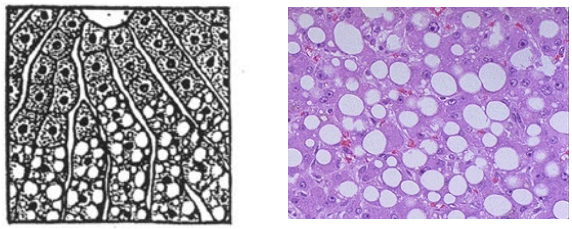
Gross Appearance

- Organs affected by lipidosis may be enlarged with rounded borders.
- Depending on the volume of excess fat being stored, the organ may appear slightly paler than normal or prominently discoloured cream to yellow or yellow-orange.
- Affected parenchyma is softer and more friable than normal, and may have a greasy bulging cut surface.
- If lipidosis is severe, the affected tissue may float in water and in formalin.

Microscopic Appearance

- Lipid initially accumulates as small, clear cytoplasmic globules (**Figure 3**, left)
- Cells with severe lipidosis are distended by a single, large, clear, cytoplasmic vacuole that may displace the nucleus to the cell periphery (**Figure 3**, right)
- Lipid vacuoles always have sharply delineated margins due to the hydrophobic interface between the lipid and cytoplasmic water.
- That the vacuoles contain lipid can be confirmed by use of a fat-soluble dye (e.g. Oil Red O) on frozen tissue sections

Figure 3 – Microscopic Appearance of Fatty Degeneration



Swollen liver cells containing globules of fat

Reference: "Pathology Illustrated" - - A.D.T. Govan, P.S. Macfarlane, R. Callander, 4th edition, Churchill Livingstone, Edinburgh (1995)

Fate of Affected Cells

- Cells that have undergone fatty change may or may not be dysfunctional.
- If fatty change is referable to cell injury, it is still **potentially reversible.**
- If the cause can be removed and the cell injury repaired, affected cells can return to normal appearance and function.
- However, severe and prolonged fatty change can lead to cell death and/or to tissue fibrosis (scarring) and architectural remodelling that can be irreversible.

INTRACELLULAR ACCUMULATION OF GLYCOGEN

- Glycogen is normally stored in the cytoplasm of **skeletal myocytes** and **hepatocytes.**
- Excess glycogen may be stored intracellularly if there is an abnormality in glucose or glycogen metabolism
- Large amounts of glycogen may also be seen in health in hepatocytes of recently fed animals, young growing animals and well-nourished animals.
- Excess glycogen storage is therefore **not necessarily indicative of cell injury** and **Cell function is not necessarily compromised.**
- The following are examples of pathological accumulation of glycogen.

Steroid Hepatopathy in Dogs

- Accumulation of excess glycogen in hepatocytes is **common in dogs with hyperadrenocorticism** and referred to as **steroid hepatopathy**
- E.g. Dogs with *spontaneous hyperadrenocorticism* (due to a functional ACTH-producing pituitary tumour or a functional cortisol-producing tumour in an adrenal cortex) or with *iatrogenic hyperadrenocorticism* (caused by excessive use of corticosteroids)

- Glucocorticoids induce transcription of glycogen synthetase -> excessive storage of glycogen in hepatocytes.

- Affected hepatocytes are often markedly swollen with irregular cytoplasmic clearing.
- Usually the nucleus of the cell is not displaced from its central position (c.f. lipid vacuoles)
- The nature of the stored material can be confirmed as being glycogen by performing histochemical stains (glycogen stains positively with periodic acid Schiff (PAS) stain and the positive staining is lost after addition of diastase)

- The affected liver may be grossly enlarged and pale and may be friable.

- Glycogen storage in steroid hepatopathy is **usually NOT responsible for dysfunction of hepatocytes** and is a **reversible change** if the underlying cause is controlled.

