DECALCIFICATION& CARBOHYDRATES

Lab 9 &10

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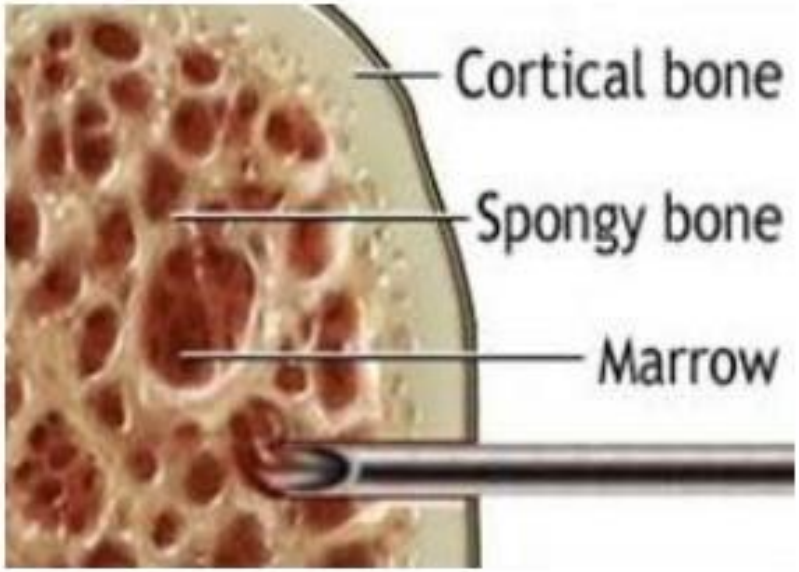
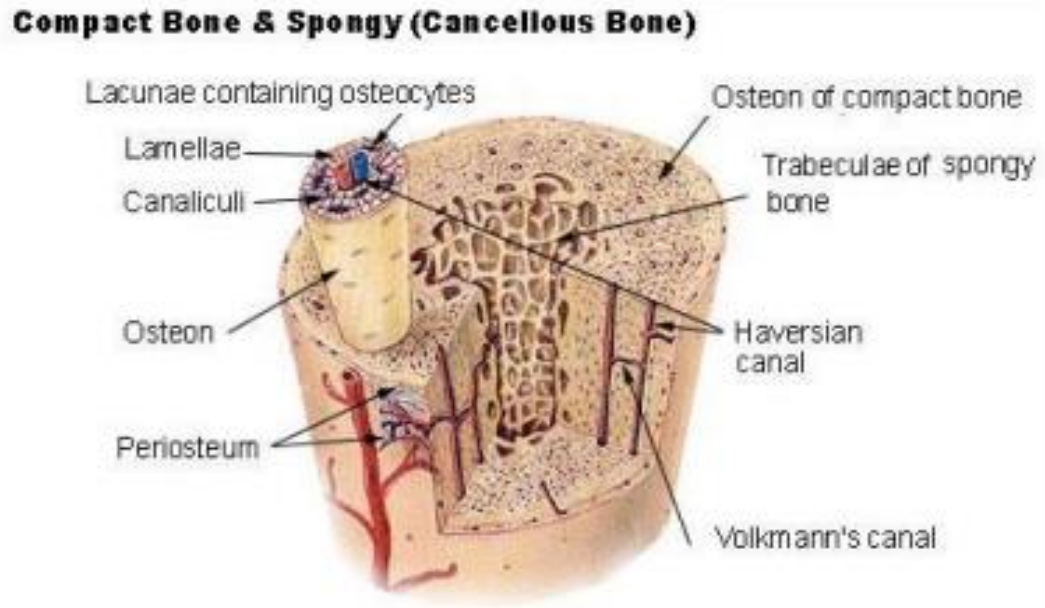
Preparation of Bone sections

- Tissues must be fixed adequately before decalcification. The Selected bone is

placed in 10% neutral buffered formalin for 24-48hrs .

- The ideal fixative for bone marrow is Zenker's (Modified, Zinc Chloride).
- For tooth specimens, 15% formic acid is mostly preferred.





Decalcification

Decalcification is the process of removing inorganic calcium (mineral) content of

the bone /tissue before processing the specimen after fixation.

There are four methods of decalcification

1. Simple dilute mineral acids:

a) Weak organic acid.

b) Strong inorganic acid.

