SECTION

I

Oral Histology

CHAPTER

1

Preparation of Oral Tissues for Microscopic Examination

- Study of dental hard tissues
  - Decalcified sections
  - Ground sections
- Study of oral soft tissues
  - Routine soft tissue processing

A sound knowledge of the structures of oral tissue is very much essential for understanding of this region. The knowledge about these structures gives the clinician an insight into the understanding of the disease mechanism that may develop in these tissues and predict the reaction of tissues to various factors, etc.

Microscopic examination is the method adopted to study the histological structures of the oral tissues. To study the histological structure, the tissue should be appropriately prepared for microscopic examination.

STUDY OF DENTAL HARD TISSUES

The structure of dental hard tissues can be studied using ground sections or decalcified sections. The exception is enamel, where decalcified sections are not of much use because 96% of enamel is mineralized, which is lost while decalcification.

Decalcification

Decalcification is the process by which calcium in the mineralized tissue is removed so that the tissue becomes soft enough to make sections. Decalcification is achieved by using acids, chelating agents or by electrolysis. The commonly used method is acid decalcification.

Frequently used acid for decalcification is 5% nitric acid. 15% Formic acid may also be used. Nitric acid may cause yellowing of the tissue, that may interfere with further staining procedure. To avoid this 0.1% urea is added to nitric acid.

Procedure

Hard tissue to be decalcified should be fixed in 10% formalin or formal saline. To reduce the time for decalcification tissue may be cut into smaller pieces. Then place the tissue in a container with 5% nitric acid. The acid should be changed daily for a few days. The specimen is then tested for completion of decalcification.

Methods to check the completion of decalcification

1. **Checking the consistency of the tissue:** Completely decalcified tissue will be soft without any hardness being felt. (Experienced hand can tell by the feel of the tissue.)
2. **Piercing the tissue with a needle:** If it enters the tissue without resistance, the tissue is completely decalcified. This is not recommended because it may cause damage to the tissue.

3. **Judicious bending or trimming of the tissue:** This can be done to ensure completion of decalcification.
4. **Taking radiograph of the specimen:** In the radiograph, if radiopaque specks are found, tissue is not completely decalcified.
5. **Chemical test:** The basis of this test is to identify calcium in the decalcifying solution in which the specimen was kept. Sodium hydroxide or strong ammonia is added to 5 ml of decalcifying fluid, to neutralize the solution. Then 5 ml of saturated ammonium oxalate solution is added. After this, check for turbidity. Absence of turbidity after 5 minutes indicates the fluid is free from calcium and thereby decalcification is complete. Turbidity is observed due to precipitation of calcium. If precipitation is observed after addition of sodium hydroxide, it indicates large amount of calcium is present in fluid. Precipitation seen only after addition of ammonium oxalate suggests decalcification is nearly complete.

Checking the end point of decalcification is important because incomplete decalcification makes further cutting of specimens difficult. Prolonged decalcification is also not desirable because it may affect the staining procedure. Once the decalcification is complete the tissue should be washed in running water to remove all acids. Then the steps of processing can be continued like soft tissue processing which includes dehydration, embedding, sectioning and staining.

Ground Sections

Ground sections are of particular importance in the study of structure of dental hard tissues especially enamel. In this method the tooth is made into thin sections by grinding, using abrasive stones.

Procedure

The tooth to be examined should be cut into 2–3 sections using dental hand piece and diamond impregnated or carborundum disc. These sections should be ground using an Arkansas stone or by simply rubbing on a glass plate using abrasive slurry. Grinding should be continued till it is approximately 25–50 microns thick. Fine abrasives should be used for the final polishing. Most suitable abrasive is domestic scouring powders followed by soapy water. Once the desirable thickness is attained, the section should be washed and dehydrated and mounted on a glass slide using synthetic resin or Canada balsam as mounting medium and is allowed to dry.

Grinding of the tooth can also be done using a laboratory lathe. Initial grinding is done by holding the tooth in fingers and pressing it against the rotating coarse abrasive wheel of a lathe. When the tooth is thin, it is difficult to hold with fingers. Therefore, a wooden block wrapped with adhesive plaster with sticky side directed outward can be used. Stick the tooth on to the plaster and press the wooden block to the rotating wheel of the lathe so that the tooth is ground thin. Then change the coarse wheel to fine wheel and continue grinding till the section is sufficiently thin. To remove the adhesive plaster the sections can be soaked in water. The section removed from the plaster is then mounted on a glass slide using a mounting medium.

Precision equipment like hard tissue microtomes are now available for the preparation of ground sections.

STUDY OF ORAL SOFT TISSUES

The most commonly used method of preparing soft tissue for the light microscopic study is by embedding the tissue in paraffin, and cutting and mounting the section on slides and staining.

Preparation of Sections of Paraffin Embedded Specimens (Soft Tissue Processing)

1. **Obtaining the specimen:** Specimens for microscopic study are obtained through either biopsy or autopsy.

   Biopsy is the removal of tissue from a living organism for the purpose of microscopic examination and diagnosis. If tissue is taken for the same purpose from dead organisms, it is called autopsy.

2. **Fixation:** After the removal, specimen should be kept in fixative solution at the earliest. The amount of fixative should be approximately 25 times the size of the specimen. Depending on the size and density of the specimen, the fixation time can vary from a few hours to days. Usually 24-hour is sufficient for small specimens.

   Commonly used fixative is 10% buffered formalin. Other fixatives used are methyl alcohol, ethyl alcohol, etc.

   The aims of fixation are
   - To preserve the cells and tissue constituents in life like condition as closely as possible without loss or derangement.
   - To prevent the process of autolysis and bacterial action or putrefaction of tissues.
   - To coagulate the proteins, thus reducing the change in shape or volume during further processing of tissue and to make the tissue more readily permeable to the subsequent application of reagents.

   After fixation the specimen is washed in running water.

3. **Dehydration of specimen:** Dehydration is the process by which the water content from the tissue specimen is removed to allow the penetration of paraffin. Dehydrating agents commonly used are alcohol and acetone. Gradual dehydration is done using increasing grades of alcohol (40, 60, 80 and 95% and absolute alcohol). To ensure complete removal of water, the specimen is placed in 2–3 changes of absolute alcohol. After dehydration the water in the specimen is completely replaced by alcohol.

4. **Clearing:** Alcohol is not miscible with paraffin. So impregnation of the tissue by paraffin is not possible unless alcohol is replaced by a fluid that is miscible with both alcohol and paraffin. The fluid used for this purpose is xylene. Xylene is an organic solvent, miscible with alcohol and paraffin. This process is called clearing because tissue becomes transparent or clear after the treatment with xylene. This occurs because the refractive index of xylene is similar to that of proteins in the specimen. Other clearing agents used are toluene, chloroform, benzene, etc.

5. **Impregnation of specimen with paraffin:** After clearing the specimen should be transferred to a dish with molten wax and this should be kept in an oven where temperature is adjusted to around 60°C. The xylene in the specimen is gradually replaced by paraffin. To ensure complete impregnation 2–3 changes of molten paraffin is required.

6. **Embedding:** The specimen is embedded in paraffin after impregnation. For this a paper box or Leuckhart's 'L' pieces fixed to form a cube can be used. Fill the cube with molten wax and the specimen can be embedded in to this with the help of a warm forceps. Allow the wax to cool and solidify. When the 'L' forms are removed, the tissue is found embedded in a cube of solidified wax block.

7. **Cutting the specimen:** The tissue specimen embedded in the paraffin wax is clamped to a rotary microtome to take sections of desirable thickness of 4–10 microns.

8. **Mounting the cut sections on microscopic glass slides:** Sections taken using a microtome is floated on warm water and mounted on to a microscopic slide coated with adhesive. The slide is then placed on a slide warmer adjusted to a temperature of 40°C which helps to adhere the sections to the slide.

9. **Staining the section:** The sections are now ready for staining. Routinely used stain is hematoxylin and eosin. After staining mounting is done using a mounting medium. Commonly used mounting medium is Distrene dibutylphthalate polystyrene xylene (DPX). After mounting it is allowed to dry, before microscopic examination.

CHAPTER

2

Cells, Tissues and Stains

- Epithelial tissue
  - Stratified squamous epithelium
  - Pseudostratified ciliated columnar epithelium
- Connective tissue
  - Fibroblasts and fibrocytes
  - Fat cells or adipocytes
  - Bone cells
  - Cartilage cells
  - Endothelial cells
  - Striated Muscle
  - Defence cells
  - Giant cells
- Cells of odontogenic apparatus
- Routine stain/ Hematoxylin and Eosin
- Special stains

Cells are the basic structural units of the body. A group of cells performing the same function forms a tissue. Two important basic tissues of the body are epithelium and connective tissue.

EPITHELIAL TISSUE

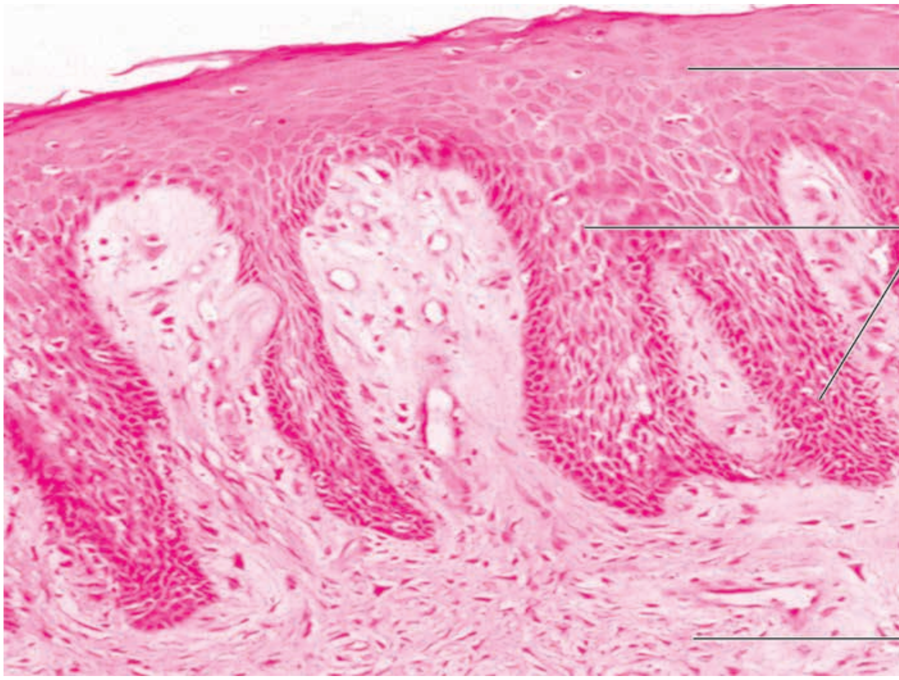
Epithelium is an ectoderm derived tissue that forms a protective covering of connective tissue. Although there are different types of epithelia in the human body, the main types important for students of dentistry are stratified squamous epithelium that forms the lining of oral cavity and pseudostratified ciliated columnar epithelium lining the maxillary sinus and respiratory tract.

Stratified Squamous Epithelium (Fig. 2.1)

In stratified squamous epithelium the cells are arranged in different layers or strata. The basal cells are cuboidal in shape with central nucleus, arranged in single layer on the basement membrane. The superficial cells are squamous or polyhedral in outline with centrally placed nucleus. All these cells are attached to each other by desmosomal junctions.

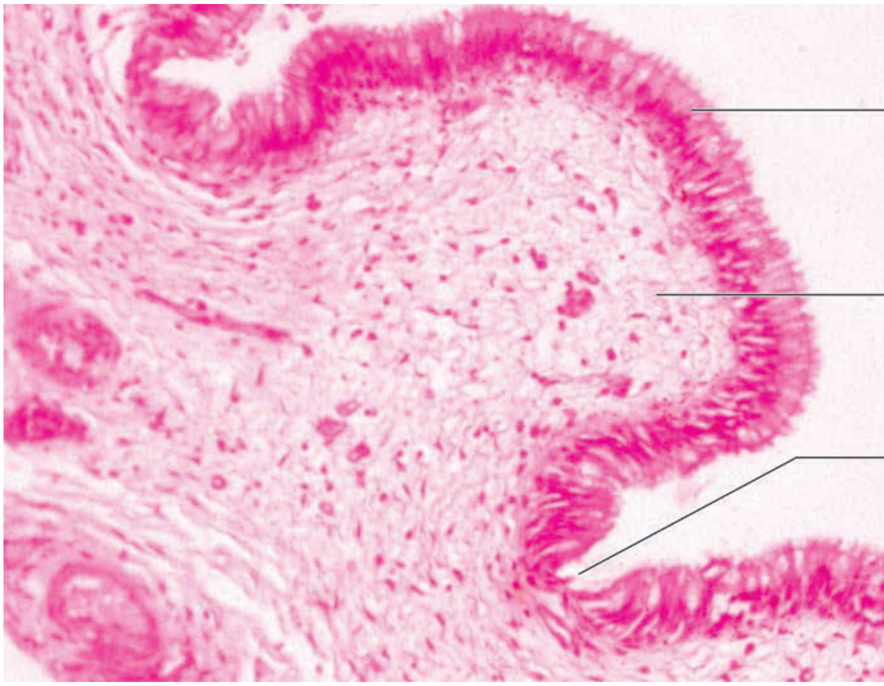
Pseudostratified Ciliated Columnar Epithelium (Fig. 2.2)

The cells of the ciliated columnar epithelium are columnar in shape, of varying sizes arranged in a single layer on a basement membrane. The nuclei of the cells are placed at different levels giving the erroneous appearance of stratification. The cells on the superficial aspect have cilia which help in the movement of the mucous secretions. Among the columnar cells unicellular secretory organs called goblet cells are also noticed. Goblet cells are goblet shaped with a basally placed nucleus and apical cytoplasm filled with secretory products.



Stratified squamous epithelium (photomicrograph 10X)

Fig. 2.1: Stratified squamous epithelium



Pseudostratified ciliated columnar epithelium (photomicrograph 10X)

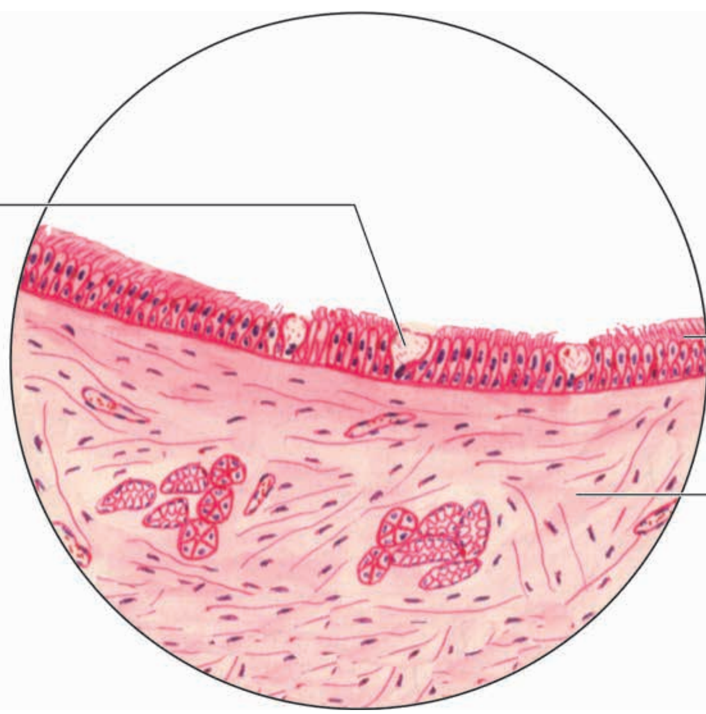


Fig. 2.2: Pseudostratified ciliated columnar epithelium

CONNECTIVE TISSUE

Connective tissue is of mesodermal origin and comprises fibers, ground substance and cells. The primary groups of fibers are collagen fibers. Collagen fibers are arranged to form bundles and stain pink in H and E stained sections. Collagen fibers and cells are embedded in ground substance, rich in proteoglycans and glycoproteins. Fibers and ground substance together constitute the extracellular component. Cellular components are given as follows:

Fibroblasts and Fibrocytes (Fig. 2.3)

These are the primary cells of the connective tissue. **Fibroblasts** are ovoid or star-shaped cells with multiple cytoplasmic processes. The cells have large, round and vesicular or open face nucleus and abundant cytoplasm exhibiting slight basophilia indicating that they are highly active in protein synthesis. **Fibrocytes** are spindle shaped with flat deeply staining, close face nucleus. Fibroblast and fibrocytes are arranged parallel to the collagen fibers.

Fibroblasts are the synthetic cells that produce collagen fibers and ground substances. They also help to degrade these components, thereby helping in remodelling of connective tissue. Fibrocytes are resting cells in connective tissue.

Fat Cells or Adipocytes (Fig. 2.4)

These are the cells those synthesize and store fat. These cells are spherical or ovoid with a flattened nucleus displaced to the periphery. These cells filled with fat are usually seen as groups where they are compressed with each other giving rise to polyhedral shape. In routine H and E stained histological sections fat cells appear as empty cells because fat is dissolved during processing. Fat cells can be stained by a special stain called Sudan III. Fat cells are distributed in the submucosal tissue.

Osteoblasts, Osteocytes and Osteoclasts (Fig. 2.5)

Osteoblasts are synthetic cells of bone that help in the formation of bone, by matrix deposition and mineralization. They are cuboidal or ovoid cells with centrally placed ovoid open face nucleus. Osteoblasts are arranged along the periphery of the bony trabeculae, forming a lining or rimming of the trabeculae.

Osteocytes are resting cells found entrapped in the bone. They occupy spaces called lacunae. Osteocytes have a cell body and processes called canaliculi. Cells are ovoid or flat with close faced nucleus and scanty cytoplasm.

Osteoclasts are the cells which resorb the bone. Osteoclasts are derived from circulating monocytes. They are large giant cells with multiple nuclei. These cells occupy irregular resorption bays called Howship's lacunae.

Chondroblasts and Chondrocytes (Fig. 2.6)

Chondroblasts are cartilage forming cells. They appear as flattened or elliptical cells and are located at the periphery of cartilage parallel to the surface.

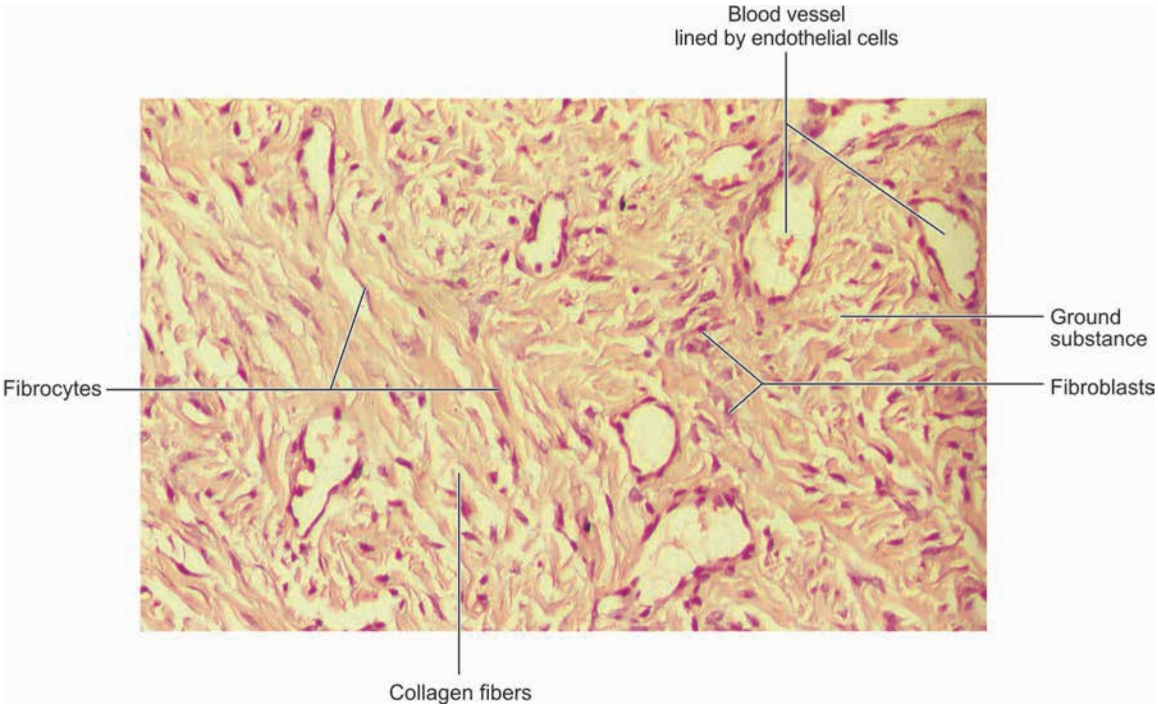
Chondrocytes are the cells entrapped in the cartilaginous matrix. They are located in spaces called lacunae. Usually the chondrocytes are seen as groups of 2–4 cells and is described as 'cell nests'.

Endothelial Cells (Fig. 2.3)

Endothelial cells are the cells lining the blood capillaries. They form a single layer flattened cells having flattened nucleus resting on the basement membrane.

Striated Muscle (Fig. 2.7)

Striated muscle is seen as highly eosinophilic cylinder like structures in a hematoxylin and eosin stained sections. Each muscle fiber is composed of many myofibrils. Fibers show characteristics transverse striations. The



Fibroblasts, fibrocytes and endothelial cells (photomicrograph 10X)

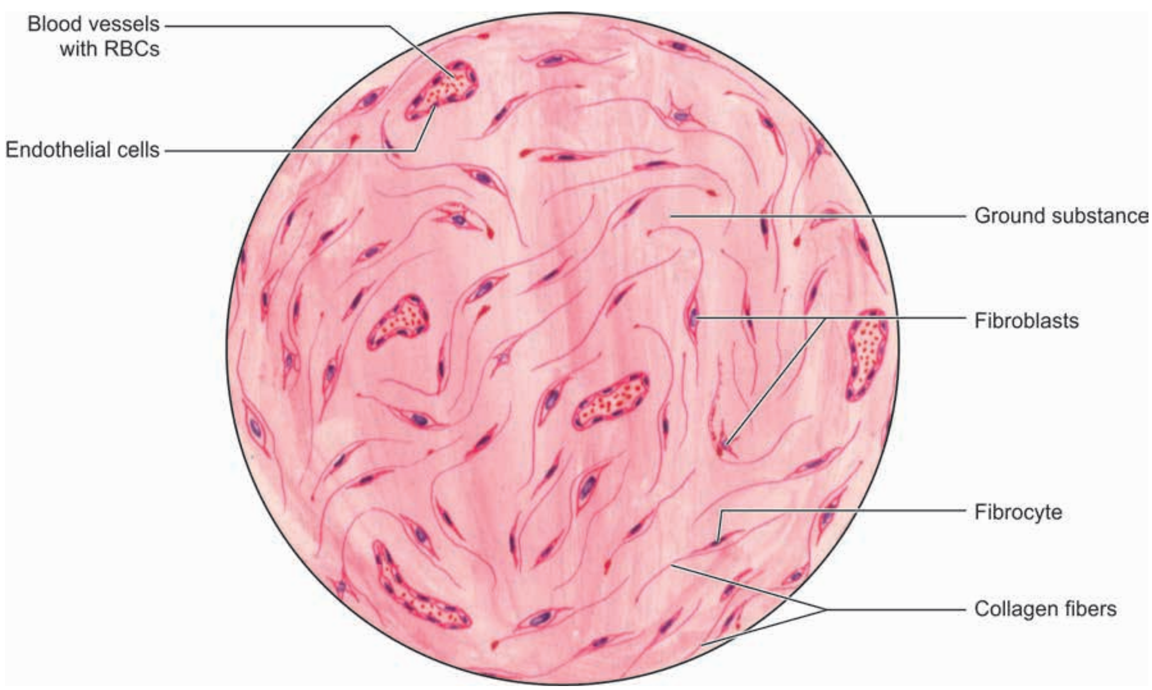
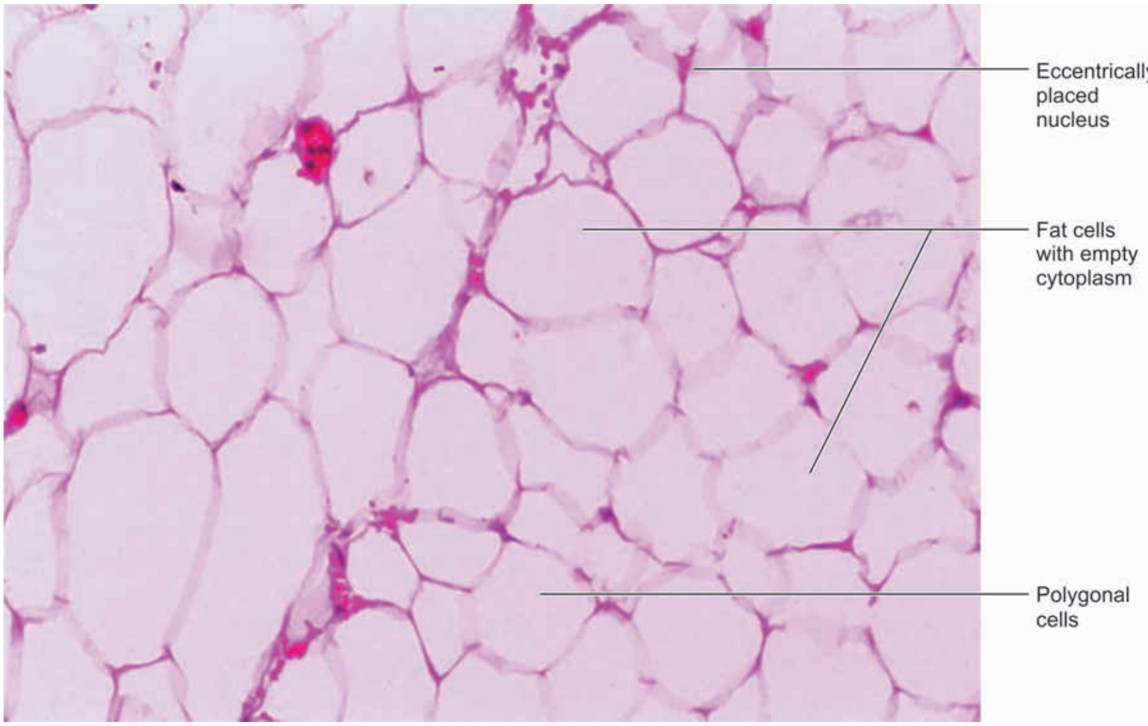