Glycogen Storage in Diabetes Mellitus

- **Diabetes mellitus** = a metabolic disease characterised by persistent hyperglycaemia (high blood glucose concentration) and a generalised catabolic state due to an absolute or relative deficiency of insulin
- In severe diabetes of rapid onset, may see intracellular glycogen storage in hepatocytes (usually overshadowed by fatty change), pancreatic islets of Langerhans, bile duct and pancreatic duct epithelial cells, and in epithelial cells lining the renal tubules (distal convoluted tubules and loops of Henle)

Glycogen Storage Disorders

- Intracellular accumulation of glycogen can also occur in the rare **inherited glycogen storage disorders** (**glycogenoses)**, particularly in skeletal and cardiac muscle fibres (see Lysosomal Storage Disorders below)

INTRACELLULAR ACCUMULATION OFPROTEINS

- Occasionally, cells may accumulate excess proteins intracellularly.
- The proteins usually appear as eosinophilic (pink-orange) cytoplasmic bodies in H&E-stained sections
- Sometimes referred to as **hyaline droplets.**
- Accumulation of proteins is **not necessarily indicative of cell injury** and **cell function is not necessarily compromised.**

- E.g. **Absorbed colostrum proteins** accumulate within small intestinal villous intestinal epithelial cells in neonatal animals.

- E.g. **Resorbed protein droplets** accumulate within the cytoplasm of renal proximal tubular epithelial cells in animals with glomerular damage and leakage of protein into the glomerular filtrate.

- E.g. Excess immunoglobulins (**Russell bodies**) accumulate within the cytoplasm of aged plasma cells that have been persistently stimulated to produce antibodies.

- E.g. Cells may accumulate **misfolded proteins** that are not eliminated via the ubiquitin-proteasome pathway (e.g. Various inclusion bodies within the cytoplasm of neurons in certain neurodegenerative disorders; e.g. Mallory bodies within damaged hepatocytes)

LYSOSOMAL STORAGE DISORDERS

- Lysosomal storage disorders (LSD) are conditions in which **substrates derived from normal cell catabolism accumulate within lysosomes** rather than being degraded by lysosomal enzymes.
- Substrate accumulation is progressive -> cell swelling, cytoplasmic vacuolation, cell dysfunction and occasionally cell death.

Inherited Lysosomal Storage Disorders

- With few exceptions, LSD are **inherited** in an **autosomal recessive pattern.**
- There is a gene dose effect - recessive homozygotes manifest the disease whereas heterozygotes are phenotypically normal but have reduced lysosomal enzyme activity (approximately 50% of normal)

- Lysosomal hydrolases tend to be **exoenzymes** which sequentially break linkages at the ends of large molecules but cannot break links within them.
- If the sequence is blocked at some point, further digestion cannot proceed.
- Failure of substrate degradation may be because of total absence of an enzyme, a defective or unstable enzyme, absence of a specific activator protein required by some enzymes

for activity etc.

- Substrate accumulation begins *in utero* and may be well advanced at birth but the age of onset of clinical signs and the severity and rate of progression of the clinical signs depend on the degree of compromise of enzyme activity.
- Affected animals are usually normal at birth, with onset of progressive clinical signs (often neurological) in the first few months of life.
- **Neurons** and **myocardial fibres** are most vulnerable to LSD as they are post-mitotic cells that can continue to accumulate the undigested substrate throughout life -> cell dysfunction.
- However, depending on the substrate, storage may also occur in such cells as renal tubular epithelium, macrophages, pancreatic acinar cells and hepatocytes.
- In the **glycogenoses**, the substrate accumulates within **skeletal** and **cardiac myofibres** -> muscle weakness and cardiac failure

- **Inherited LSD documented in animals** include:

- **Glycoproteinoses** - defective catabolism of the carbohydrate component of N-linked glycoproteins - e.g. A-mannosidosis in Angus, Murray Grey and Galloway cattle andcats

- **Sphingolipidoses** - defective catabolism of glycosphingolipids (components of cell membranes) - e.g. Galactocerebrosidosis in Cairn terriers, West Highland white terriers, cats and sheep

- **Glycogenoses** - defective lysosomal catabolism of glycogen - e.g. A-1,4-glucosidase deficiency in Shorthorn and Brahman beef cattle

- **Ceroid-lipofuscinosis** - defective catabolism of oxidised phospholipids +/- complexed proteins - e.g. Siamese cats, Devon cattle, South Hampshire sheep, many dog breeds including Border collies

- Identification of the specific type of LSD may be achieved by ultrastructural examination of the product stored in the lysosomes and/or by assays of lysosomal enzyme activity in skin fibroblasts or blood leukocytes etc.

