Decalcification and Clearing of Bone eg. Fibula

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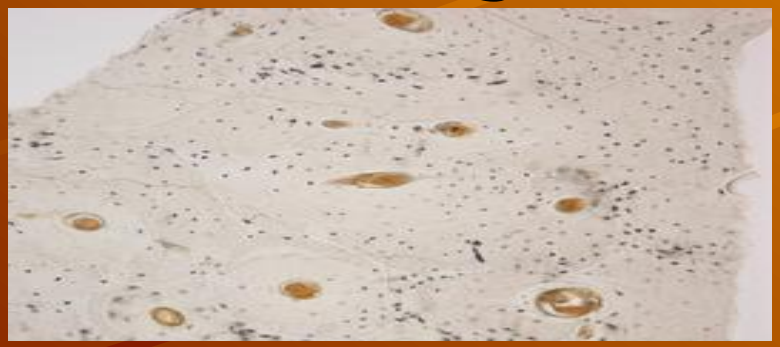
Bone composition

- Bone consists of cells (osteocytes) surrounded by a calcified matrix containing collagen fibres.
- In this matrix calcium is in the form of crystals deposited in between fibrous elements.
- These crystals are dissolved during decalcification leaving dense fibrous tissue.



Preparation for decalcification

- Debone- manual removal of soft tissue from bones
- Macerate- cooking of bones to a stage when the soft tissue is easily removed of the bones and immersion of a whole organ or tissue on concentrated acid
- Defat-removal of fat from bones using Trichloroethylene

Decalcification

- Decalcification describes the technique for removing of inorganic salts (calcium) from bone.
- It is carried out after the bone is prepared thoroughly.
- 3 main types of decalcification agents.
  - a) strong acids
  - b) weak acids
  - c) chelating agents

Decalcifying agents

- Strong acids- Nitric acid(HNO_3) is the most rapid in action but prolonged exposure will cause damage to bone.
- Weak acids – formic acid with formalin is slower acting but gentler in action.
- Chelating agents- EDTA works by capturing the calcium ions from the surface of the crystal, slowly reducing its size.

Factors influencing rate of Decalcification

- Concentration- larger volume of decalcifier increases calcium removal.
- Temperature- increase in temperature speeds up decalcification.
- Fluid renewal- Fresh decalcifier renewal enhances diffusion and penetration into the bone increasing ionization and removal of calcium ions.

Method

- Debone,macerate and defat fibula.
- Place in 10% formalin and add 5% concentrated nitric acid.
- Add 0.1% urea and this removes the brown stain.
- Decalcification takes between 3-6 weeks.
- Check by inserting needle through the bone for hardness.
- Leave for another week and then was in running water for 3-5 days to remove the acid.
- If bone still brown immerse in 10% hydrogen peroxide. H_2O_2 is a strong oxidizer.
- Finally allow to dry and ready for demonstration.

Principle of action

- Acid releases calcium ions from its chemical combinations with phosphates and carbonates in the bone through ion exchange.
- Calcium in bone migrates into the acid solution.
- Peroxides bleach and restore the colour by removing brown sediment.

Decalcified Fibula

- Figure 9: A decalcified paraffin section of fibula (H&E). Note the size of the specimen. It was decalcified with a formic acid agent using a chemical endpoint test then processed to paraffin using an extended schedule. The 13 y/o male sustained a pathologic fracture of his right fibula after a fall. X-ray showed bony enlargement of the mid-shaft of the (R) fibula. Pathologic diagnosis was bone cyst with pathologic fracture. The section was prepared in the bone laboratory of the Armed Forces Institute of Pathology (USA) in 1983.

Clearing

- Clearing then proceeds after decalcification.
- Clearing is replacement of the dehydrating fluid with mixture that is totally miscible with both the dehydrating fluid and the medium.
- Types of clearing agents used are ethanol, benzene and methyl salicylate.

Clearing agents

- Ethanol and denatured ethanol (methylated spirits) is hyrophilic and mixes well with water and used sequence of solutions that gradually increase in concentration and this reduces damage and shrinkage of bone.
- Benzene is fast acting and does not over harden the tissue but must be used with caution as its toxic and volatile.

Method

- Before the bone dries start dehydrating in methylated spirits(denatured ethanol).
- Follow by 3 changes of ethanol at intervals of 3 weeks.
- Replace bone in dessicator to remove traces of ethanol.
- Place bone in 2 changes of benzene using dessicator.
- Place in methyl salicylate and clearing commences rapidly gradually slows down
- Leave here for 3-5 months and remove when sufficiently cleared.