Fig. 2.3: Fibroblasts, fibrocytes and endothelial cells



Fat cells (photomicrograph 40X)

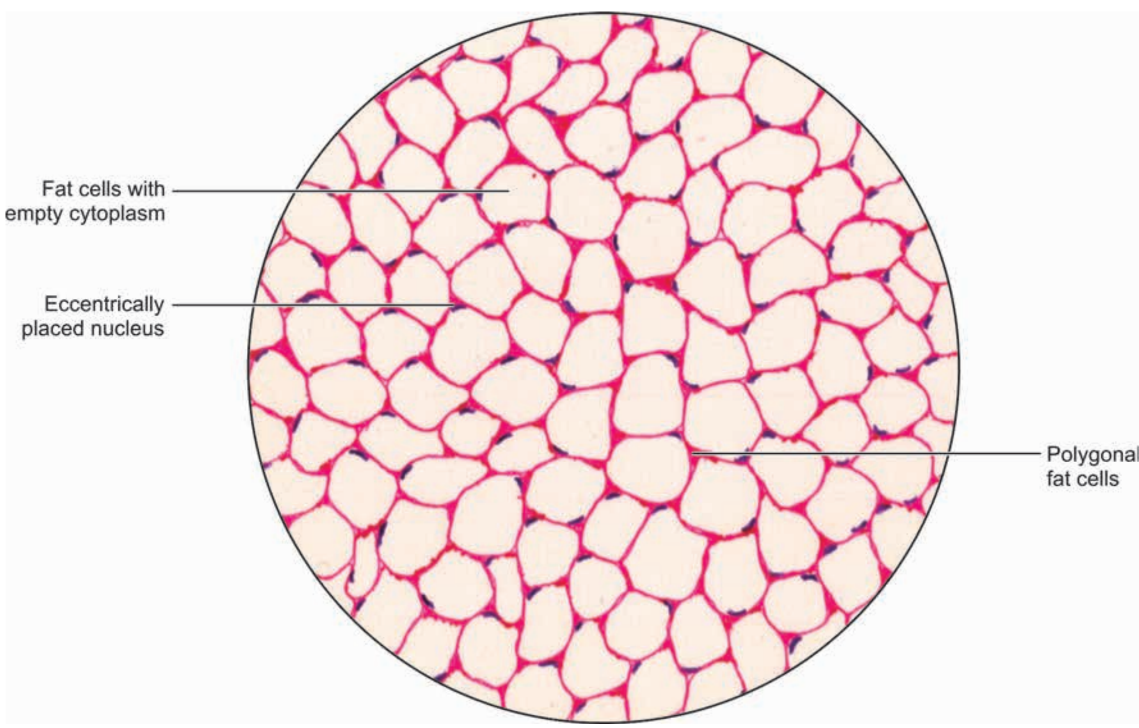
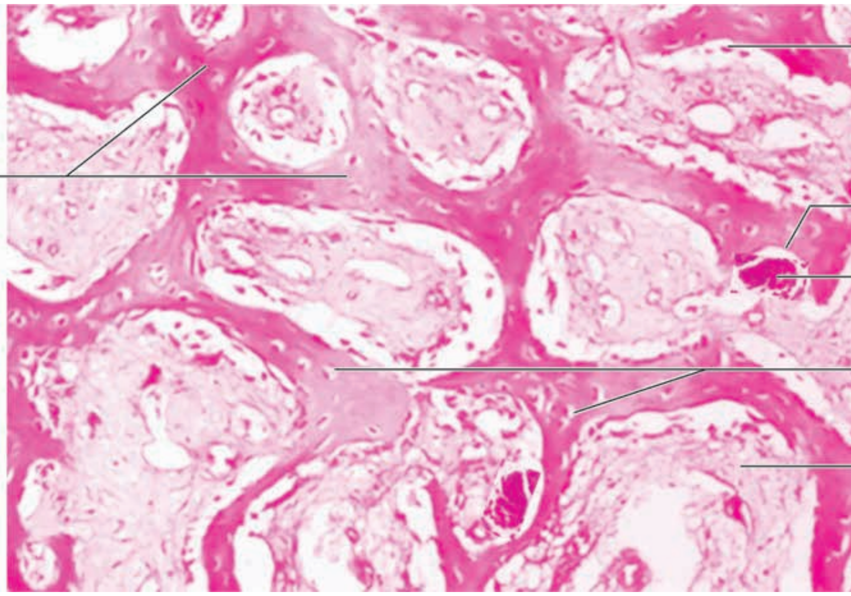


Fig. 2.4: Fat cells



Osteoblasts, osteocytes and osteoclasts (photomicrograph 10X)

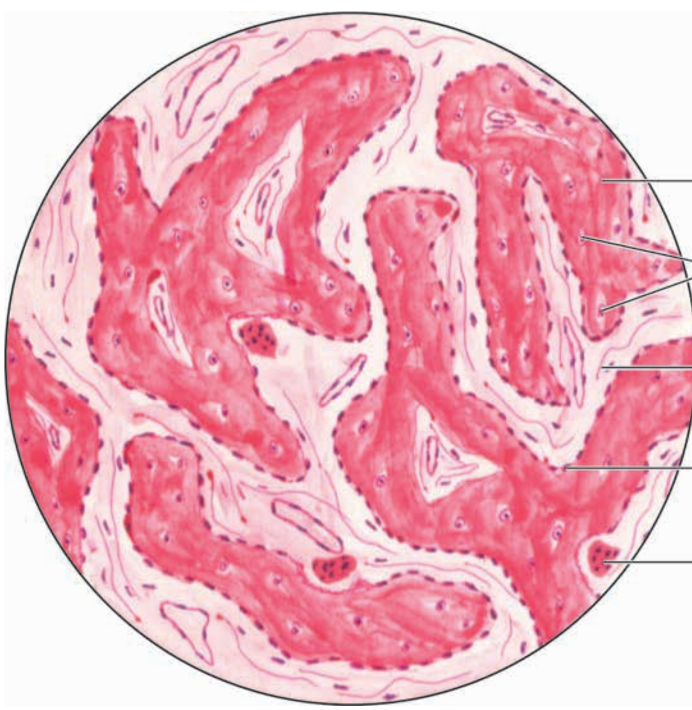


Fig. 2.5: Osteoblasts, osteocytes and osteoclasts

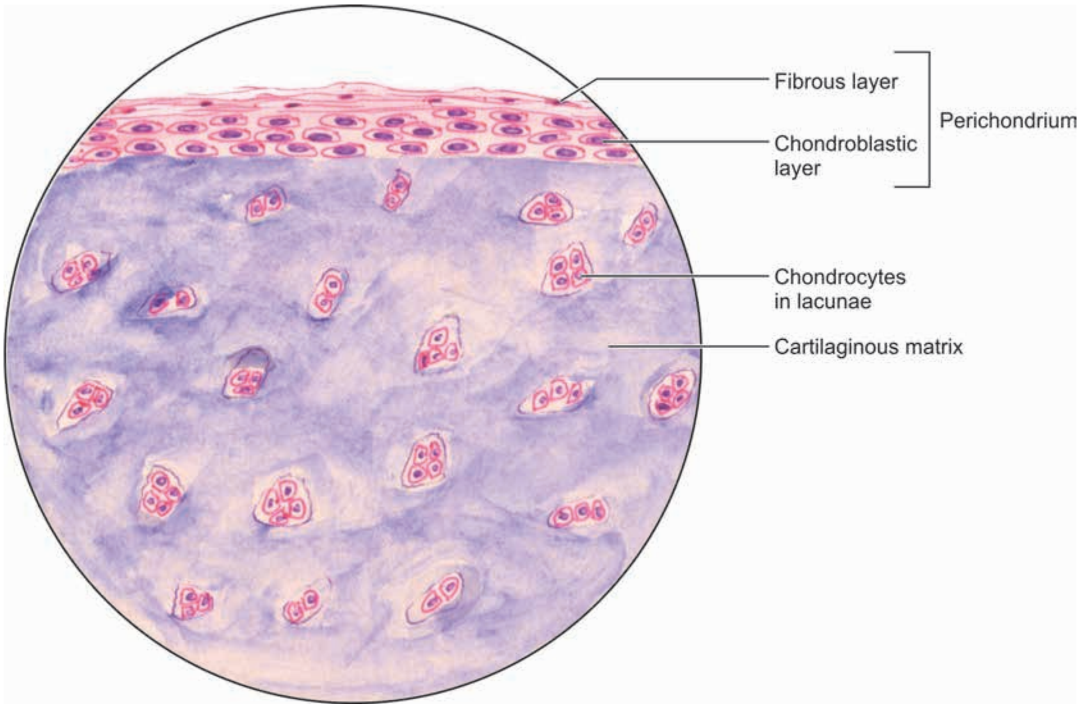


Fig. 2.6: Chondrocytes and chondroblasts

cytoplasm of muscle or sarcoplasm is rich in cytoplasmic organelles. Nuclei are flattened, multiple and are located at the periphery. The muscle as a whole is enclosed in connective tissue called epimysium. This connective tissue extends inwards dividing muscle into fasciculi. These extensions are called perimysium, from which again septa (endomysium) extend that invests individual muscle fibers.

Neutrophils (Fig. 2.8)

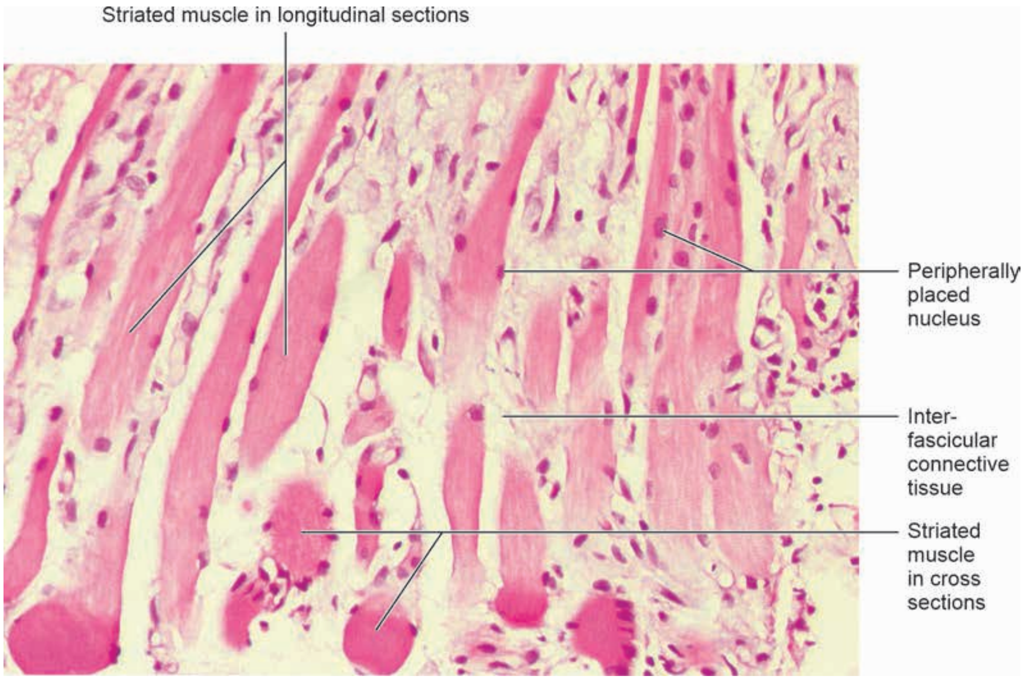
Neutrophils are defence cells of the body functioning as the first line of defence against invading microorganisms. They are active in acute infections and thereby belong to the group of acute inflammatory cells. Neutrophils are 7 to 9 microns in diameter, have three to five lobes of nuclei and cytoplasmic granules, containing various enzymes.

Plasma Cells (Fig. 2.8)

Plasma cells are derived from B-lymphocytes and are specialized for the synthesis of antibodies (immunoglobulin), thereby imparting resistance to the body against diseases. They are ovoid in shape with basophilia of cytoplasm and eccentric, oval or round nucleus. The nucleus shows chromatin condensation in a radial pattern giving rise to a 'cartwheel' or 'clock face' appearance. Plasma cells are seen in connective tissue of the oral mucosa. These cells belong to the group of chronic inflammatory cells. Sometimes collection of immunoglobulin is seen as eosinophilic globules called Russel bodies in close proximity to groups of plasma cells.

Lymphocytes (Fig. 2.8)

These are defence cells of the body belonging to the group of chronic inflammatory cells. Lymphocytes can be large or small according to their size. Small lymphocytes are round cells with 6–8 microns diameter. Nucleus is round occupying the major part of cytoplasm. Only a thin rim of cytoplasm is seen around the nucleus. Large lymphocytes are larger than



Striated muscle (photomicrograph 10X)

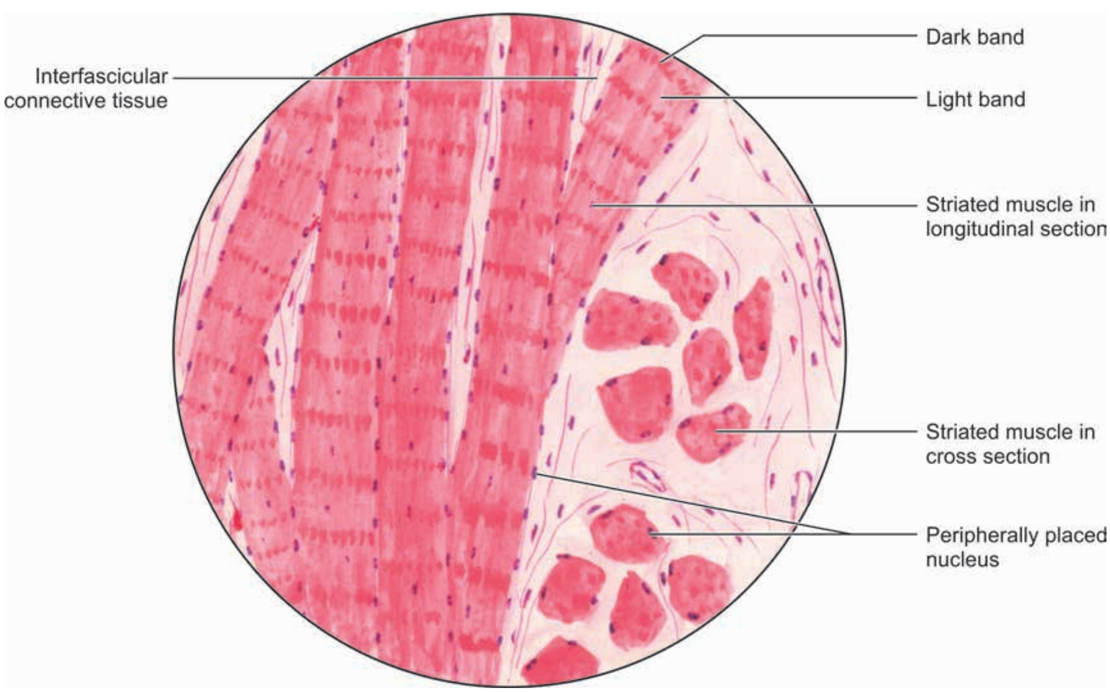
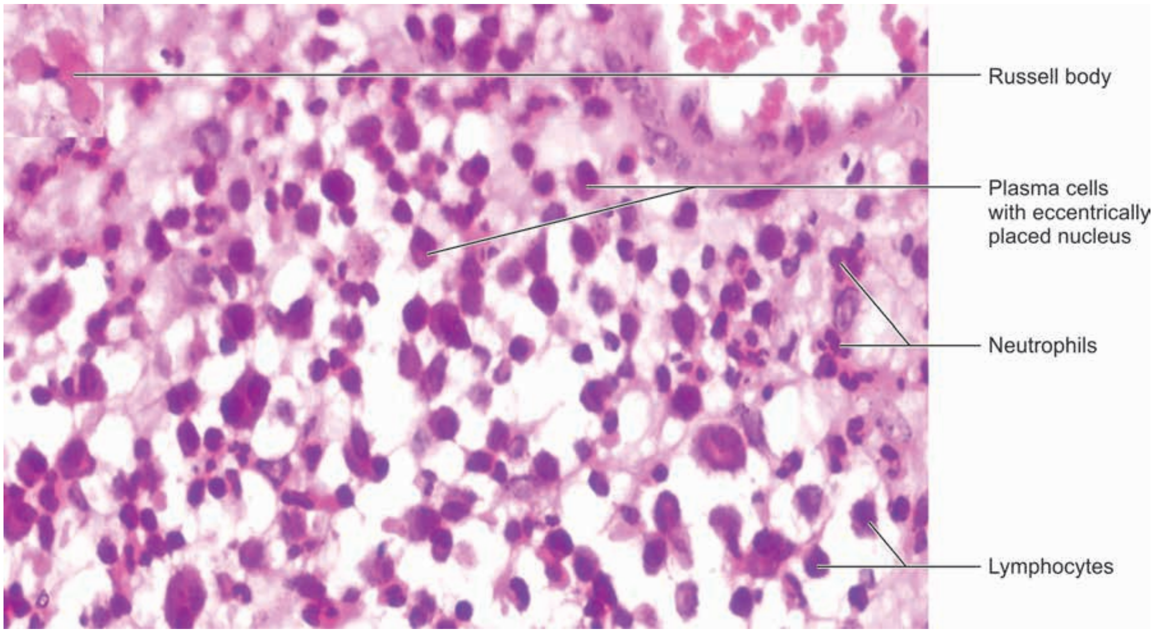


Fig. 2.7: Striated muscle



Inflammatory cells (lymphocytes, plasma cells and neutrophils: Photomicrograph 40X)

Fig. 2.8: Inflammatory cells (lymphocytes, plasma cells and neutrophils)

small lymphocytes with greater amount of cytoplasm.

Mast Cells (Fig. 2.9)

These connective tissue cells are widely distributed in the oral mucosa. Mast cells are round or ovoid with small centrally placed nucleus. The cytoplasm contains granules rich in histamine, heparin and serotonin that have important role in allergic reaction. These cells can be seen in sections stained by toluidine blue as cells filled with purple/violet colored coarse granules. Mast cells are concerned with inflammation and immune response.

Giant Cells (Fig. 2.10)

Large and/or multinucleated cells are called giant cells. Giant cells can be seen in physiological or pathological conditions. Osteoclast is an example for giant cells seen in physiological conditions. Commonest giant cells in pathological conditions are foreign body giant cells. They are seen in chronic inflammatory reactions in relation to a foreign body. These giant cells are large cells having multiple nuclei dispersed in the cytoplasm.

Cells of Odontogenic Apparatus

Ameloblasts are enamel forming cells which differentiate from the cells of inner enamel epithelium of enamel organ. They are columnar in shape with approximately 40 microns in length and diameter of 4 to 5 microns. Ameloblasts show reversal of polarity with nucleus located at the proximal end (away from the basement membrane). During formative stage, ameloblasts develop a conical projection at the basal portion which is termed Tomes' process.

Odontoblasts are the cells which form dentin. These cells differentiate from the dental papilla of the tooth germ. Odontoblasts are located in the pulp adjacent to the predentin with the cell body in the pulp and cell processes in the dentinal tubules. These cells are 5 to 7 microns in diameter and 25 to 40 microns in length. In the root region the odontoblasts are ovoid or spindle shaped.

Cementoblasts and Cementocytes

Cementoblasts are the cells forming cementum and are derived from dental follicle of the tooth germ. These cells are cuboidal in shape and line the outer surface (periodontal ligament surface) of cementum.

Cementocytes are the cells found entrapped in cellular cementum. They are spider-shaped cells which lie in spaces called lacunae. These cells have a cell body and numerous processes or canaliculi radiating from it. The canaliculi are directed towards the periodontal ligament which is the source of their nutrition.

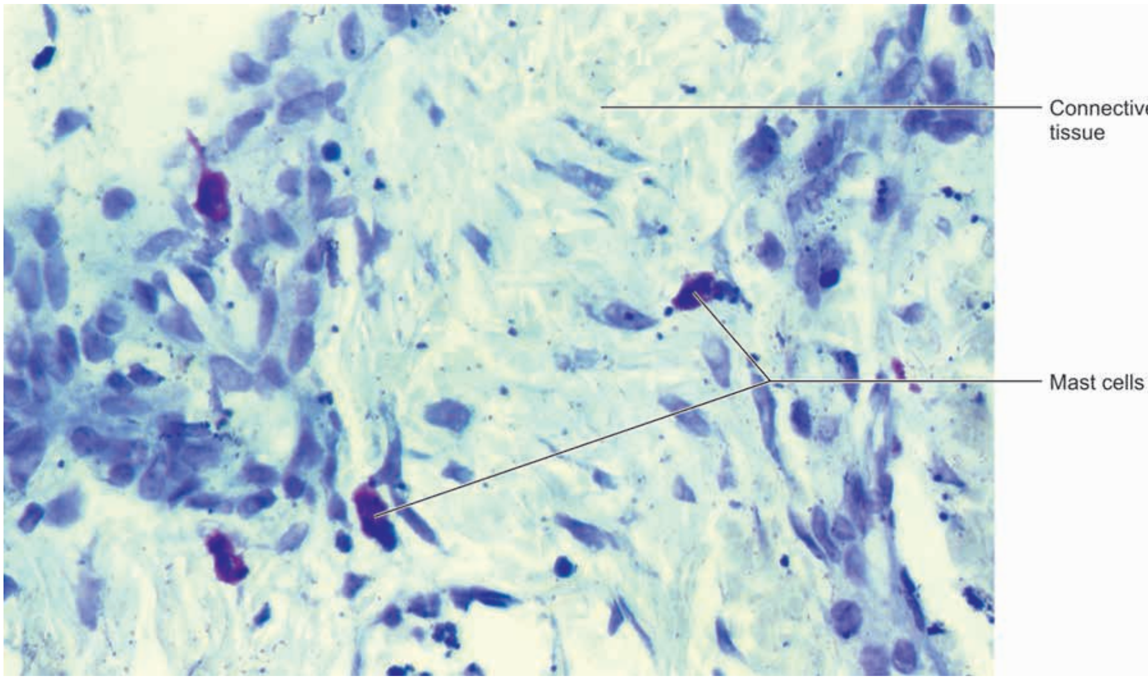
STAINS USED IN HISTOPATHOLOGY LABORATORIES

Staining of otherwise transparent tissue sections, is highly essential to demonstrate various cellular and tissue components and tissue structure and is a basic requirement for studying histologic structure of various tissues and also for making accurate diagnosis of a disease condition. Therefore, routine and special stains have a critical role in histology and histopathology.

In the histopathology laboratory, the term routine staining refers to the hematoxylin and eosin (H & E) stain that is used as a basic staining technique performed routinely with all tissue specimens to reveal the underlying tissue structures and conditions. Special stains are alternative staining techniques that are used when the H & E does not provide adequate information. A pathologist or researcher requires special stains to obtain additional information for more detailed analysis, for differentiating two morphologically similar disease conditions.

