Induced lesions of the musculoskeletal system

STPI, Pune, October 2016

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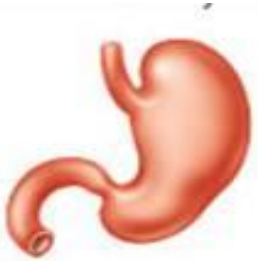
Skeletal Muscle

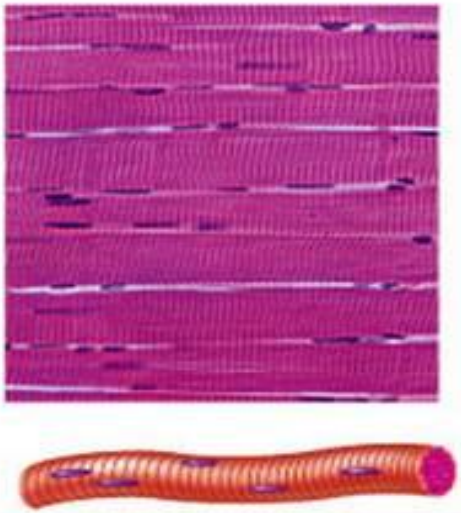
Muscle types

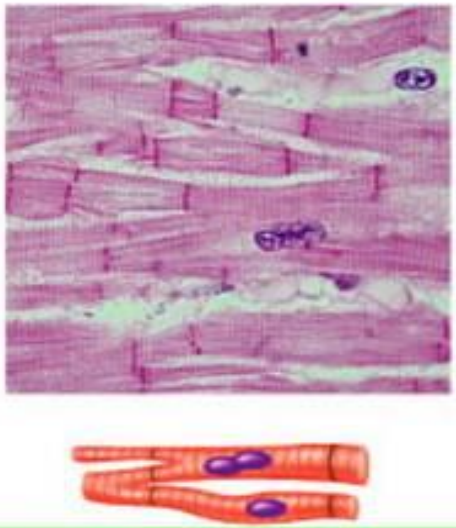
Single, very long, cylindrical, multinucleate cells with very obvious striations

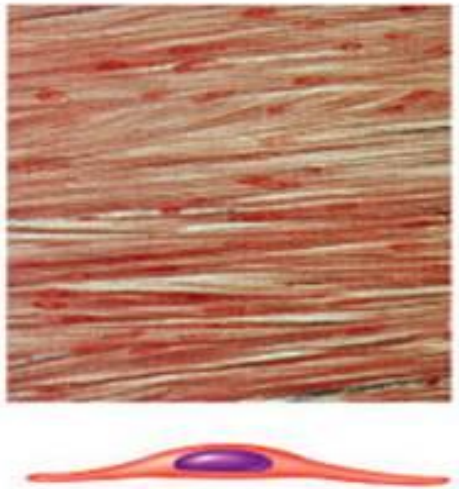
Branching chains of cells; uni- or binucleate; striations



Single, fusiform, uninucleate; no striations



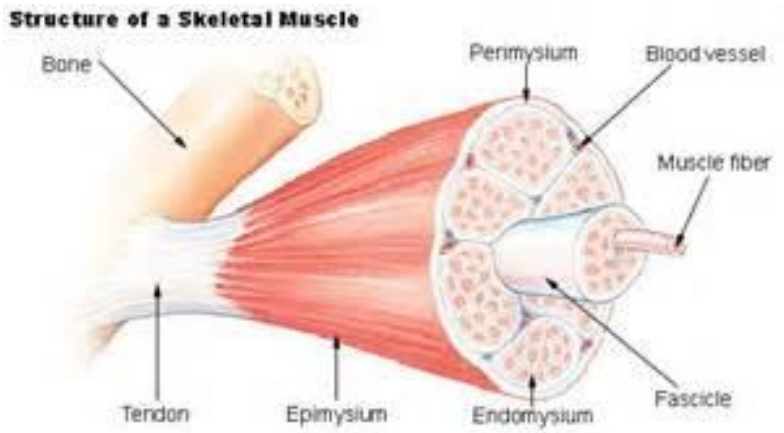


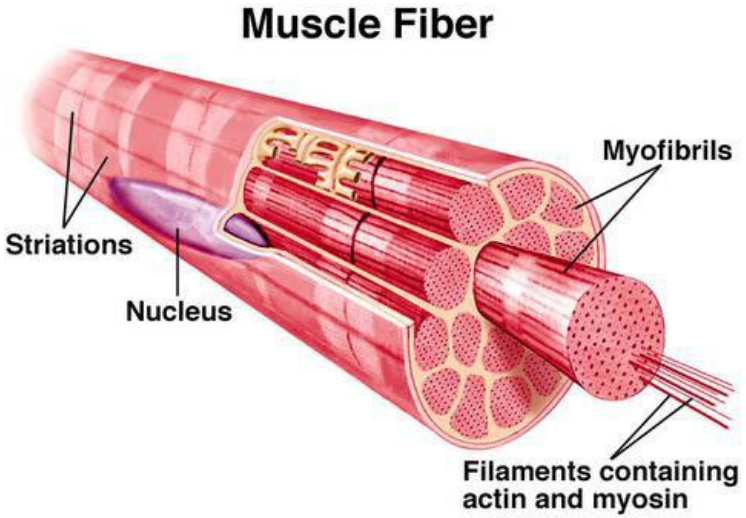


Muscle structure

Numerous classifications depending on

1. Muscle fibre type
2. Number of heads
3. Number of bellies
4. Shape
5. Others





Various Properties of Different Fiber Types [3]

Properties	Type I fibers	Type IIA fibers	Type IIB fibers
Motor Unit Type	Slow Oxidative (SO)	Fast Oxidative/Glycolytic (FOG)	Fast Glycolytic (FG)
Twitch Speed	Slow	Fast	Fast
Twitch Force	Small	Medium	Large
Resistance to fatigue	High	High	Low
Glycogen Content	Low	High	High
Capillary Supply	Rich	Rich	Poor
Myoglobin	High	High	Low
Red Color	Dark	Dark	Pale
Mitochondrial density	High	High	Low
Capillary density	High	Intermediate	Low
Oxidative Enzyme Capacity	High	Intermediate-high	Low
Z-Line Width	Intermediate	Wide	Narrow
Alkaline ATPase Activity	Low	High	High
Acidic ATPase Activity	High	Medium-high	Low

Muscle - Responses to Toxicity

+ Toxicity can be local or systemic
+ Severity and nature of injury depend on muscle type
+ Muscle fibres have a limited number of responses

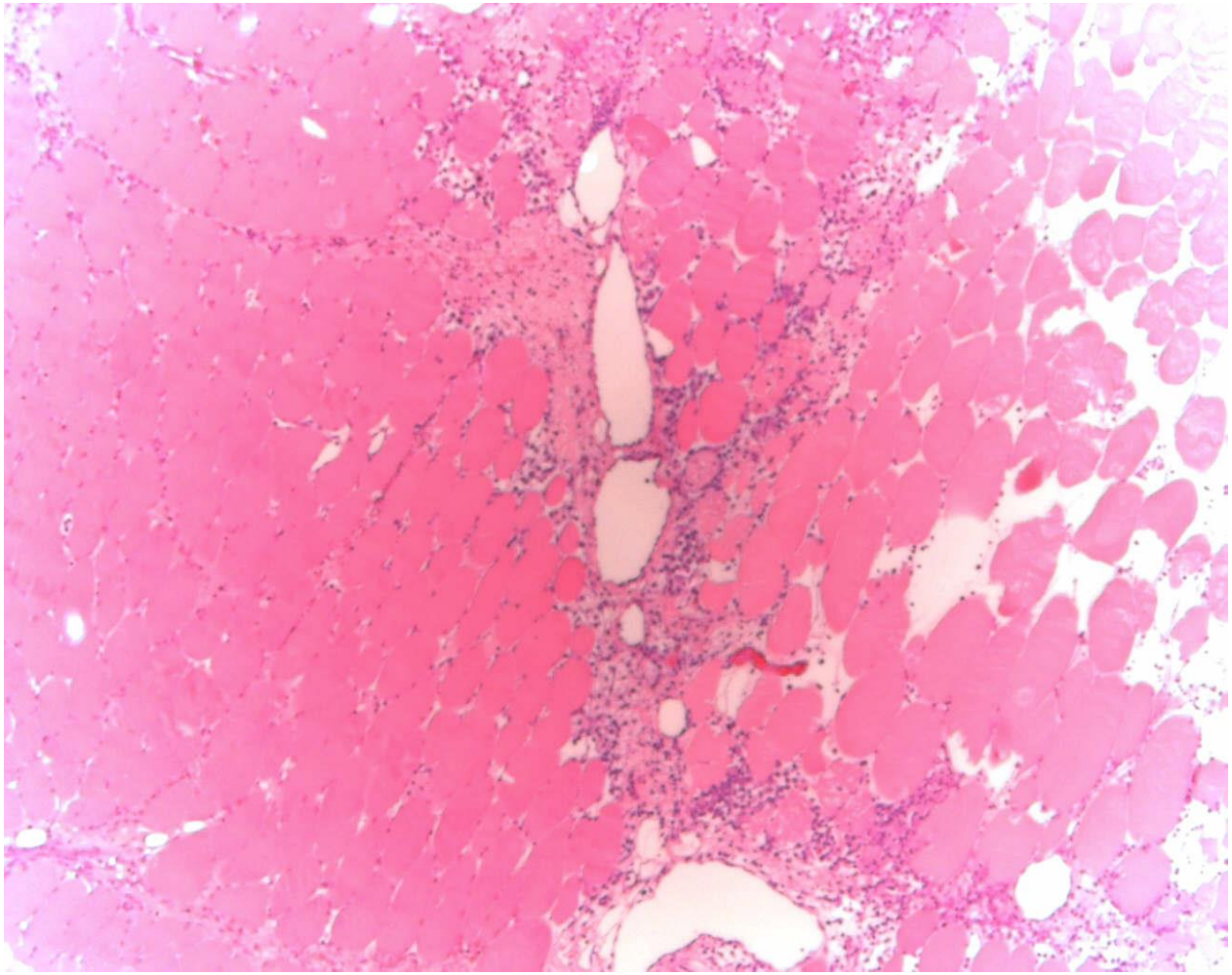
1. Increase or decrease in myofibre size
2. Eosinophilia
3. Vacuolation
4. Inflammation
5. Degeneration
6. Necrosis
7. Regeneration
8. Fibrosis

Localised Toxicity

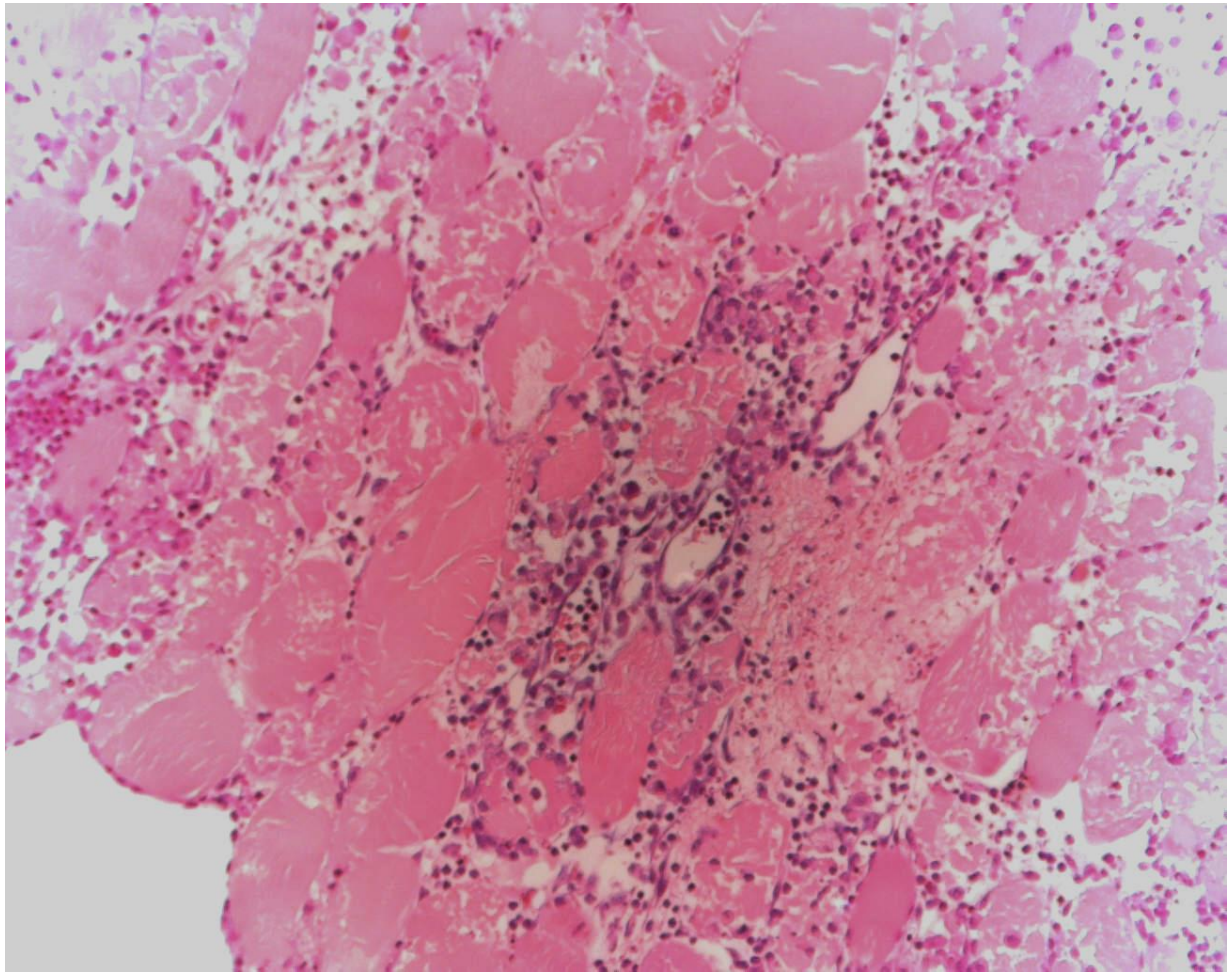
- Often associated with subcutaneous or intramuscular injection of various materials.
- Can be caused by vascular occlusion
- Manifests as localised inflammation, vacuolation, degeneration, necrosis and fibrosis of myofibres
- Often accompanied by regenerative changes
- Wide range of injected materials used I/M in animals and man

1. Antibiotics (penicillin, streptomycin)
2. Adjuvants/vaccines
3. Analgesics (pentazocine)
4. Anaesthetics (bupivacaine, lignocaine)

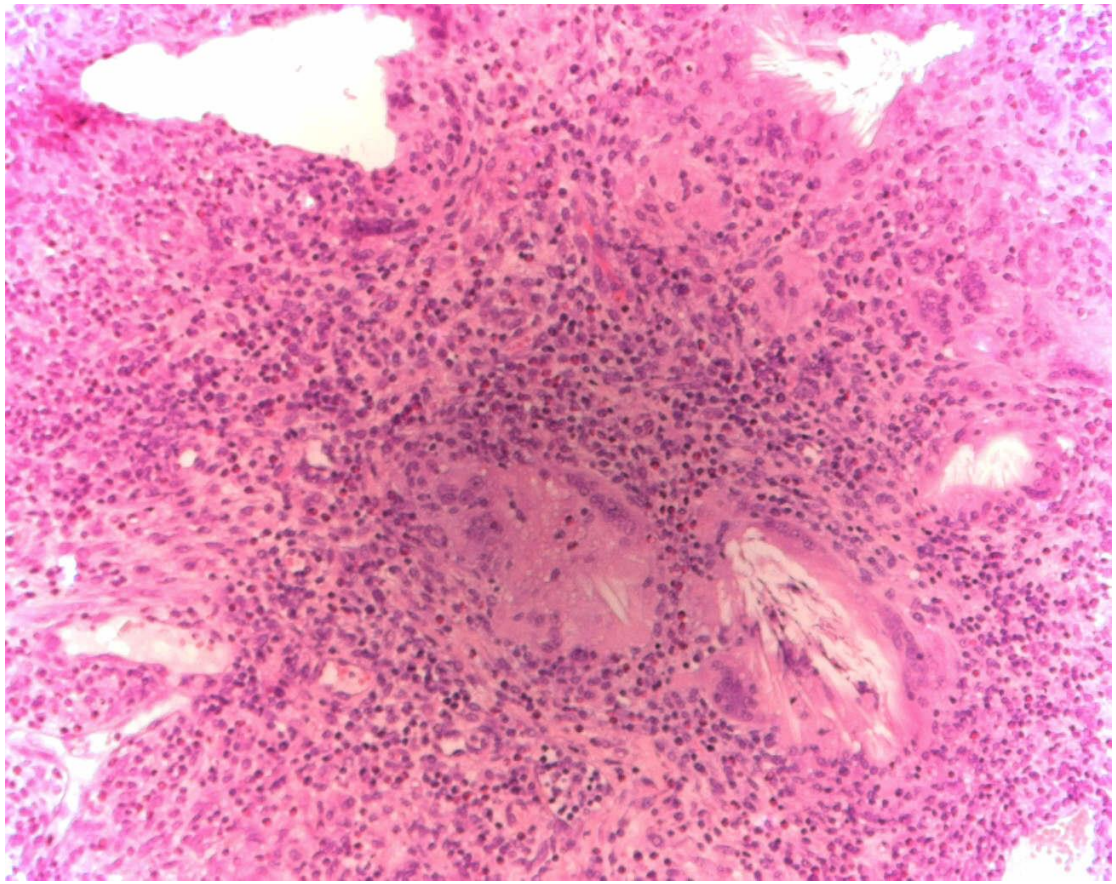
Skeletal muscle – injection site



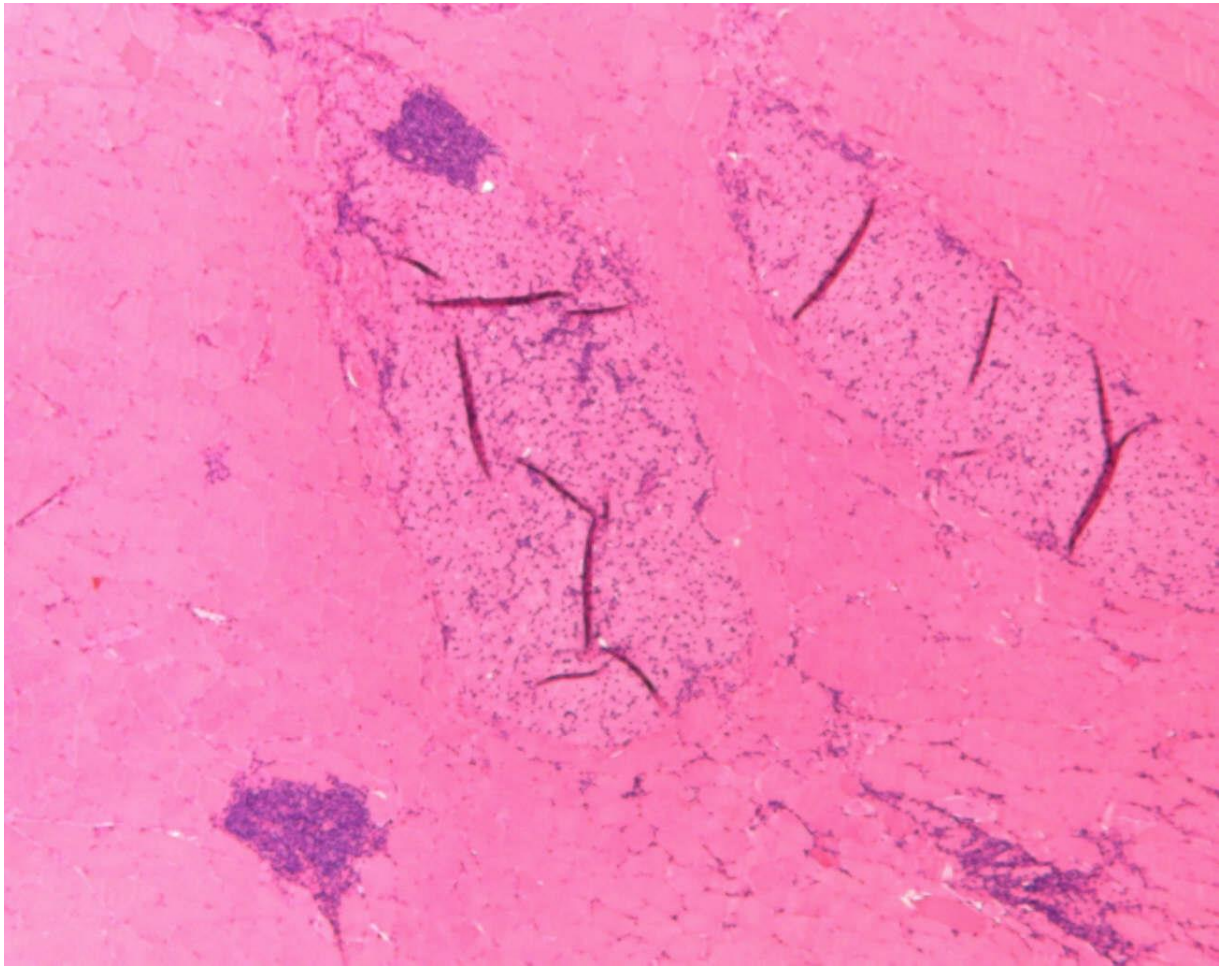
Skeletal muscle – injection site



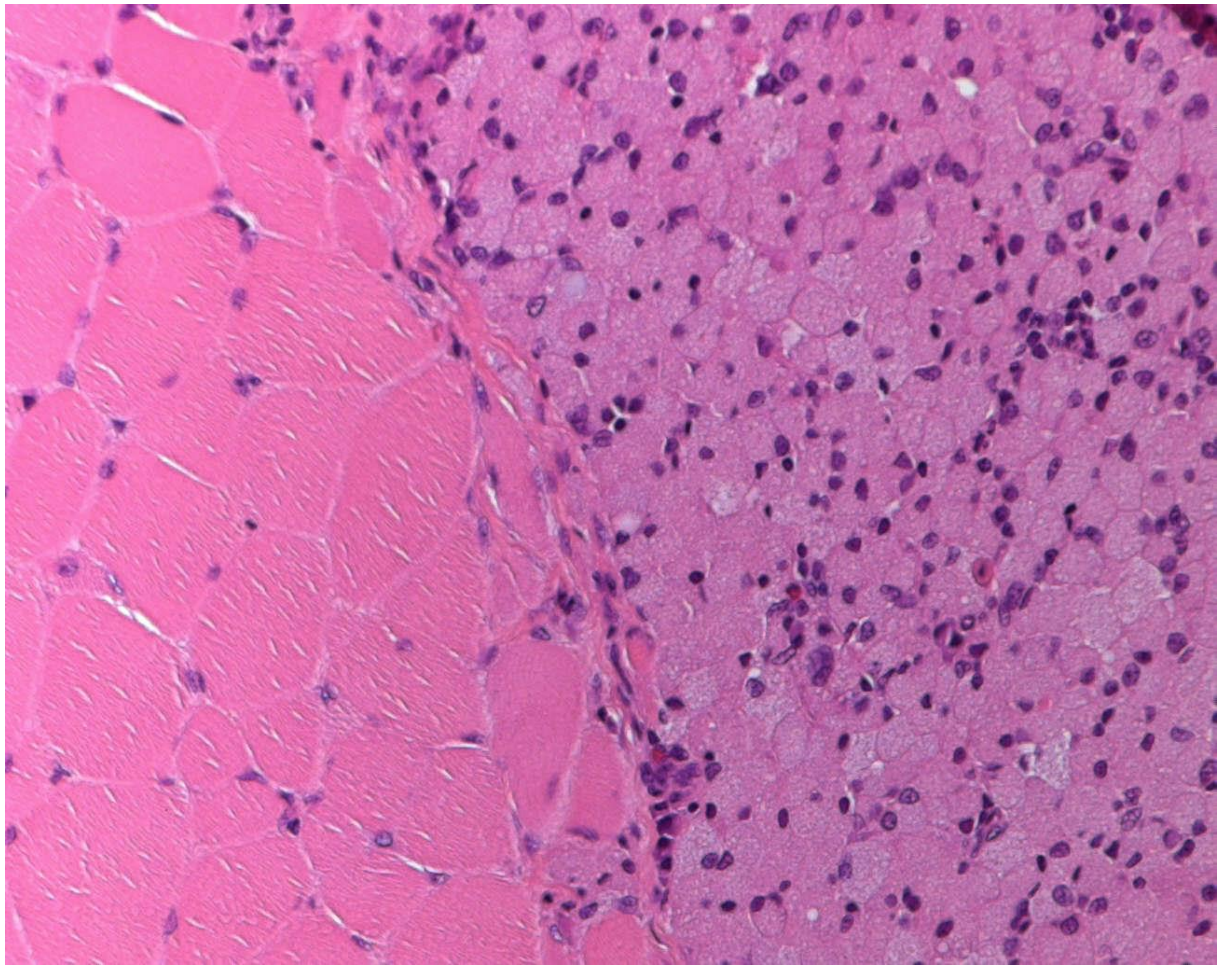
Skeletal muscle – injection site, foreign material