Acquired Lysosomal Storage Disorders

- Lysosomal enzymes may also be inhibited by exogenous toxins -> **acquired LSD**
- E.g. **Swainsonine** is an indolizidine alkaloid contained within *Swainsona* species of Australian plants (e.g. Darling pea) and within the locoweeds (*Astragalus* and *Oxytropis* species) of North America; it inhibits lysosomal α–mannosidase -> clinical disease comparable to inherited α-mannosidosis

DEGENERATION OF EXTRACELLULAR TISSUES

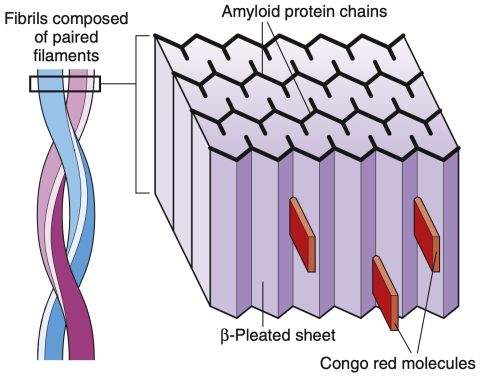
- In the following disorders (**amyloidosis**, **fibrinoid change** and **collagenolysis**), the degenerative process is centred on **extracellular** tissues.

AMYLOIDOSIS

Amyloid = an insoluble, extracellular, fibrillar glycoprotein deposit
Amyloidosis = disease resulting from localised or generalised (systemic) tissue deposition of amyloid

- Although there are different precursor molecules and hence chemical types of amyloid, amyloid is always composed of non-branching linear fibrils (7.5-10.0 nm in diameter) that form β-pleated sheets (**Figure 4**)

Figure 4 – Structure of Amyloid



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- The β-pleated conformation renders the deposits resistant to enzymatic degradation (e.g.by proteolytic enzymes of macrophages)
- All forms of amyloid are mainly (approximately 95%) composed of these fibrillar proteins but they also contain some serum amyloid P protein (a serum acute phase protein produced by hepatocytes), proteoglycans and glycosaminoglycans.
- **Serum amyloid P protein (APP)** may contribute to amyloidosis by stabilising the fibrillar proteins and decreasing their susceptibility to enzymatic proteolysis.

AL Amyloid

- In **humans**, the most common type of amyloid is **AL amyloid**, derived from antibody (immunoglobulin) light chains (especially λ light chains) produced by plasma cells

- This form may develop in **domestic animals**, either as a localised process in chronic plasma cell-rich inflammatory lesions or especially as a localised or generalised process due to neoplasia of plasma cells (e.g. Multiple myeloma arising within the bone marrow; e.g. Plasma cell tumours arising in the skin)

AA Amyloid

- In **domestic animals**, the most common type of amyloid is an insoluble fragment of the acute phase protein, **serum amyloid A (SAA)**, which is normally produced by the liver and found in circulating blood.
- Deposition of this type of amyloid (**AA amyloid**) is often referred to as **secondary** or **reactive amyloidosis** because increased hepatic synthesis and release of SAA occurs as a response to active inflammation and/or tissue damage anywhere in the body.
- Amyloid deposition is usually generalised, especially involving the liver, spleen and kidneys, +/- lymph nodes, adrenal glands and myocardium.
- Most animals with increased blood concentrations of SAA do **NOT** develop amyloidosis
- **AA amyloid deposition** may therefore involve defective enzymatic degradation of SAA by macrophages, or the synthesis of an aberrant SAA protein that is resistant to degradation and prone to forming insoluble deposits in tissues.
- AA amyloid can be identified by its loss of affinity for Congo red stain by pre-treatment (oxidation) with potassium permanganate.
- A diagnosis of reactive amyloidosis always warrants a hunt for an underlying disease process that is promoting **increased serum amyloid A production.**
- However, some forms of AA amyloidosis in animals are **inherited** or **familial.**
- E.g. Amyloidosis in Shar Pei dogs and in Oriental breeds of cats (e.g. Abyssinian and Siamese)