Hematoxylin and Eosin (Fig. 2.11)

Hematoxylin and Eosin (H & E) staining is used routinely in histopathology laboratories as it has ability to demonstrate a wide range of cell and tissue components and allows very detailed view of the tissue. As indicated in the name, this staining technique involves



Mast cells (photomicrograph 40X—toluidine blue stain)

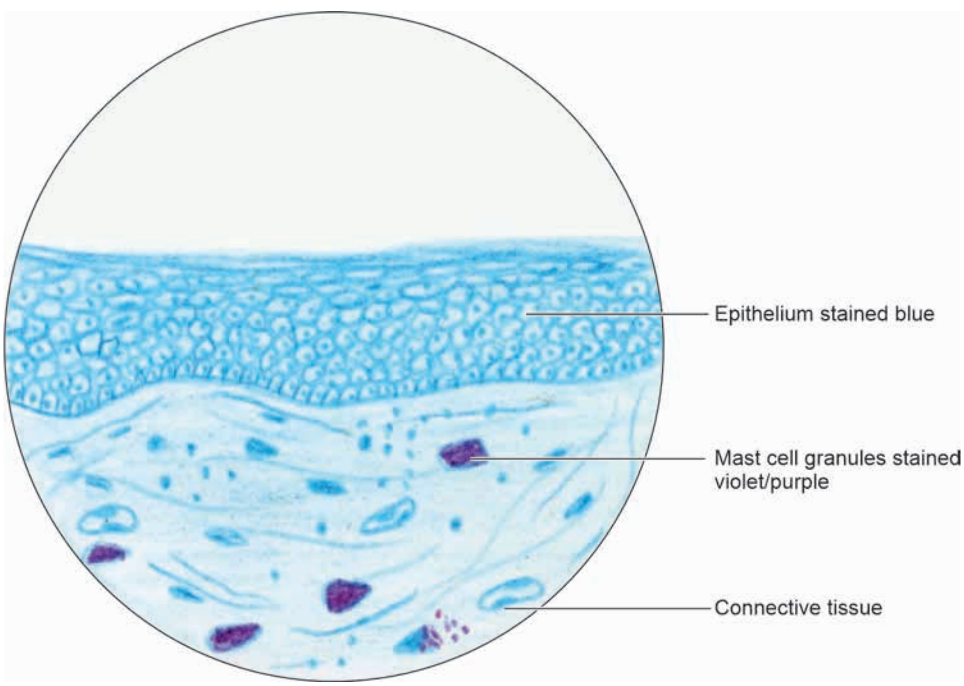
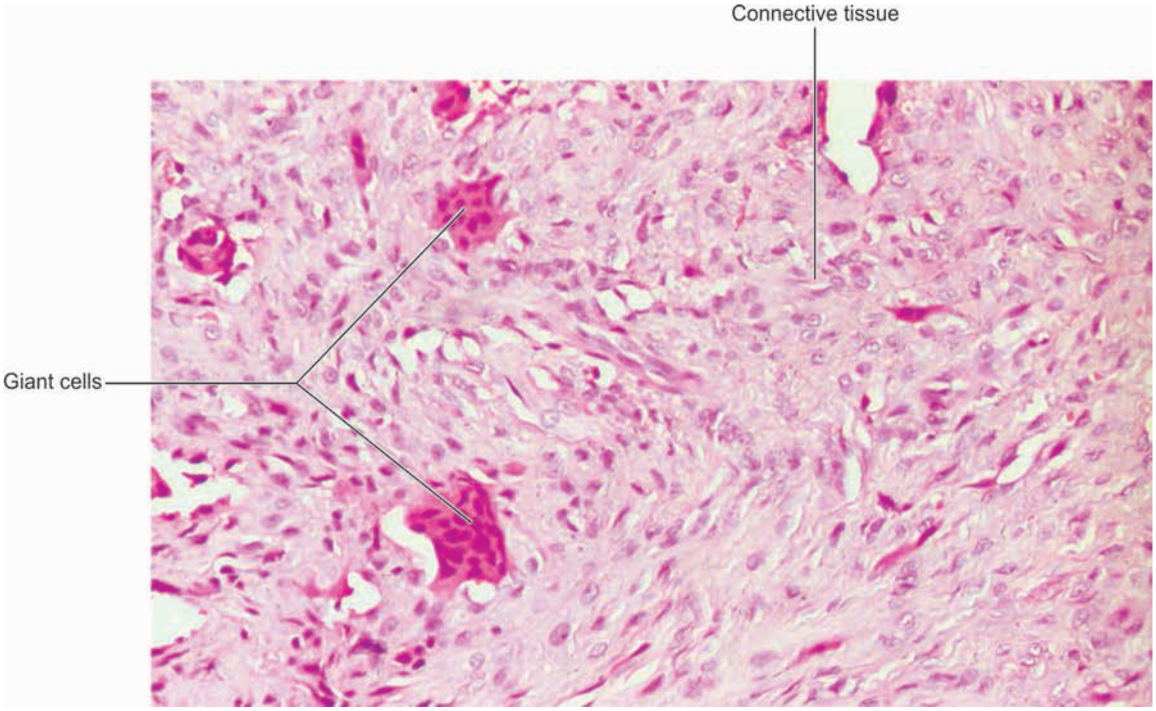


Fig. 2.9: Mast cells



Giant cells (photomicrograph 10X)

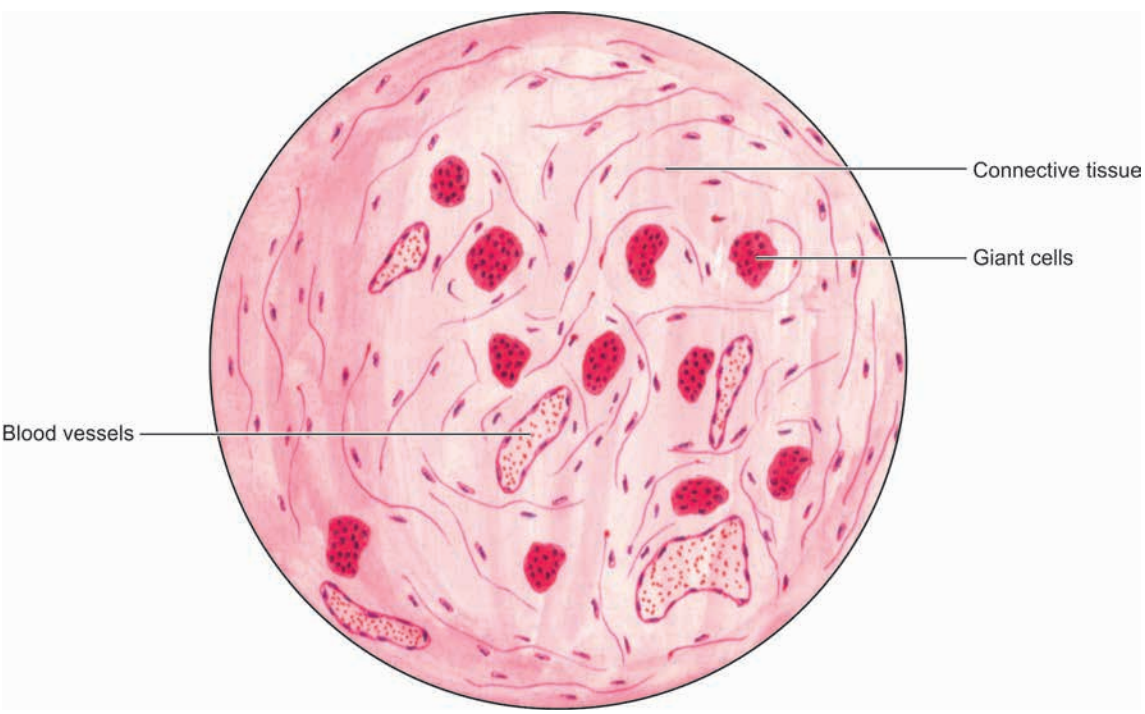


Fig. 2.10: Giant cells

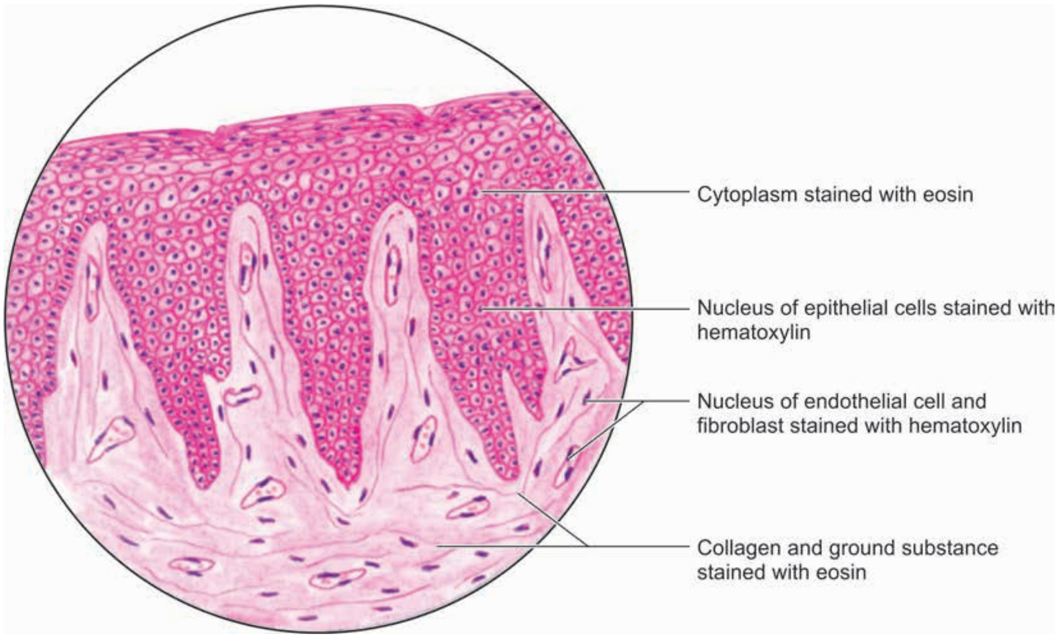


Fig. 2.11: Hematoxylin and Eosin stain

application of two different components: (i) A basic dye hematoxylin, which stain basophilic structures of the tissue mainly cell nuclei with blue color, (ii) eosin which stain eosinophilic structures (cytoplasm, organelles and extra-cellular components) pink or red.

This information obtained with H & E stain is often sufficient to observe basic histologic structures. As this staining technique reveals organization/disorganization of the cells and tissues and also abnormalities such as nuclear changes, it is widely used in medical diagnosis and still remains as the gold standard for diagnosis.

Special Stains

In a histopathology laboratory, special or advanced stains are ordered, subsequent to routine H & E stain, if additional information is needed for more detailed analysis.

Special Stains for Connective Tissue

Trichrome Stains

Trichrome stains are a group of staining techniques that can be used for differential demonstration of connective tissue components such as collagen fibers, muscle tissue, erythrocytes, etc. This is called trichrome stain as three dyes are used of which one may be nuclear stain.

The trichrome staining technique uses different dyes with varying molecular weight and the basic principle is that when used to stain a section, smaller molecules penetrate and stain various tissue elements. Further, when large dye molecules are allowed to penetrate the same tissue elements, larger ones replace the smaller one. This helps to selectively stain different tissue components.

a. **van Gieson stain** (Fig. 2.12): This is one of the commonly used special stains to demonstrate collagen. When paraffin embedded tissue sections are stained using this staining technique: **Collagen takes up red color; nucleus, blue/black and other tissues, yellow.**

b. **Masson trichrome stain** (Fig. 2.13): This trichrome stain is used to differentially stain collagen and muscle. When this technique is employed: **Cytoplasm, muscles and**

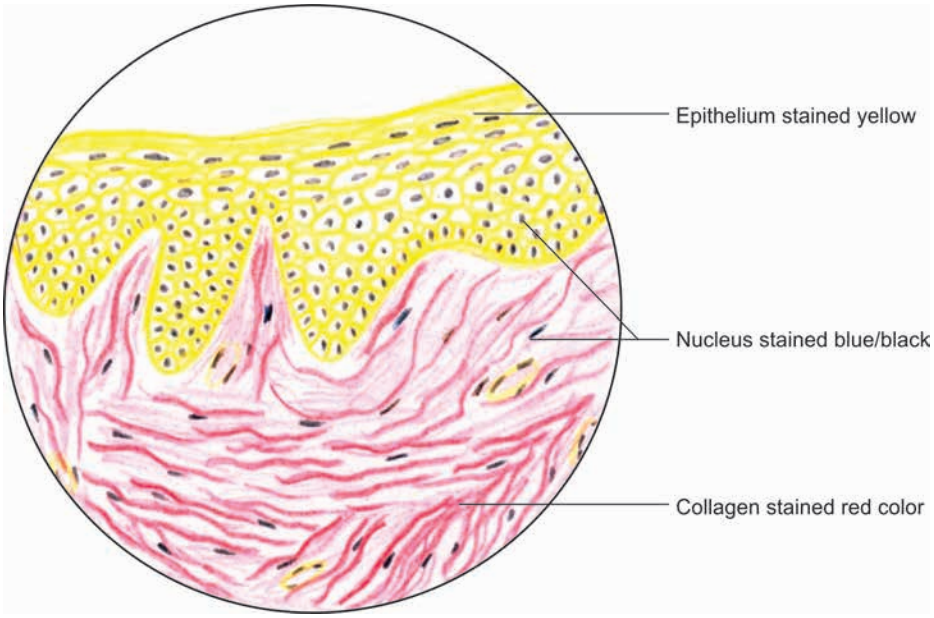


Fig. 2.12: van Gieson stain

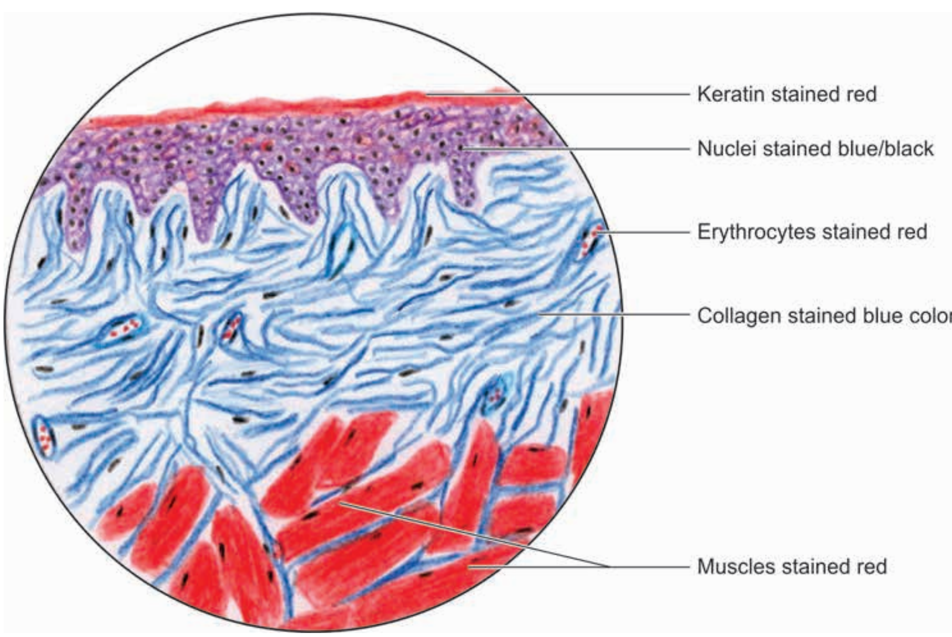


Fig. 2.13: Masson trichrome stain

erythrocytes stain red while collagen takes up blue color and nuclei, blue/black.

Special Stains for Demonstrating Carbohydrates

Demonstration of carbohydrate (CHO) is essential in many situations in a histopathology laboratory as it helps in differentiating and characterizing various pathological conditions. Pure CHO or CHO conjugated with other molecules such as proteins or lipids occur in cells and tissues and demonstration of CHO or glycol-conjugates such as glycogen or mucin help in confirming histopathology diagnosis. A number of staining techniques are used to demonstrate different types of mucopolysaccharides components in tissue specimens in histopathology laboratory which include periodic acid–Schiff (PAS) stain, Alcian blue, colloidal iron, mucicarmine, or metachromatic dyes.

a. **Periodic acid–Schiff (PAS) stain** (Figs 2.14 and 2.15): This is the most popular technique used in histopathology laboratories to demonstrate glycogen or mucin. This special stain is used to demonstrate basement membrane as Schiff reagent used in this technique reacts with glycoprotein present in basement membrane separating epithelial compartment from connective tissue. As the periodic acid, another component of this stain reacts with polysaccharides present in wall of fungi, this technique is also employed for demonstrating fungi such as *Candida albicans*. When this stain is used, **glycogen and various glycoproteins take up magenta color and nuclei stain blue.**
b. **Mucicarmine stain:** This stain is used to demonstrate mucin, specific for acidic epithelial mucin and therefore of great importance in diagnosing salivary gland tumors, particularly adenocarcinomas. **Epithelial mucin appears deep rose or red in mucicarmine stained tissue sections while other tissue elements stain light yellow and nuclei, black** (Fig. 2.16).

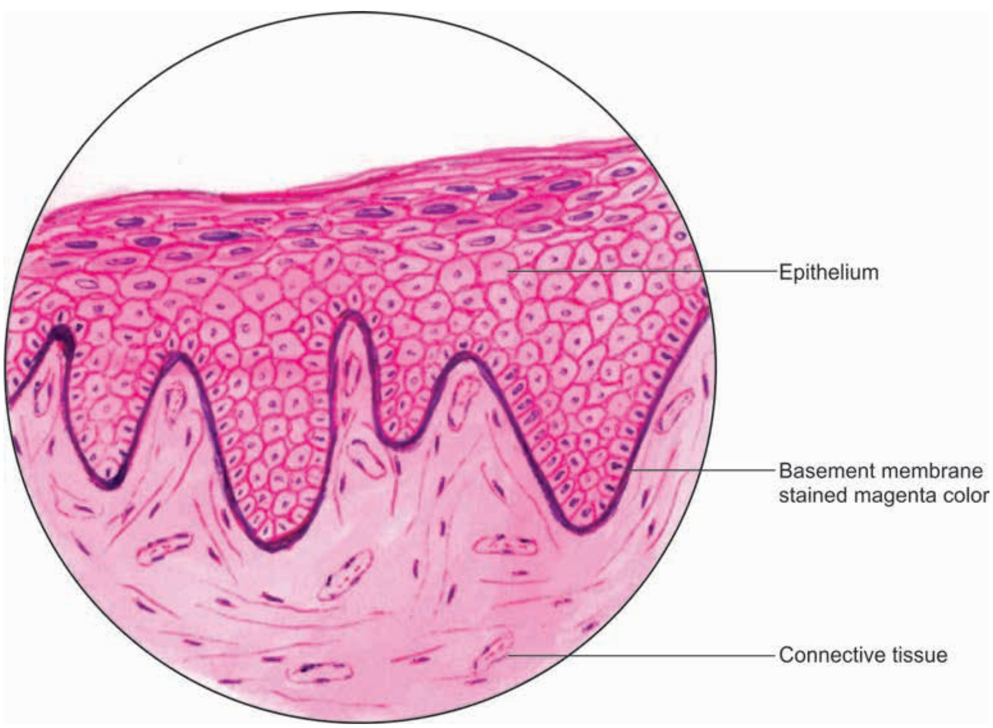


Fig. 2.14: PAS stain for basement membrane

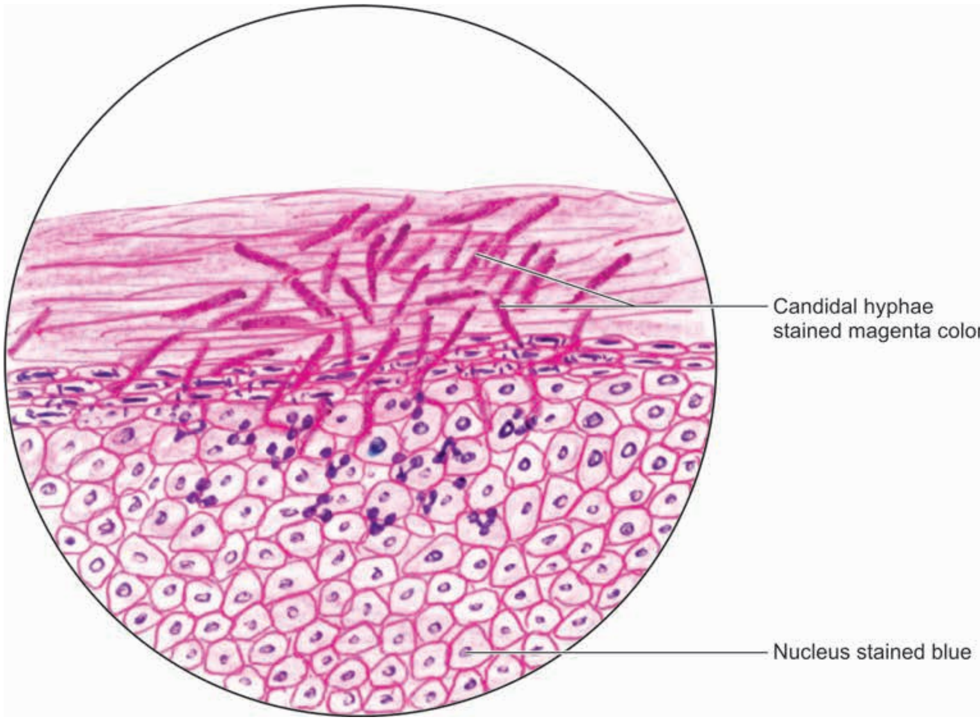


Fig. 2.15: PAS stain for Candida

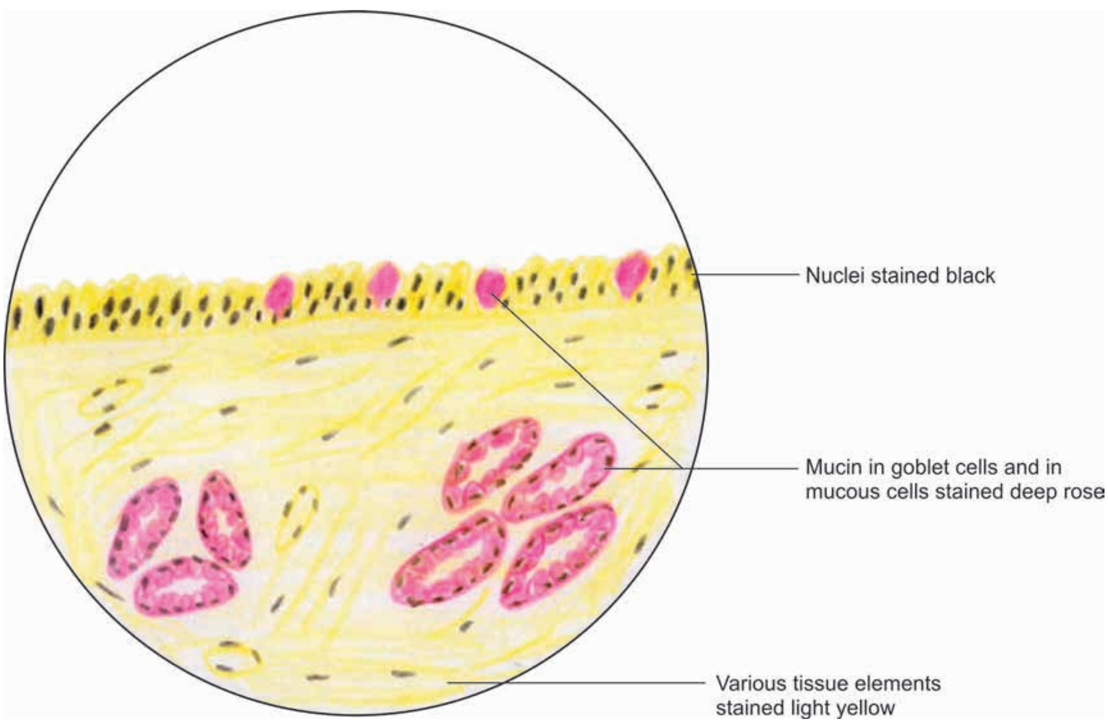


Fig. 2.16: Mucicarmine stain

Special Stains for Demonstrating Melanin (Fig. 2.17)

Melanin is a light brown to black pigment, produced by melanocytes, normally found in skin, hair, eyes and to some extent in oral mucosa. Melanin in protein bound form is present in cytoplasm of melanocytes as melanin granules and demonstration of it helps identifying melanin producing cells. Extensive melanin pigmentation resulting either from increased activity/proliferation of melanin producing cells is characteristic feature of pigmented lesions. In inflammatory conditions, melanin is found in phagocytic cells referred to as melanophages.

Demonstration of melanin in a tissue section is of particular importance in diagnosis of benign conditions like nevus and malignant diseases like malignant melanoma. Melanin can be demonstrated using a number of techniques, which Masson-Fontana is a commonly used and reliable method. This method is based on capability of melanin to reduce silver solution. In this method formalin fixed tissue sections are treated with 10% silver nitrate solution and further counterstained with neutral red. **In Masson-Fontana stained sections, melanin pigments stain black and cytoplasm and nuclei of cells red.**

Special Stain for Mast Cells

Toluidine blue is special stain used to demonstrate mast cells. It is a polychromatic dye that stains tissues via the phenomenon known as metachromasia; which means a dye stains some tissue components, a different color from the dye solution and the rest of the tissue. When tissue sections are stained with toluidine blue, mast cell granules and polysaccharides will stain violet/purple, while the rest of the tissue stain blue (Fig. 2.18).