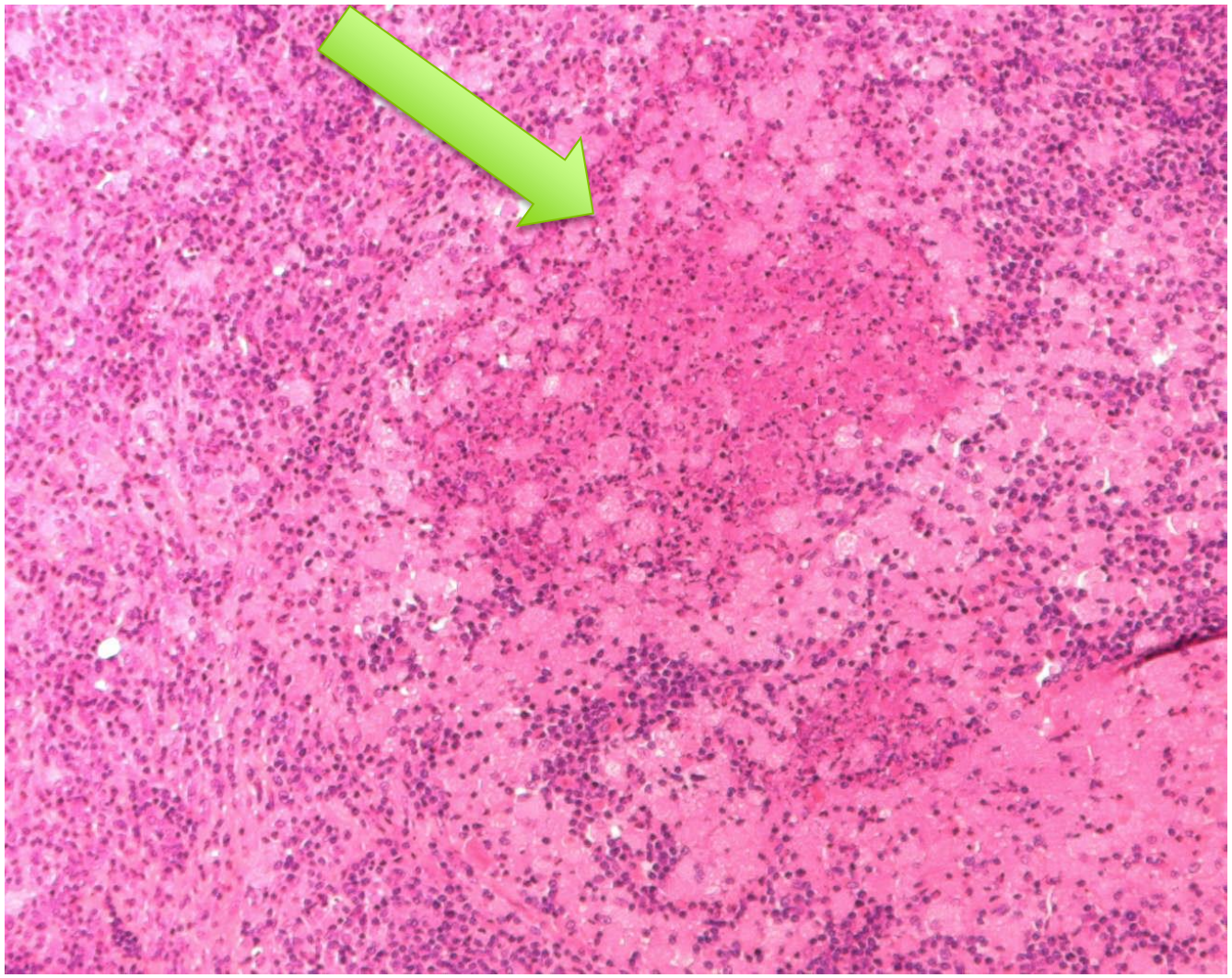
Injection site – granulomatous inflammation



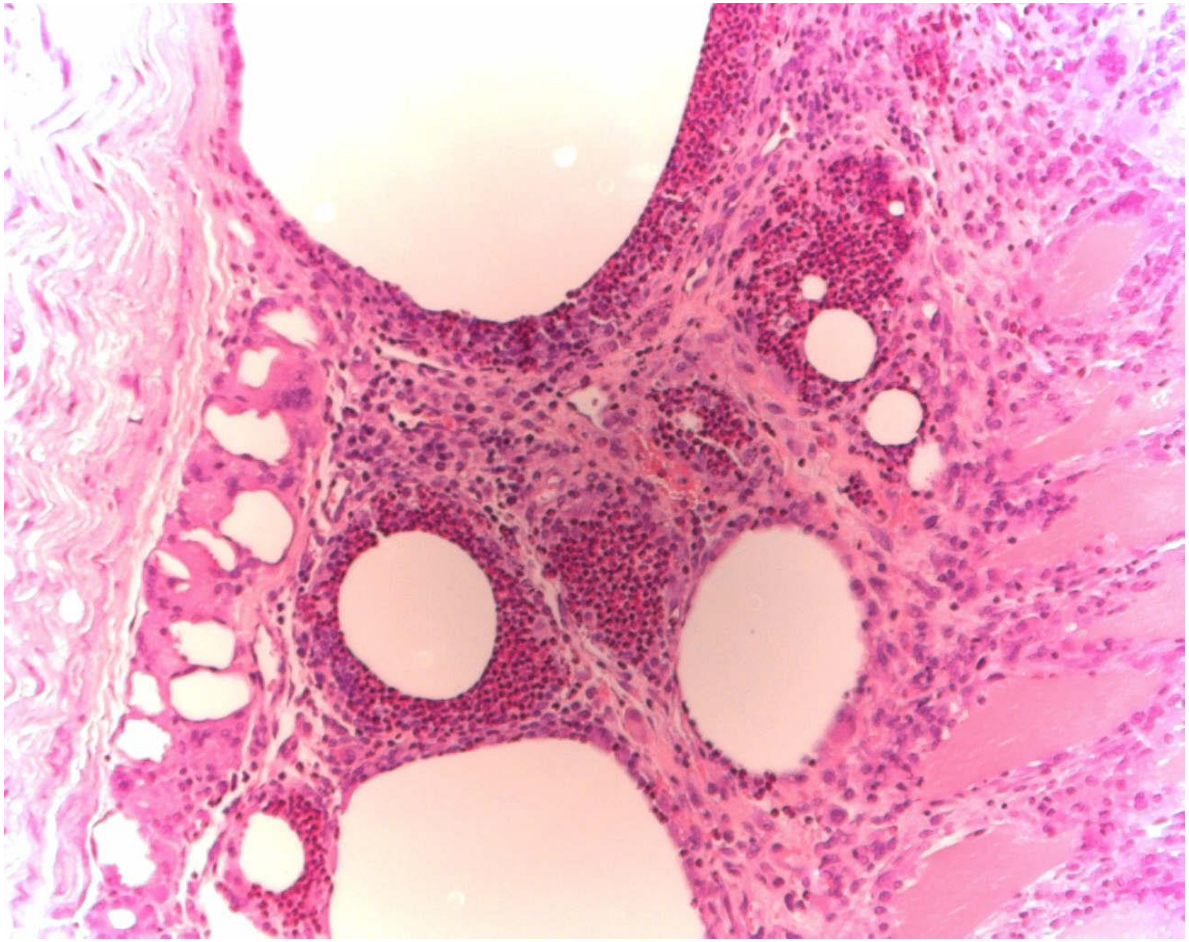
Injection site – granulomatous inflammation



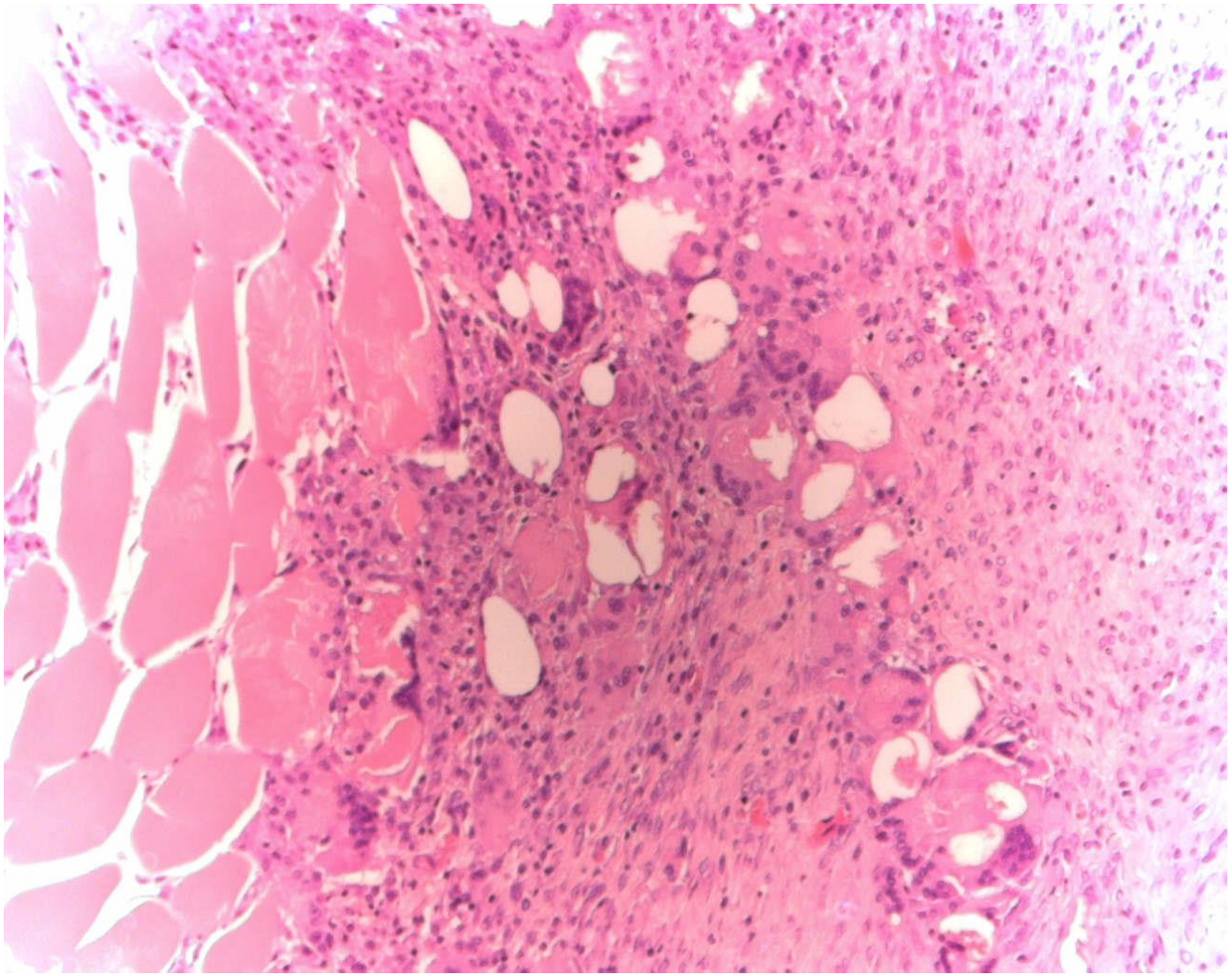
Injection site – granulomatous inflammation, central necrosis



Granulocytic inflammation, vacuolation



Inflammation, fibrosis, vacuolation



Systemic Toxicity

+ Atrophy/Hypertrophy

1. Altered protein synthesis – atrophy or hypertrophy
2. Fasting/starvation/cachexia of chronic disease
3. Disuse/high level of exercise
4. Hormones – steroids, growth hormone
5. Neurogenic/neurotransmitters - atrophy

+ Vacuolation – lysosomal, genetic or induced
+ Dystrophy – genetic, induced, dietary

1. Sarcolemmal impairment
2. Microtubule disruption
3. Mitochondrial impairment
4. Calcium imbalance

Atrophy

+ Reduction in myofibre size caused by a reduction in protein synthesis.

1. Drug-induced eg, steroids
2. Malnutrition
3. Disruption of enervation
4. Disuse e. g due to orthopaedic immobilisation of a limb following trauma

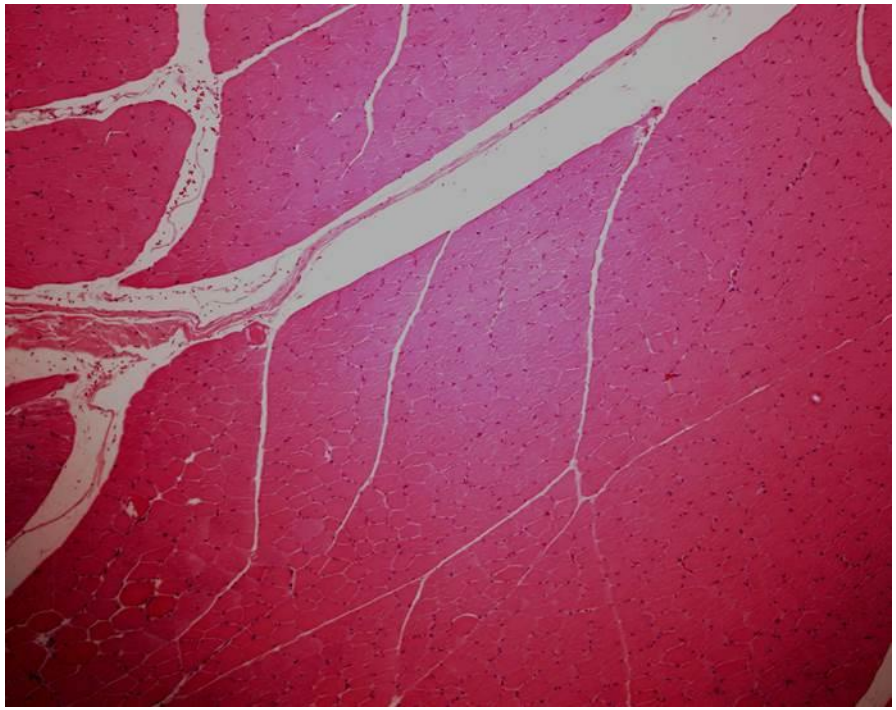
+ Myofibres decreased in diameter, with myofibre nuclei appearing prominent.
+ Myofibers may be evenly or unevenly affected
+ Atrophy occurs spontaneously in the ageing rat, often secondary to age-related nerve fibre degeneration in spinal cord and sciatic nerve.

Altered protein synthesis

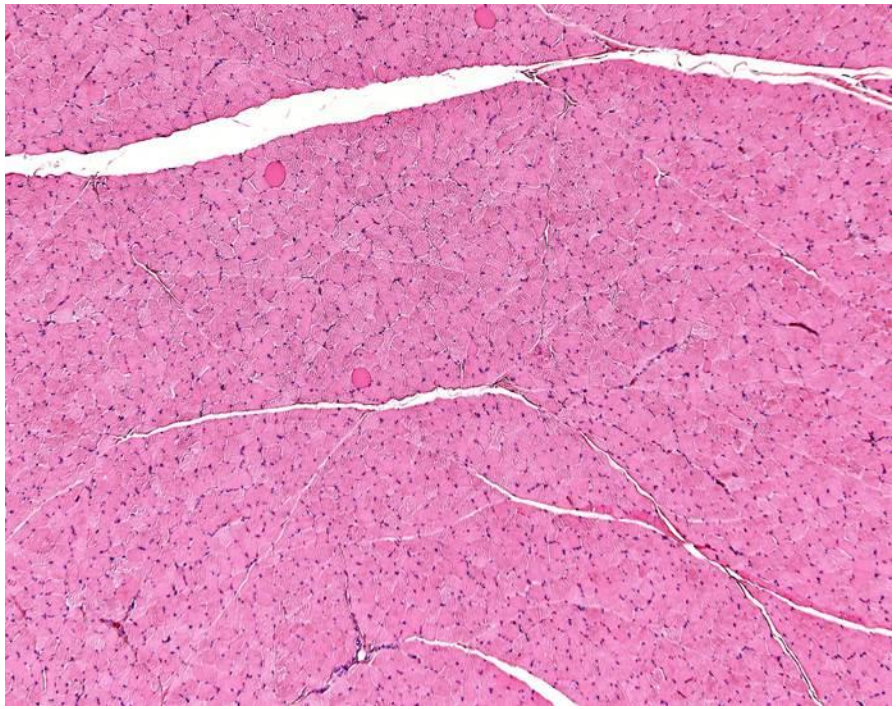
- **Corticosteroids** such as **Betamethasone** and **Dexamethasone** are thought to inhibit protein synthesis resulting in atrophy and muscular weakness with selective atrophy of type II fibres.
- Tyrosine kinase inhibitors
- A similar effect occurs in protein deficiency through malnutrition.

Skeletal muscle – myofibre atrophy, corticosteroid

Control



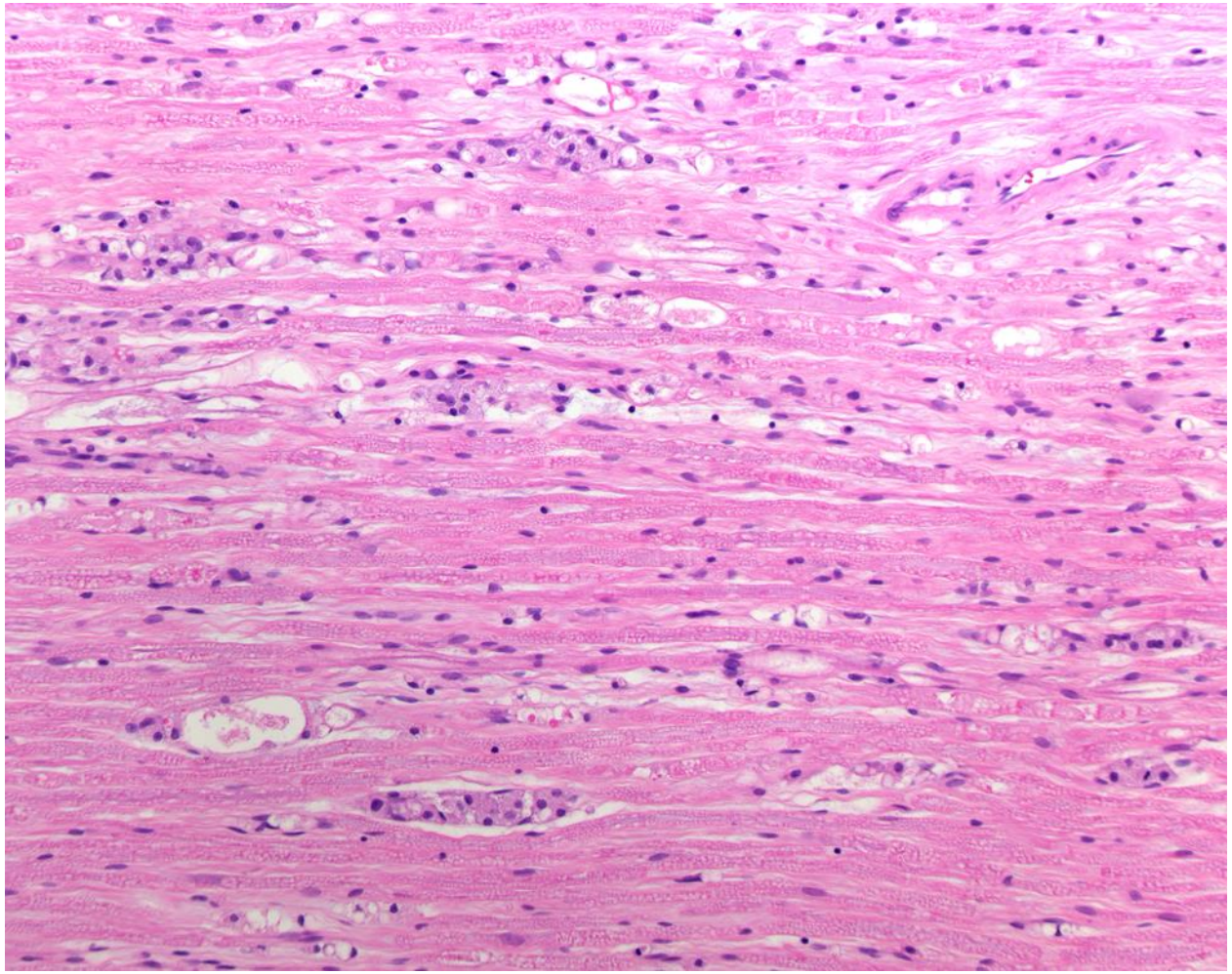
Treated



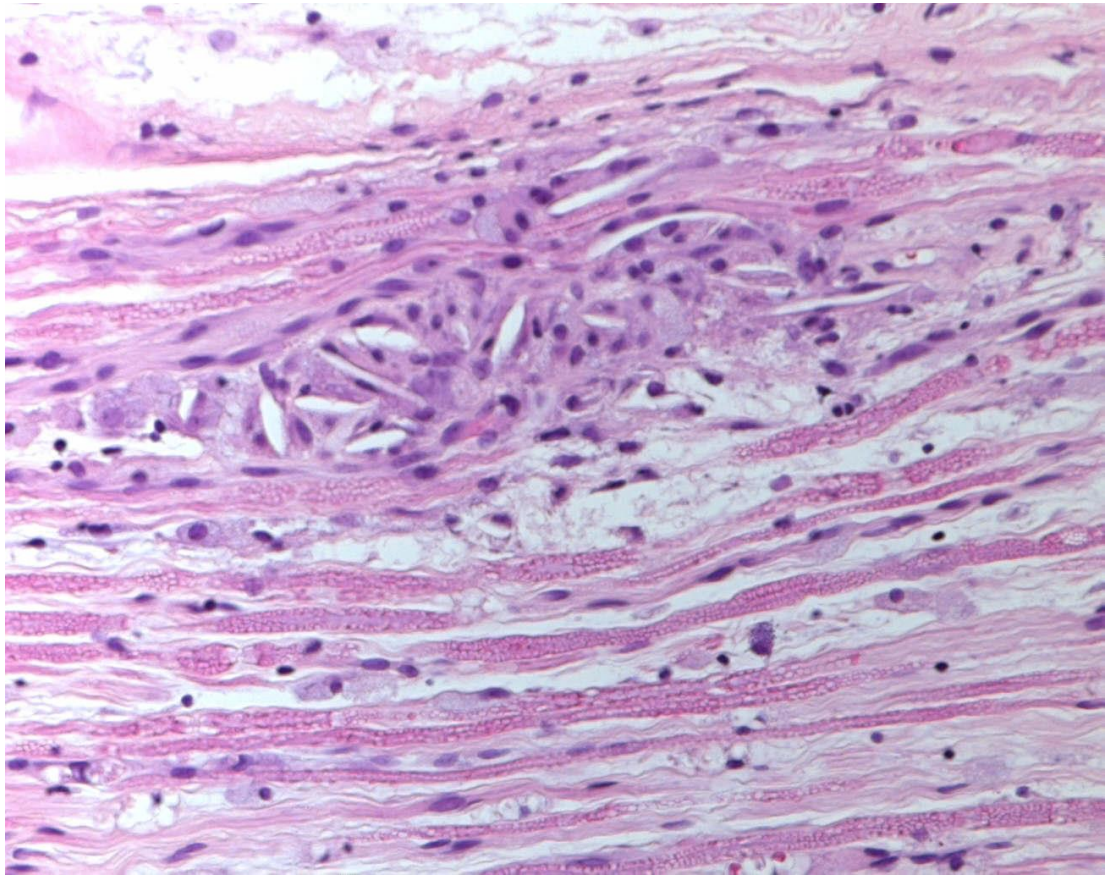
Neurogenic Toxicity

- Can result from denervation or excessive stimulation
- Interruption of or damage to the nerve supply can produce muscle atrophy and loss of use of a limb
- Interference with neural transmission by blocking the neurotransmitter **acetylcholine - anticholinergics**
- Inhibition of acetylcholinesterase by compounds such as **organophosphates** and **carbamates** which result in an accumulation of a high concentration of acetylcholine leading to increased contractile activity of the muscle itself.