2. Chelating agents.

3. Ion exchange resins with acid decalcifying fluids.

4. Electrolytic decalcification.

1_Simple dilute mineral acids

This method of decalcification dissolves the calcium salts in an acid solution called decalcifying fluids used either singly or mixture such as nitric acid, formic acid and trichloro acetic acid.

1. Simple dilute mineral acids- Strong inorganic acid

• Nitric acid solution (5-10%). Is strong acid recommended for the decalcification of small pieces of bone which must be processed rapidly.

• Over-exposure to nitric acid destroys nuclear staining.

• Used in urgent biopsy specimens.

• Strong acids are more damaging to:

1. Tissue antigens for immunohistochemical staining

2. Enzymes may be completely lost.

2. Simple dilute mineral acids- Weak organic acid

• Weak organic acid such as Formic acid, Trichloro acetic acid & Picric acid

• Formic acid (5%) it is much slower than nitric acid but considerably less damaging to tissue structures and staining.

• 10% Formic acid in D.W is recommended for (2days) to small pieces of bone and for (20days) for to larger pieces of dense compact bone.

T.C.A. used as 5% aqueous solution rather quicker in action than the same concentration of formic acid with good staining results.

End-Point of Decalcification

There are several methods for testing the completion of decalcification:

1) Radiological method: It is the best method; very quick it is not practically used because it is not available and very expensive.

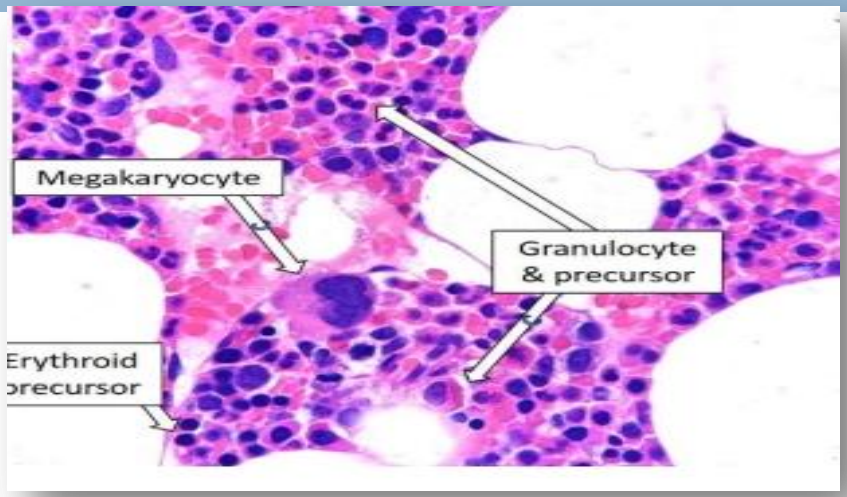
2) Physical method: It is a very bad method; damage the cells Structure.

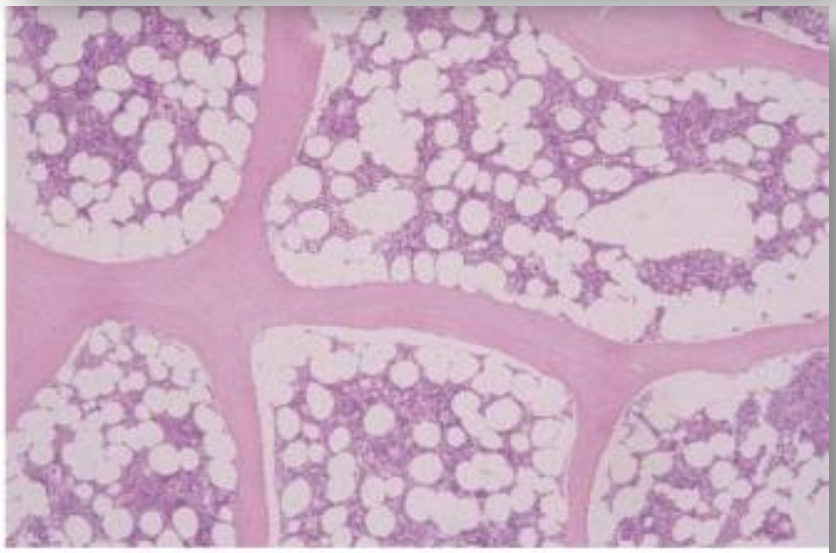
✓ The physical tests include bending the specimen or inserting a pin, razor, or scalpel directly into the tissue, or twisting the sample by fingers.