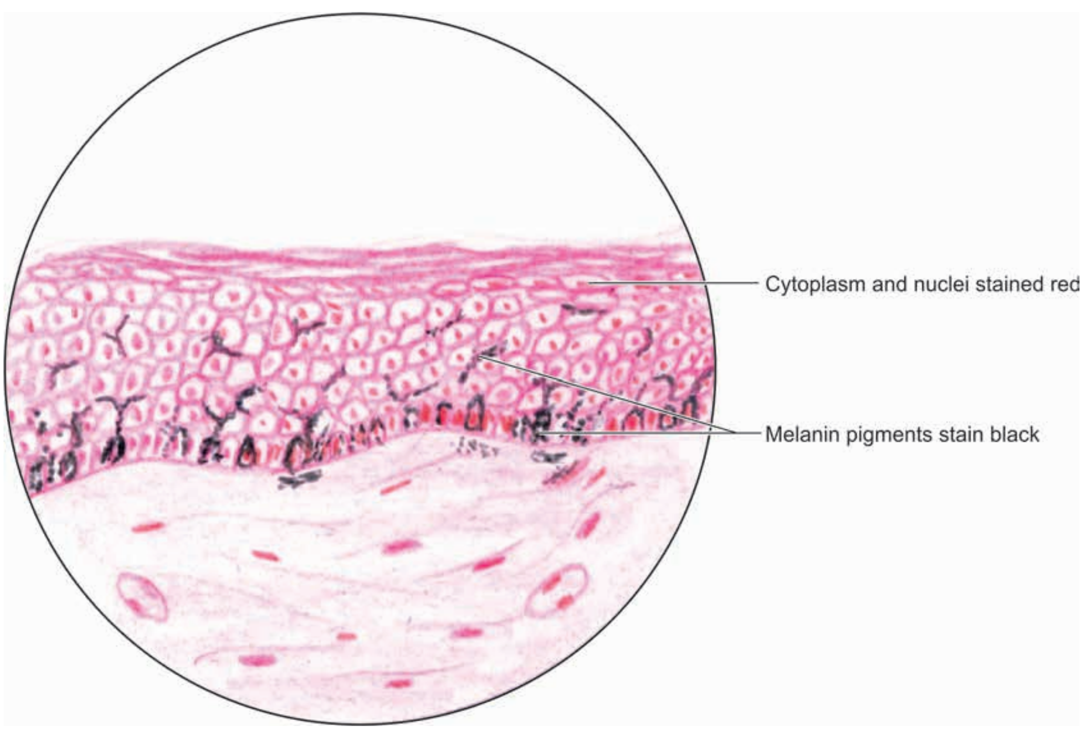


Fig. 2.17: Masson-Fontana stain

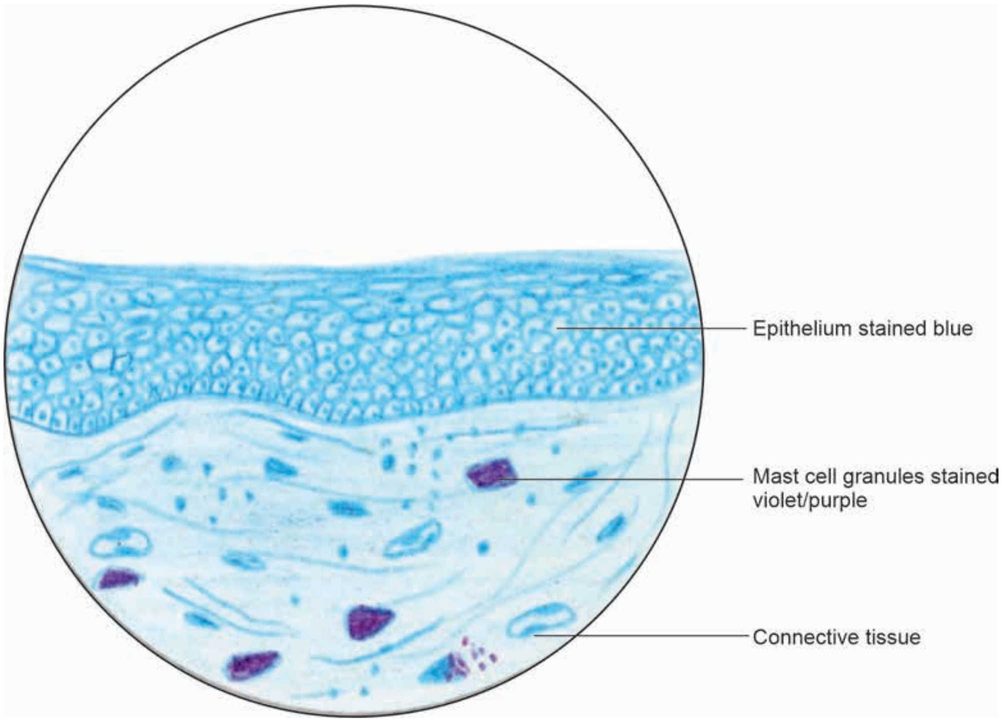


Fig. 2.18: Toluidine blue stain

CHAPTER

3

Development of Teeth

- Stages of tooth development
  - Bud stage
  - Cap stage
  - Early bell stage
  - Advanced bell stage
- Development of root

Development of tooth is a complex process. The various tissues of the tooth and its supporting structures develop from tooth germ. The term tooth germ thus includes all the formative tissues for the entire tooth and its supporting structures. Tooth germ has three main components.

1. *Enamel organ:* The ectodermal component that gives rise to enamel.
2. *Dental papilla:* The ectomesenchymal component that gives rise to dentin and pulp.
3. *Dental follicle or dental sac:* The ectomesenchymal component giving rise to cementum, periodontal ligament, and part of the alveolar socket.

STAGES OF TOOTH DEVELOPMENT

Based on the shape of enamel organ during the development of tooth, developmental stages of the tooth are divided into three morphological stages.

1. Bud stage
2. Cap stage
3. Bell stage
   - Early
   - Late or advanced

Bud Stage (Fig. 3.1)

- **Enamel organ** is bud shaped (round or ovoid) with peripheral cuboidal cells and central polyhedral cells. Peripheral cells of enamel organ are separated from ectomesenchymal components by a basement membrane. All the cells are attached to each other by desmosomal junctions.
- Ectomesenchymal condensation adjacent to enamel organ forms the **dental papilla**.
- Marginal condensation of ectomesenchymal cells enclosing dental papilla and enamel organ is called **dental follicle or dental sac.**

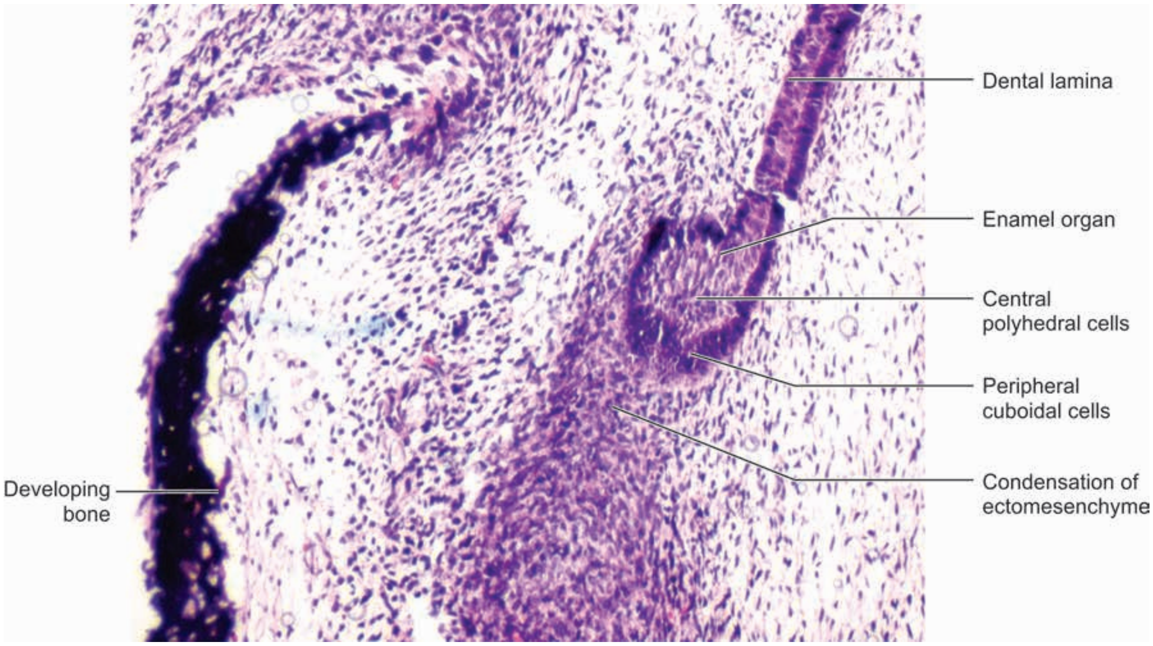
Identification Points (Fig. 3.1)

Bud Stage

- Round or ovoid (bud shaped) enamel organ
- Peripheral cuboidal and central polyhedral cells
- Dental papilla adjacent to enamel organ
- Dental follicle surrounding dental papilla and enamel organ

Cap Stage (Fig. 3.2)

Enamel organ increases in size and attain the shape of a cap by invagination of the deep portion of the bud. Cells undergo change in



Bud stage of tooth development (photomicrograph 10X)

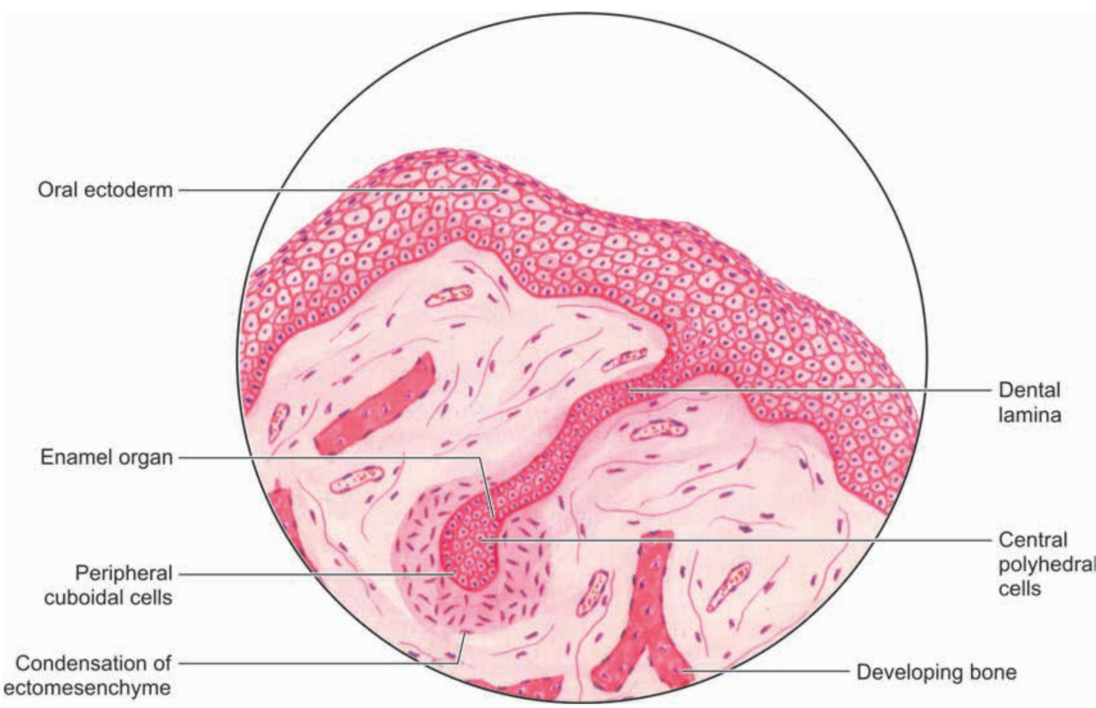
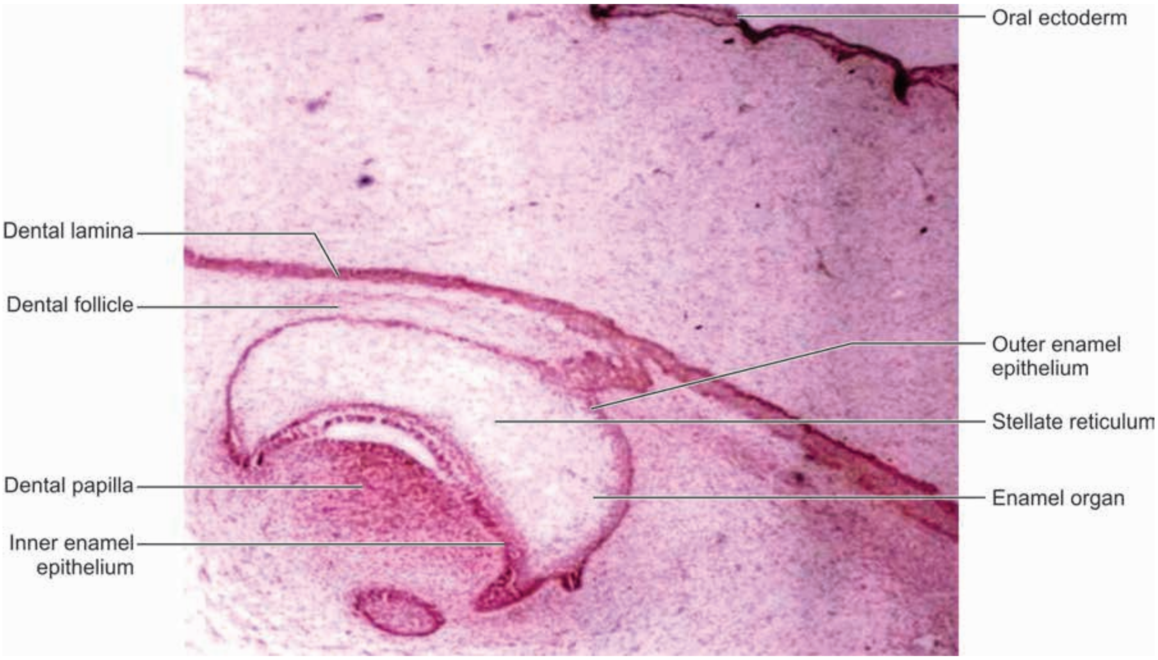


Fig. 3.1: Bud stage of tooth development



Cap stage of tooth development (photomicrograph 10X)

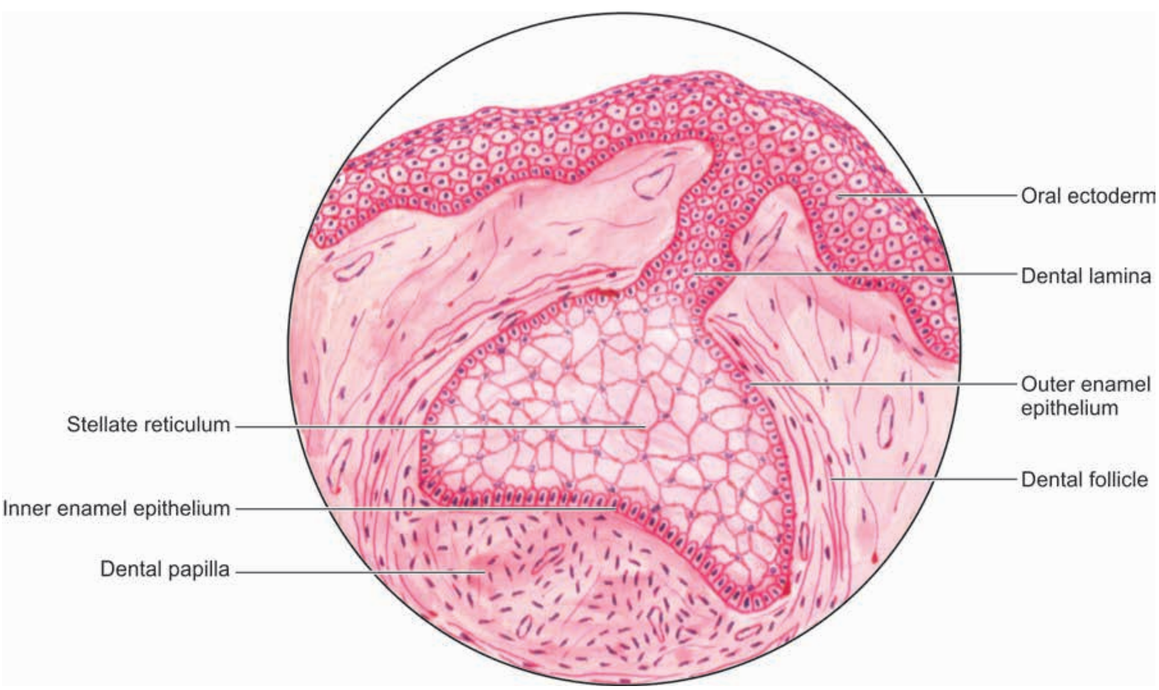


Fig. 3.2: Cap stage of tooth development

shape so that **three separate cell groups** can be identified. Cells lining the convexity or the periphery of the cap are cuboidal in shape and are called outer enamel epithelium. The cells lining the concave or invaginated portion change to columnar cells named inner enamel epithelium. The central polyhedral cells transform into a network of star-shaped cells called stellate reticulum.

Dental papilla gets partially enclosed by the invaginated portion of enamel organ. Cells of dental papilla undergo proliferation and further condensation.

Dental follicle shows further condensation of ectomesenchymal cells. It becomes more fibrous and denser in cap stage.

Identification Points (Fig. 3.2)

Cap Stage
- Cap-shaped enamel organ
- Three layers in enamel organ—inner enamel epithelium, stellate reticulum and outer enamel epithelium
- Dental papilla with condensation of ectomesenchyme and budding capillaries
- Dense fibrous dental follicle

Bell Stage (Fig. 3.3)

Enamel organ enlarges further and invagination deepens changing the shape to that of a bell in a longitudinal section. In bell stage **four different layers** of cells are seen in the enamel organ.

Cells lining the invaginated portion, the inner enamel epithelium is composed of single layer of tall columnar cells that differentiate to ameloblasts (enamel forming cells). During bell stage a new layer appears which is called stratum intermedium. This layer is located between inner enamel epithelium and stellate reticulum and is composed of two to three layers of squamous cells. Stellate reticulum expands further in early bell stage. Cells of outer enamel epithelium lining the periphery of enamel organ flatten to low cuboidal cells. All the cells are attached to each other by desmosomal junctions. At the cervical region of the enamel organ outer enamel epithelium loops inward to join with inner enamel epithelium. This is called cervical loop.

During early bell stage enamel organ loses its connection to oral ectoderm due to degeneration of dental lamina. Remnants of the dental lamina are called cell rests of Serres. Successional lamina develops at this stage which is the primordium for the permanent successor.

Dental papilla: The dental papilla is fully enclosed in the invaginated portion of the enamel organ in this stage. Peripheral cells of dental papilla differentiate into odontoblasts (dentin forming cells) under the organizing influence of inner enamel epithelial cells.

Dental follicle becomes more fibrous, with three layers, i.e. inner cellular, outer fibrous layer and middle loose connective tissue.

Identification Points (Fig. 3.3)

Early Bell Stage
- Enamel organ having bell shape
- Four layers in enamel organ—inner enamel epithelium, stratum intermedium, stellate reticulum and outer enamel epithelium
- Dental papilla with peripheral cells differentiating to odontoblasts
- Distinct dental follicle

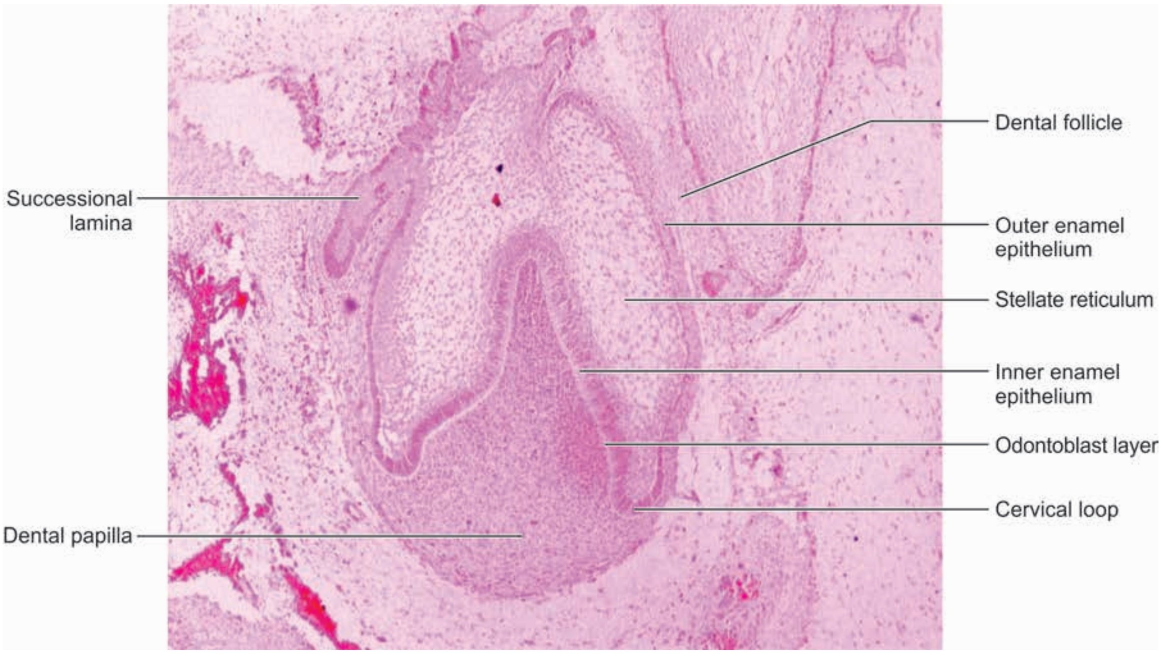
Advanced Bell Stage (Fig. 3.4)

Differentiating feature between early and advanced bell stage is formation of hard tissues.

Enamel organ shows four different layers, inner enamel epithelium (ameloblasts), stratum intermedium, stellate reticulum and outer enamel epithelium.

Histological differences from early bell stage are

- Hard tissue (enamel and dentin) formation.
- Collapsed stellate reticulum and folding of outer enamel epithelium bringing capillaries of the dental follicle nearer to the ameloblasts.