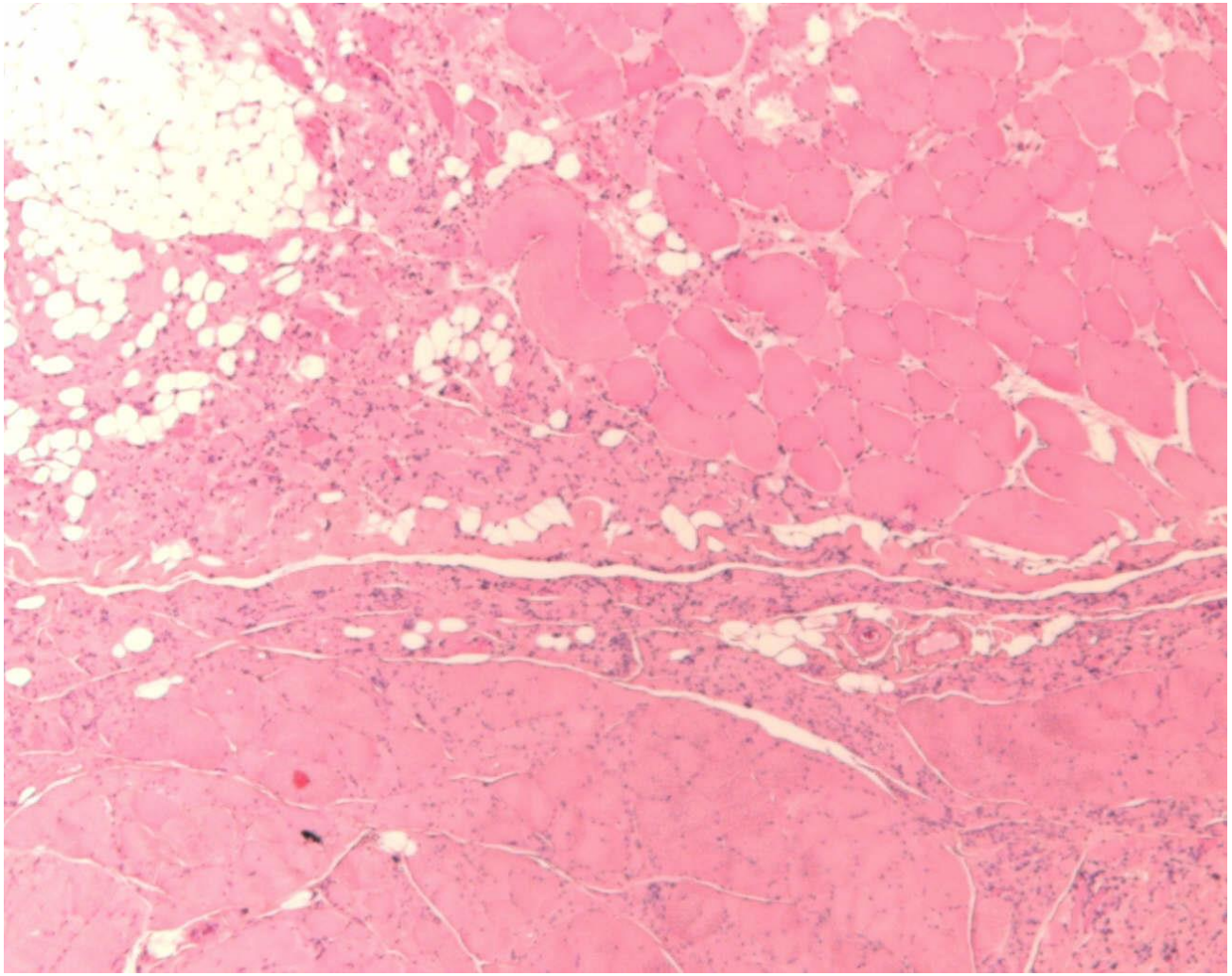
Rat - sciatic nerve, axonal atrophy



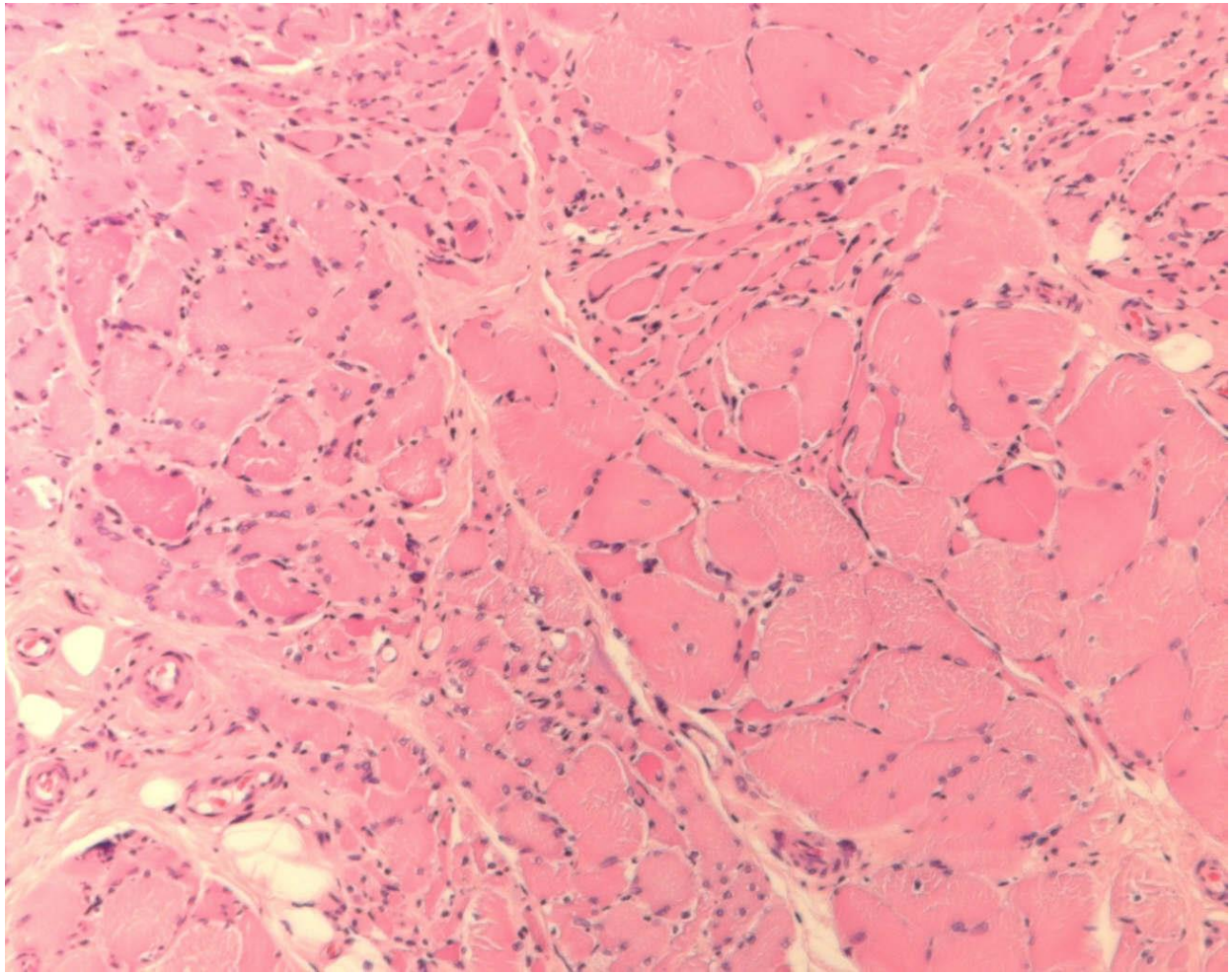
Sciatic nerve - axonal degeneration, clefts



Myopathy –fibre atrophy, adipose replacement



Muscle atrophy – variation of fibre diameter and shape



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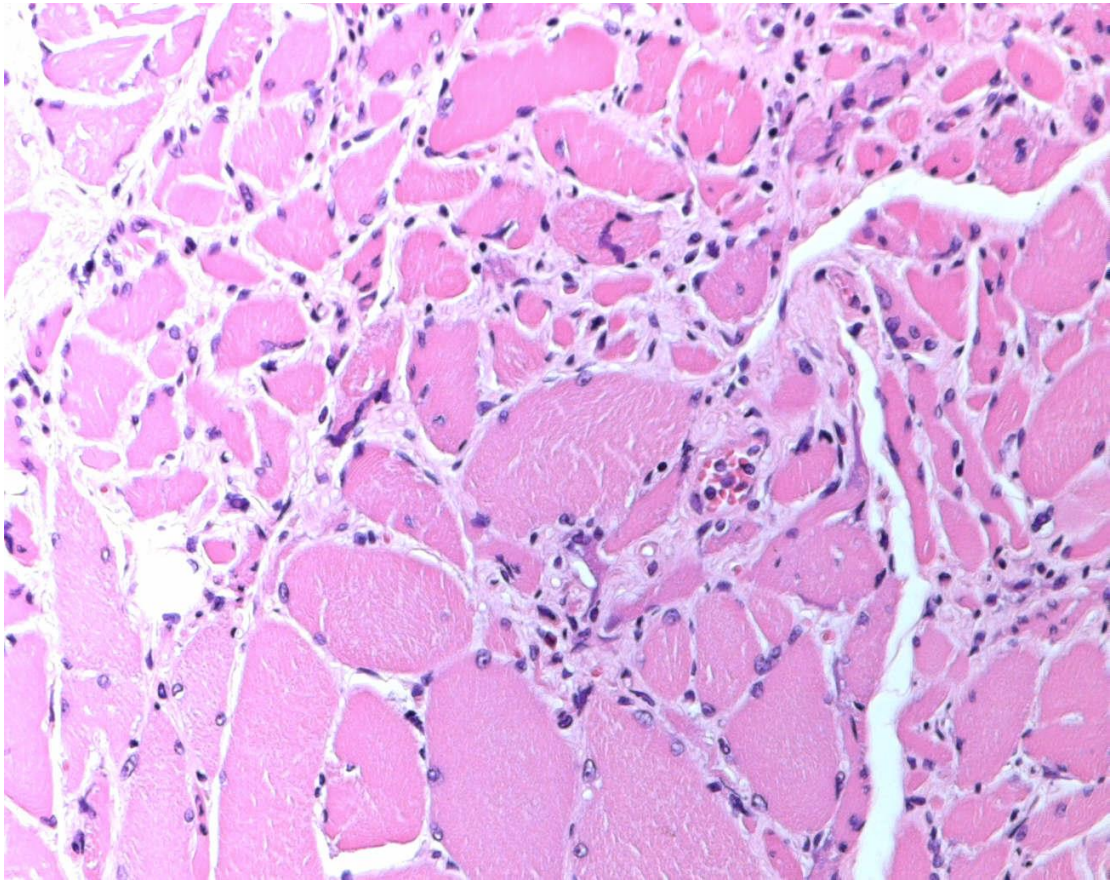

Hypertrophy

- An increase in myofibre size due to the addition of more myofibrils and sarcomeres within the myofibres.
- May be due to increased workload - physiologic
- Direct effect of drugs with anabolic potential eg. growth hormones, somatomedins, insulin-like growth factors, anabolic steroids

Dystrophy

- A term used for a group of hereditary primary muscle disorders that lead to progressive muscle weakness. Duchenne's MD X-linked in man
- The nerve supply is normal but there is a lack of effective regeneration of damaged fibres leading to degeneration and atrophy.
- This may be induced by compounds which inhibit the synthesis of DNA and RNA, e.g. **6-mercaptopurine**, an immunosuppressant, which produces a muscular dystrophy in the neonatal rat - teratogenic effect

Skeletal Myopathy in Transgenic Mice Carrying Human Prototype c-Ha-*ras* Gene

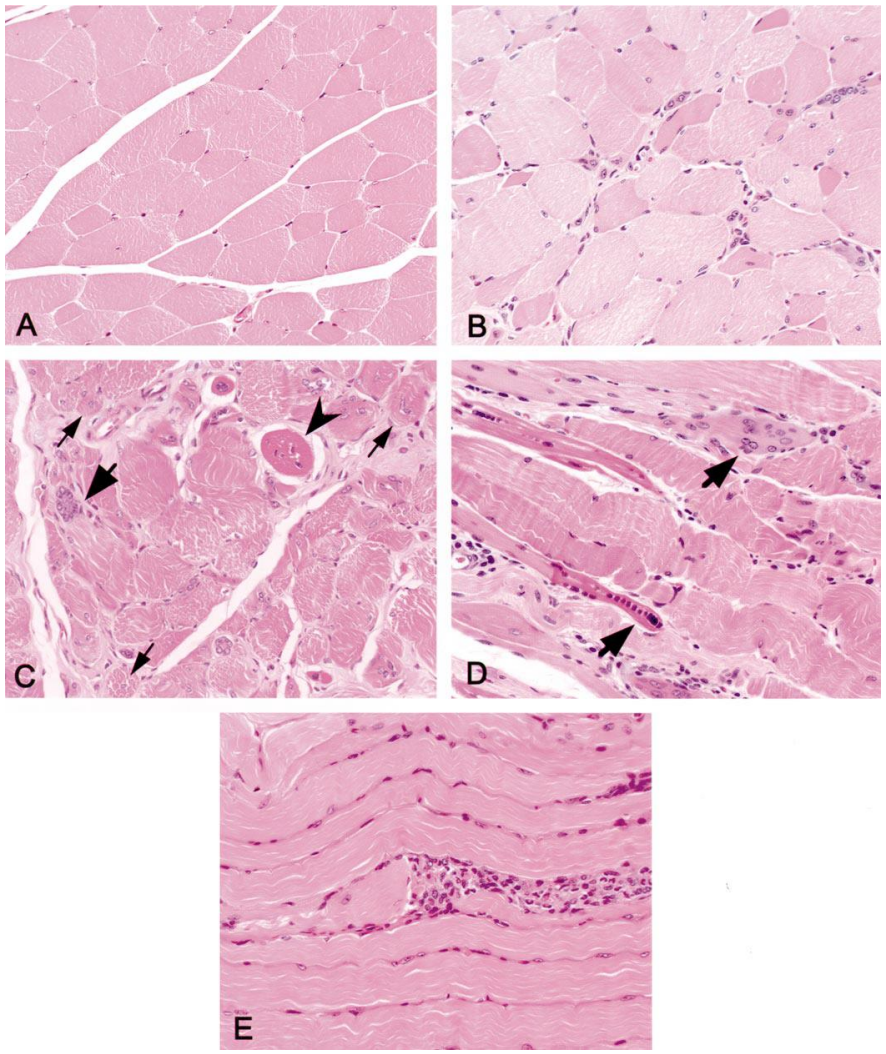

FIGURE 1.—Muscle femoralis from a 34-week-old male non-Tg mouse (A) and rasH2 mice of the same age (B, C, D, and E), H&E.
The variation of muscle
fiber size is evident in the transverse section of the muscle fibers, and some nuclei in regenerating fibers were enlarged (B).
Hyaline degeneration (arrowhead), central placed nuclei (small arrow) and regenerating fibers (large arrow) are apparent (C).
Regenerating fibers (large arrow) with proliferation and long chain of the nuclei and basophilic cytoplasm are evident (D).
Notice the infiltration of granulocytes and macrophages, suggesting reaction to degeneration or phagocytosis of necrotic muscle fibers (E).

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Degeneration and Necrosis

+ Degeneration may lead to irreversible necrosis or recovery. A wide range of agents can cause this change. Several forms of degeneration have been reported based on morphological appearance.

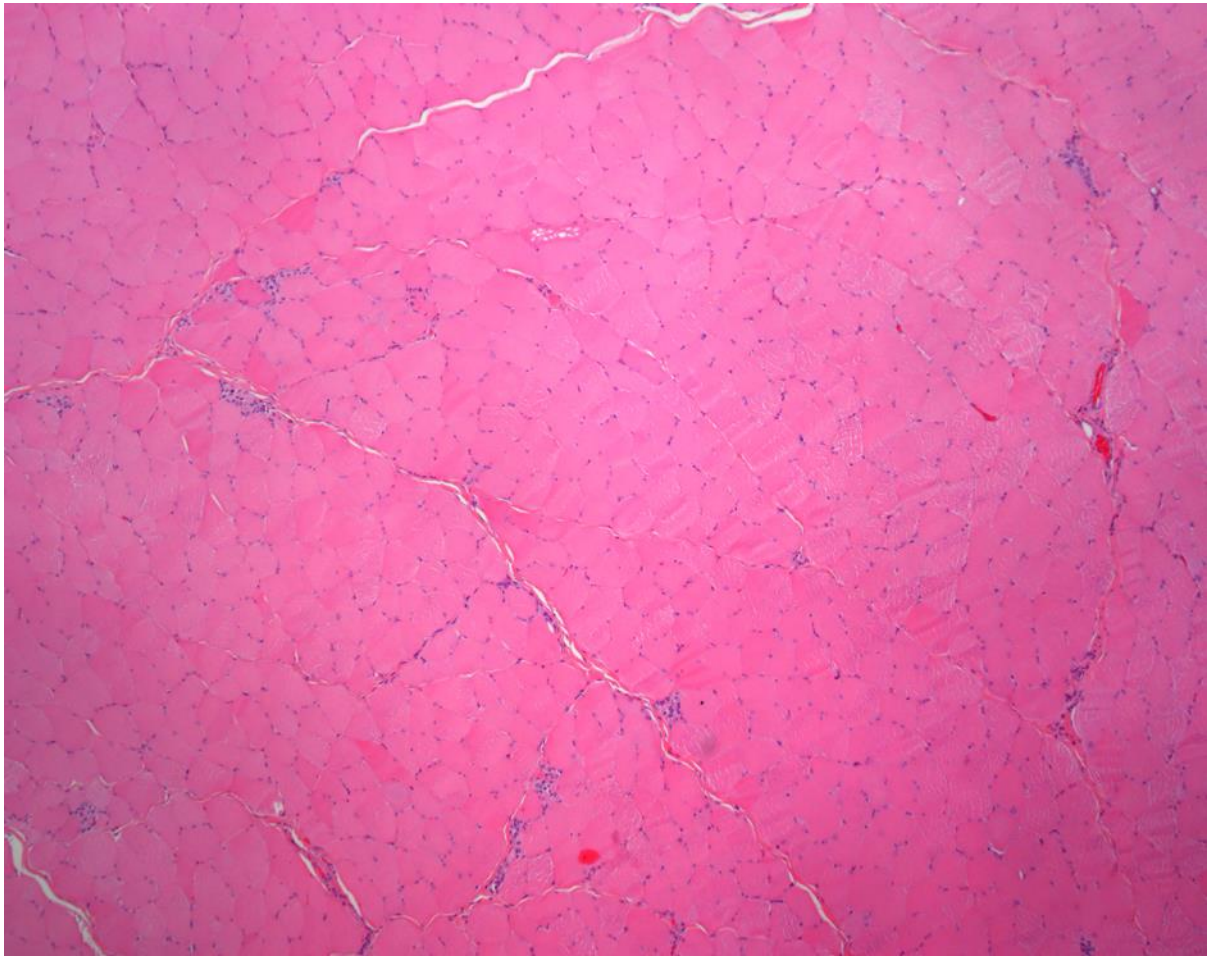
1. Hyaline
2. Granular

Hyaline Degeneration

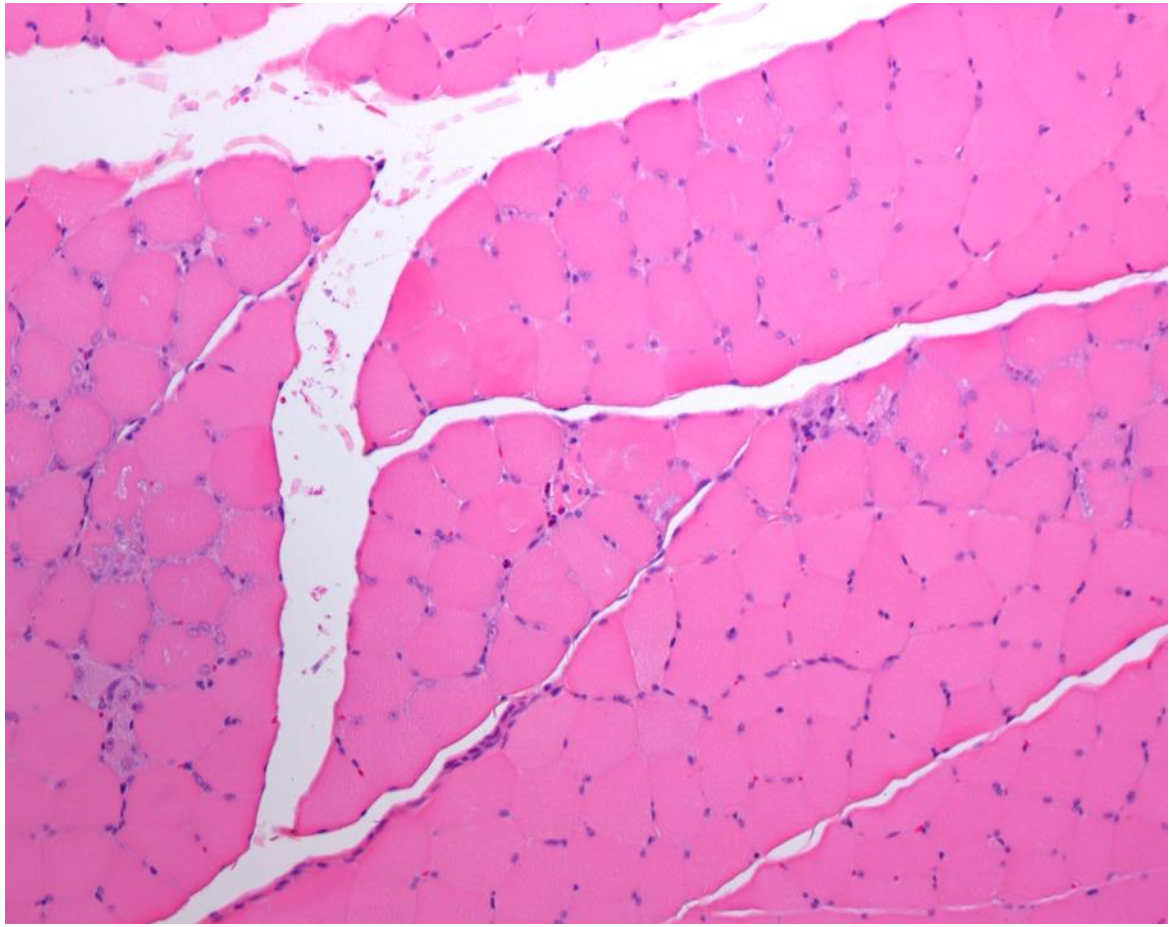
Affected parts of the myofibre appear swollen and eosinophilic with loss of cross-striations. The actin and myosin becomes fragmented into blocks or discs within the “tube” of persisting lamina externa of the fibre.

A wide range of compound classes induce myofibre degeneration eg. statins, reverse transcriptase inhibitors,

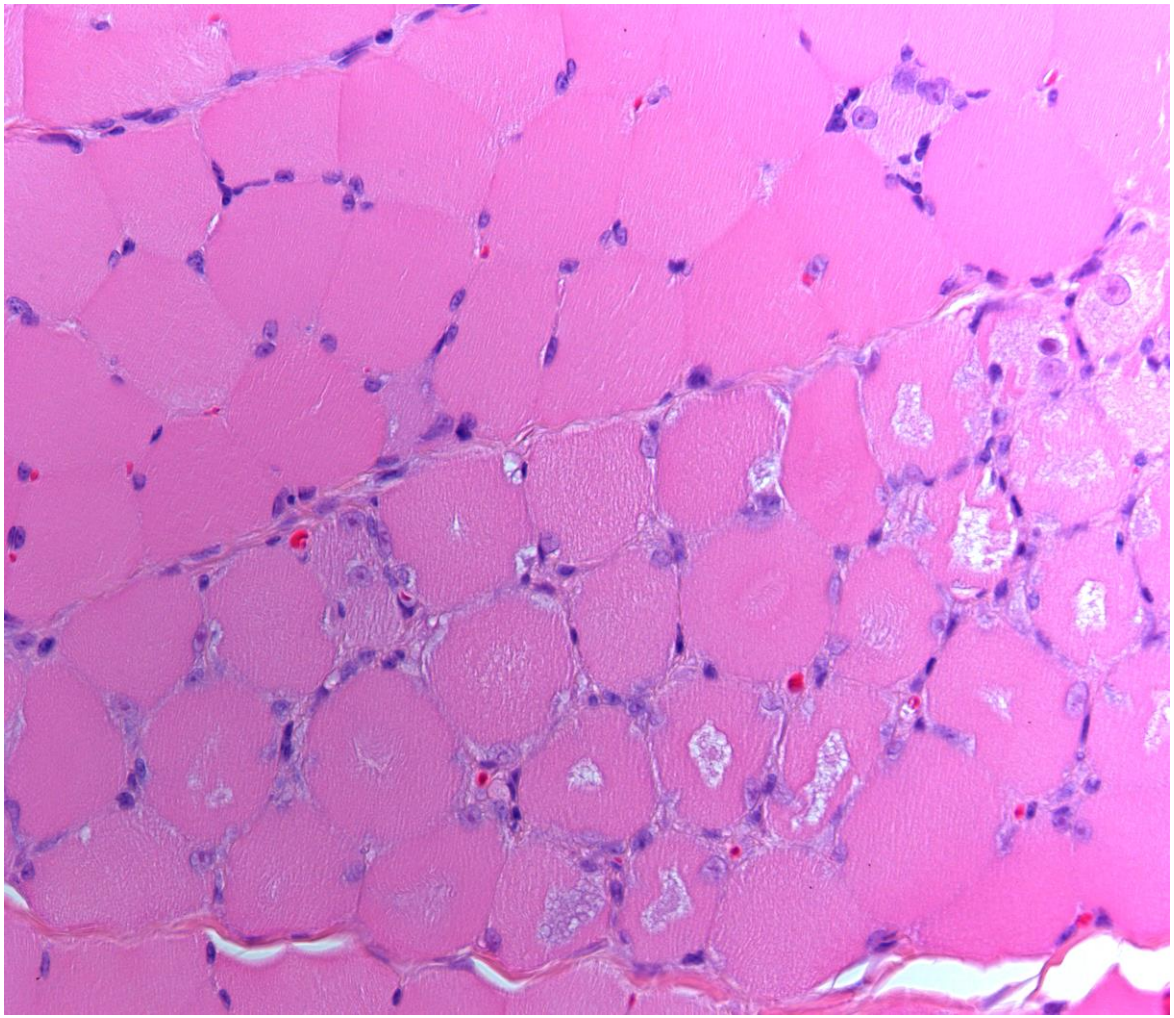
Monkey – skeletal muscle, myopathy



Monkey – skeletal muscle, myopathy



Monkey – vacuolation and early degeneration



Granular Degeneration

The sarcoplasm takes on a granular basophilic appearance. This is associated with nutritional disorders, mycotoxic drugs and plants, and certain metabolic disorders.