IAPP Amyloid

- Many cats and humans with **Type 2 diabetes mellitus** develop deposits of amyloid in the pancreatic islets of Langerhans (**islet amyloidosis**)
- Islet amyloid is derived from the hormone, **islet amyloid polypeptide** (IAPP), which is normally co-secreted with insulin by -> islet cells and antagonises the action of insulin by stimulating breakdown of muscle glycogen (-> increased blood glucose)
- In animals with Type 2 diabetes, there may be hypersecretion of both IAPP and insulin.
- The amyloid deposits can damage the -> cells, reducing their capacity to secrete insulin

Amyloid Derived from Misfolded Prion Proteins

- In some of the **transmissible spongiform encephalopathies** (TSE), amyloid deposits composed of misfolded proteins may develop in the brain
- E.g. New variant Creutzfeldt-Jacob disease in humans, scrapie in sheep, and chronic wasting disease in North American deer and elk
- In these disorders, the amyloid is thought to be due to aberrant post-translational misfolding (β-pleating) of a normal α-helical host cell membrane sialoglycoprotein (prp^c), caused by exposure to a prion (a proteinaceous infectious particle) (prp^{sc})
- It is currently thought that prp^{sc} acts as a template on which prp^c is refolded, thereby progressively converting the host prp^c into a likeness of itself

Gross Appearance

- Gross changes will only be apparent if amyloidosis is severe.
- Affected tissues are enlarged, firm and pale, with a waxy appearance.

Microscopic Appearance

- In H&E-stained tissue sections, amyloid appears as **amorphous, Homogeneous, eosinophilic (pink) extracellular material**
- It stains positively (orange-red) with Congo red stain (Figure 4) or Sirius red stain
- It appears green and birefringent when stained with Congo red and viewed with polarised light ("apple-green fluorescence")

Effects of Amyloid Deposition

- Mild to moderate forms of amyloidosis may be asymptomatic (e.g. Splenic arteriolar amyloidosis in middle-aged to older dogs)
- However, amyloid deposits can -> physical compression of adjacent cells and compromised vascular perfusion -> atrophy or cell degeneration (with decreased cell function) or cell death.
- **Severe hepatic amyloidosis** -> risk of spontaneous liver rupture -> potentially fatal haemoperitoneum (haemorrhage into the peritoneal cavity)
- Animals with **renal amyloidosis** often succumb to renal failure and those with renal glomerular amyloidosis may suffer from hypoproteinaemia due to loss of circulating proteins through the damaged glomeruli into urine.

FIBRINOID CHANGE

Fibrinoid change = an extracellular degenerative phenomenon observed in damaged blood vessels, especially small arteries and arterioles

- Previously termed **fibrinoid necrosis** (this terminology is inappropriate for an extracellular change)

- Injury to the vascular endothelium of a blood vessel -> **entry and accumulation of plasma proteins** (including polymerised **fibrin** derived from circulating fibrinogen), **+/- complement** and/or **immunoglobulins** (antibodies) in the tunica intima and media +/- perivascular connective tissues.
- Fibrinoid change is only visible **microscopically** but may be accompanied by vascular thrombosis (= inappropriate intra-luminal blood clot formation within blood vessels) and/or haemorrhage and oedema that may be visible grossly
- The extravasated plasma proteins (especially fibrin) appear deeply eosinophilic in H&E- stained sections.

- E.g. Renal failure - due to endothelial injury by circulating toxins.
- E.g. Systemic hypertension
- E.g. Vasculitis
- E.g. Selenium/vitamin E deficiency in pigs - due to endothelial injury by reactive oxygen species
- E.g. Oedema disease in pigs - due to endothelial injury by circulating *E. Coli* toxins

FLAME FIGURES

- A **microscopic lesion** caused by the degranulation of eosinophils.
- Granules and released compounds adhere to collagen fibrils and these appear

amorphous and eosinophilic in H&E-stained sections.
- Collagen fibres are surrounded by brightly eosinophilic, granular to amorphous material representing massed eosinophils and their released granules.

- E.g. Insect bite hypersensitivity reactions
- E.g. Mast cell tumours
- E.g. Eosinophilic collagenolytic granulomas in cats and horses