Early bell stage of tooth development (photomicrograph 10X)

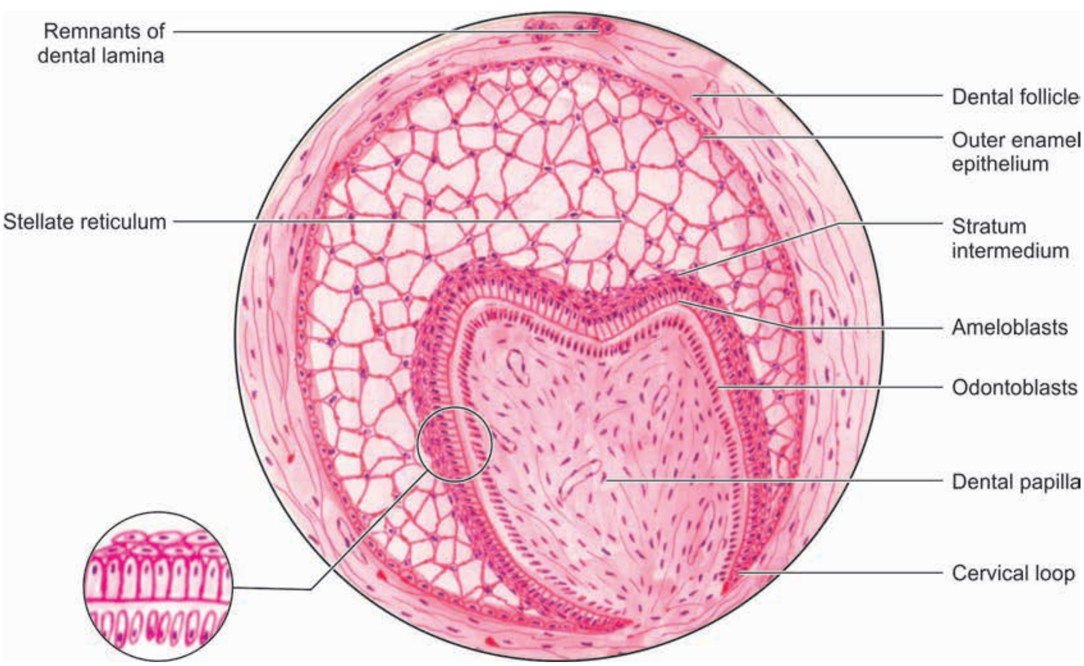
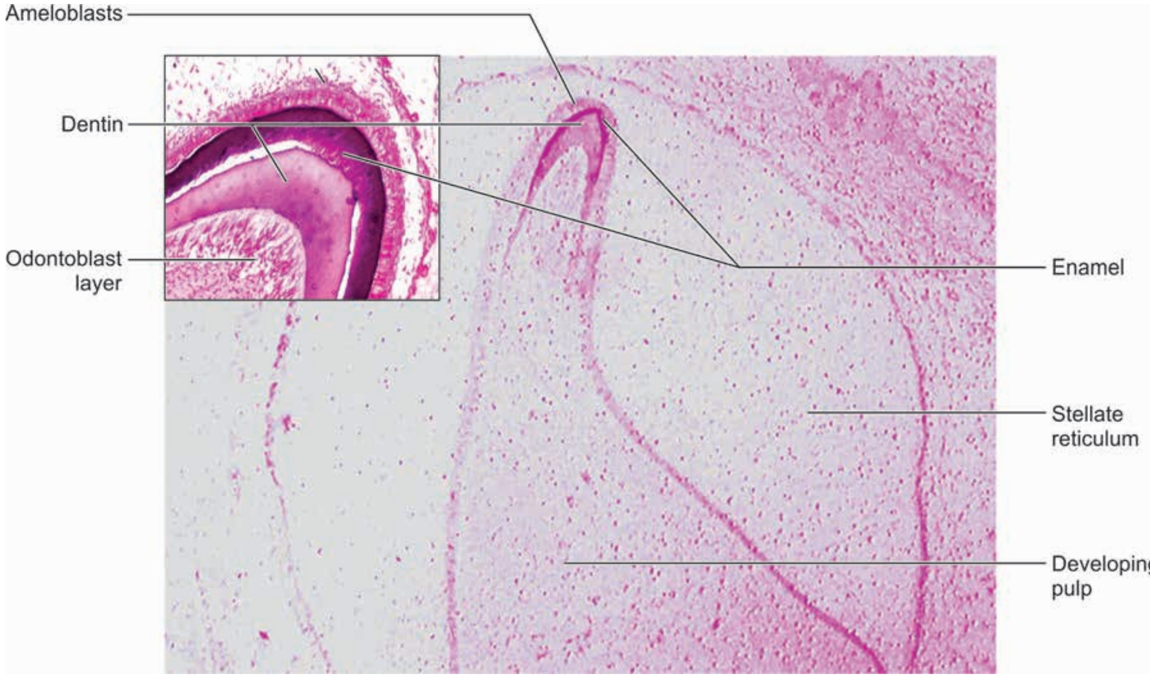


Fig. 3.3: Early bell stage of tooth development



Advanced bell stage of tooth development (photomicrograph 4X)

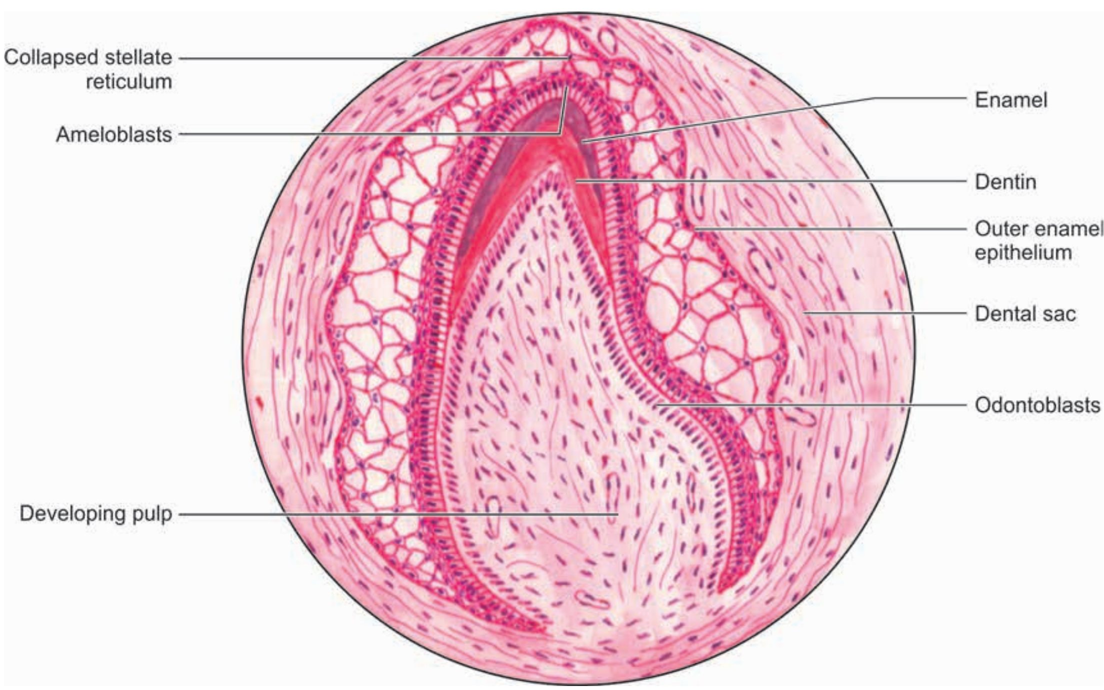


Fig. 3.4: Advanced bell stage of tooth development

Ameloblasts are brought closer to the dental follicle which now becomes their source of nutrition.

Dental papilla shows differentiated odontoblast at the periphery.

Dental follicle is distinct enclosing enamel organ and dental papilla.

Identification Points (Fig. 3.4)

Advanced Bell Stage

- Dentin and enamel formation
- Four distinct layers of enamel organ, collapse of stellate reticulum
- Distinct layer of odontoblasts
- Distinct dental follicle

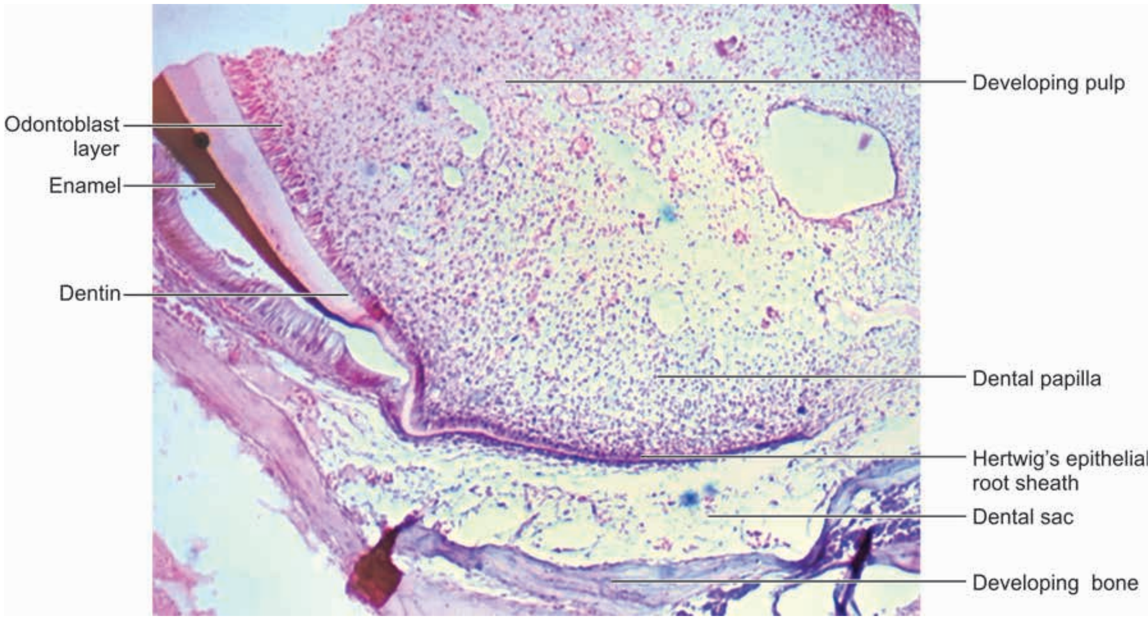
Development of Root (Fig. 3.5)

Root development occurs in advanced bell stage after the enamel and dentin formation reaches the cervical region of tooth. The cervical loop which is composed of outer and inner enamel epithelium, proliferates to form Hertwig's epithelial root sheath (HERS) which determines the size, shape and number of roots to be formed. Inner cells of HERS exert an organizing influence on dental papilla cells to differentiate into odontoblasts that deposit radicular dentin. Once the root dentin is formed, HERS degenerates allowing the dental follicle cells to come in contact with dentin. The cells of dental follicle differentiate to form cementoblasts and lays down cementum over the root dentin. As the cementum formation progresses, the rest of the dental follicle becomes more fibrous and develops into periodontal ligament. The remnants of HERS remain in periodontal ligament and are called 'cell rests of Malassez'. Under pathological conditions these cell rests may proliferate giving rise to odontogenic cysts or tumors.

Identification Points (Fig. 3.5)

Development of Root

- Formation of Hertwig's epithelial root sheath
- Formation of radicular dentin
- Degeneration of Hertwig's epithelial root sheath
- Formation of radicular cementum
- Orientation of periodontal ligament fibers



Development of root (photomicrograph 4X)

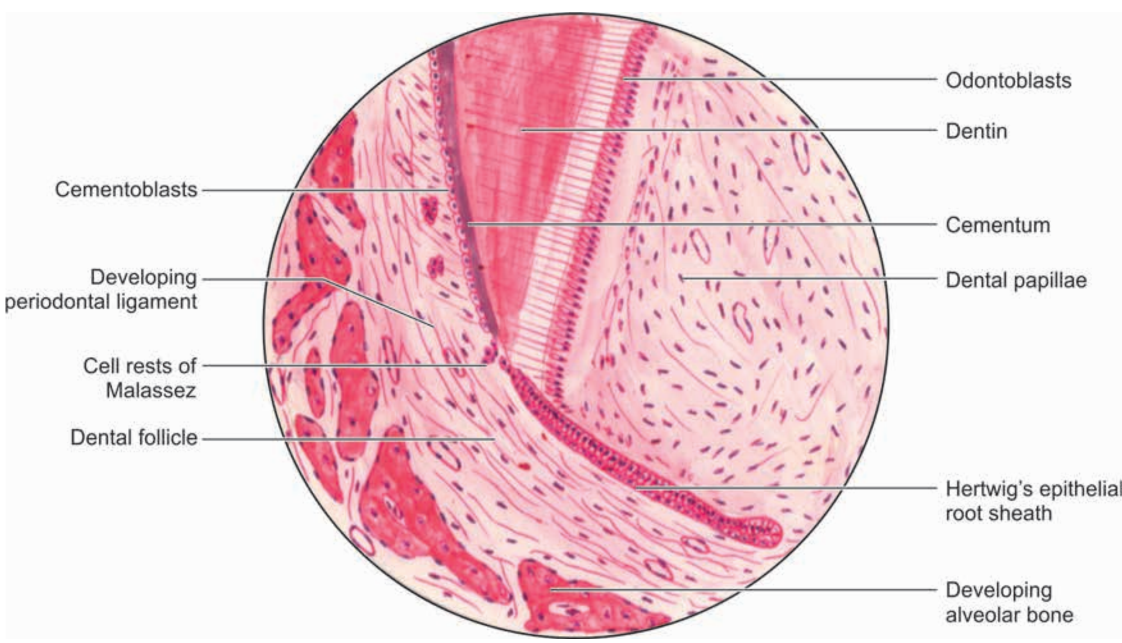


Fig. 3.5: Development of root

CHAPTER

4

Enamel

- Enamel Rod
- Striae of Retzius
- Neonatal line
- Enamel lamellae
- Enamel tufts
- Enamel spindle
- Hunter-Schreger bands
- Gnarled enamel

Enamel is the hardest calcified tissue of the body covering the anatomic crown of the tooth. Enamel, in contrast to other calcified structures of the body, is ectodermal in origin, has 96% inorganic component, and unique organic constituent which does not contain collagen. Structure of enamel is studied using ground sections. Decalcified sections are not of much use because enamel is lost during decalcification due to its high mineral content.

Enamel Rods (Figs 4.1 and 4.2)

Enamel rods are the basic structural units of enamel. They run from the dentinoenamel junction to the outer surface of enamel and follow somewhat tortuous course. In a longitudinal section (Fig. 4.1) enamel rods appear to be divided into segments by dark lines. These dark lines across the enamel rods are called cross striations. These cross striations are separated by a distance of 4 microns making each segment 4 microns which is the increment of enamel deposited daily.

Identification Points (Figs 4.1 and 4.2)

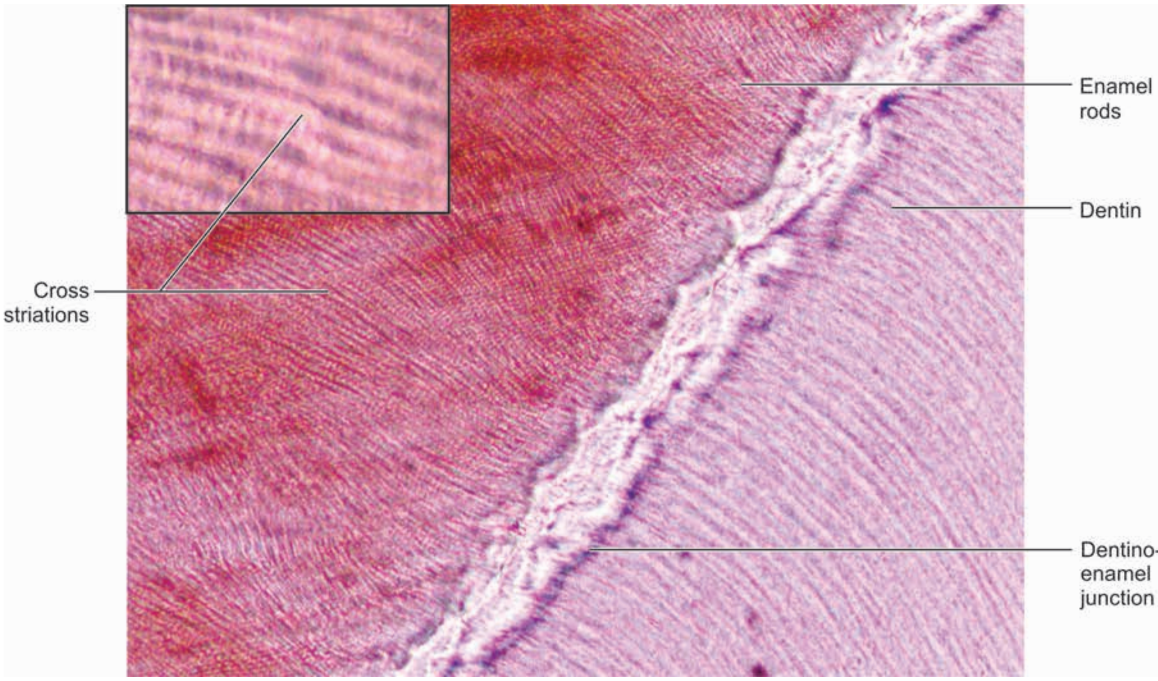
Enamel Rods

- Basic structural units of enamel
- Rod-shaped in longitudinal sections
- Resemble fish scale or keyhole in cross section

In cross section (Fig. 4.2) enamel rods may resemble a fish scale or keyhole pattern with a head and a tail. Head represents the rods and tail represents the inter rod region. The head portion is directed towards the occlusal aspect and tail to the cervical region of the tooth.

Striae of Retzius (Fig. 4.3)

Striae of Retzius are the incremental lines of enamel representing the successive apposition of enamel. These structural lines appear as brownish bands in ground sections. In the region of incisal edge and cusps (a) they surround the dentin while in cervical region they are seen as oblique lines extending from DEJ towards the outer surface deviating in an occlusal direction (b). In transverse sections of teeth incremental lines are seen as concentric rings. These lines are hypocalcified and reflects variation in structure and mineralization.



Enamel rods: Longitudinal section (photomicrograph 10X)

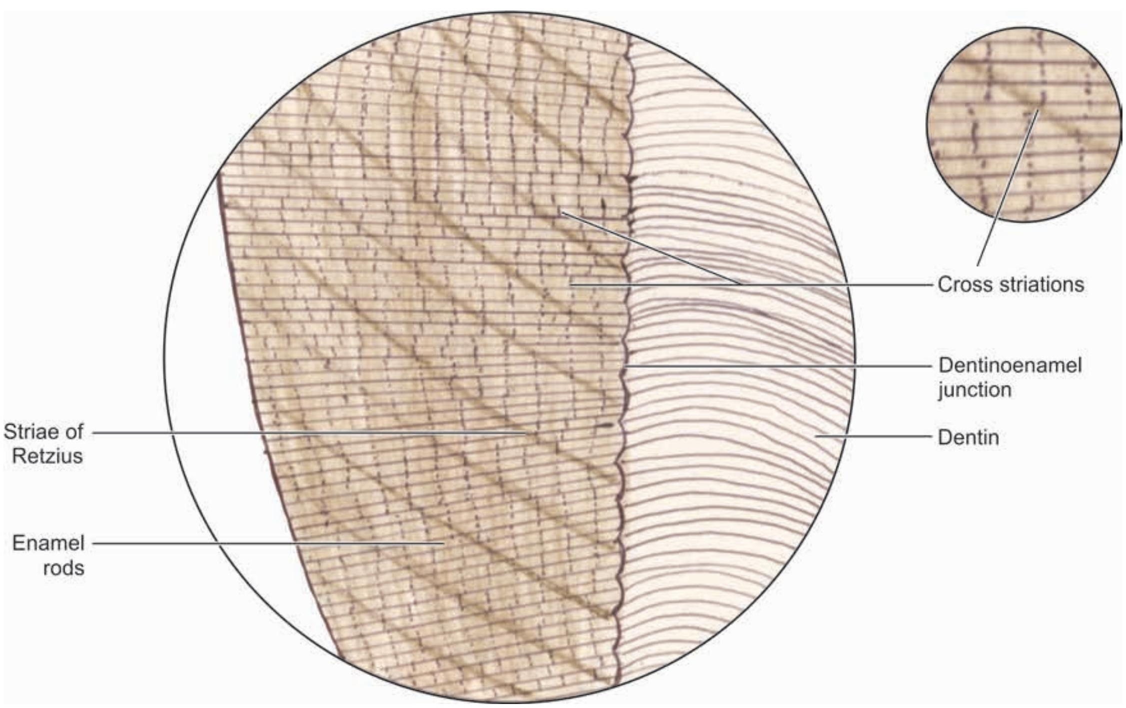


Fig. 4.1: Enamel rods: Longitudinal section

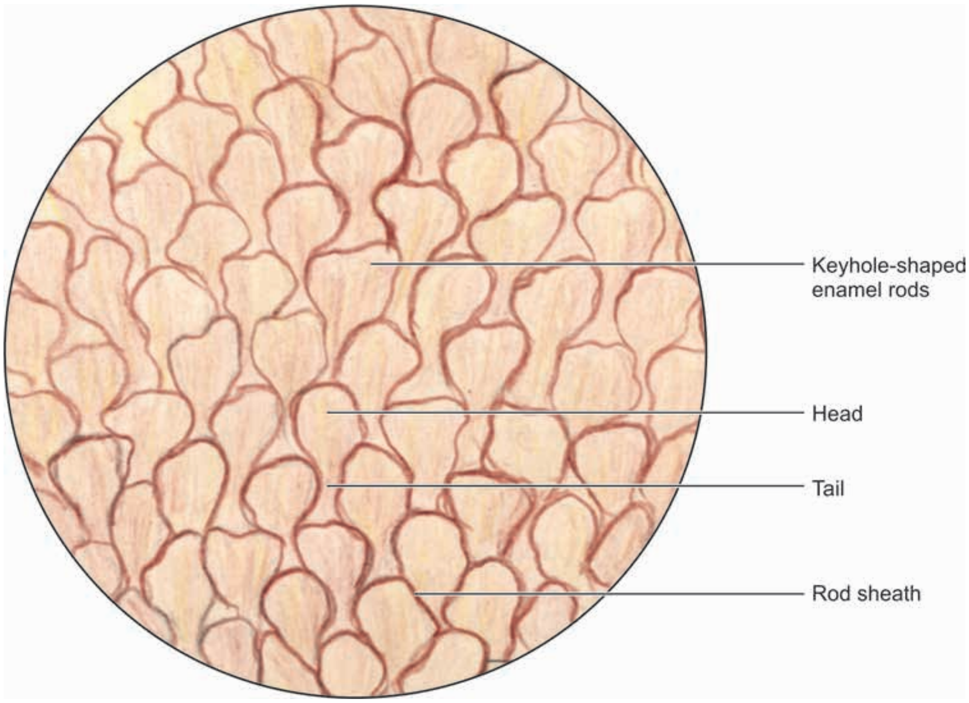


Fig. 4.2: Enamel rods—transverse section

Identification Points (Fig. 4.3)

Stage of Retzius

- Incremental lines of enamel
- Appears as brownish bands in ground section
- Hypocalcified structures

Neonatal Line (Fig. 4.4)

Neonatal line is prominent incremental line that separates the enamel that is formed before birth (prenatal enamel) and after birth (postnatal enamel). The incremental line becomes prominent because of abrupt change in the environment that occurs at the time of birth. The neonatal line is seen in all deciduous teeth and first permanent molars where enamel is formed partly before birth and partly after.

Identification Points (Fig. 4.4)

Neonatal Line

- Accentuated incremental line
- Separates pre- and postnatal enamel
- Seen in all deciduous teeth and first permanent molars

Enamel Lamellae (Fig. 4.5)

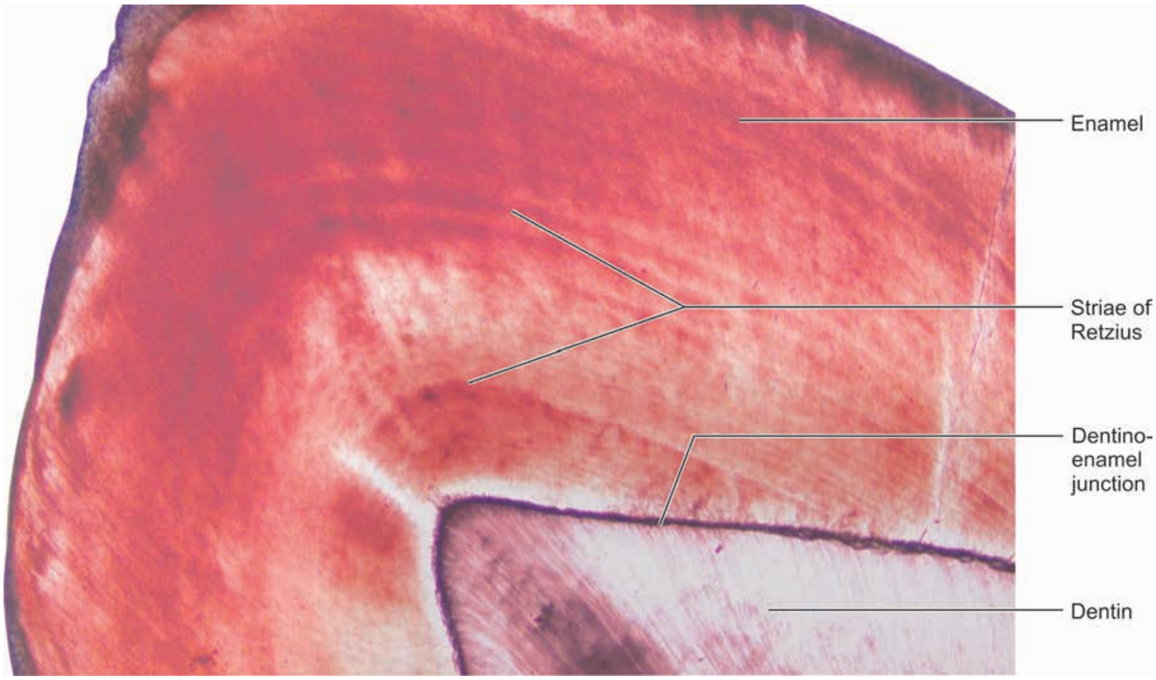
Enamel lamellae are leaf-like structures that extend from the outer surface of enamel towards the dentin. These are hypocalcified structures and are formed in planes of tension.

Three types of lamellae are seen.