Impaired Sarcolemmal Function

The sarcolemma may exhibit increased excitability as a result of altered blood potassium levels resulting in either myotonia (muscle contraction) if elevated or muscle weakness if reduced

Clofibrate and statins that lower plasma cholesterol levels have been linked with myotonia and myofibre necrosis in man and rats. The mechanism is uncertain.

Herbicides, alcohol also associated with myopathy

Microtubule and microfilament Disruption

Anti-mitotic compounds such as **Colchicine** and **Vincristine** disrupt the microtubules of the cell cytoskeleton. This produces effects in the peripheral nervous system and in skeletal muscle.

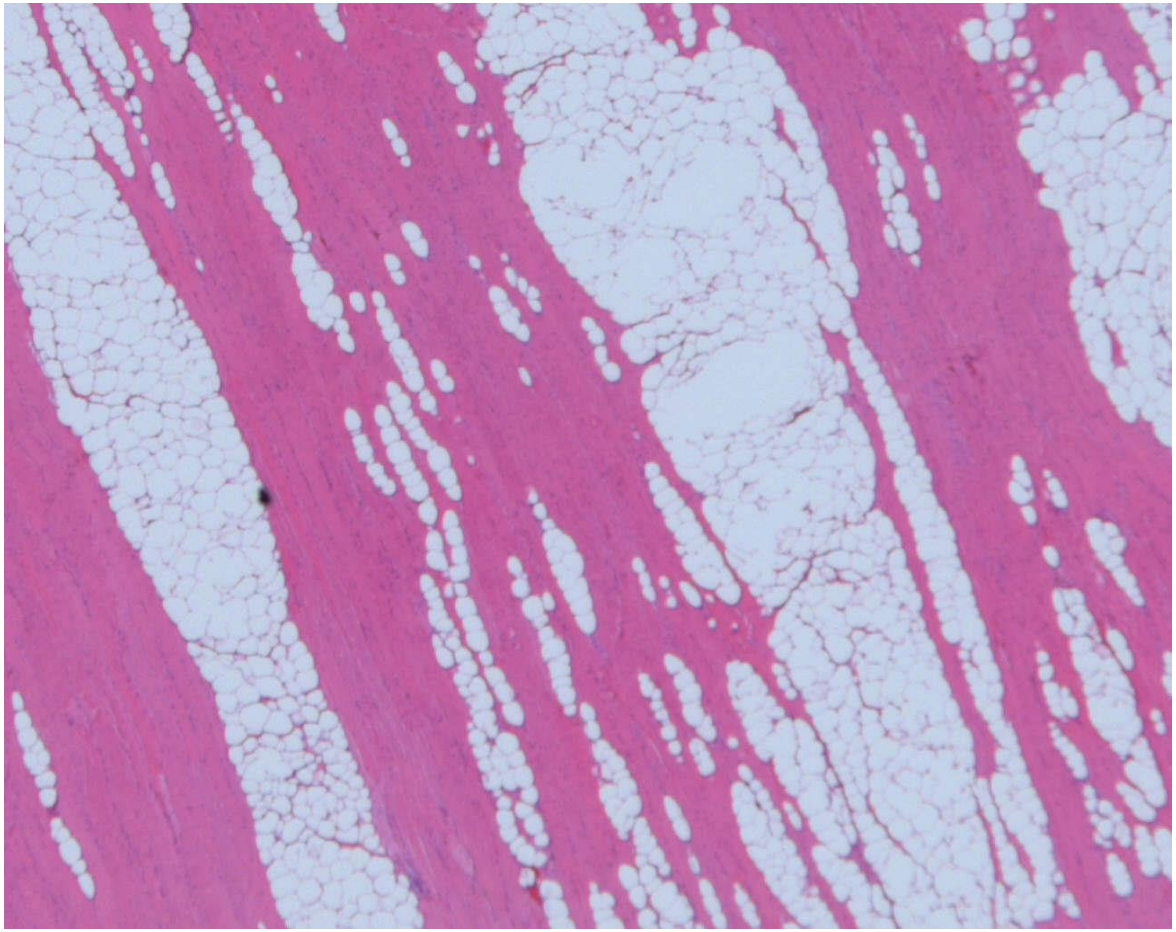
Plasmocid affects myofilaments, especially those of muscles in continuous use eg. diaphragm

Emetine damages intermediate filaments, resulting in disruption of Z discs initially, leading to myofilament degeneration

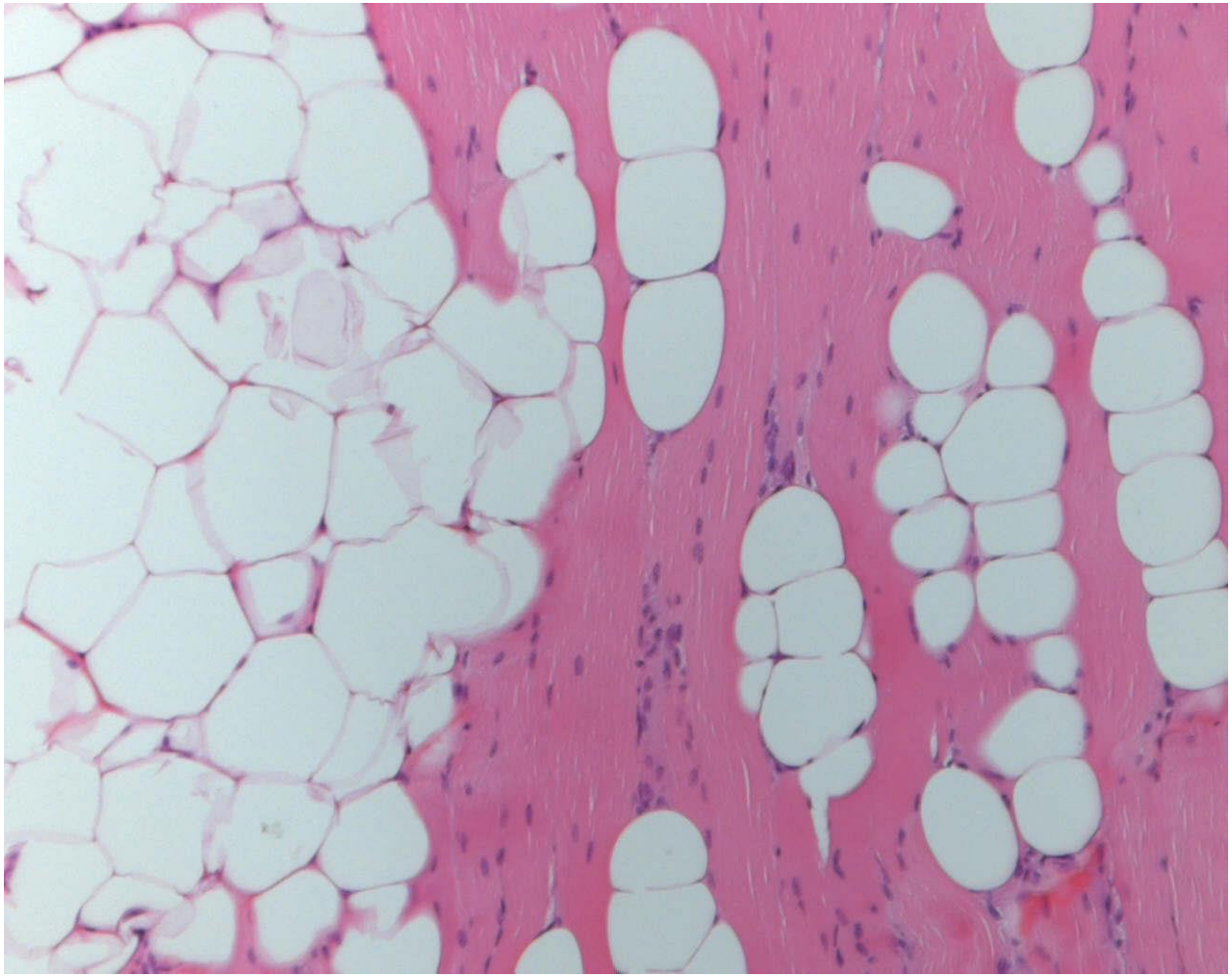
Lysosomal Myopathies

- Drugs such as **Amiodarone, Chlorphentermine, Chloroquine, Tamoxifen** and **Chlorcyclizine** form stable intracellular drug-lipid complexes which resist lysosomal degradation.
- This results in generalised phospholipidosis characterised by accumulation of undegradable phospholipid membranous material in lysosomes.
- This produces muscle damage either by accumulation in sarcolemmal lysosomes or by direct effect on the sarcolemma.

Muscle – adipose replacement of fibres



Muscle – adipose replacement of fibres



Miscellaneous Myopathies

Agents such as **2,4-dinitrophenol** uncouple oxidative metabolism in mitochondria producing eosinophilia of the Type I fibres

Anaesthetics lead to increased intracellular calcium, resulting in muscular rigidity, myoglobinuria and metabolic acidosis eg. malignant hyperthermia in pigs

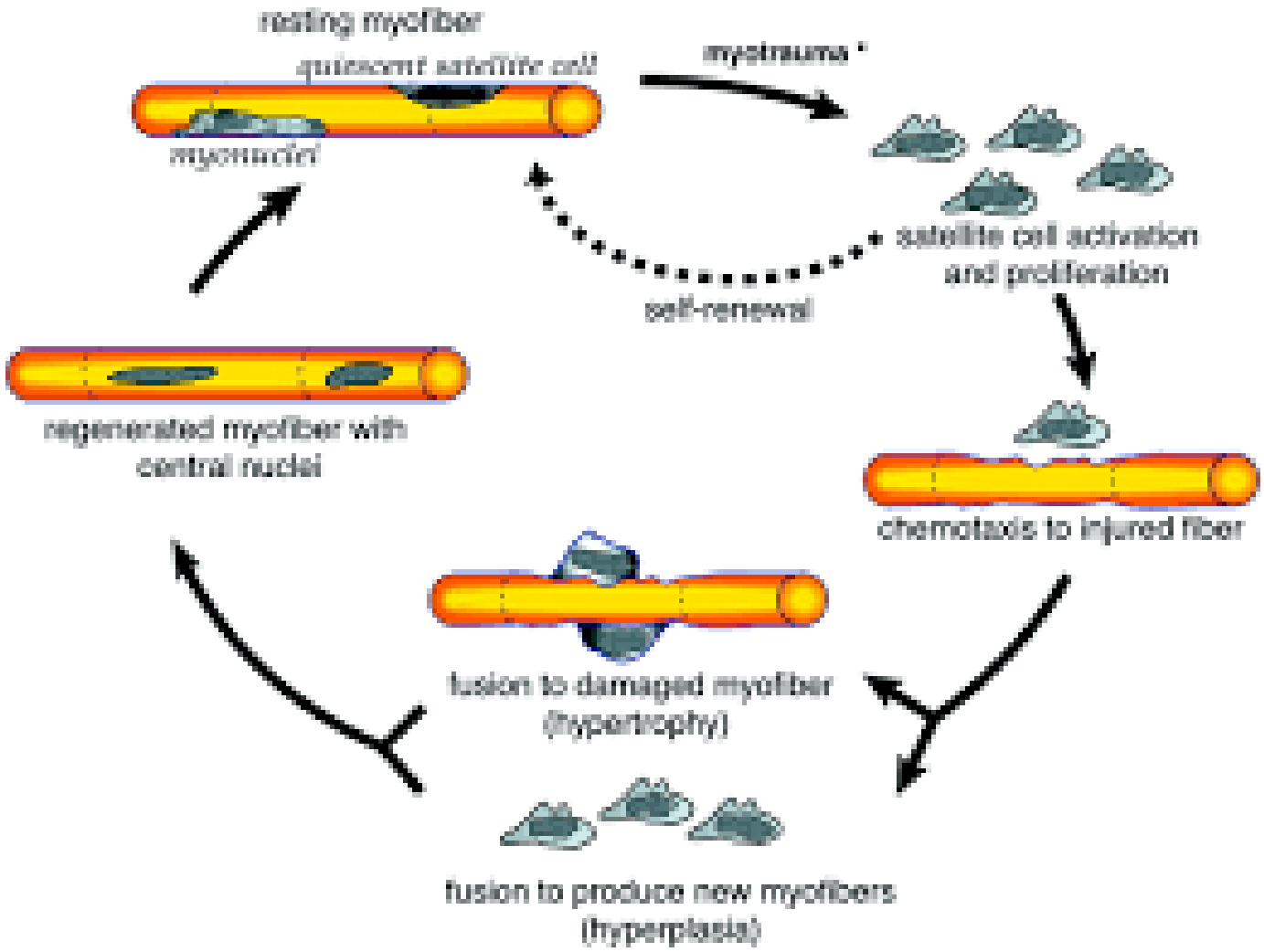
Altered Vascular Supply

Vasoactive agents such as **serotonin** and **adrenalin** produce damage to type I and some type II fibres dependent upon oxidative metabolism. This effect is attributed to oxygen deficiency.

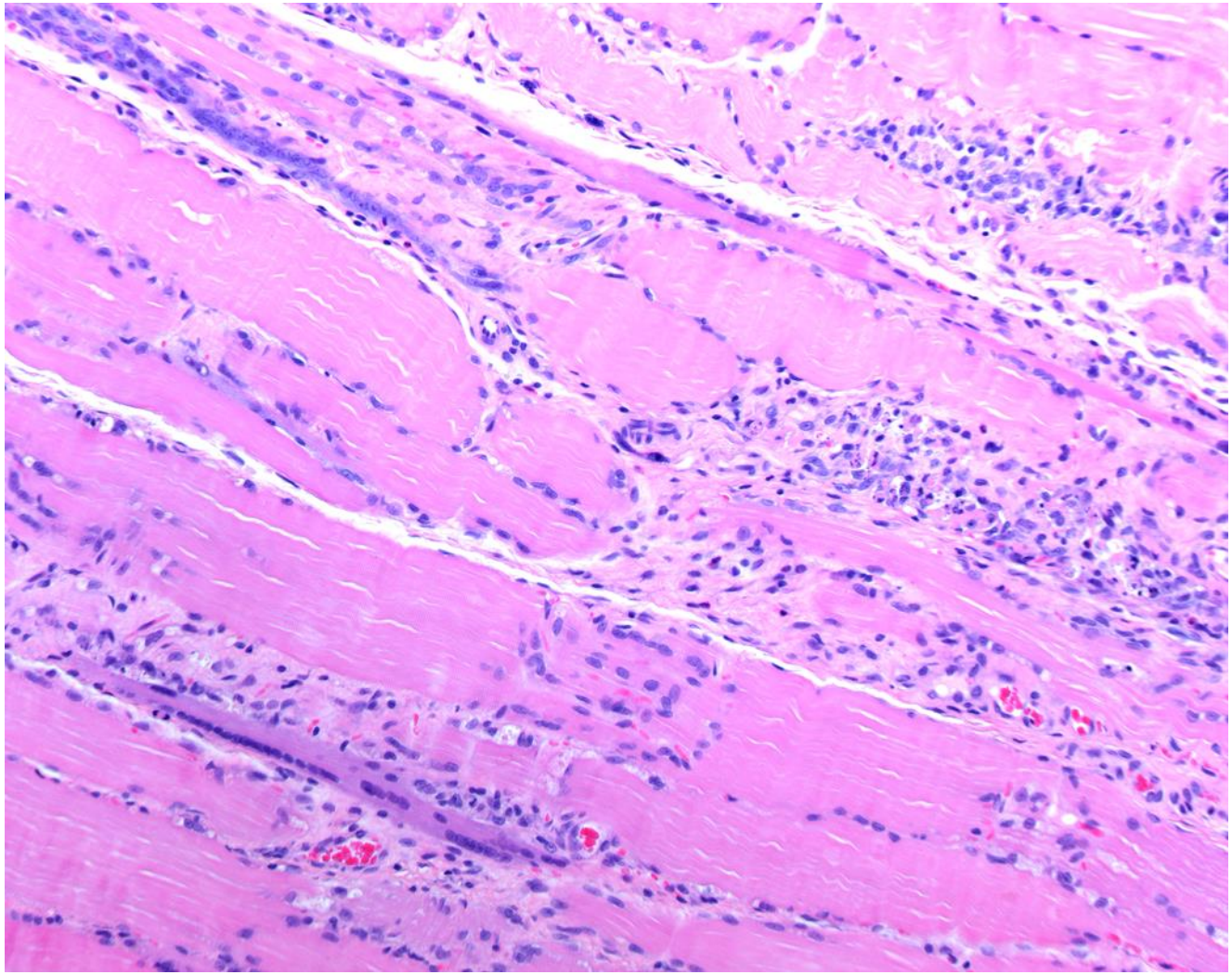
Myofibre regeneration and repair

- Myofibre degeneration and necrosis can be produced by a variety of agents and often only affects a portion of the cell.
- Regeneration is possible provided the framework of the lamina externa and the innervation and blood supply to the affected area are intact.
- A reserve population of highly resistant undifferentiated satellite cells lie between the lamina externa and the sarcolemma differentiate into myoblasts.
- These myoblasts then fuse to form multinucleate myotubes.
- These then undergo normal cellular organisation to form replacement muscle fibres.
-

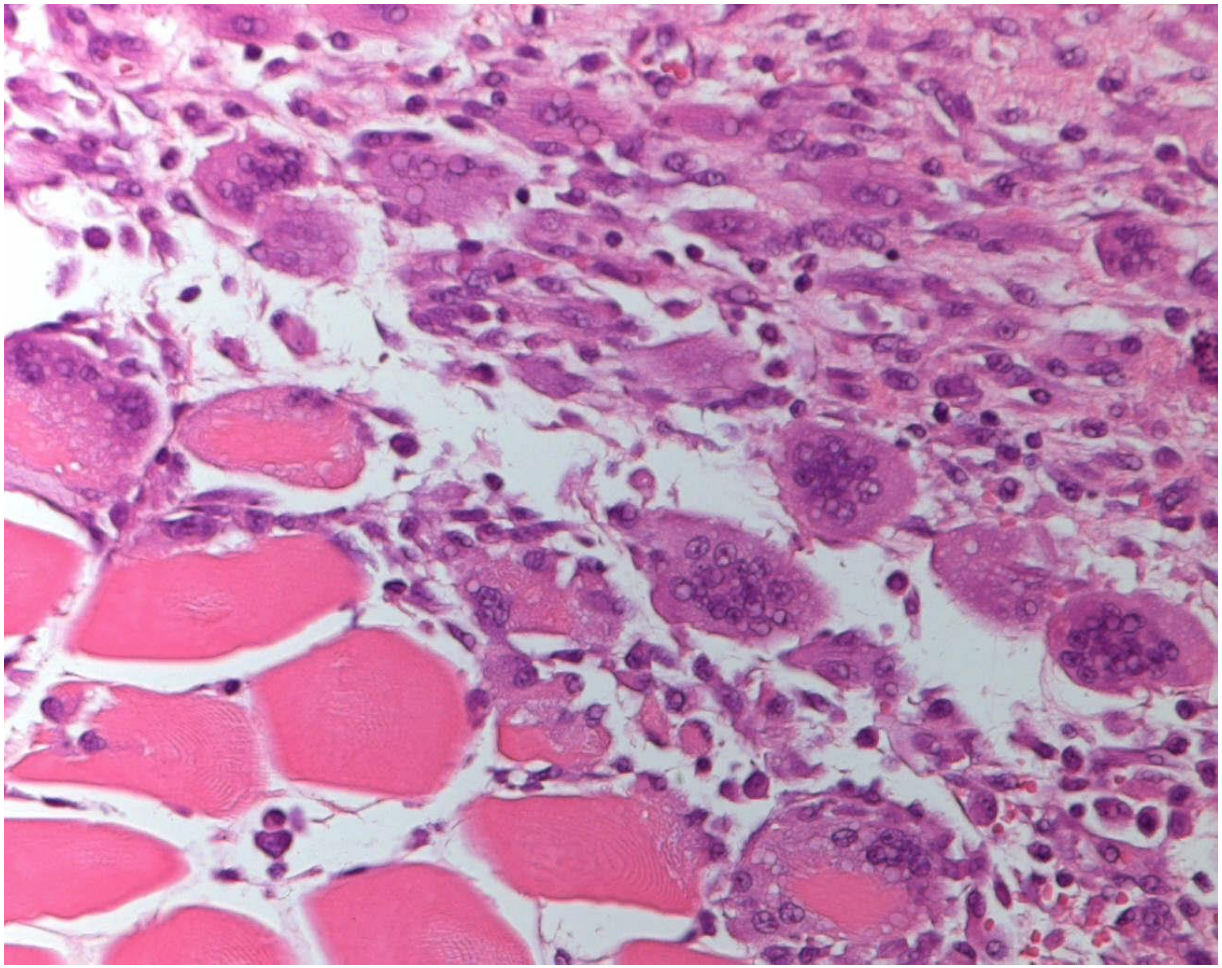
Regeneration and Repair



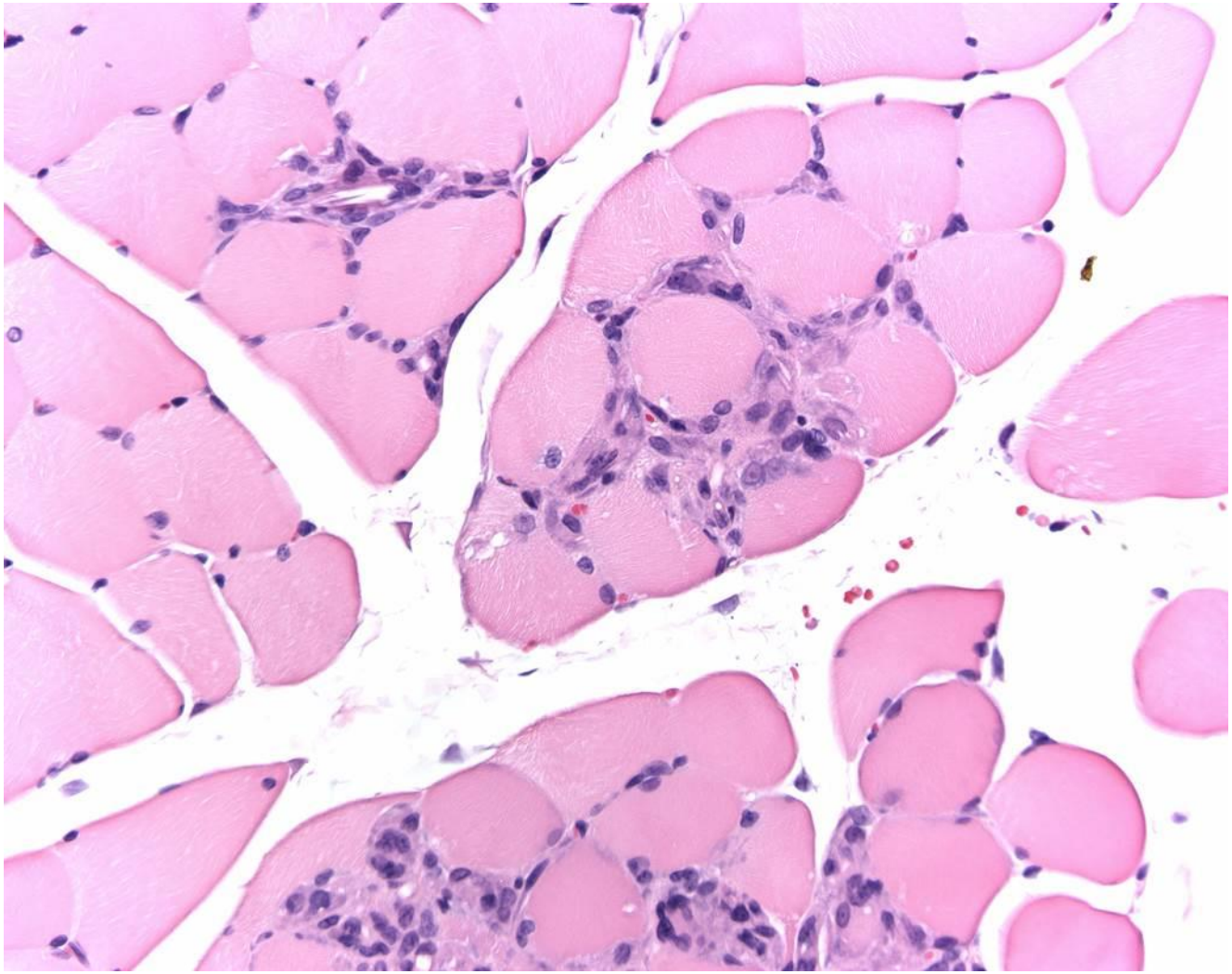
Rat – skeletal muscle, early regeneration, satellite cell migration