3) Chemical method: It is the suitable method used in the routine histopathology lab.

✓ The following solutions are needed to chemically test for residual calcium.

Principle (Ammonium Oxalate reacts in alkaline pH with calcium salts to give calcium oxalate)

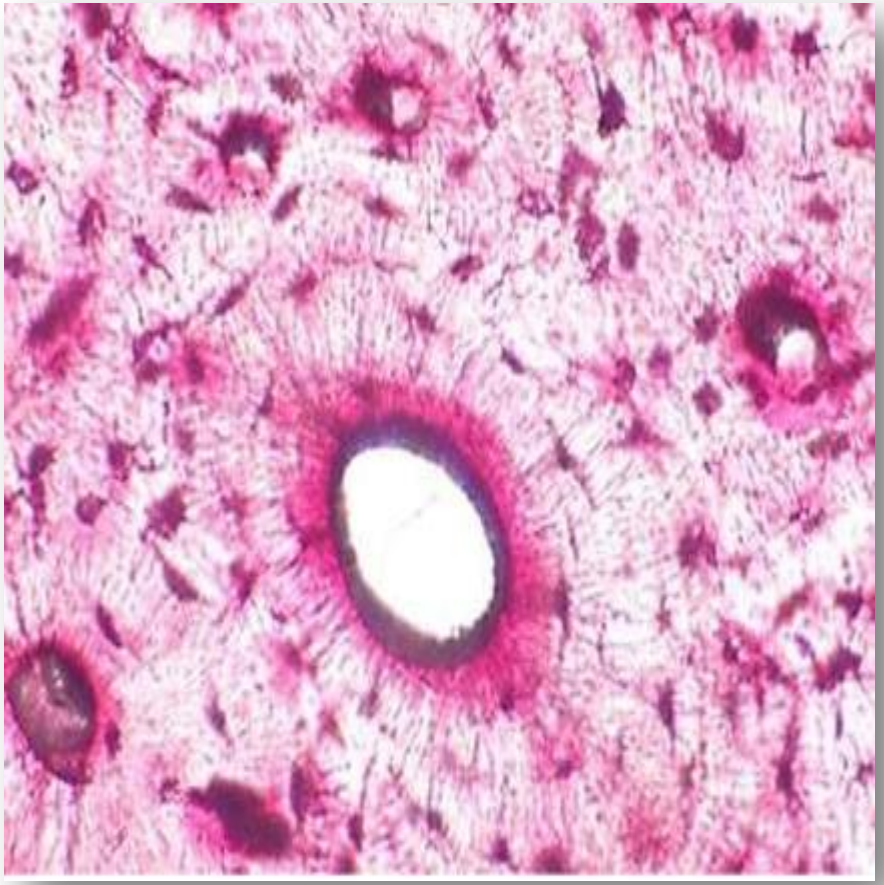




normal bone structure

Factors affecting rate of decalcification:

1. Concentration of decalcifying solution- Increase conc fastens the reaction.
2. Temperature- Increase temp. Fastens the reaction
3. Density of the bone-harder bone takes longer time.
4. Thickness of tissue - small tissue piece decalcify earlier.
5. Agitation-which increases the rate of decalcification



Special stains for: protein, carbohydrates, lipid, mucosubstances, pigments minerals & microorganisms

Special stains are used to identify certain normal and abnormal substance present in the cells and tissue. Which cannot be identified on routine Hematoxylin & Eosin staining or are better appreciated on special stain.

Classification of stains

1. Stains for carbohydrates
- **Periodic Acid Schiff (PAS) stain**
- **Enzymatic digestion technique**
- **Alcian blue**

2. Stains for amyloid
- **Congo red stain**
- **Crystal/Methyl violet stain**

3. Nucleic acid stains
- **Feulgen stain**
- **Methyl green pyronin stain**

4. Lipid stains
- **Oil red o stain**
- **Sudan Black B stain**

5. Stains for microorganisms
- **Ziehl-Neelsen stain**
- **Gomori methenamine silver stain**

6. Connective tissue stains
- **Reticulin stain**
- **Masson's Trichrome**
- **Van Gieson stain**

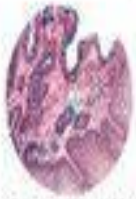
7. Stains for pigments and minerals
- **Perl's stain**