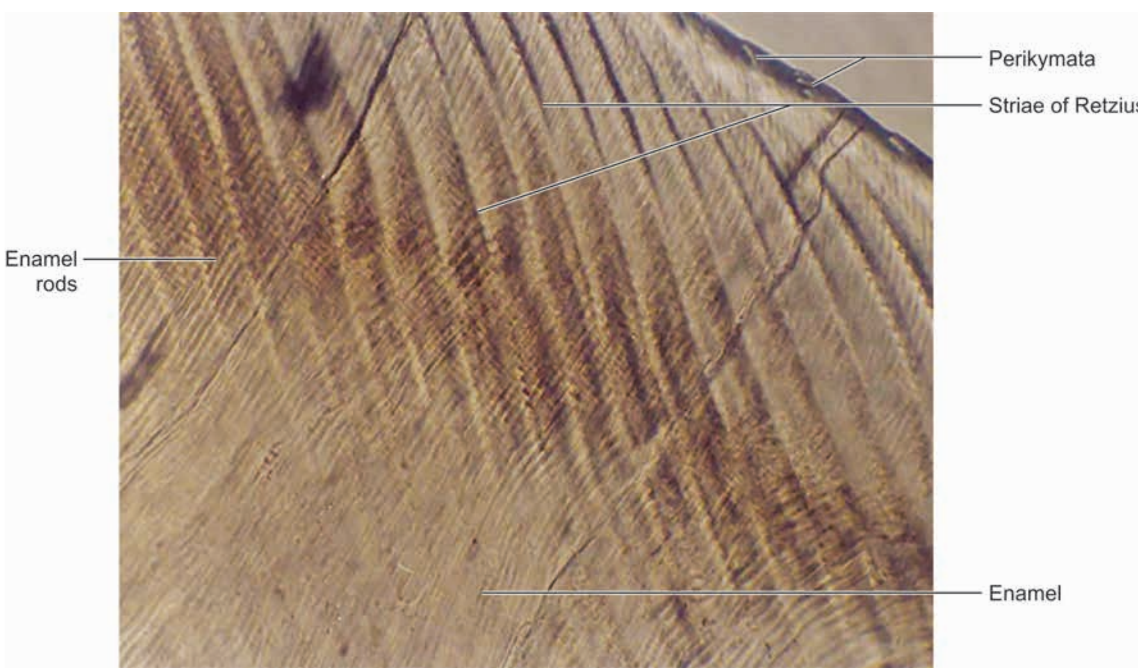
Type A: Composed of poorly calcified enamel rods. This type is restricted to enamel.

Type B: Consists of degenerated cells and may extend into dentin.

Type C: Filled with organic matter derived from saliva. This type is formed after the eruption of the tooth and may be extended into the dentin.



(a) Striae of Retzius in cuspal region (photomicrograph 4X)



(b) Striae of Retzius with perikymata

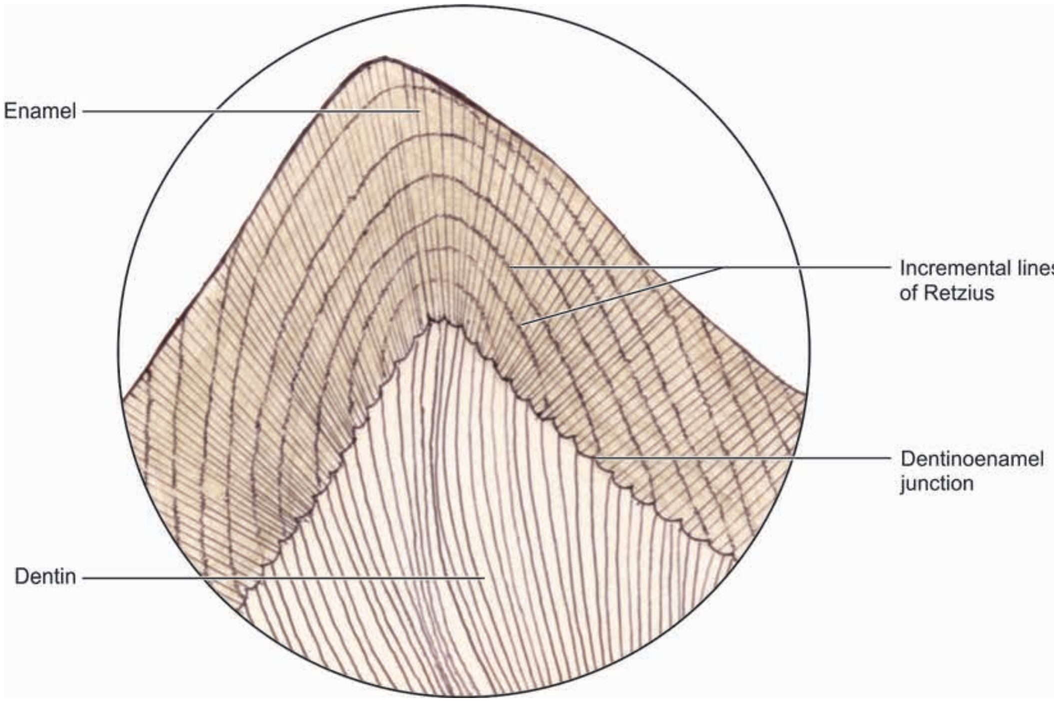


Fig. 4.3: Striae of Retzius

Identification Points (Fig. 4.5)

Enamel Lamellae
- Hypocalcified structures of enamel
- Extend from outer surface of enamel towards the dentin
- Types A, B, or C can be seen

Enamel Tufts
- Hypocalcified structure of enamel
- Ribbon-like structures extending from DEJ to enamel to a distance of one-third to one-fifth of enamel thickness
- Resemble tufts of grass in ground section

Enamel Tufts (Fig. 4.5)

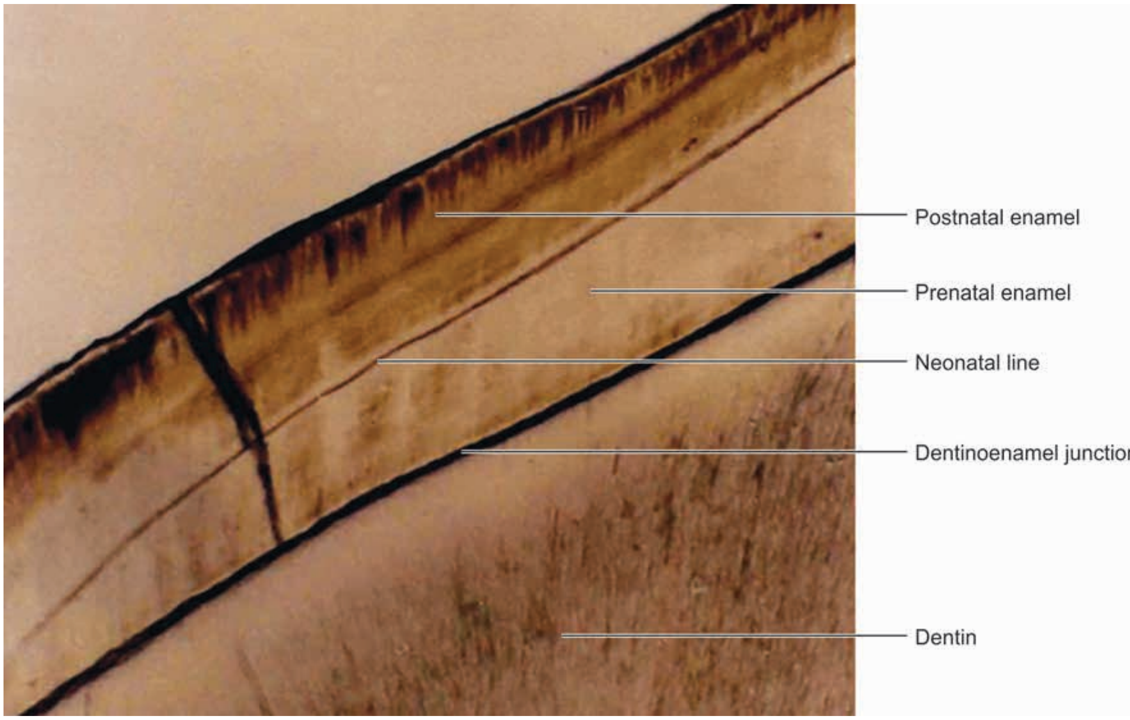
Enamel tufts are ribbon-like structures extending from dentinoenamel junction into enamel to a distance of one-third to one-fifth of enamel thickness. In thick sections these ribbon-like structures arising from different planes are projected to one plane giving the appearance of tuft of grass. These are hypocalcified structures containing greater concentration of organic components. Enamel tufts are better visualized in transverse sections.

Enamel Spindle (Fig. 4.6)

The enamel spindles are the odontoblastic processes crossing the dentinoenamel junction and extending to the enamel. These are spindle-shaped structures extending from dentinoenamel junction to enamel to a distance of approximately 10 microns. They appear dark in a ground section under

Identification Points (Fig. 4.6)

Enamel Spindles
- Odontoblast processes extending to enamel
- Appears as dark spindle-shaped structure
- Found more in the cuspal region



Neonatal line of enamel (photomicrograph 4X)

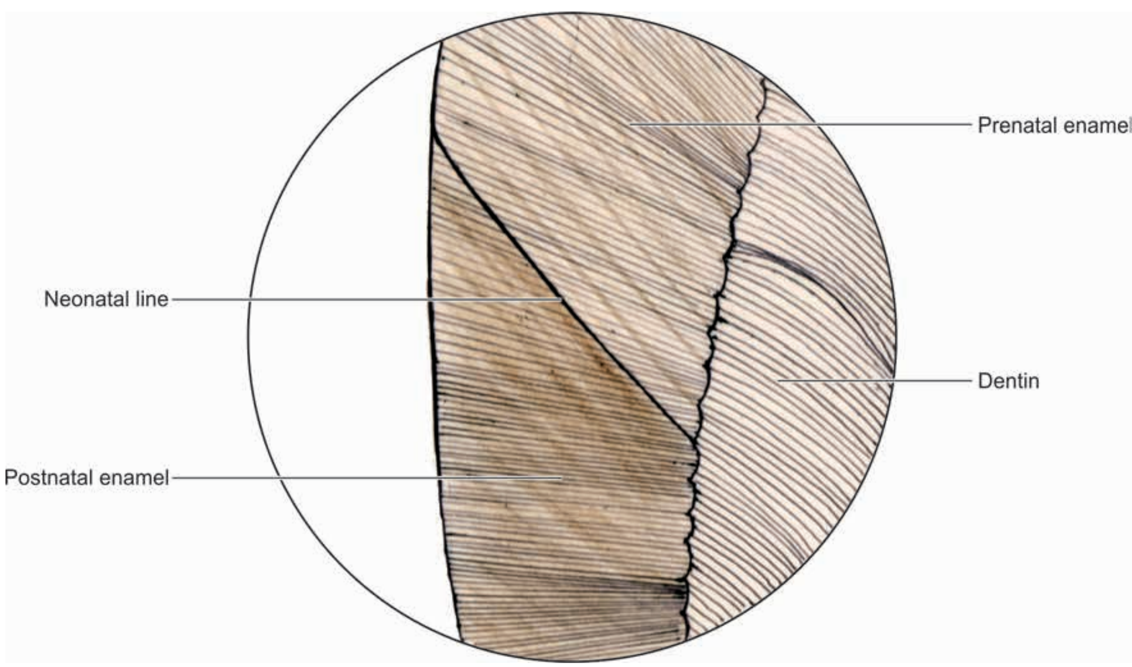
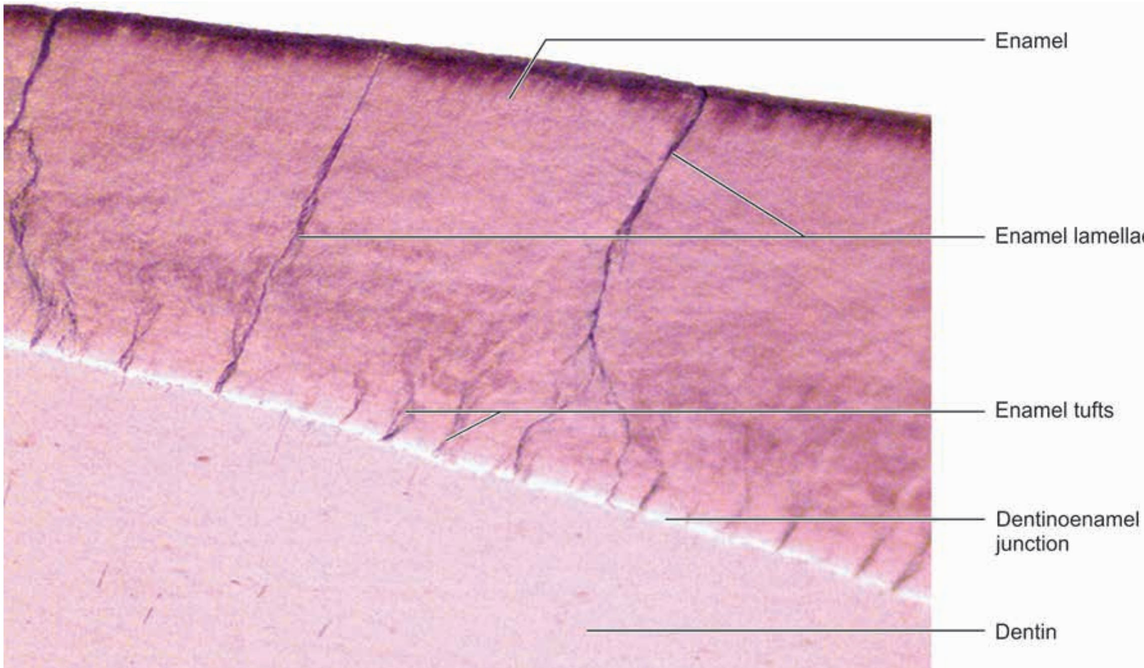


Fig. 4.4: Neonatal line of enamel



Enamel lamellae and enamel tufts (photomicrograph 4X)

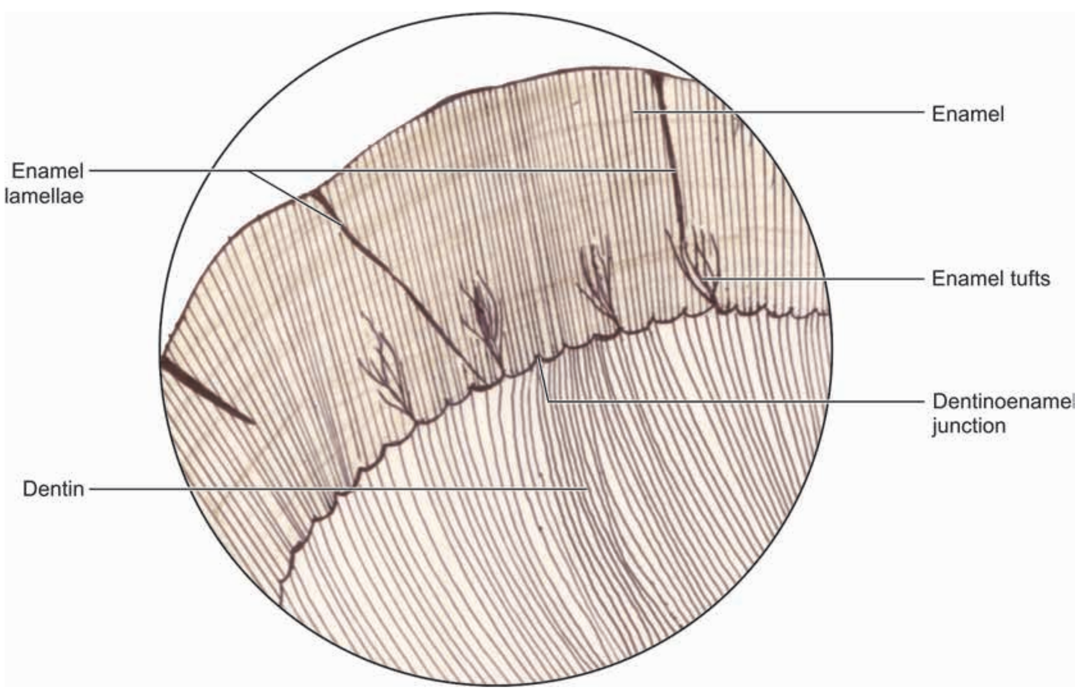


Fig. 4.5: Enamel lamellae and enamel tufts

Enamel spindle (photomicrograph 4X)

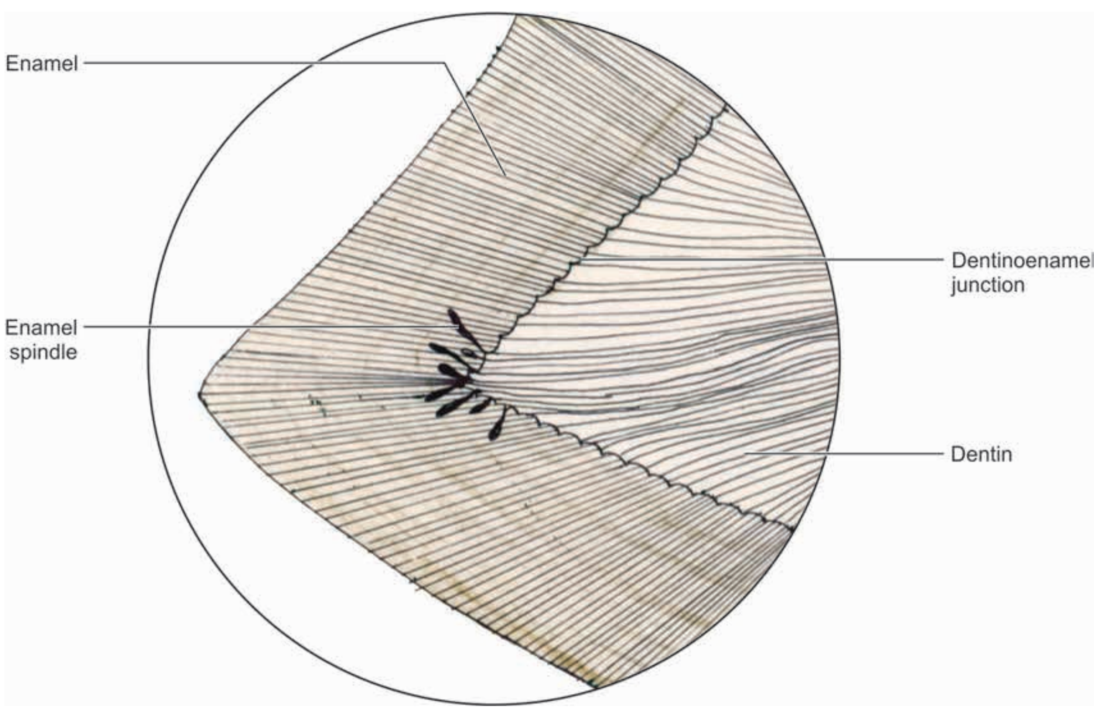
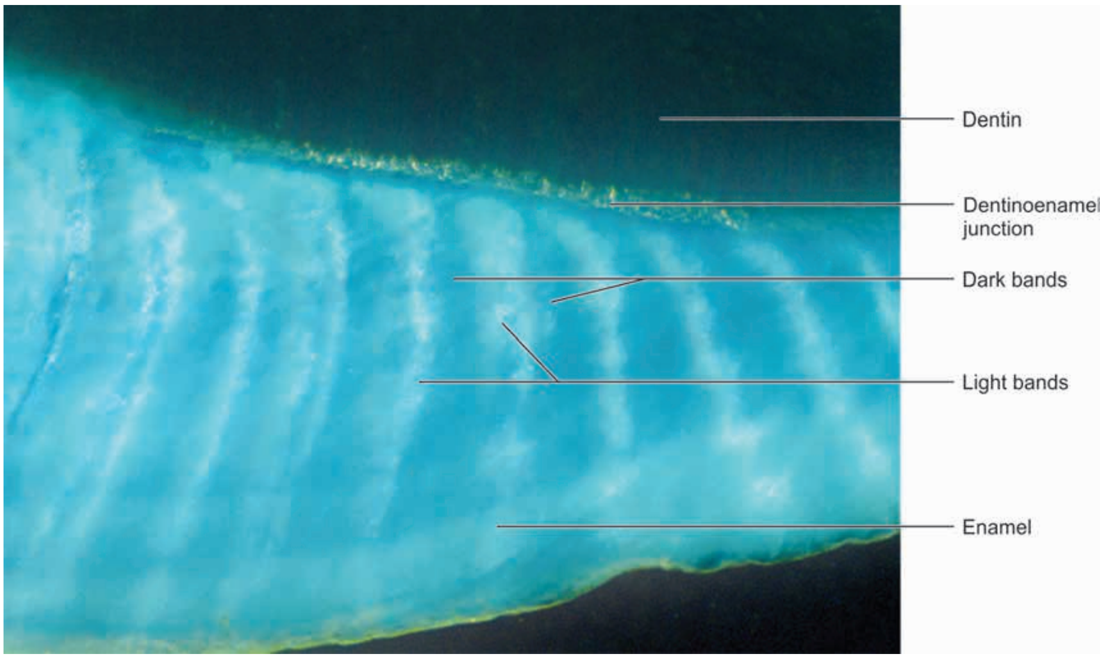


Fig. 4.6: Enamel spindles

transmitted light because the organic content of spindle is lost and is replaced by air. Enamel spindles are seen more in the region of cusp tip.

Hunter-Schreger Bands (Fig. 4.7)

Hunter-Schreger bands (HS bands) are alternate dark and light bands of varying width observed in enamel when a longitudinal



Hunter-Schreger bands (photomicrograph 4X)

Fig. 4.7: Hunter-Schreger bands

Identification Points (Fig. 4.7)

Hunter-Schreger Band
- Appear as alternate light and dark bands.
- Seen under reflected light.
- Occur due to abrupt change in direction of enamel rods.

section is viewed under reflected light. These bands arise from the dentinoenamel junction and pass outward till the inner two-thirds of enamel thickness. These bands are slightly curved with convexity directed to the cervical region. HS bands are not seen in outer one-third of enamel, because the enamel rods are straight in this region.

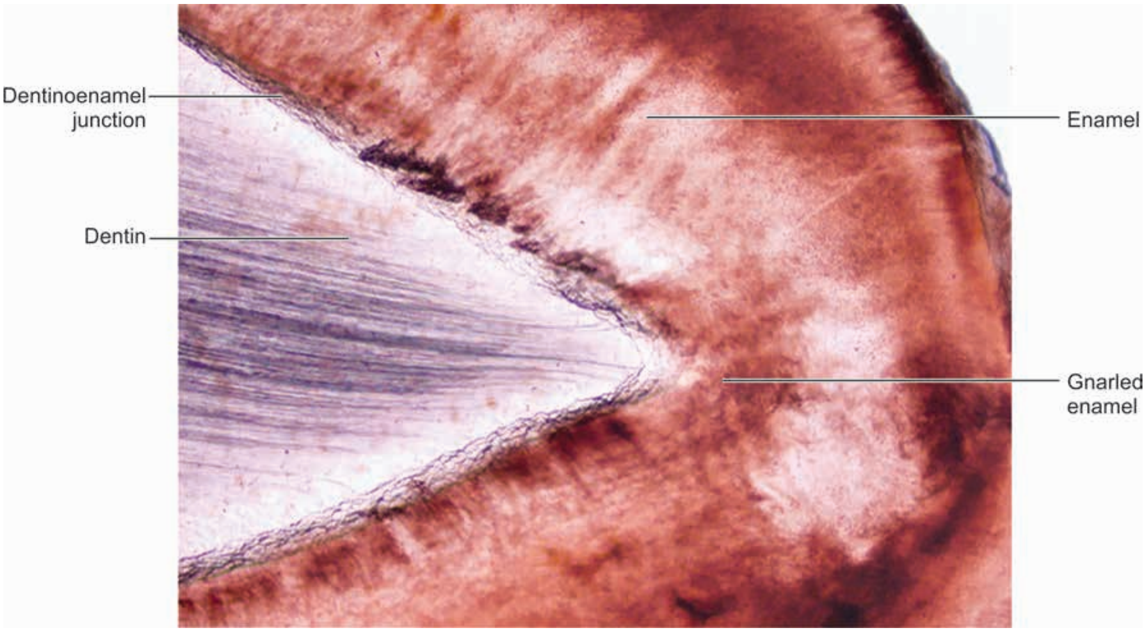
HS bands are optical effect created due to variation in course of adjacent groups of enamel rods; each group consists of 10–13 enamel rods. Because of this change in direction, when a longitudinal section is made some prisms are cut longitudinally and some transversely. When viewed under reflected light those prisms lying parallel to the light beam would reflect the light away from microscope and appear as dark bands (diazone). The prisms lying less parallel to the light would reflect the light through the microscope and appear bright (parazone). There is also an opinion that the dark and light bands of Hunter-Schreger bands may be composed of zones having altered permeability, difference in organic component or an area with variation in calcification.

Gnarled Enamel (Fig. 4.8)

Enamel rods follow a wavy course as they extend from dentinoenamel junction towards the outer surface. In the region of the cusps and incisal edges the arrangement of enamel rods is more complicated. The enamel rods are more wavy and irregular and intertwine with each other in this region especially near DEJ. This arrangement creates an optical appearance referred to as Gnarled enamel. This particular arrangement of rods in cuspal and incisal regions makes enamel stronger to withstand masticatory stress.