Regenerating myofibres, central nuclei



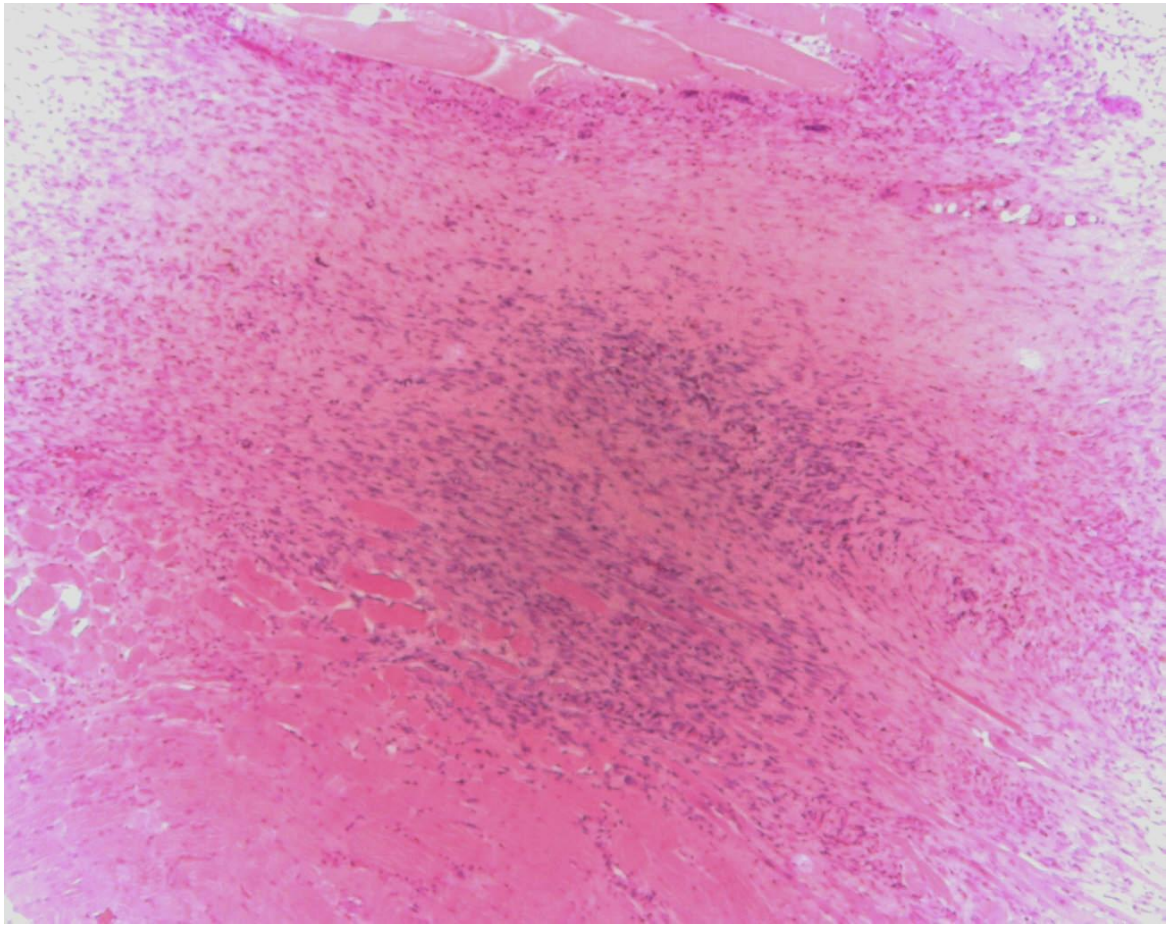
Rat, skeletal muscle, foreign body reaction



However….

- If there is extensive disruption of endomysial connective tissue and the external lamina framework is damaged then only limited regeneration can occur, accompanied by extensive fibrosis and scarring. Fatty replacement of the muscle is sometimes seen.
- This response may be seen in some intramuscular injection studies where there has been extensive necrosis, haemorrhage, trauma or infection.

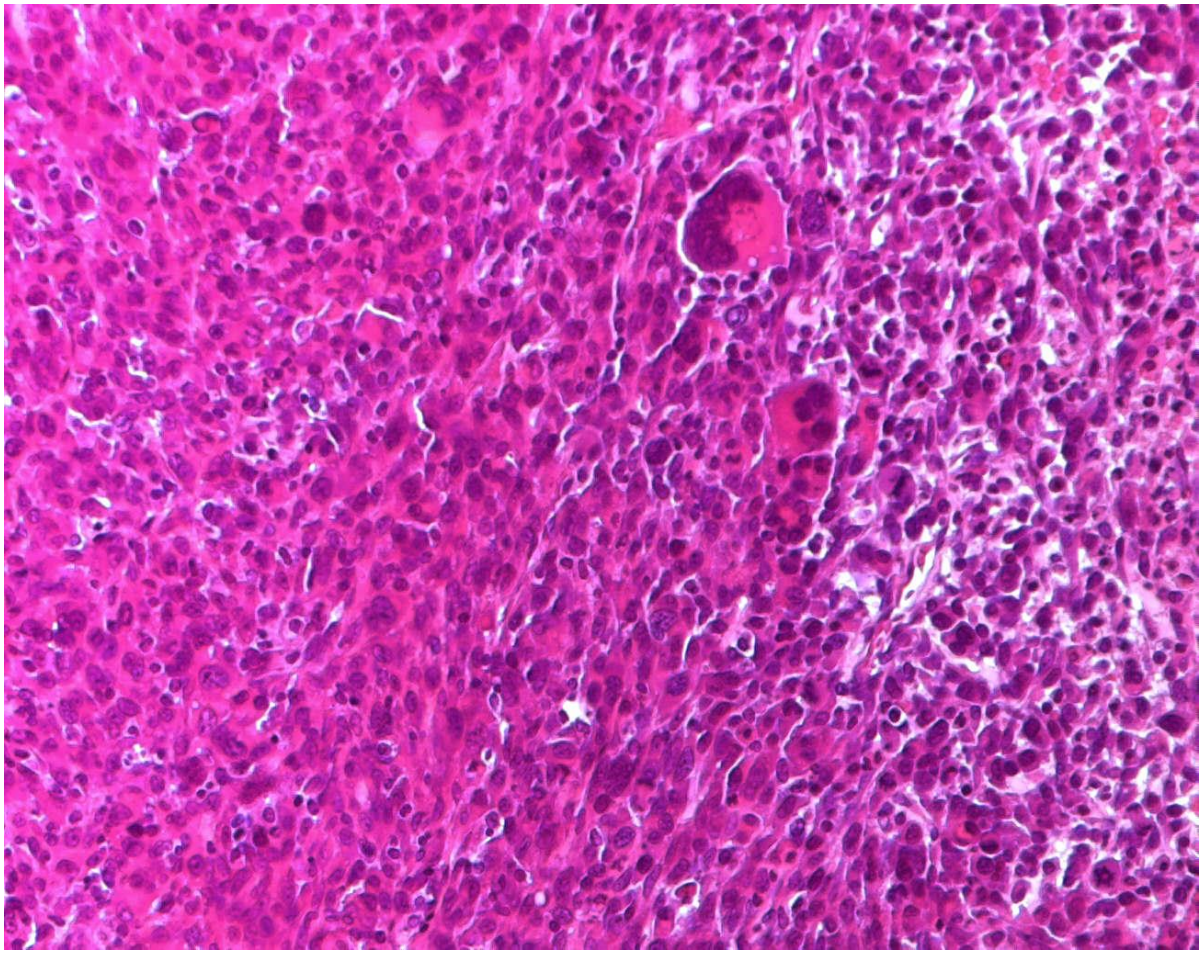
Injection site - fibrosis



Tumour Induction

- Intramuscular or subcutaneous injection of **nickel, cobalt** and **cadmium** compounds can result in the induction of malignant tumours (rhabdomyosarcoma)
- Implanted materials – bone and muscle tumours
- Microchips – occasionally associated with rhabdomyosarcomas in pets

Rhabdomyosarcoma - bizarre cells, pleomorphism, giant cells

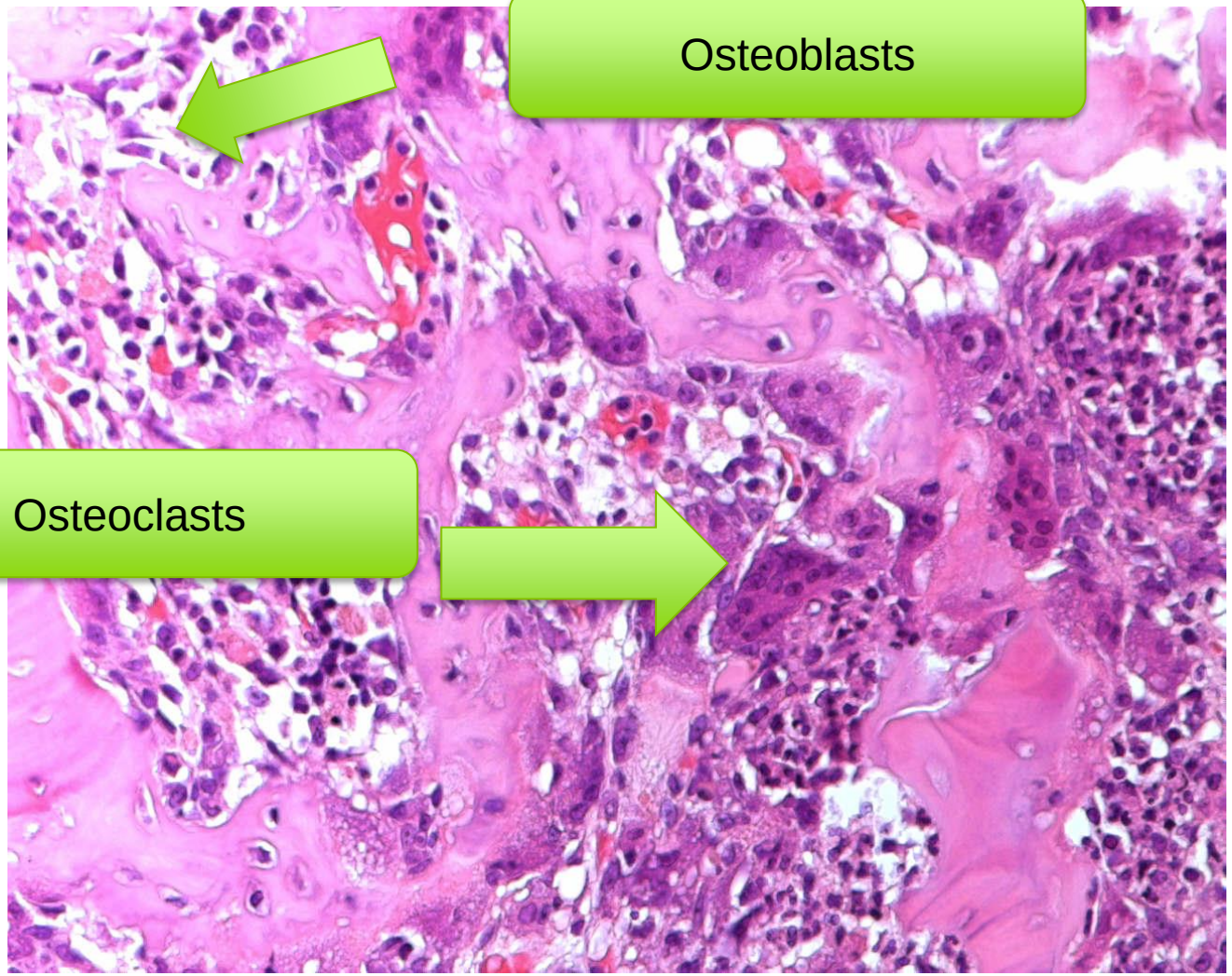


Bone

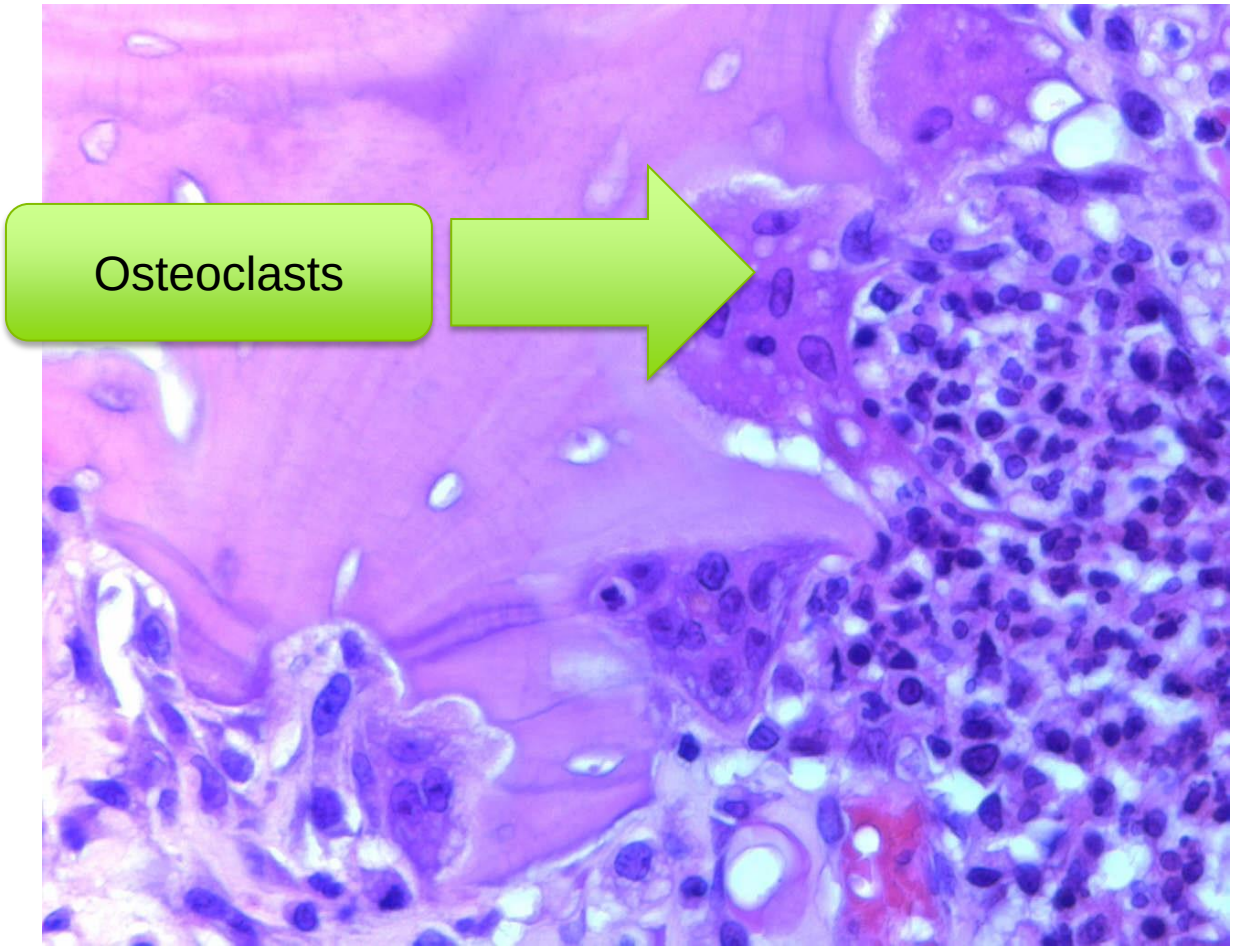
Physiology

- Osteoid formed by osteoblasts, derived from primitive pluripotential mesenchymal cells
- Bone resorbed by osteoclasts, derived from pluripotential hemopoietic stem cells (G-M colony forming units)
- Close interaction between osteoblasts and osteoclasts
- Many hormones, cytokines and factors involved in bone remodelling – growth hormones, calcitonin, parathormone, steroids etc

Bone histology



Bone osteoclasts



Bone - Responses to Injury

+ **Localised**

1. Bone implants – inflammation, necrosis, new bone formation etc
2. Fractures

+ **Systemic**

1. Mineral imbalances
2. Hormonal alterations
3. Vascular insufficiency

Local Toxicity

- This may occur as a secondary effect to the implantation of orthopaedic prostheses in fracture repair, often metal alloys such as **titanium-aluminium-vanadium** or **chromium-cobalt-molybdenum**.
- A zone of degenerate bone forms around the implant due to bone lysis initiated by these metals.
- Fractures may result secondary to the above changes
- Tumours can also develop with long-term localised exposure

Systemic Toxicity

- There are several responses to toxic insult, which can affect cartilage or bone or both.
- Bone is constantly remodelling as it responds to stress
- Areas with high remodelling eg. epiphyses, articular surfaces are more susceptible
- Growth plates stay open in rodents, close in non-rodents during maturation, so rodents better model for juveniles
- Wide range of possible mechanisms – growth factors, hormones, cytokines, physical stresses, mineral and vitamin imbalances, vascularisation, metabolic alterations,

Increased bone mass

- Hyperostosis (osteosclerosis) – increased bone formation, can be proliferative or due to failure of resorption. Location should be specified – endosteal, periosteal, peritrabecular, medullary, subperiosteal etc
- Osteopetrosis – replacement of normal bone by atypical masses of cartilage and osteoid. Seen as genetic condition, due to defective macrophage/osteoclast/haemopoietic cell function. This is due to a deficiency of osteoclasts and a lack of response to vitamin D. Seen in various species due to dietary or metabolic imbalances
- Osteophytes – periarticular, often part of joint disease

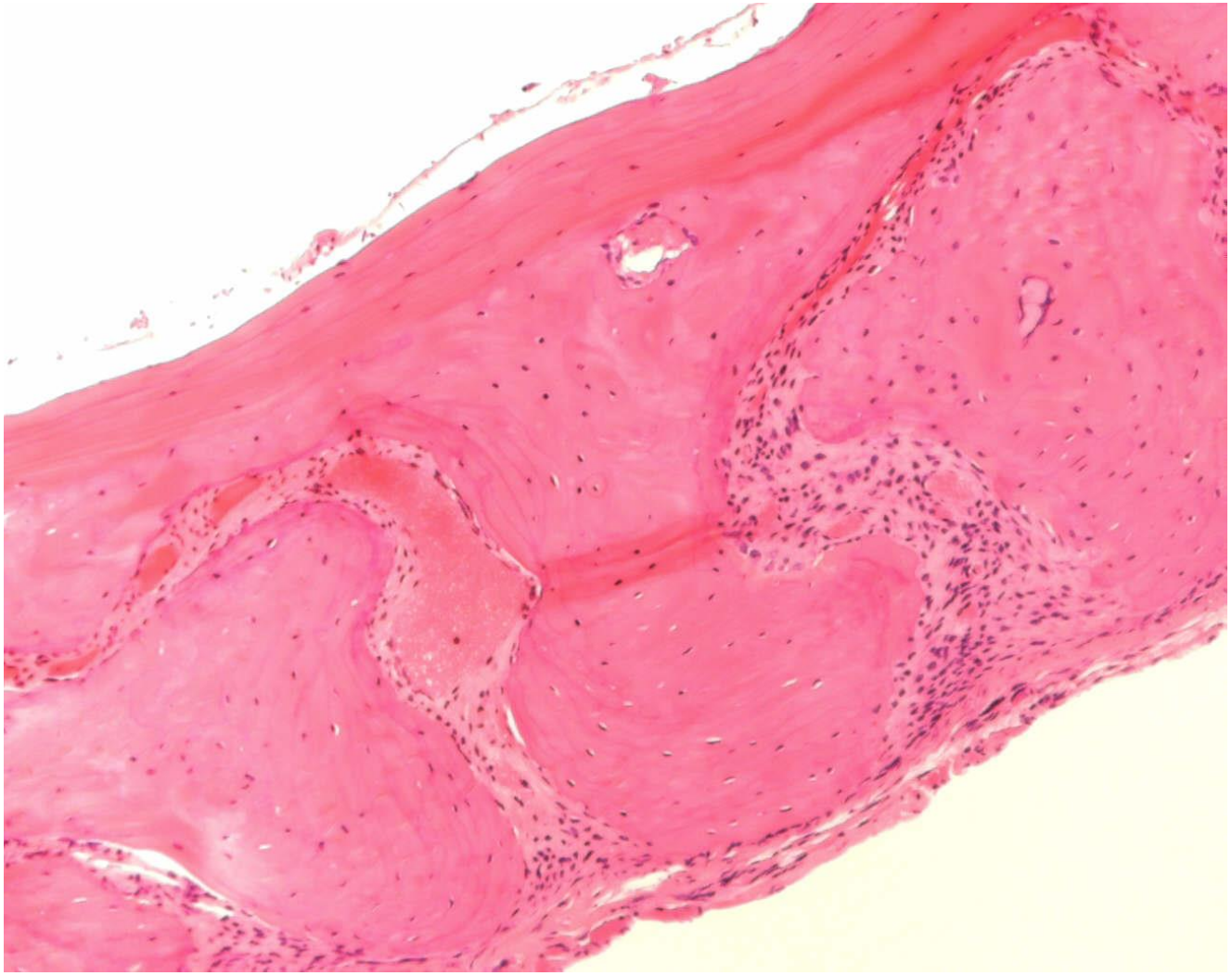
Induced increases in bone mass

- **Calcitonin** induces an increased thickness of cortical bone in rats and rabbits. This change occurs spontaneously in ageing female B6C3F1 mice and occasionally in F344 rats
- Vitamin D analogues induce increased bone deposition, particularly in the long bones
- **Zinc** is a potent inhibitor of osteoclastic activity and abolishes the effect of parathormone. Zinc also appears to regulate calcitonin from the thyroid.
- **Oestrogenic agents** increase trabeculae formation by inhibiting osteoclasts.