8. Hematoxylin and Eosin

Amyloid

Congo Red
Cat # 24614

Carbohydrates



Alcian Blue/ PAS
Cat # 25086

PAS
Cat # 24200

Rapid Mucin
Cat # 24208

Neuronal Tissue

Cresyl Violet
Cat # 21063

Bielschowsky
Cat # 25994

Luxol® Fast Blue
Cat # 24611

Triglycerides & Lipids

Oil Red O
Cat # 25962

Reticulin Fibers

Reticulin
Cat # 25094

Jones PAS-M
Cat # 25091

Connective Muscle Tissue

Picrosirius Red
Cat # 24901

Verhoeff Van Gieson
Cat # 25089

Gomori's Trichrome
Cat # 24205

Masson's Trichrome
Cat # 25088

Rapid PTAH
Cat # 25715

Pigment, Minerals & Granules

Prussian Blue Iron
Cat # 24199

Fontana Masson
Cat # 25104

Von Kossa Method
Cat # 24633

Villanueva Osteochrome Bone
Cat # 16280

Microorganisms

AFB Kinyoun
Cat # 25765

AFB Ziehl-Neelson
Cat # 24669

Auramine O
Cat # 24665

Differential Quik
Cat # 24606

Fungi-Fluor®
Cat # 17442

Grocott Methenamine Silver
Cat # 25462

TB Fluorostain
Cat # 22422

Warthin-Starry
Cat # 25093

Gram's Stain
Cat # 24668

Special Stains

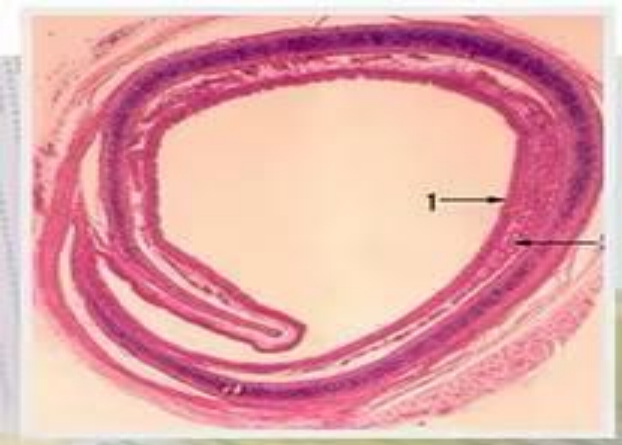
Periodic Acid Schiff (PAS) stain:

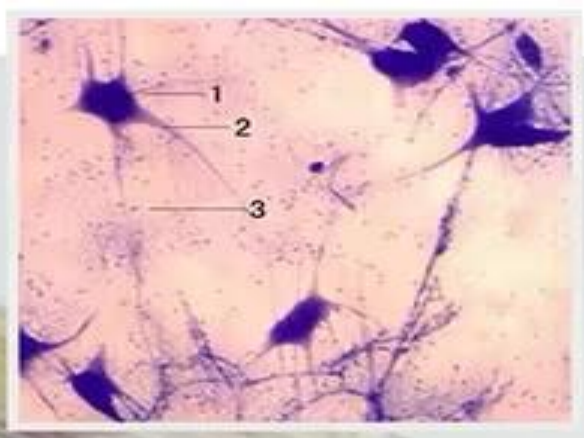
Uses of PAS:

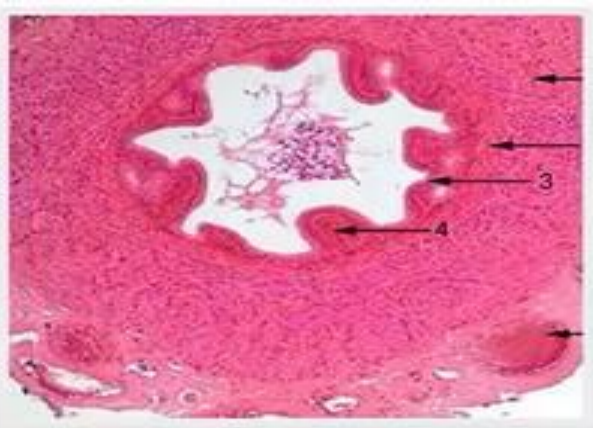
- PAS is used to demonstrate glycogen and neutral mucoprotein.
- In diagnosis of poorly differentiated adenocarcinoma of various tissues like

stomach, pancreas, and lung.

- In diagnosis of hepatocellular carcinoma.







THANK YOU