Identification Points (Fig. 4.8)

Gnarled Enamel
- Optical appearance seen in the incisal or cuspal region
- Occur due to wavy intertwining enamel rods
- This arrangement makes enamel stronger



Gnarled enamel (photomicrograph 4X)

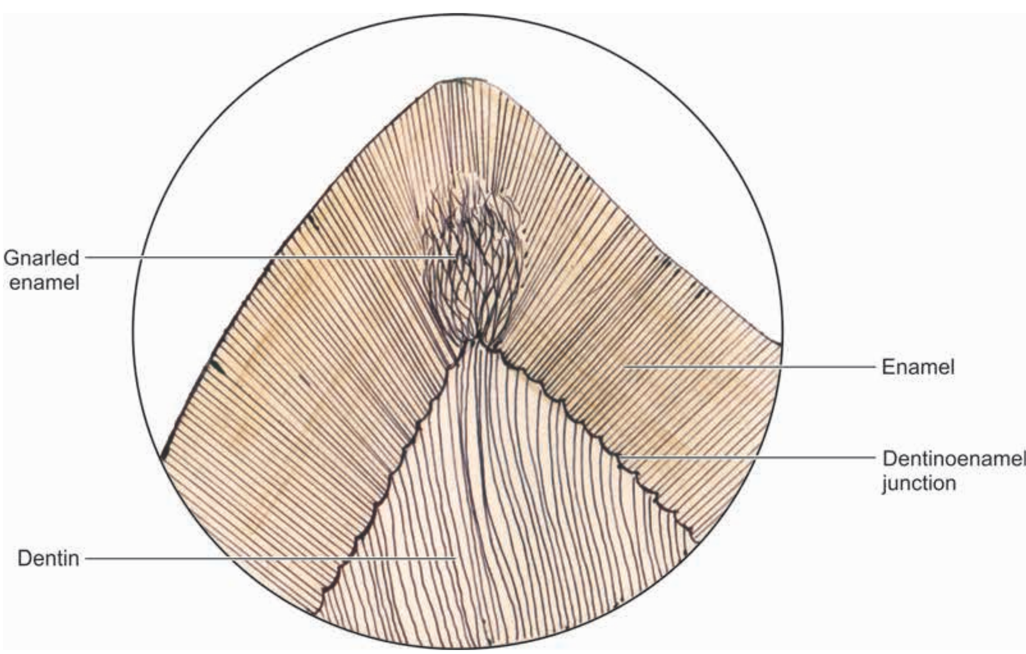


Fig. 4.8: Gnarled enamel

CHAPTER

5

Dentin

- Structure of dentinal tubules
  - S-shaped dentinal tubules
  - Y-shaped terminal branching
  - Transverse section of dentin
- Dentinoenamel junction
- Incremental lines of dentin
- Interglobular dentin
- Granular layer of Tomes
- Dead tracts

Dentin is the hard tissue component that makes up the bulk of the tooth. The structure of dentin can be studied using ground sections or decalcified sections under light microscope. The structural features seen under light microscope are dentinal tubules, which are the basic structural units of dentin, peritubular or intratubular dentin, intertubular dentin, incremental lines, interglobular dentin and Tomes' granular layer. Functional changes may be evident like dead tracts, reparative dentin and sclerotic dentin.

S-shaped Dentinal Tubules (Fig. 5.1)

Dentinal tubules extend from pulpal surface to dentinoenamel or dentinocemental junction. Dentinal tubules are S-shaped or doubly curved structures and is described as primary curvature of dentinal tubules. The first convexity from the pulpal side is directed towards the root of tooth and second convexity towards the crown. These tubules are perpendicular to pulpal surface and dentinoenamel junction. Along the length of primary curvature small oscillations will be found at intervals and are referred to as secondary curvatures.

Identification Points (Fig. 5.1)

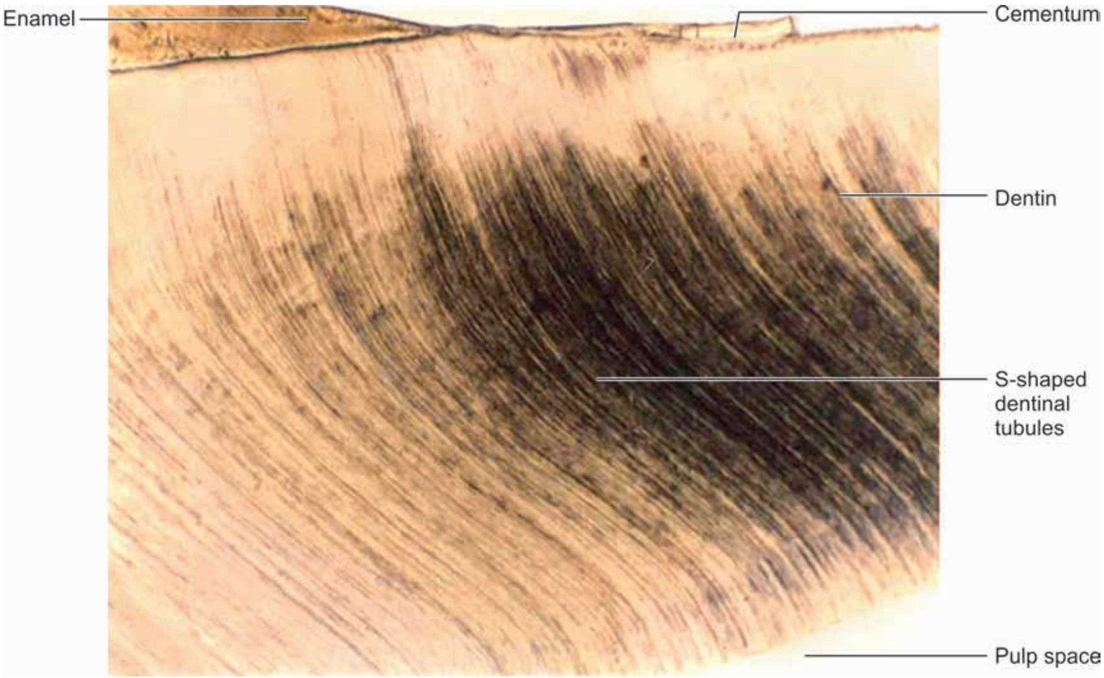
S-shaped Dentinal Tubules

- First convexity from pulpal side directed towards the root
- Second convexity directed towards the crown
- All along the course secondary curvatures are seen

Y-shaped Terminal Branches and Lateral Branches of Dentinal Tubules (Fig. 5.2)

The terminal end of dentinal tubule near dentinoenamel junction fork off from the main tubule at 45 degree to form Y-shaped terminal branching. Some of the odontoblastic processes may cross the dentino-enamel junction to form enamel spindles.

Also all along the course, the tubules have lateral branches at every 1 micrometer distance. These lateral branches are somewhat perpendicular to the main tubule and may contain odontoblast processes. Lateral branches may communicate with those of adjacent tubules or blindly end at intertubular dentin.



S-shaped dentinal tubules (photomicrograph 4X)

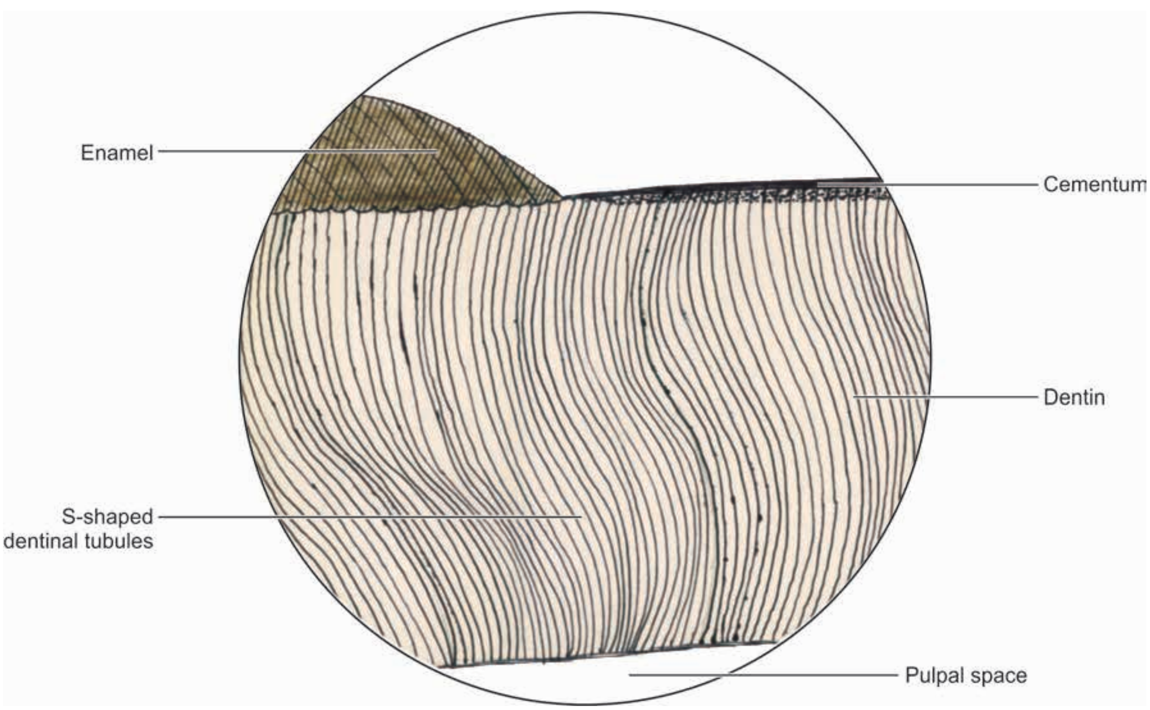


Fig. 5.1: S-shaped dentinal tubules

Primary and secondary dentin (photomicrograph 4X)

Identification Points (Fig. 5.2)

Terminal and Lateral Branches of Dentinal Tubules

- Near DEJ, terminal end of dentinal tubules shows Y-shaped branching
- Lateral branches are seen along the course
- Lateral branches may contain odontoblast process

Identification Points (Fig. 5.3)

TS of Dentin

- Dentinal tubules appear as circular structures
- Within the tubules odontoblast process is seen
- Around the tubule peritubular dentin and in between intertubular dentin are seen

Transverse Section of Dentin (Fig. 5.3)

Transverse or cross section of dentin shows numerous dentinal tubules appearing as circular structures. These tubules have odontoblast processes at the center that appear as dark spot with periodontoblast space surrounding it. The periodontoblast space contains tissue fluid named dental lymph. The inner aspect of the tubule is thought to be lined by an organic membrane called lamina limitans. The dentinal tubules are surrounded by peritubular or intratubular dentin. In between the dentinal tubules the bulk of dentin is made of intertubular dentin.

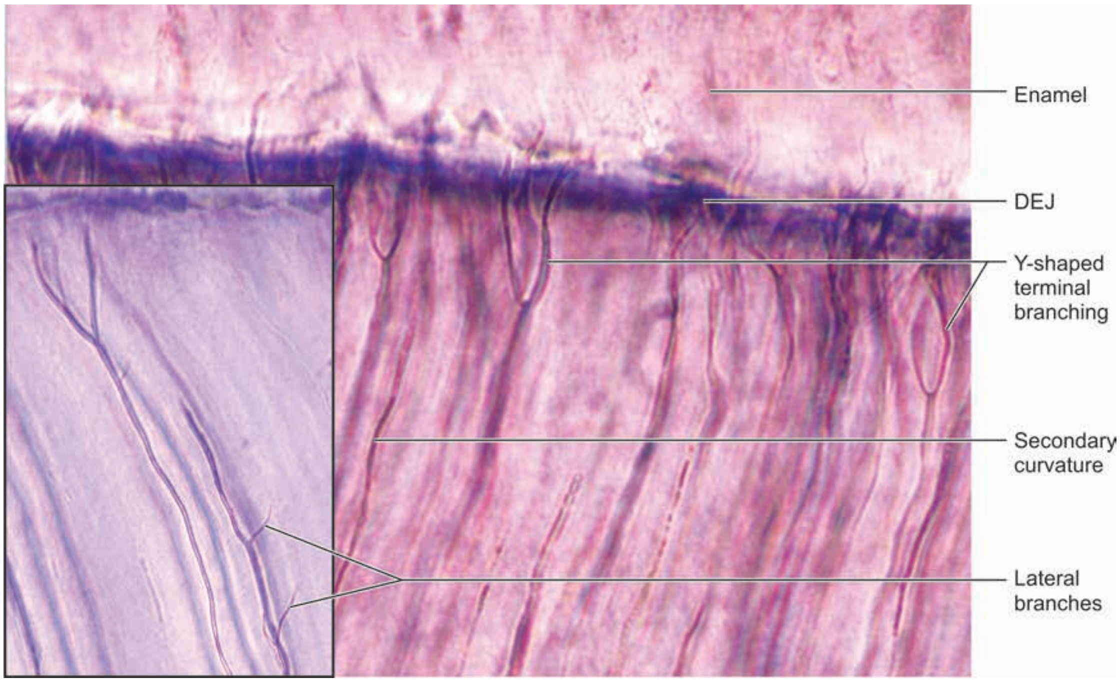
Dentinoenamel Junction (Fig. 5.4)

DEJ is scalloped with the convexity facing the dentin. This scalloped shape increases the adherence between enamel and dentin and also helps to prevent shearing of enamel during function.

Identification Points (Fig. 5.4)

Dentinoenamel Junction

- Scalloped with convexity facing dentin
- Increases surface area of contact and therefore adherence between enamel and dentin
- Prevents shearing of enamel



Terminal branches and lateral branches of dentinal tubules (photomicrograph 40X)

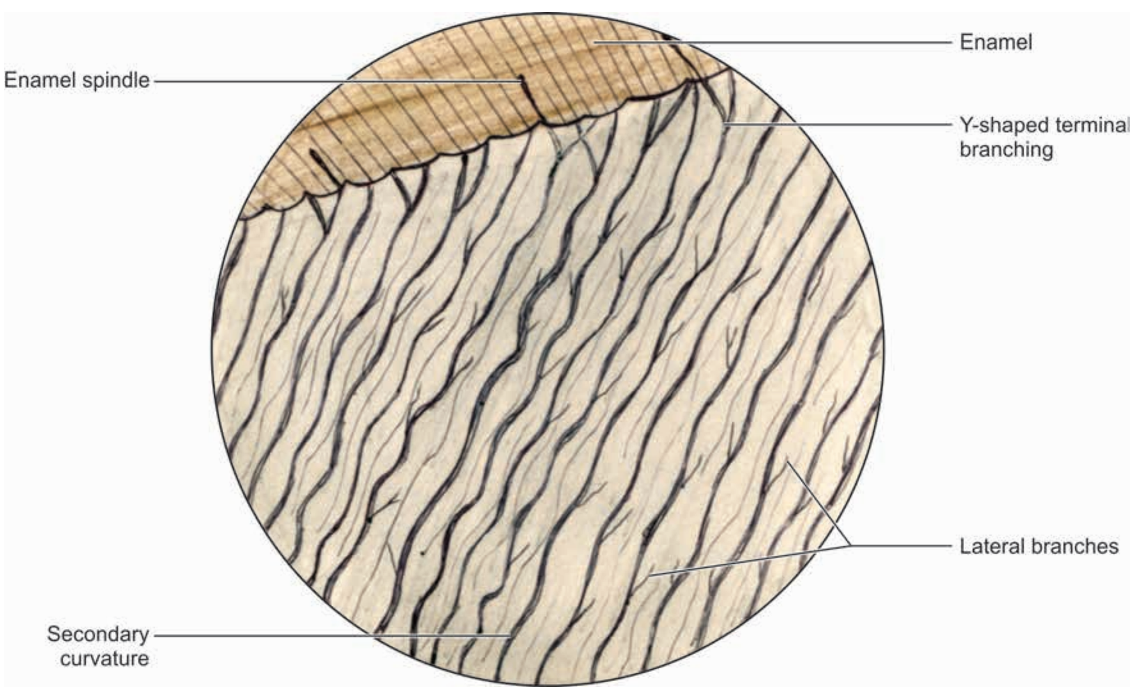
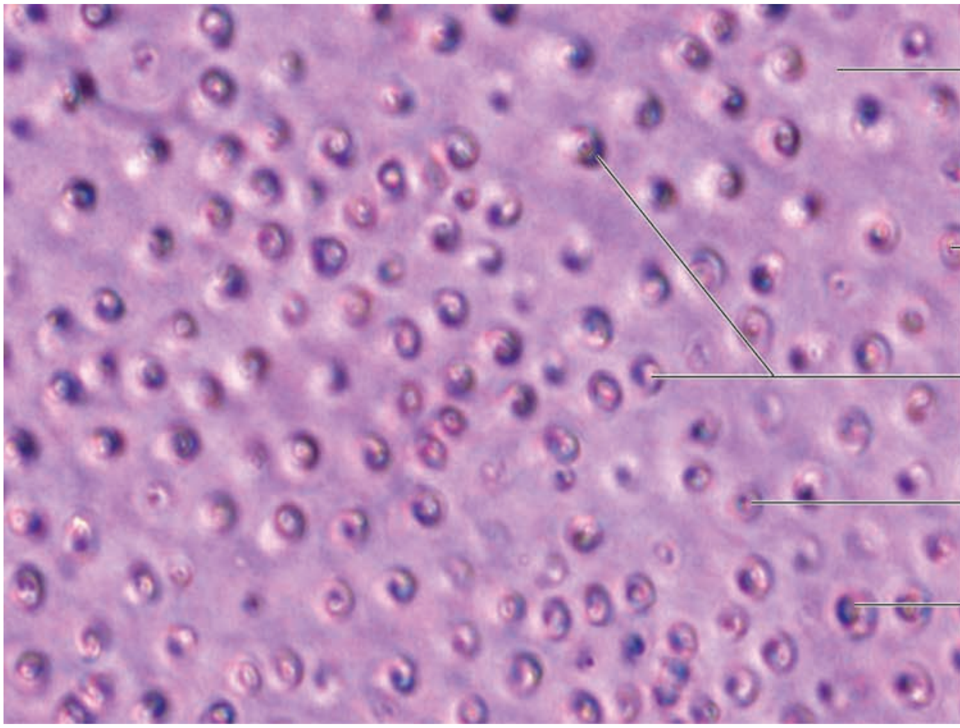


Fig. 5.2: Terminal branches and lateral branches of dentinal tubules



Transverse section of dentin (photomicrograph 40X)

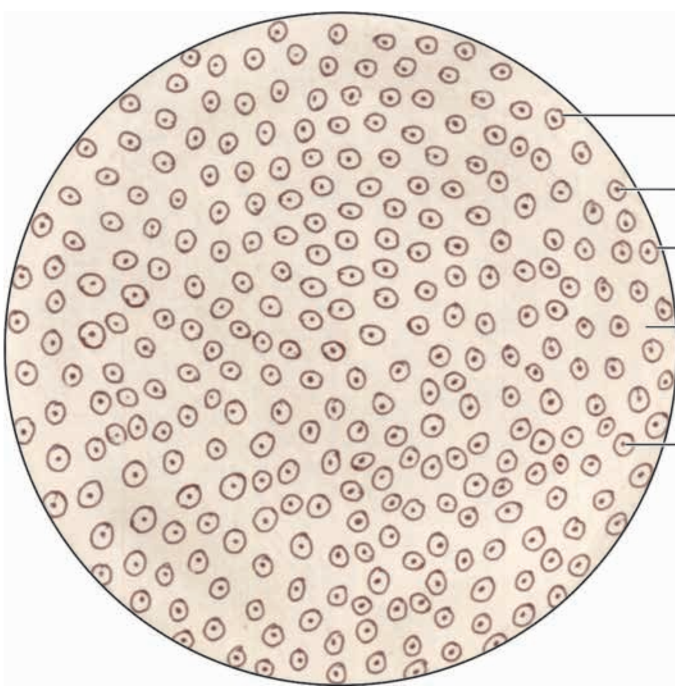
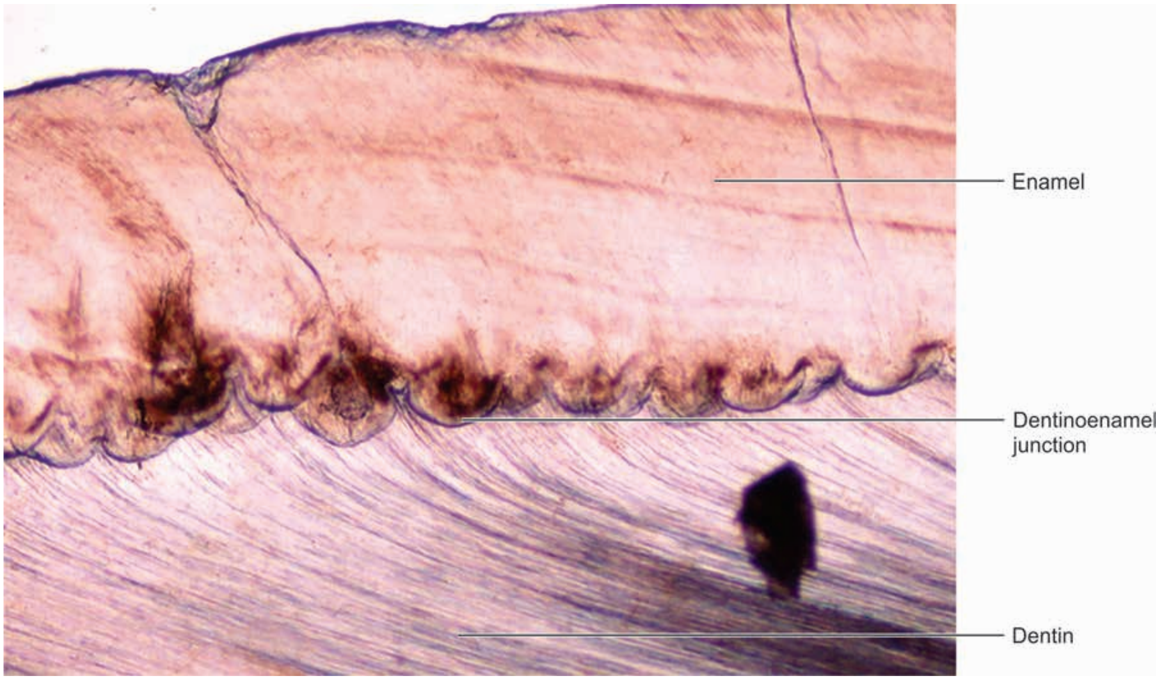


Fig. 5.3: Transverse section of dentin



Dentinoenamel junction (photomicrograph 4X)

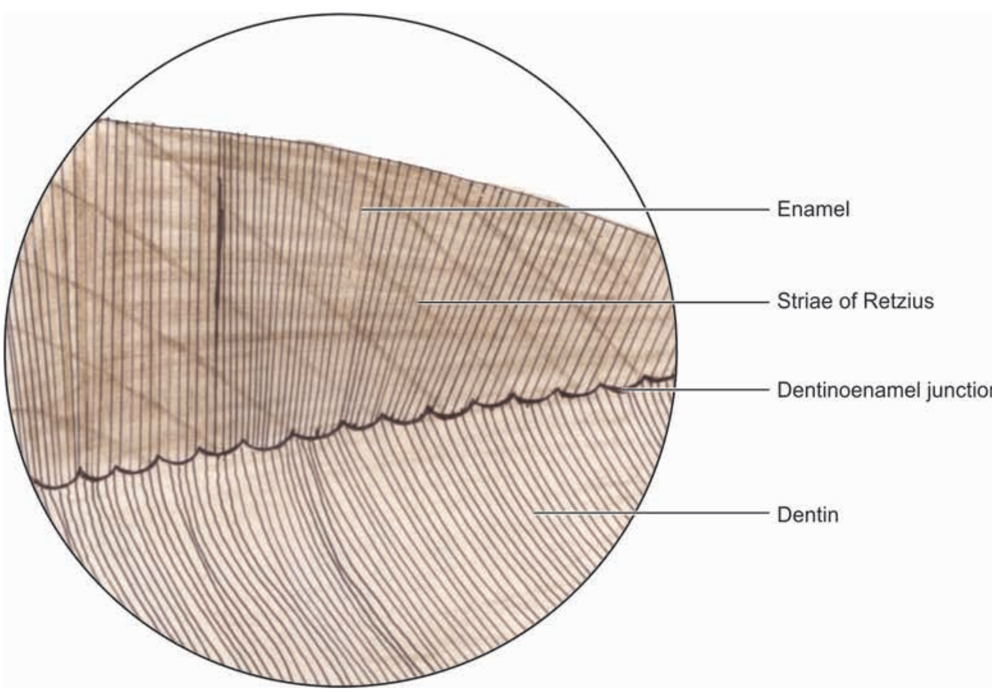


Fig. 5.4: Dentinoenamel junction

Incremental Lines of Dentin (Fig. 5.5)

Formation of dentin is a rhythmic process with alternating phases of activity and quiescence. This rhythmic deposition is indicated by incremental lines. The incremental lines of dentin are called 'lines of von Ebner' which run perpendicular to the dentinal tubules. The distance between these incremental lines are 4–6 microns and much less in radicular dentin. Sometimes due to disturbance in either matrix deposition or mineralization, these incremental lines may become accentuated and are called 'Contour lines of Owen'. The accentuated incremental line formed due to the change in environment at the time of birth is called neonatal line, and this line separates prenatal dentin from postnatal dentin.