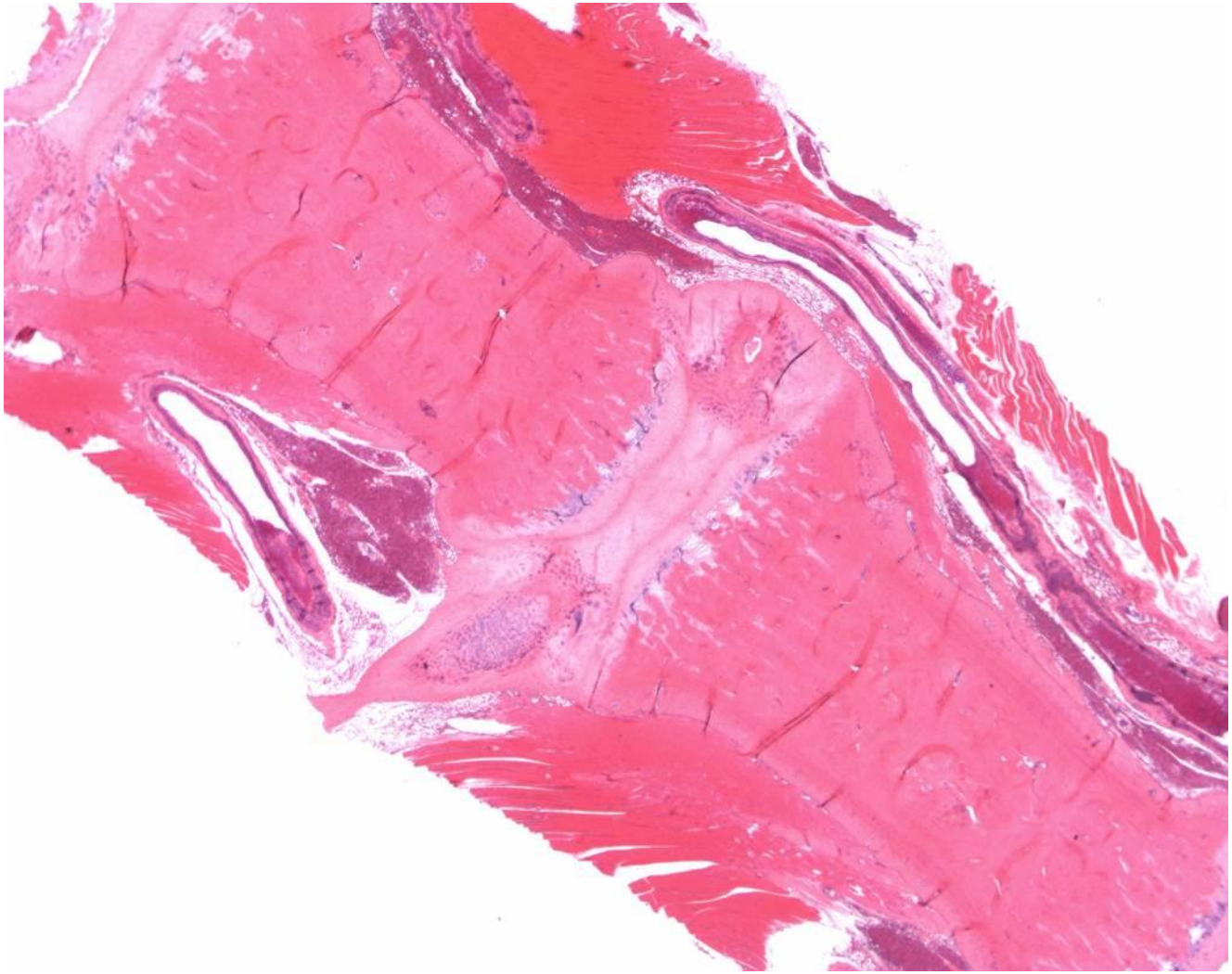
Induced increases in bone mass

- Non-steroidal anti-inflammatory drugs enhance bone formation. Non-steroidal anti-inflammatory drugs and prostaglandins enhance bone formation.
- **Bisphosphonate compounds** inhibit osteoclast activity by reducing the capacity of these cells to secrete into the resorption space and to become detached from the bone matrix. This is used to treat age-related bone loss. In the growing animals, this leads to the persistence of immature bone and to increased numbers of mineralised trabeculae in the metaphysis of the adult (**osteosclerosis**)

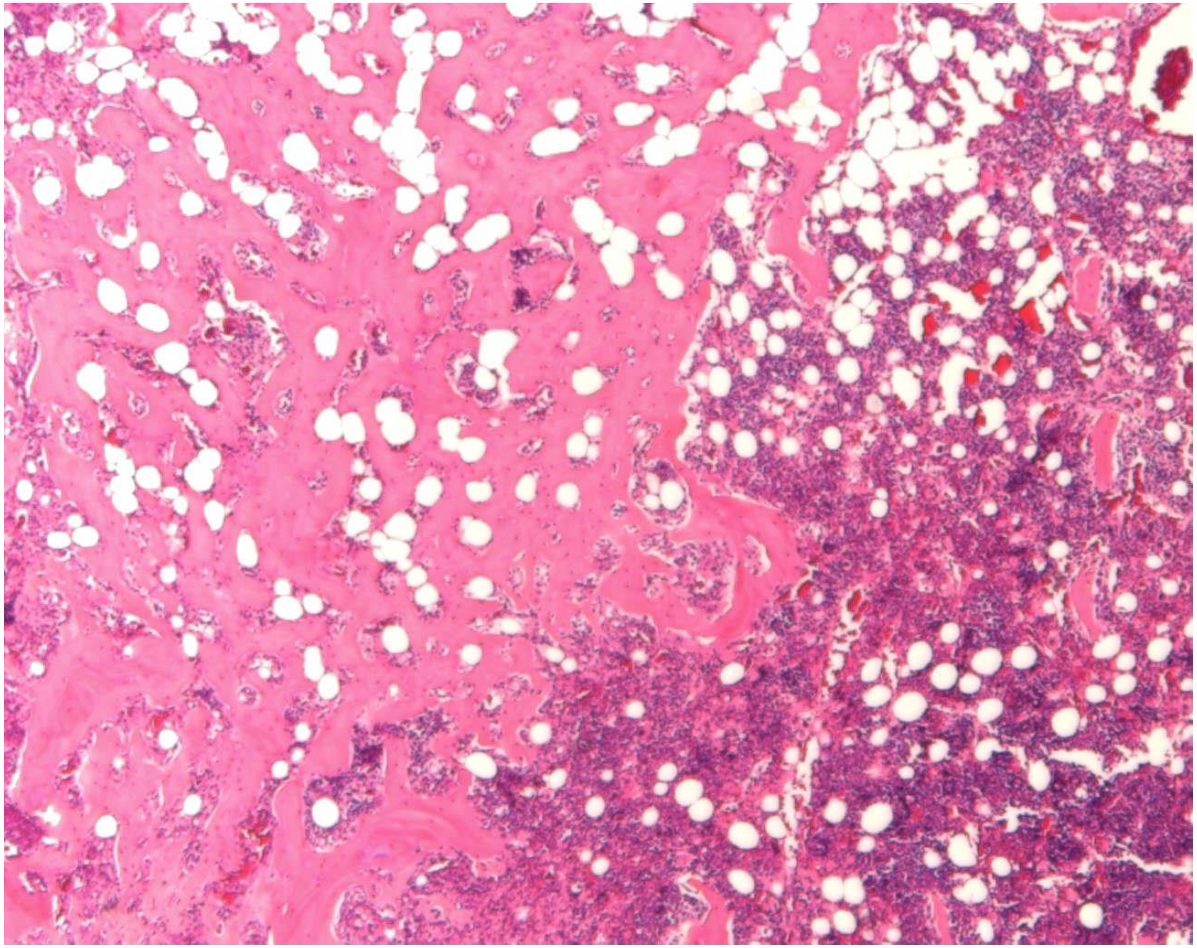
Rat - cranium



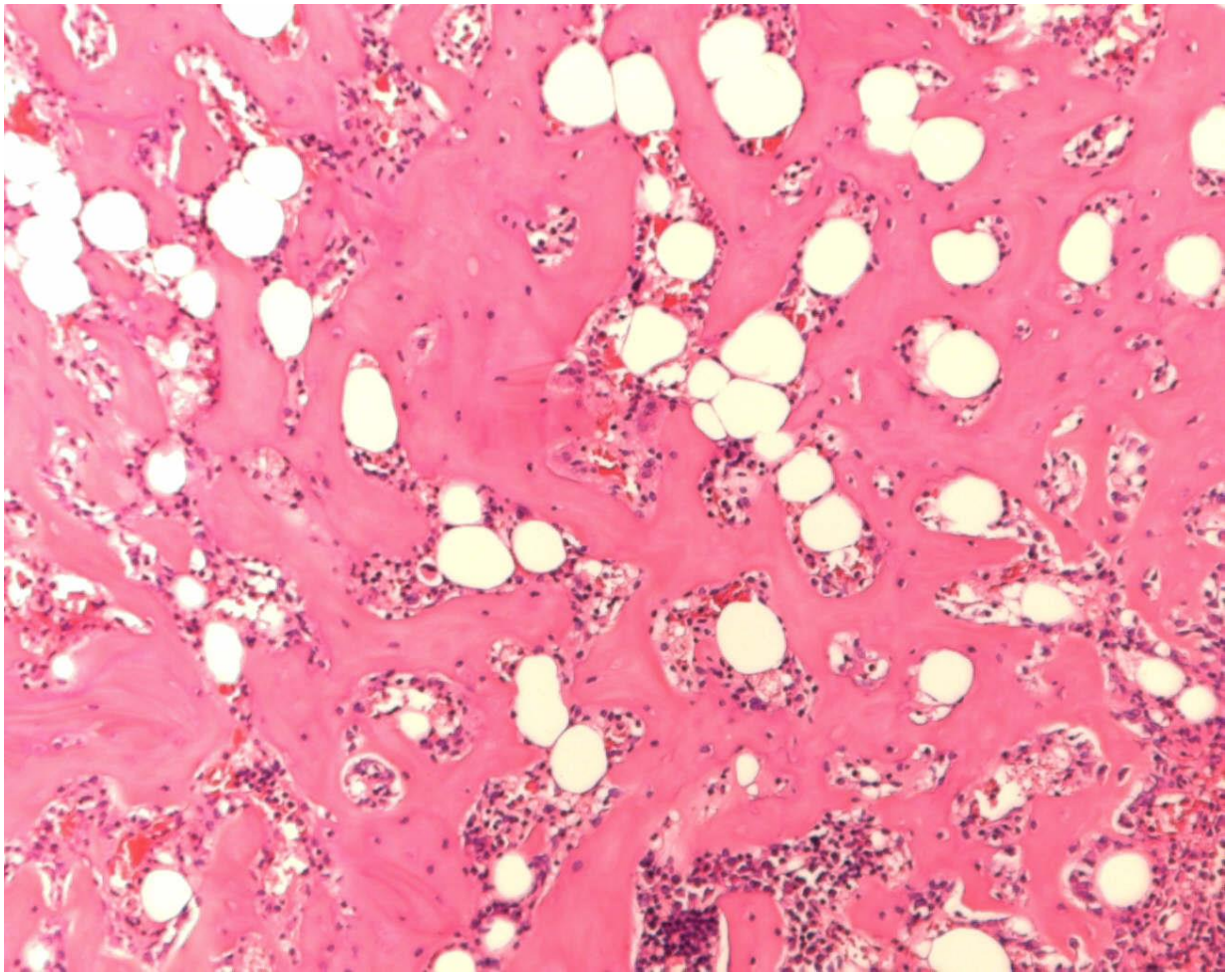
Rat sternum – endosteal hyperostosis



Osteopeterosis



Osteopeterosis



Reduced Bone Mass

- Osteoporosis – thinning, loss of mineral, common in older women due to oestrogen drop
- Defective matrix formation
- Fibrous osteodystrophy
- Altered vascular supply

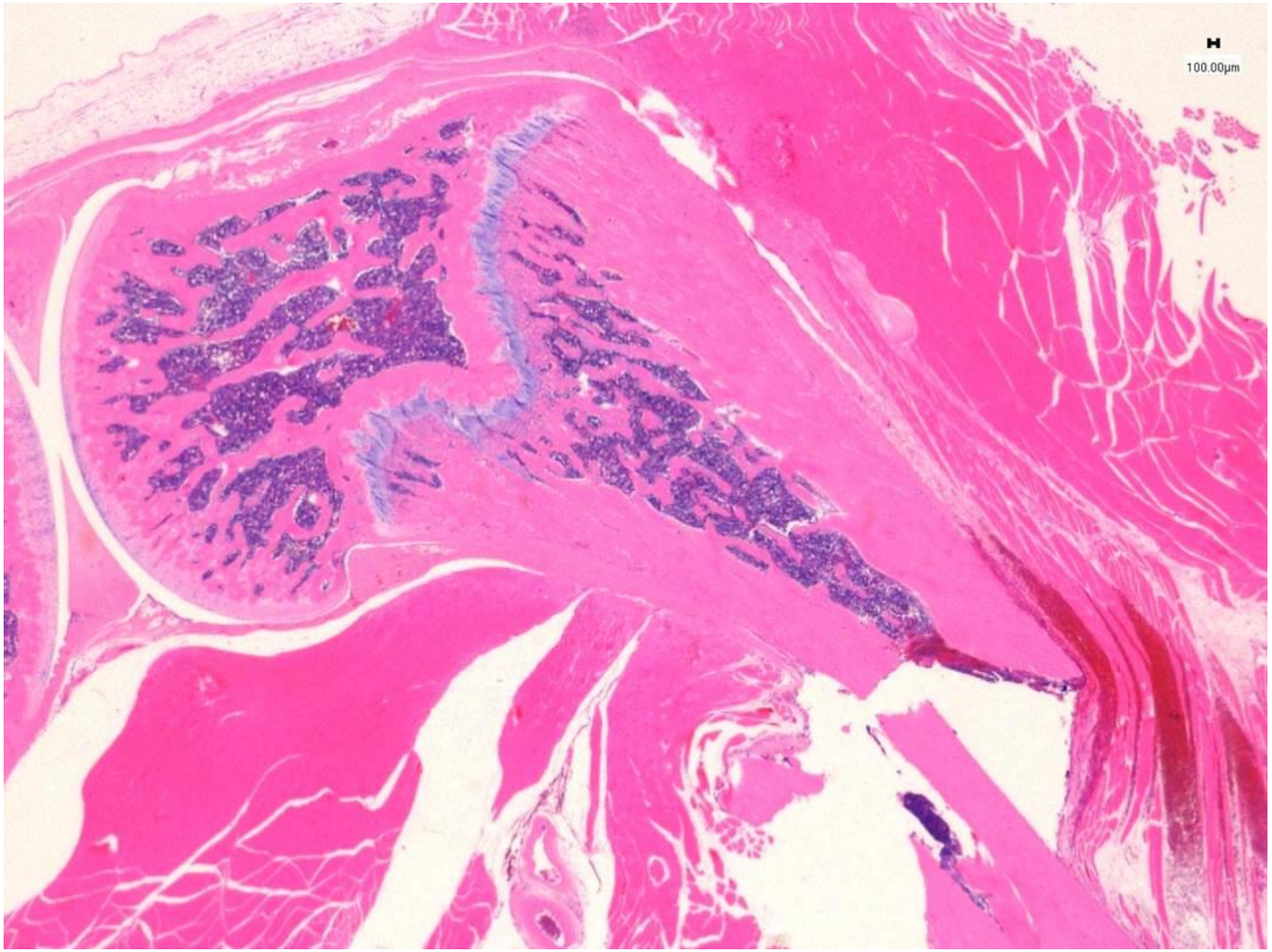
Reduced Bone Mass (Osteoporosis)

- **Lead** and **cadmium** inhibit osteoblastic activity, leading to reduced bone mass
- Enhanced osteoclastic activity induced by **corticosteroids** and deficiencies in **calcium**, **protein** and **vitamin A** lead to increased resorption of bone mass.
- Oestrogen receptor modulators eg. raloxifene
- Osteoblast inhibition by **vitamin A** and **retinoids** leads to reduced bone formation and premature growth plate closure, reduced longitudinal growth and fractures

Defective Matrix Formation

- Defective mineralisation of the matrix (**osteomalacia**) may be due to dietary deficiency of **vitamin D**, **calcium** or **phosphorus**
- **Aluminium** given by intraperitoneal injection to rats leads to increased amounts of osteoid around the cartilage of the epiphyseal growth plate
- **Fluoride** leads to reduced mineralisation and osteocyte loss

Rat femur - fracture



ENVIGO

Altered Vascular Supply

- Osteonecrosis can occur due to corticosteroid therapy, alcohol, and some lipid disorders (thought to be due to circulating lipid droplets blocking the blood supply to affected bone)
- Occurs in femoral head of spontaneously hypertensive rats, due to vessel stenosis or obstruction
- Seen in the tibia of aging ICR mice, possibly due to disturbance of blood supply
- Occurs secondary to tumours causing dysregulation of bone formation eg. myeloma

Secondary Effects on Bone

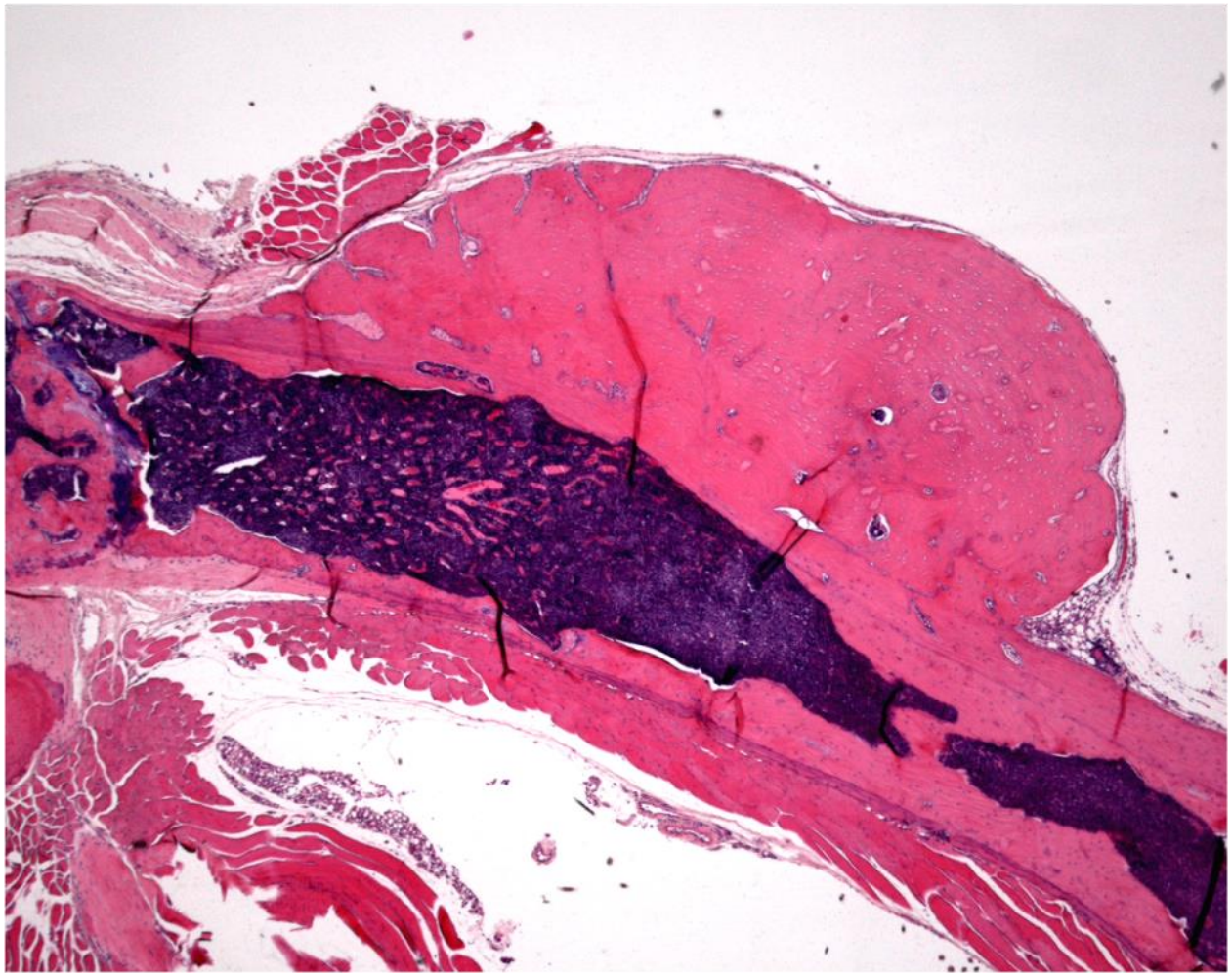
+ Hyperparathyroidism/Pseudohyperparathyroidism –

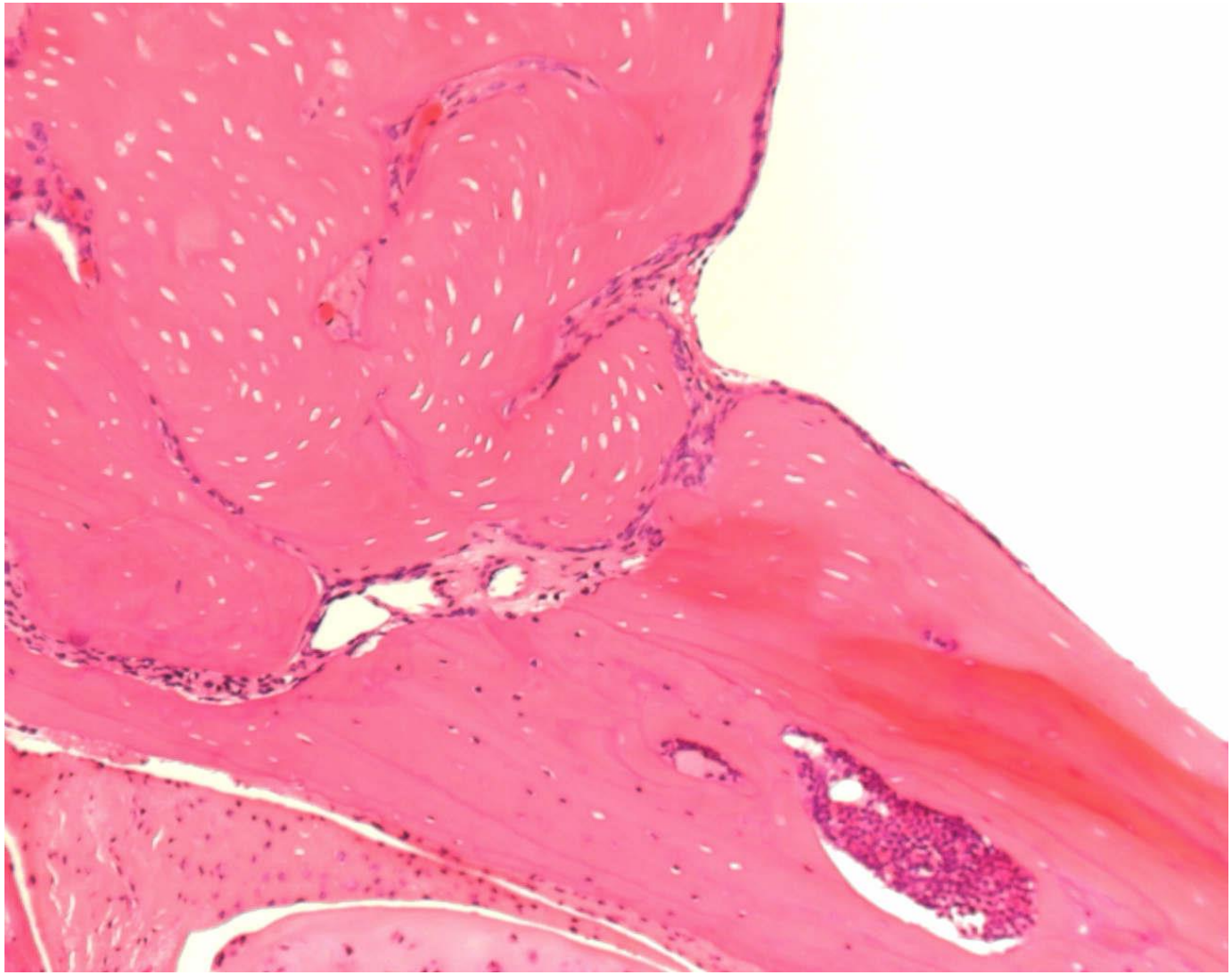
1. Falling blood calcium levels increase **parathormone** levels which stimulates osteoclast-mediated bone resorption, causing excessive absorption of matrix mineral and resulting in fibrous osteodystrophy.
2. Tumours secreting parathormone-like proteins eg. lymphoma can result in this phenomenon
3. Renal osteodystrophy in old rats secondary to CPN - parathyroid enlargement and fibrous dystrophy in the bones.
4. Altered Vitamin D metabolism – due to liver enzyme induction, seen with anticonvulsants like phenytoin

Osteoma

- Uncommon spontaneous tumours in rodents.
- Induced with oestrogens/oestrogenic compounds eg. tamoxifen
- Commoner in females
- Often multiple in some mouse strains
- Osteosarcomas can be induced by local implants, alkylating agents, polycyclic hydrocarbons, nitrosamines, nitrosoureas, acronycine, vinyl chloride and aniline dyes.
- Various morphologies and classifications in different spp – giant cell, telangiectatic, compound, fibroblastic, osteoblastic etc

Mouse femur - osteoma

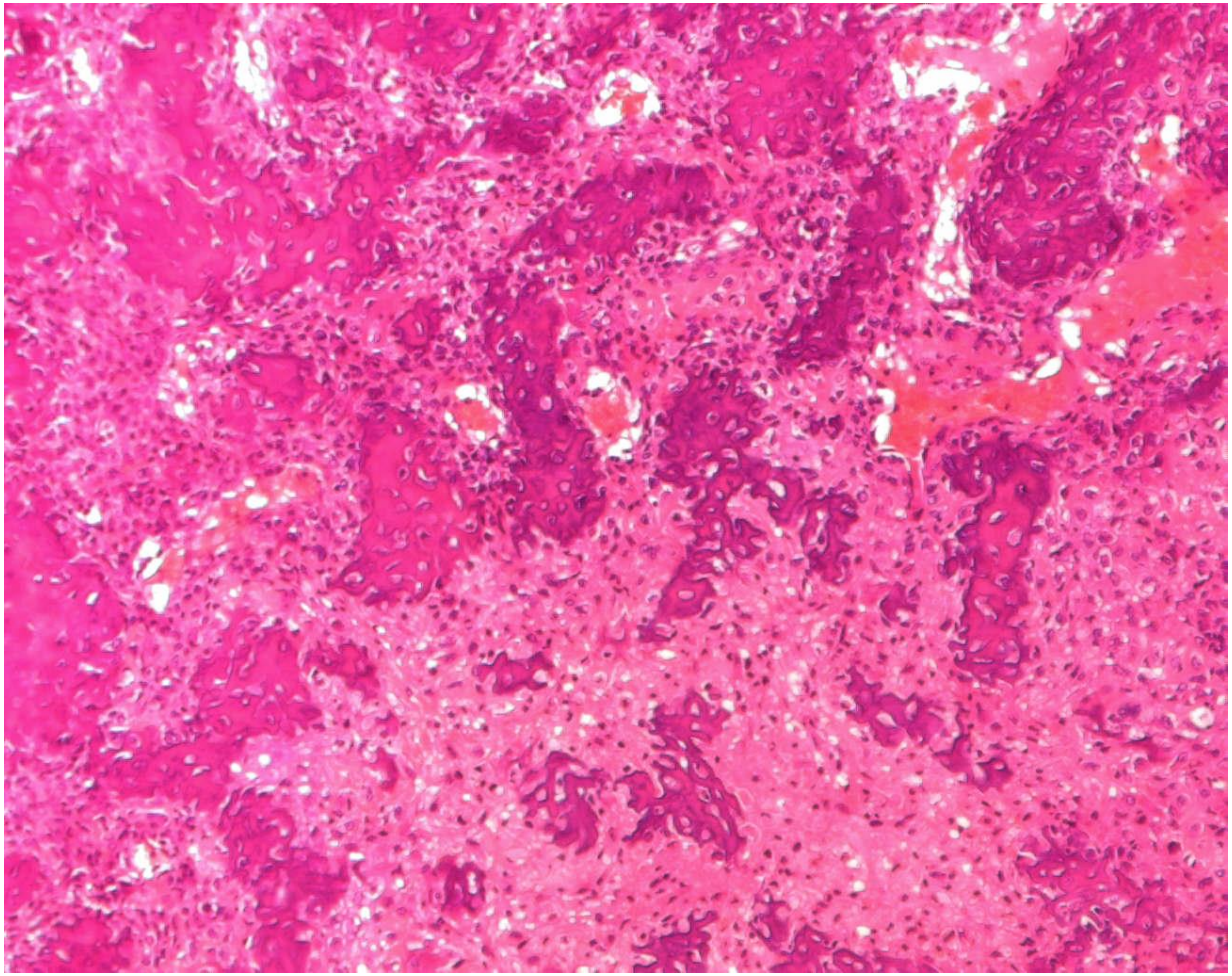




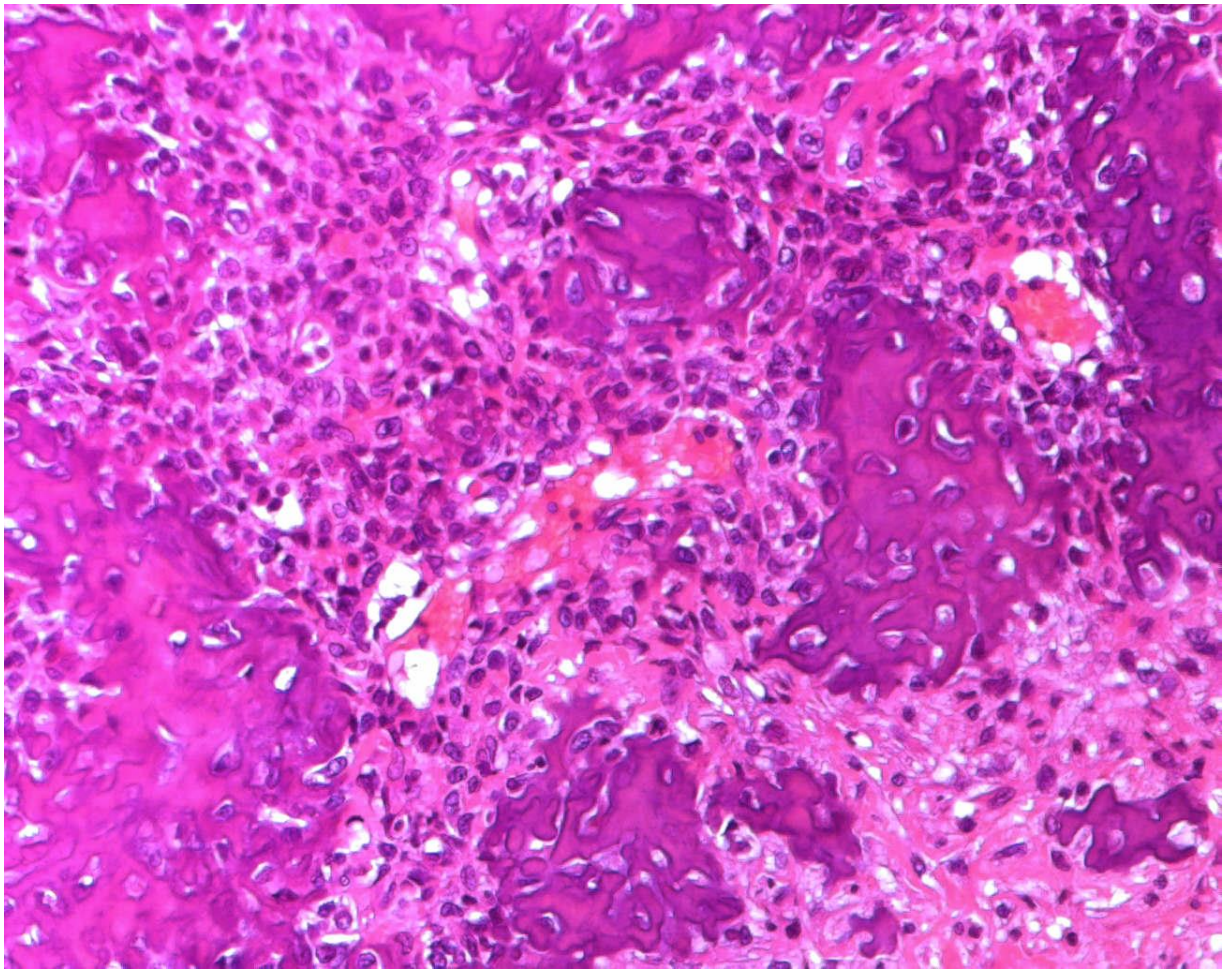
Osteosarcoma

- Seen with implants, trauma.
- Induced in models by irradiation, intratibial injection of Moloney sarcoma viruses, administration of polycyclic hydrocarbons into bone
- Systemic treatment with estrogens – probably via promotor effect rather than primary
- Teriparatide (fragment of parathormone) in rats – osteomas, osteosarcomas, osteoblastomas, probably rat-specific effect
- Several morphological classifications in different species - osteomatous, osseous, giant cell, osteoblastic, chondroblastic

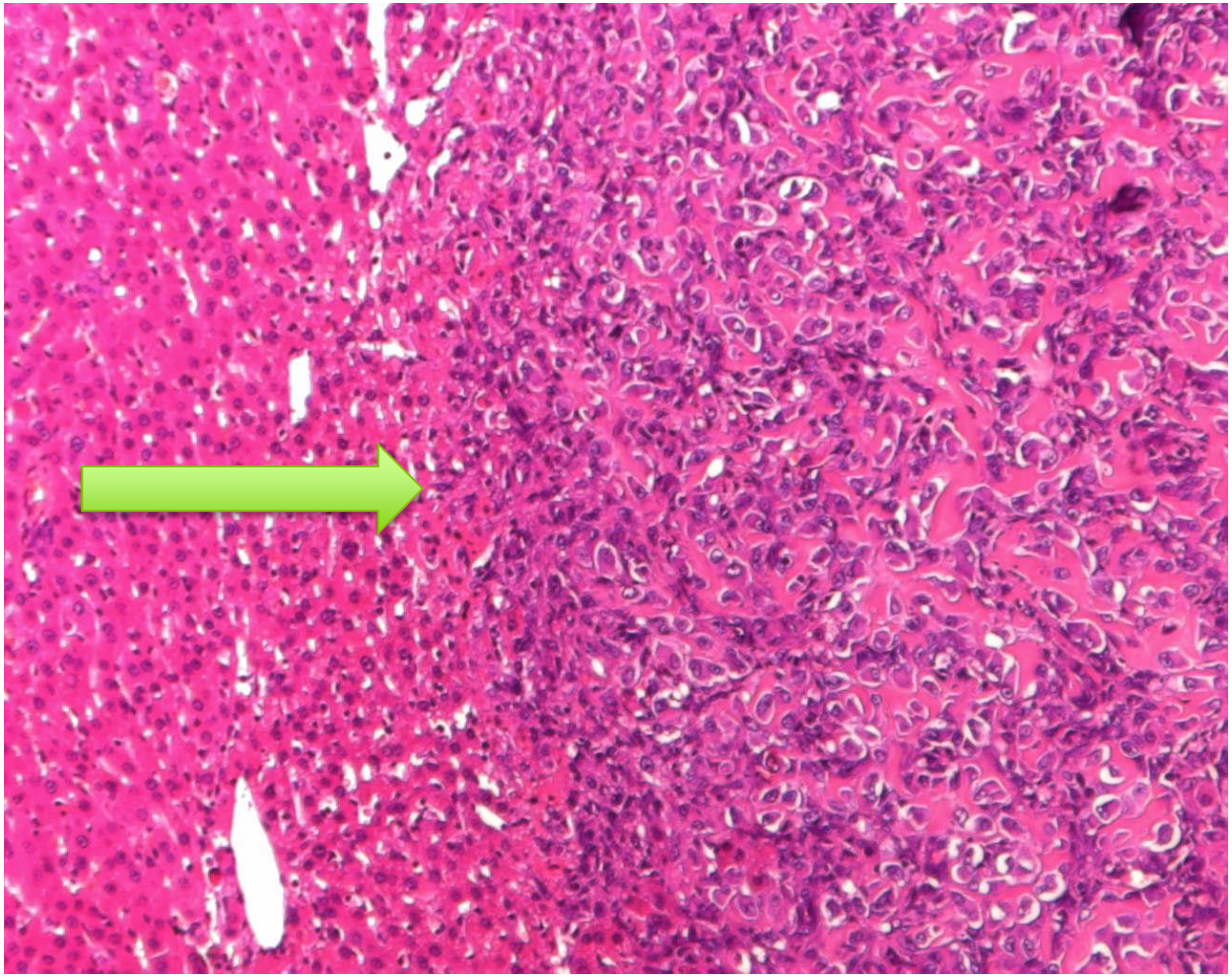
Osteosarcoma



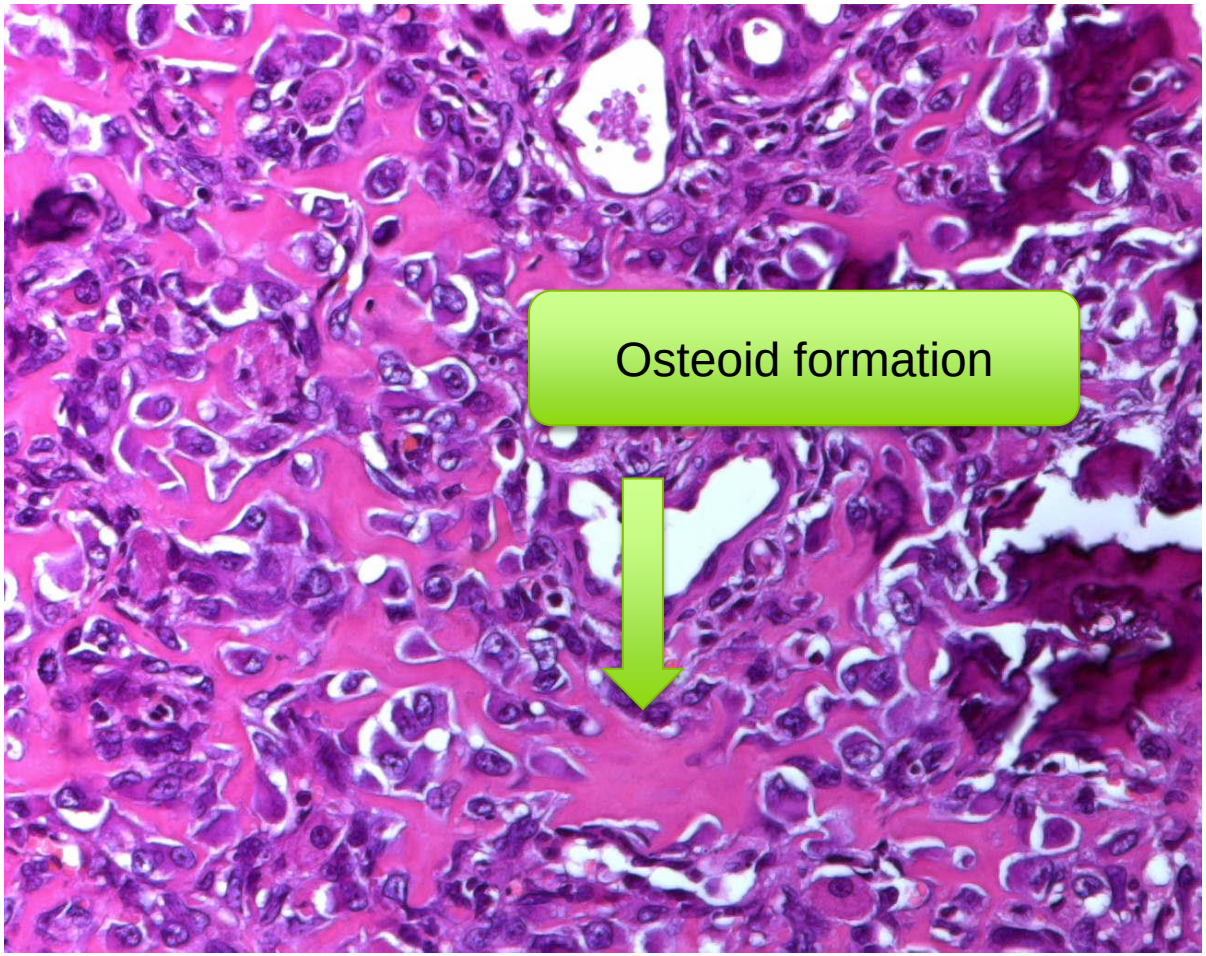
Osteosarcoma



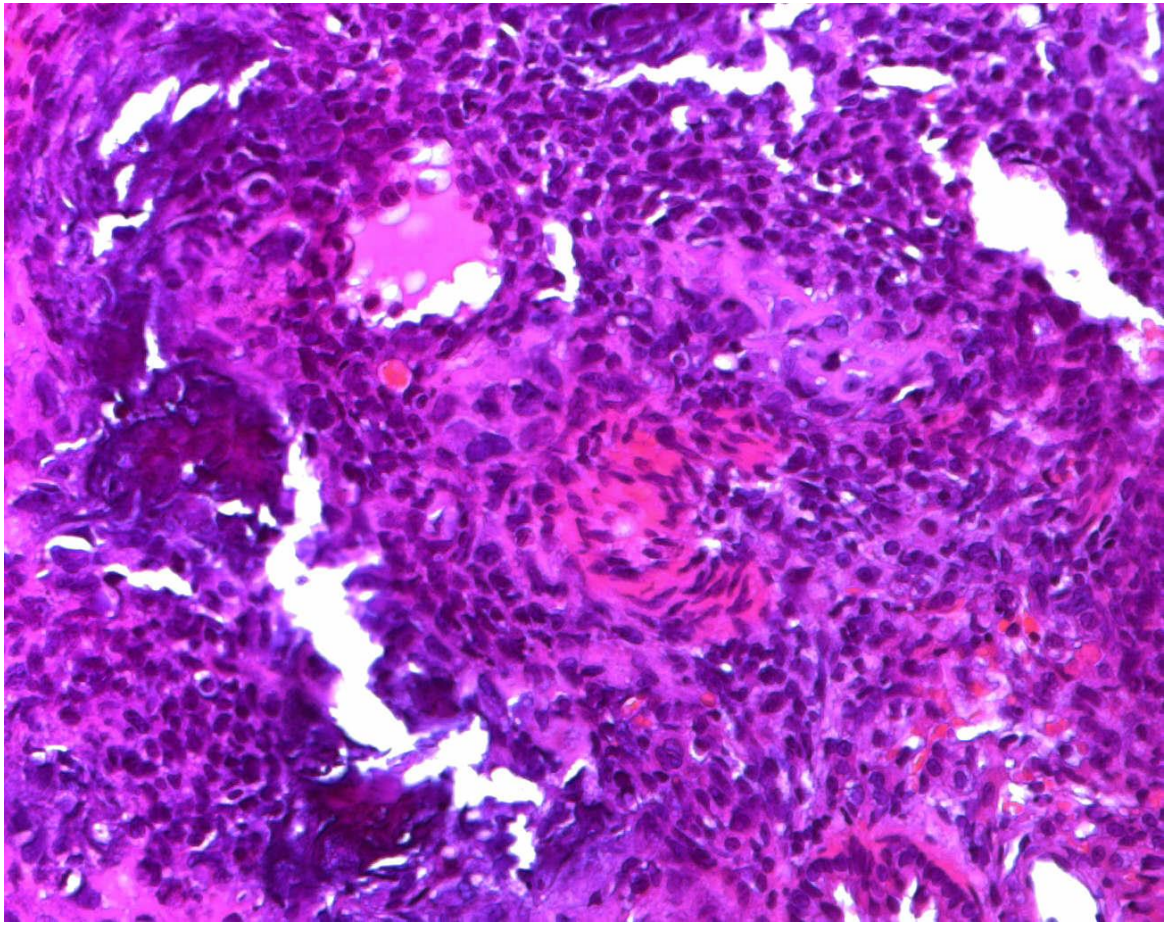
Osteosarcoma – hepatic metastasis



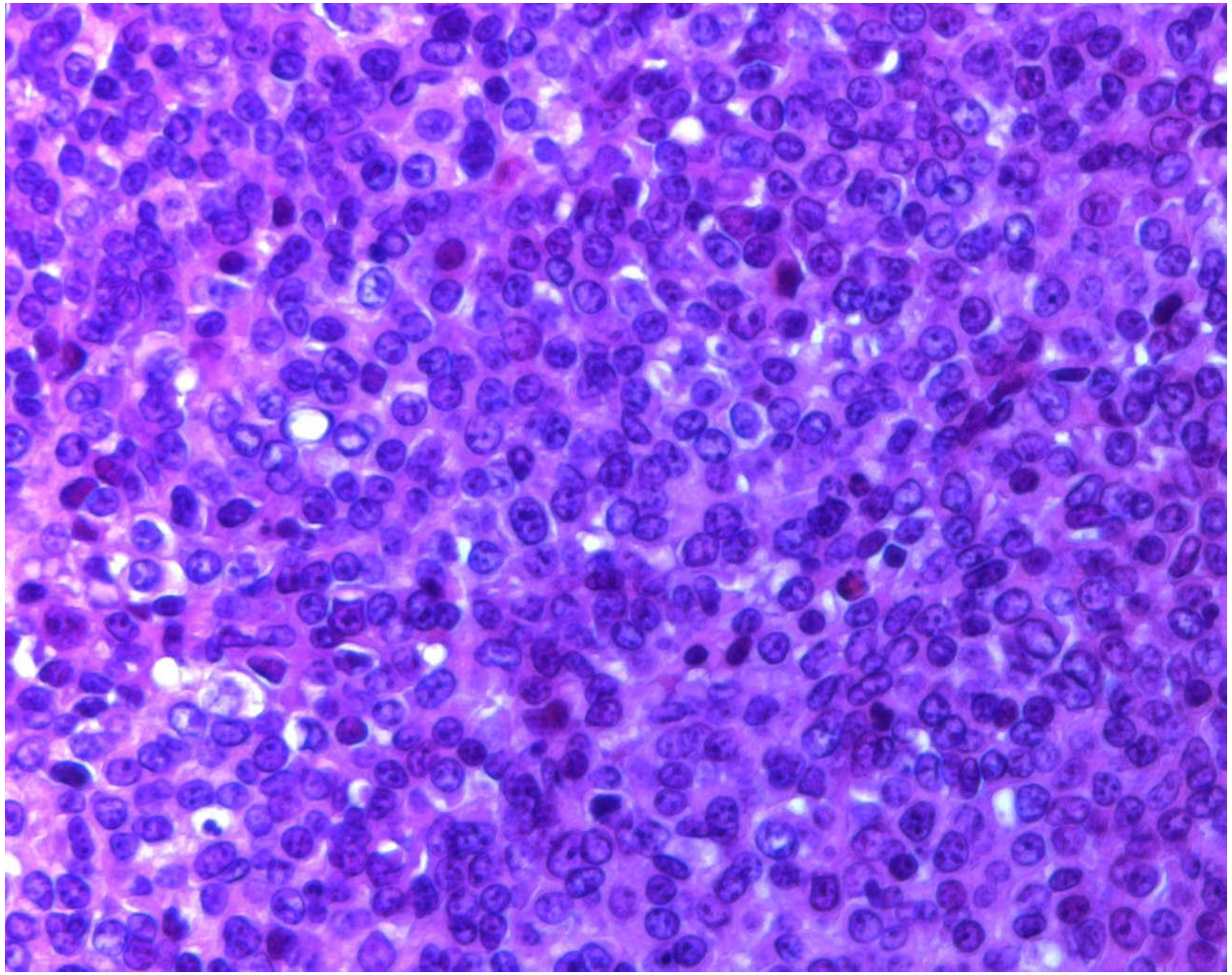
Osteosarcoma



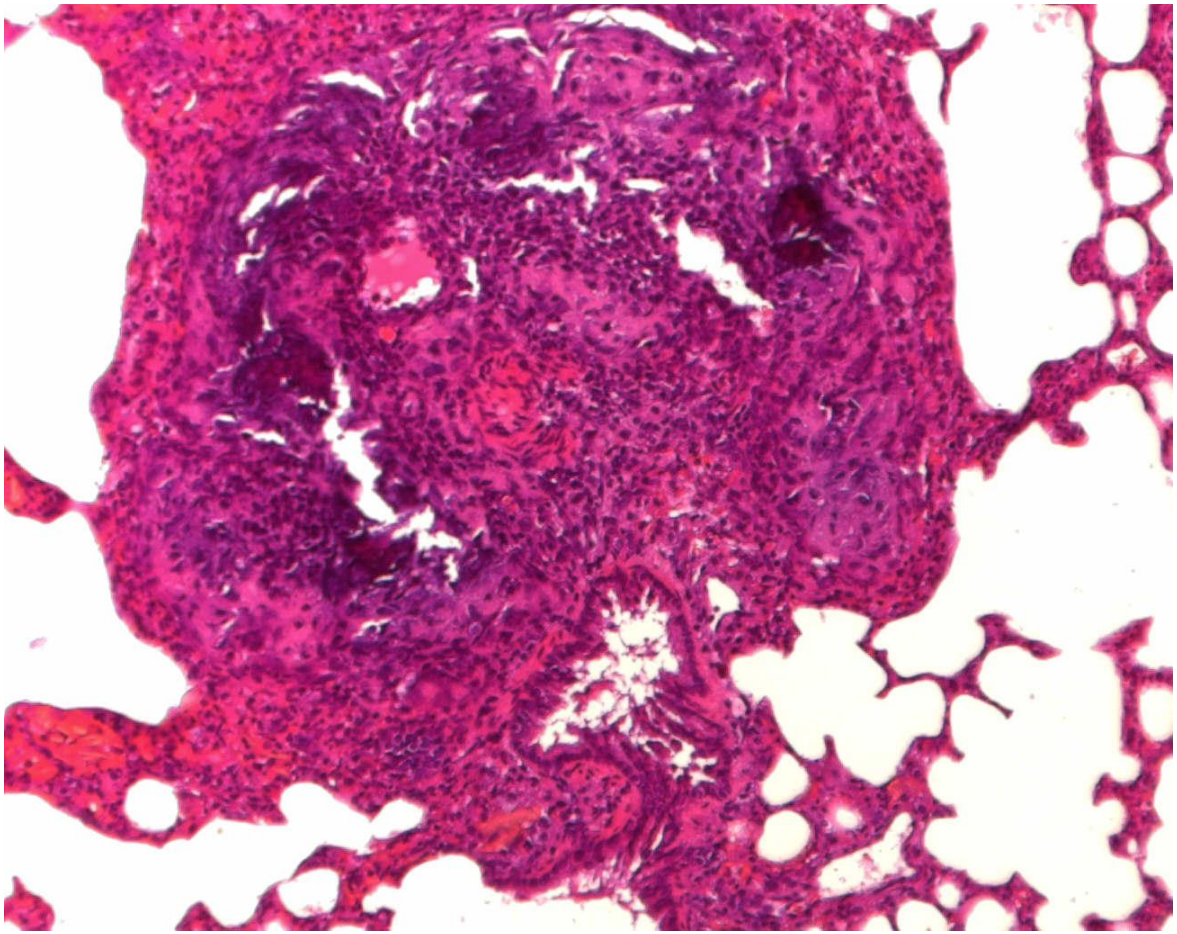
Osteosarcoma



Osteosarcoma



Osteosarcoma – pulmonary metastasis



Cartilage

Growth plate alterations

Rodents – more common

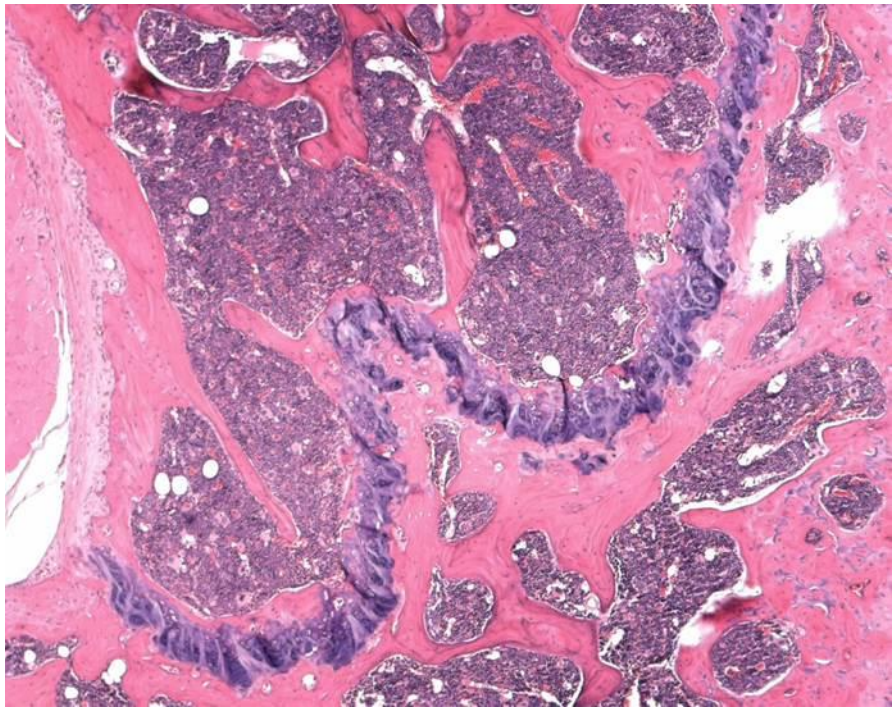
+ Thinning - steroids
+ Thickening – vEGF inhibitors
+ Disorganisation/degeneration/necrosis – quinolones, triamcinolone
+ Increased primary spongiosa - bisphosphonates

Nonrodents

+ Premature closure
+ Persistence
+ Disorganisation
+ Increased primary spongiosa

Rat epiphysis – thinning of growth plate

Control



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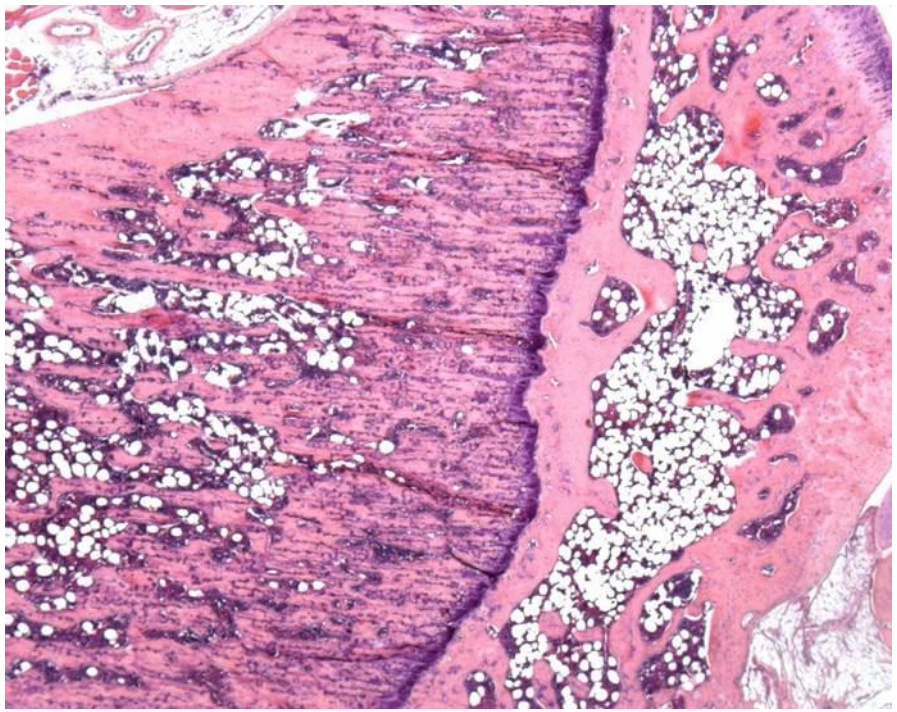
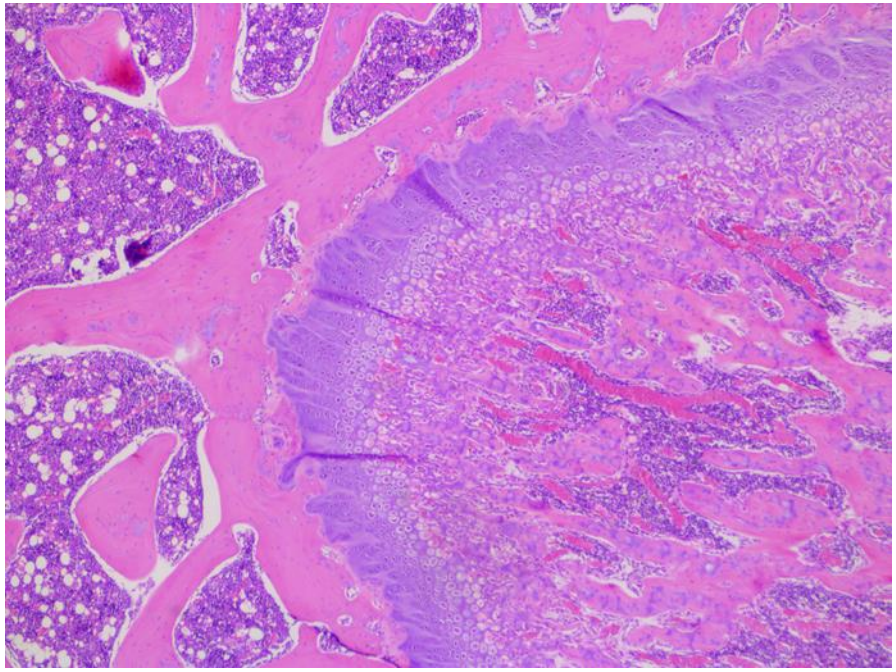


Growth plate hypertrophy

- vEGF and FGF receptor inhibitors result in widening of the growth plate due to reduced vascularisation
- Impaired mineralisation leads to increased width of primary spongiosa

Rat – growth plate hypertrophy, chondroid necrosis

Control



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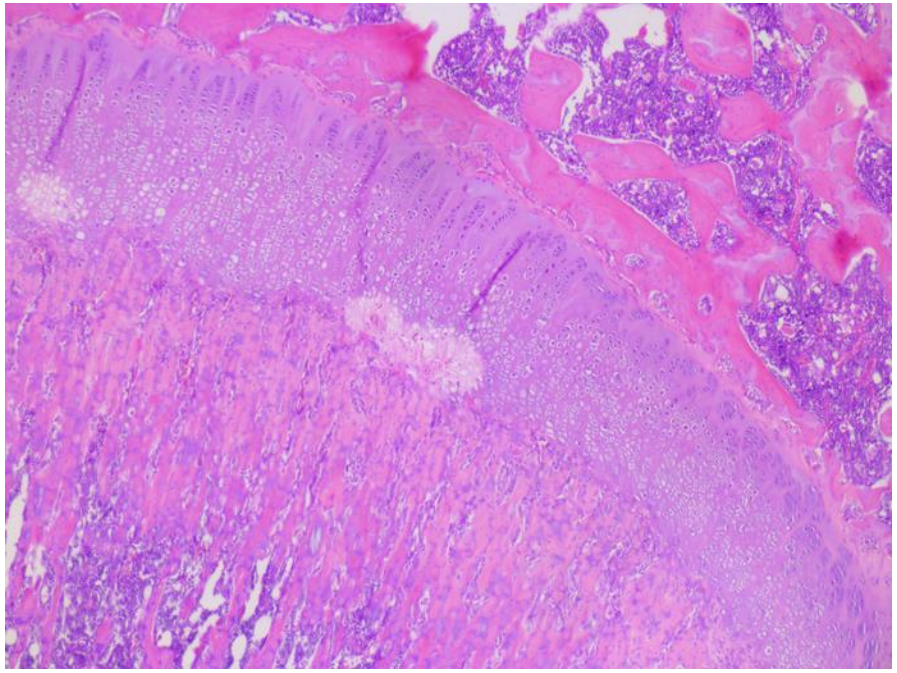
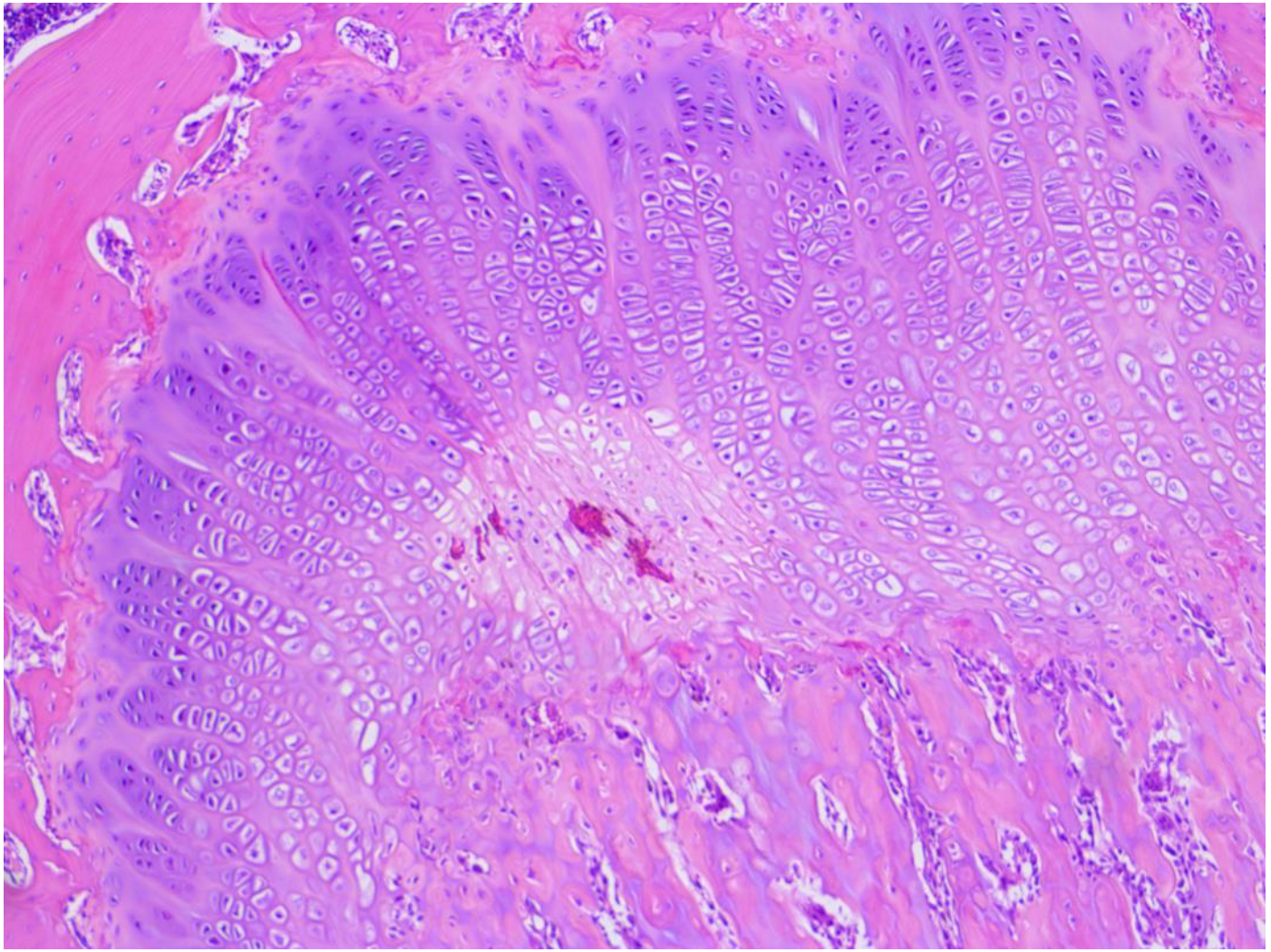
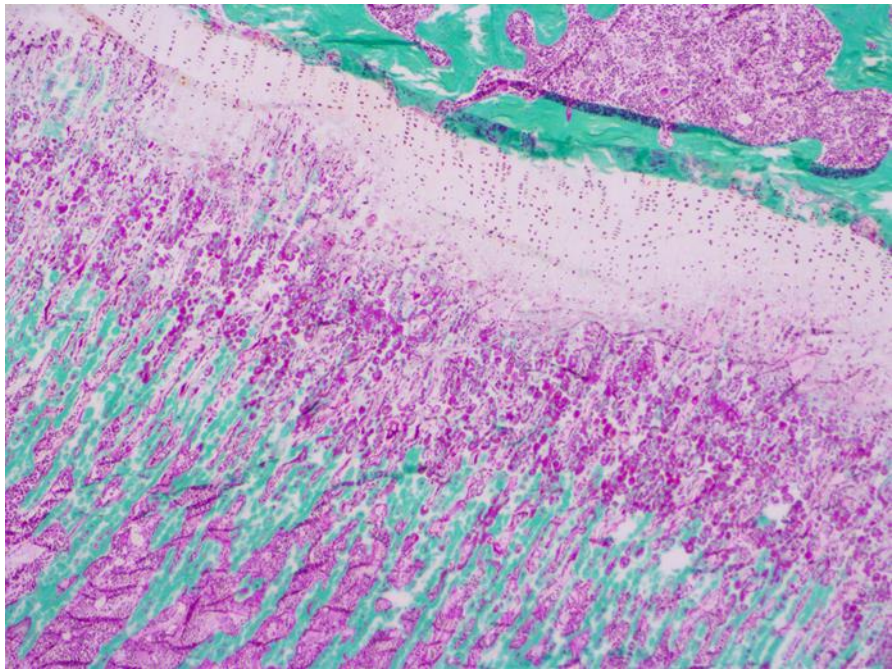


Rat – growth plate, chondroid necrosis



Rat epiphysis – increased primary spongiosa - MT

Control



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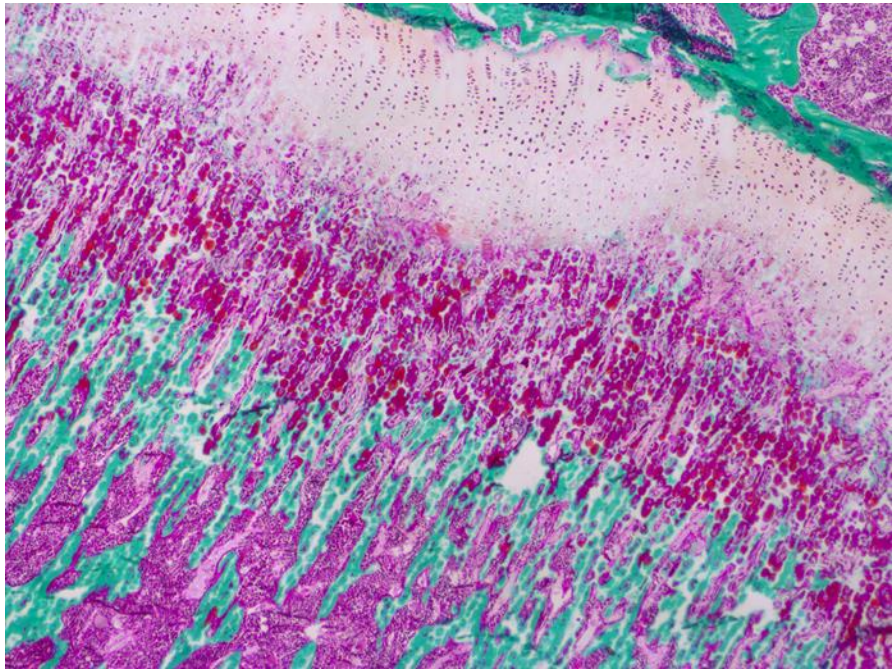
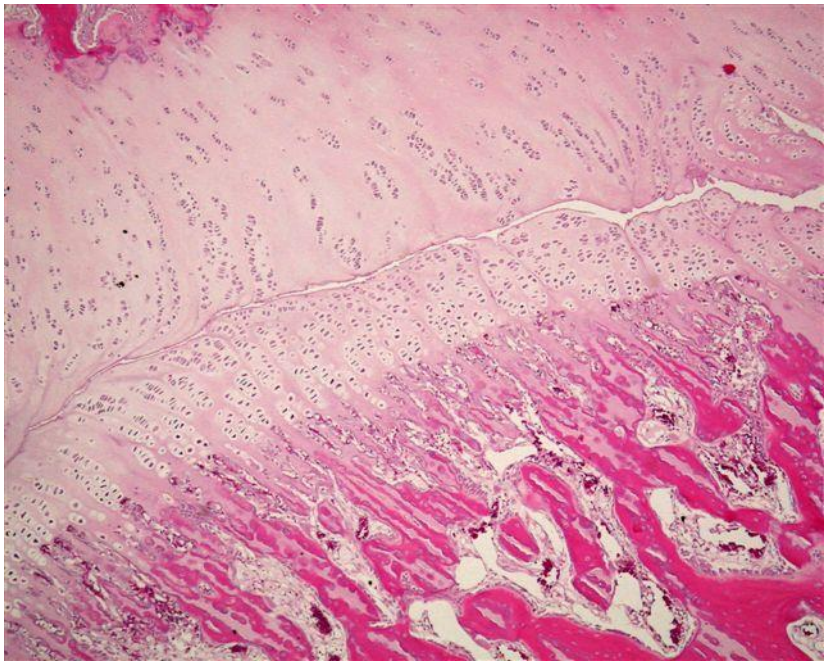
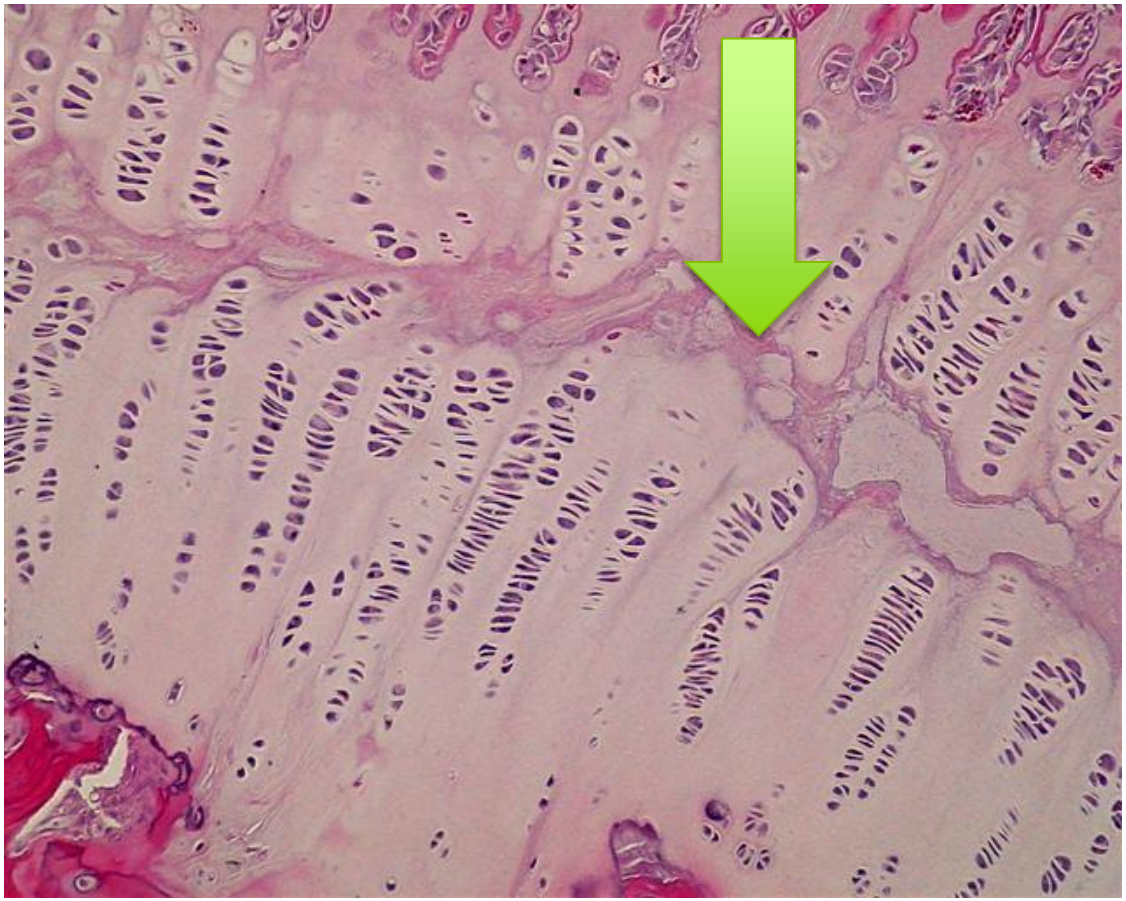


Growth plate - fissure



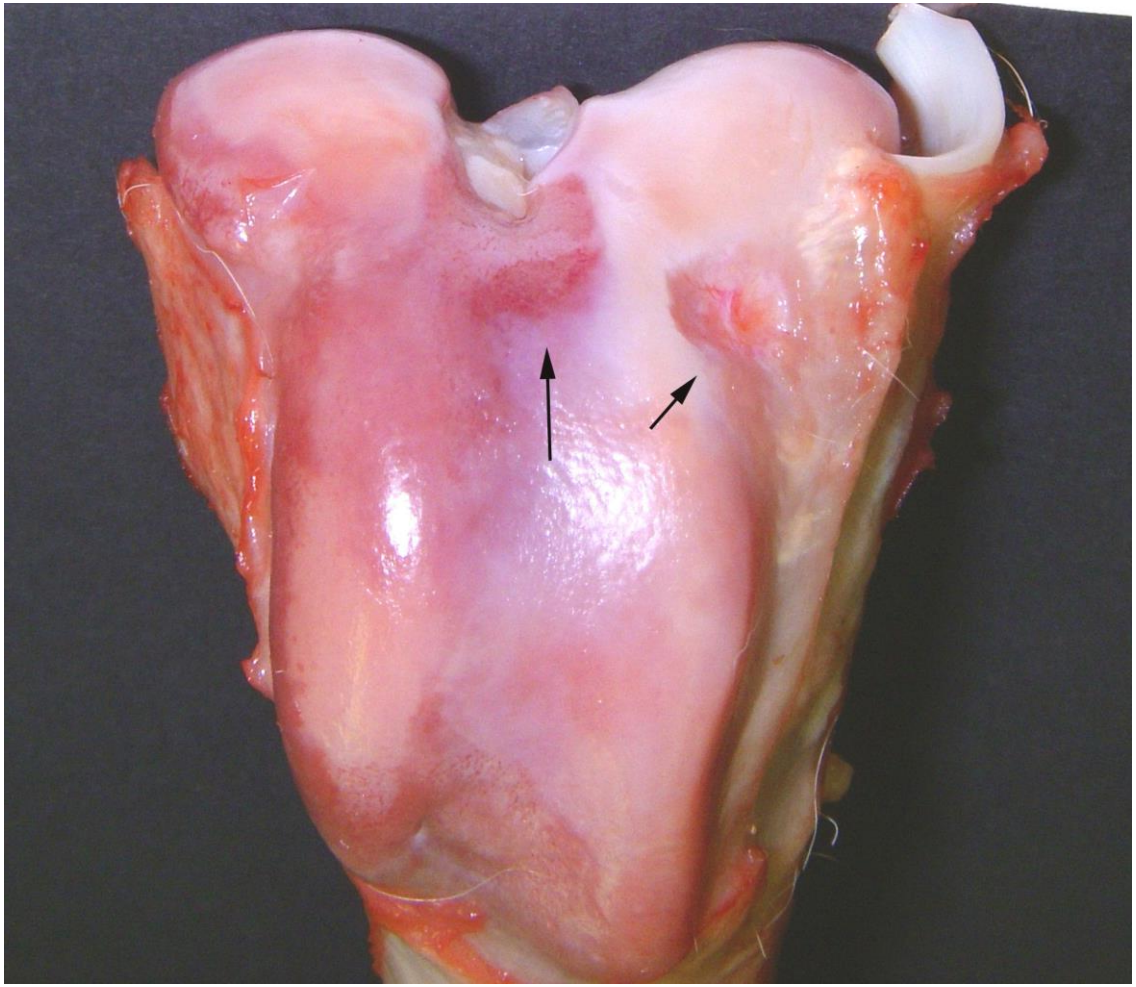
Growth plate - fissure



Quinilone Antimicrobials

Compounds such as **nalidixic acid** produce matrix rarefaction with loss of joint cartilage, particularly in the stifle joint and head of the humerus in the beagle. This may lead to severe erosion of the cartilage with extensive inflammation.

Dog – distal femur ,erosions of articular surface



Thank you