Interglobular Dentin (Fig. 5.6)

Interglobular dentin is a hypocalcified area seen in coronal circumpulpal dentin immediately below mantle dentin. These areas appear slightly dark in a ground section under transmitted light. Dentin calcification occurs in the form of globules. Failures of fusion of these globules into a homogenous mass result in the formation of interglobular dentin. They appear star-shaped with curved outline of adjacent globules. The dentinal tubules pass uninterruptedly through interglobular dentin

Identification Points (Fig. 5.5)

Incremental Lines of Dentin

- Incremental lines run perpendicular to the dentinal tubules
- Lines of von Ebner represent the rhythmic deposition
- Contour lines of Owen are accentuated incremental lines

Identification Points (Fig. 5.6)

Interglobular Dentin

- Hypocalcified structure of dentin
- Appears as star-shaped structure in circumpulpal dentin
- Develop due to lack of fusion of globules of mineralization

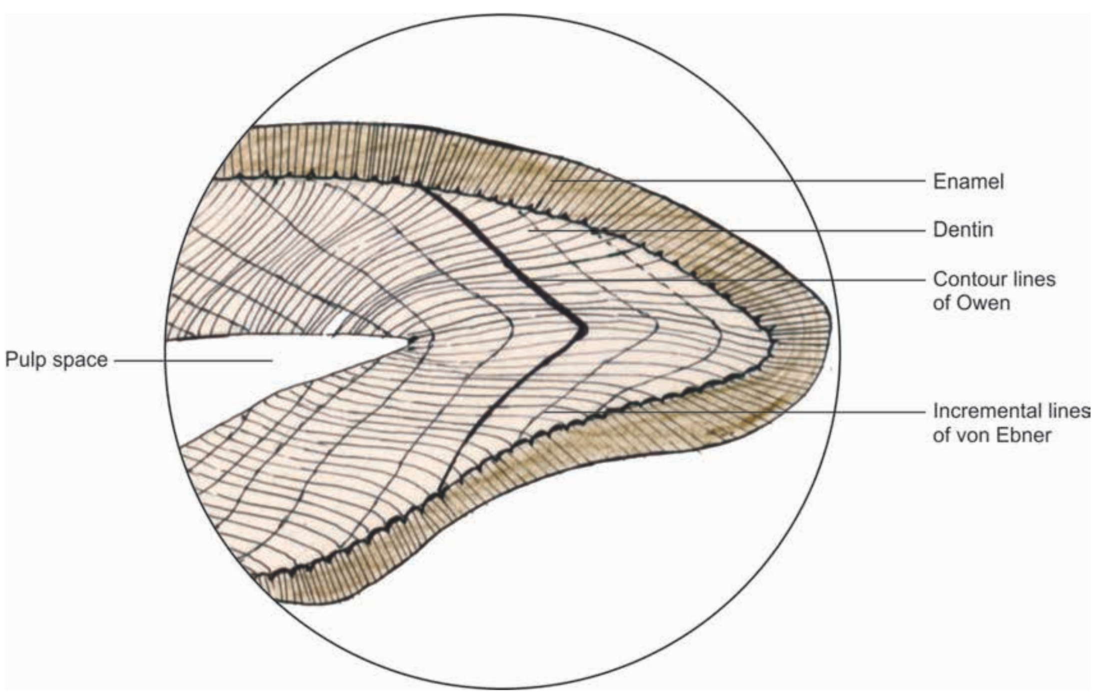
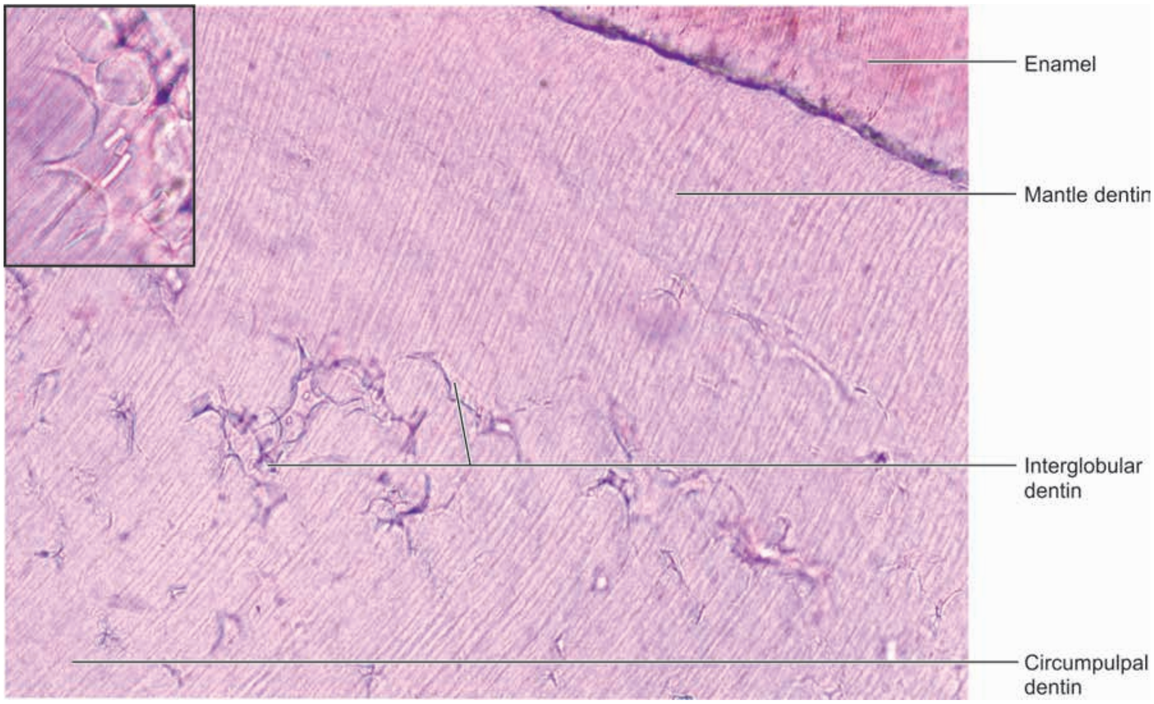


Fig. 5.5: Incremental lines of dentin



Interglobular dentin (photomicrograph 4X)

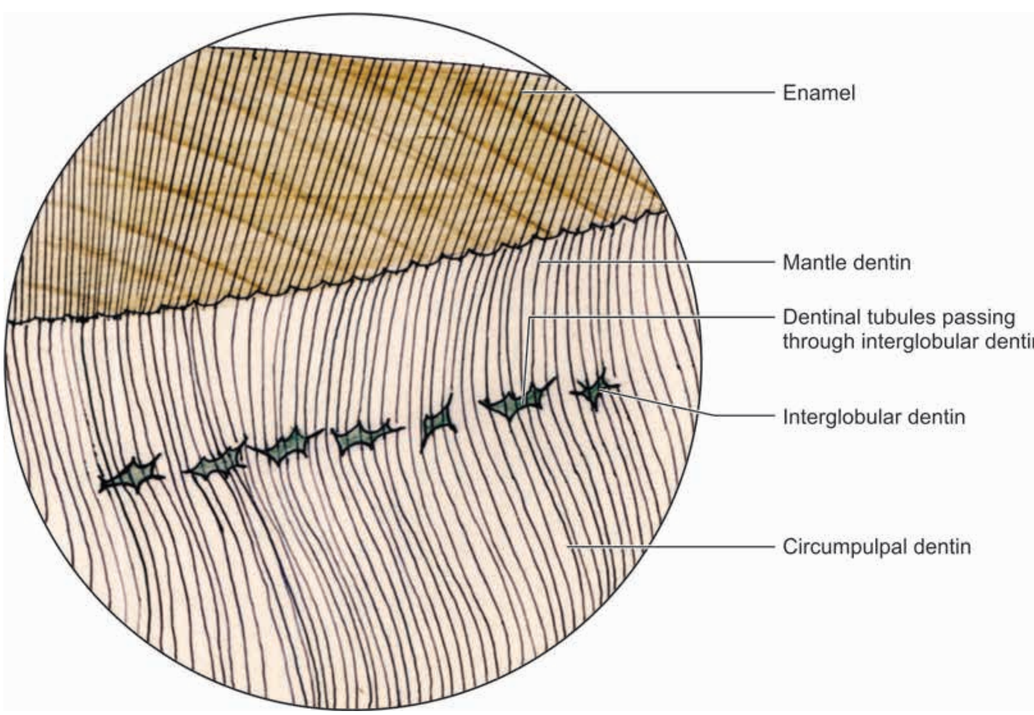


Fig. 5.6: Interglobular dentin

suggesting that this area results from defect in mineralization, and not matrix deposition.

Granular Layer of Tomes (Fig. 5.7)

It is a structure seen only in radicular dentin adjacent to cementodentinal junction. This layer appears in a ground section as dark granular structure, gradually increasing in thickness from CEJ till the apex. Tomes' granular layer is thought to be formed because of looping and coalescing of dentinal tubules near the dentinocemental junction.

Dead Tracts (Fig. 5.8)

Dead tracts are empty dentinal tubules those appear dark in ground section of dentin under transmitted light and white under reflected light. These dead tracts are formed due to degeneration of odontoblast processes in the dentinal tubules. This occurs due to exposure of dentin following attrition, abrasion or erosion. These empty dentinal tubules are filled with air; and hence appear dark under transmitted light. Dead tracts may also develop in the region of cusp or incisal edges due to death of odontoblasts occurring as a result of overcrowding. True dead tracts can be identified by the presence of reparative dentin at their pulpal end.

Identification Points (Fig. 5.7)

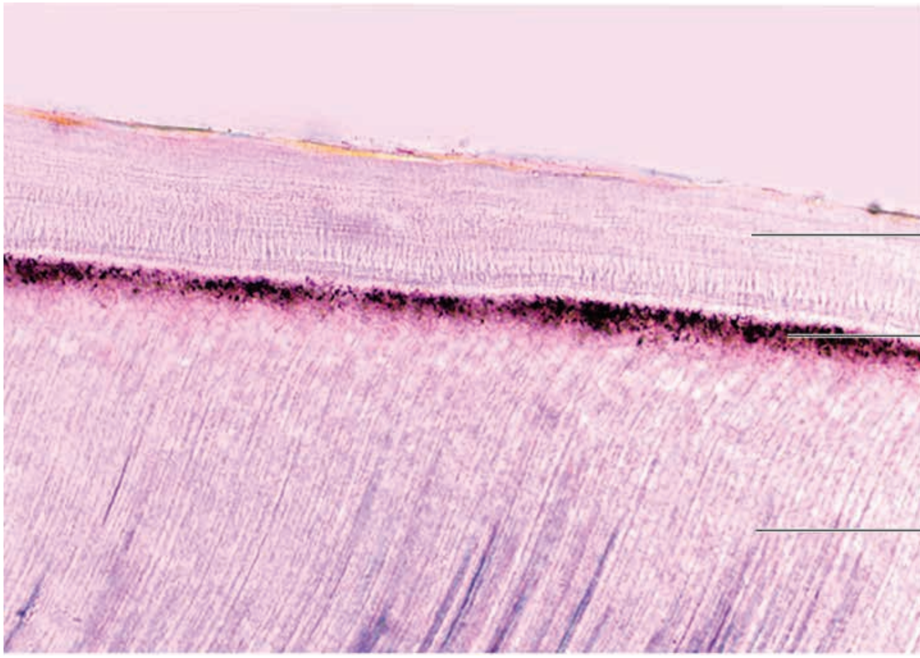
Granular Layer of Tomes

- Seen in radicular dentin adjacent to DCJ
- Appear as dark granular structure
- Formed due to looping and coalescing of dentinal tubules

Identification Points (Fig. 5.8)

Dead Tracts

- Appear dark under transmitted light and white under reflected light
- Formed due to degeneration of odontoblast processes in the tubules
- Presence of reparative dentin at the pupal end of dead tracts



Tomes' granular layer (photomicrograph 4X)

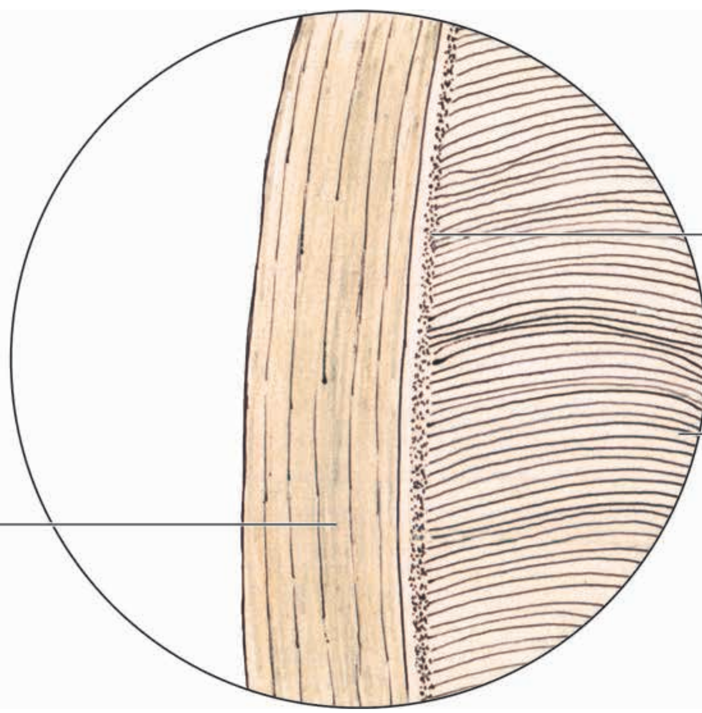
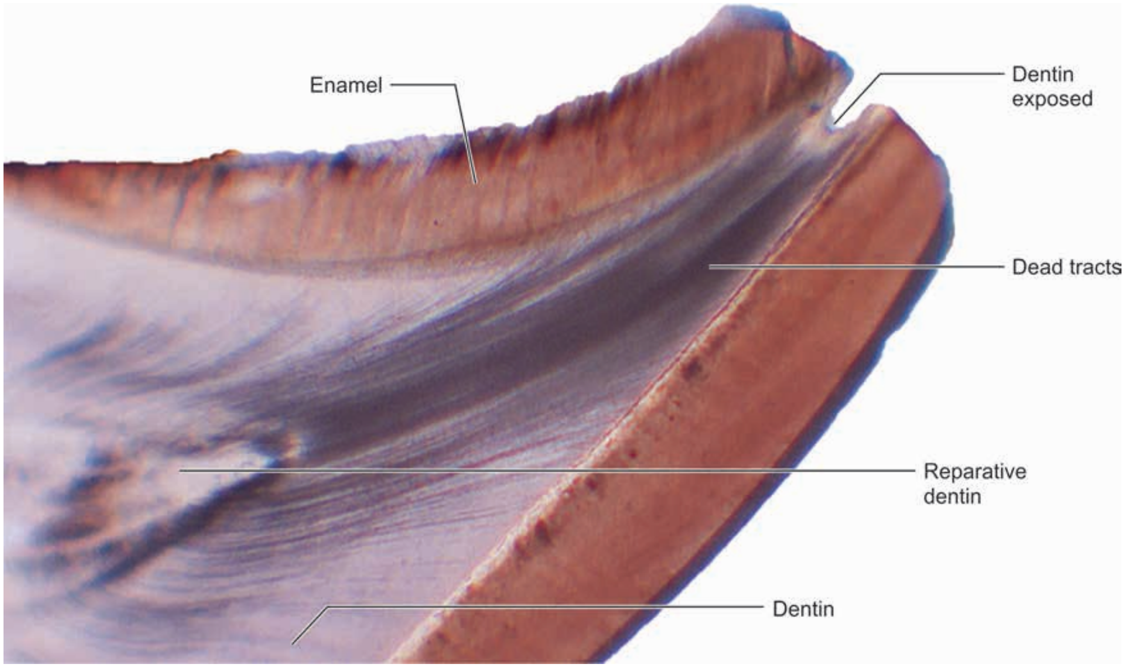


Fig. 5.7: Tomes' granular layer



Dead tracts (photomicrograph 4X)

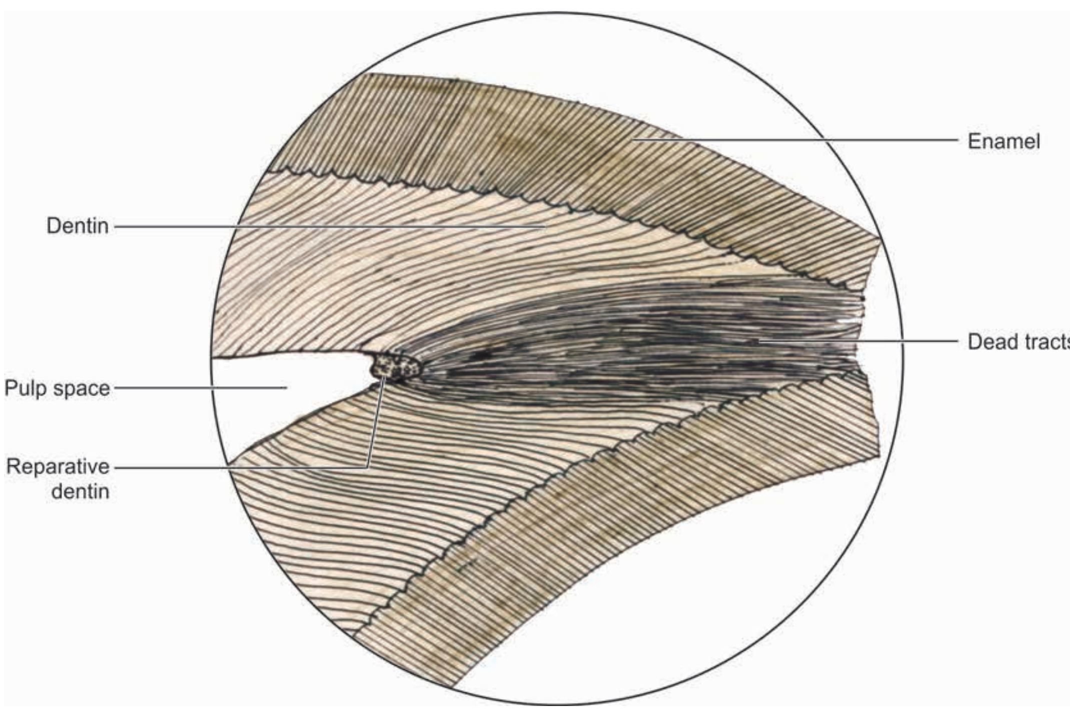


Fig. 5.8: Dead tracts

CHAPTER

6

Pulp

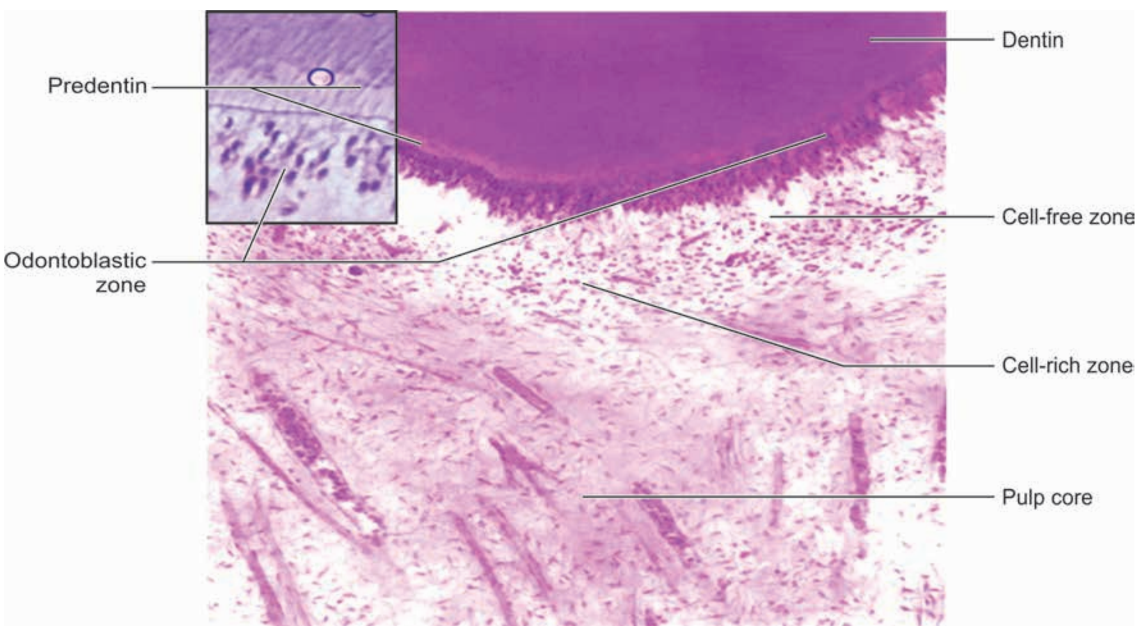
- Histology of normal pulp
- Pulp stones

Pulp is the soft tissue component of the tooth that is situated in the pulpal cavity located at the center of the tooth. Pulp is basically a connective tissue that contains formative cells of dentin, defense cells for protection, undifferentiated mesenchymal cells, and also blood vessels and nerves. Since the pulp is a soft tissue enclosed within calcified components, the structure can be studied only with the help of decalcified sections.

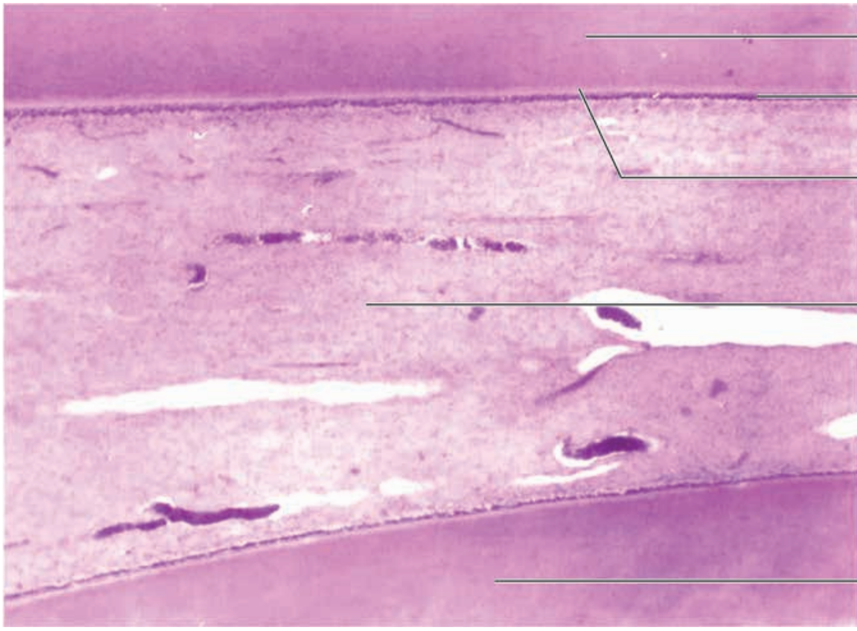
HISTOLOGY OF NORMAL PULP (Fig. 6.1)

In the pulp **four** distinct zones are seen microscopically.

Odontoblastic zone: This is the most peripheral zone of pulp seen adjacent to the predentin layer. Odontoblast cells are columnar in the crown and flattened in the root. They have process at



Histology of pulp (photomicrograph 10X)



Histology of radicular pulp (photomicrograph 4X)

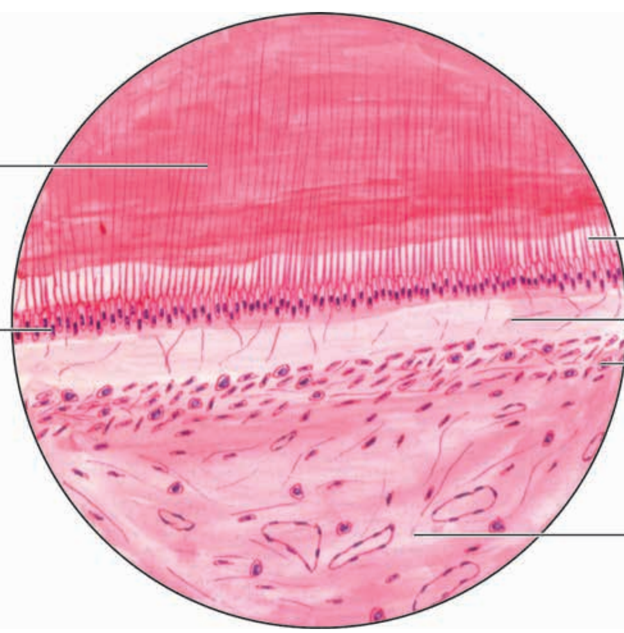


Fig. 6.1: Histology of pulp

Identification Points (Fig. 6.1)

Pulp

- Four zones are seen
- Zones are odontoblast zone, cell-free zone, cell-rich zone and pulp core
- Pulp core contains large vessels and nerves

their apical portion extending into the dentinal tubules. Cells in the coronal pulp shows a pseudo-stratified arrangement due to cell crowding.

Cell-free zone: Beneath the odontoblast layer is cell-free zone of Weil which is devoid of cells, but has fibers and nerves.

Cell-rich zone: This zone is seen beneath the cell-free zone and is rich in cells. The cells present are mainly fibroblasts and progenitor cells.

Pulp core: The central portion of pulp is called pulp core that contains cells, large blood vessels and nerves, etc. distributed in the ground substance.

PULP STONES (Fig. 6.2)

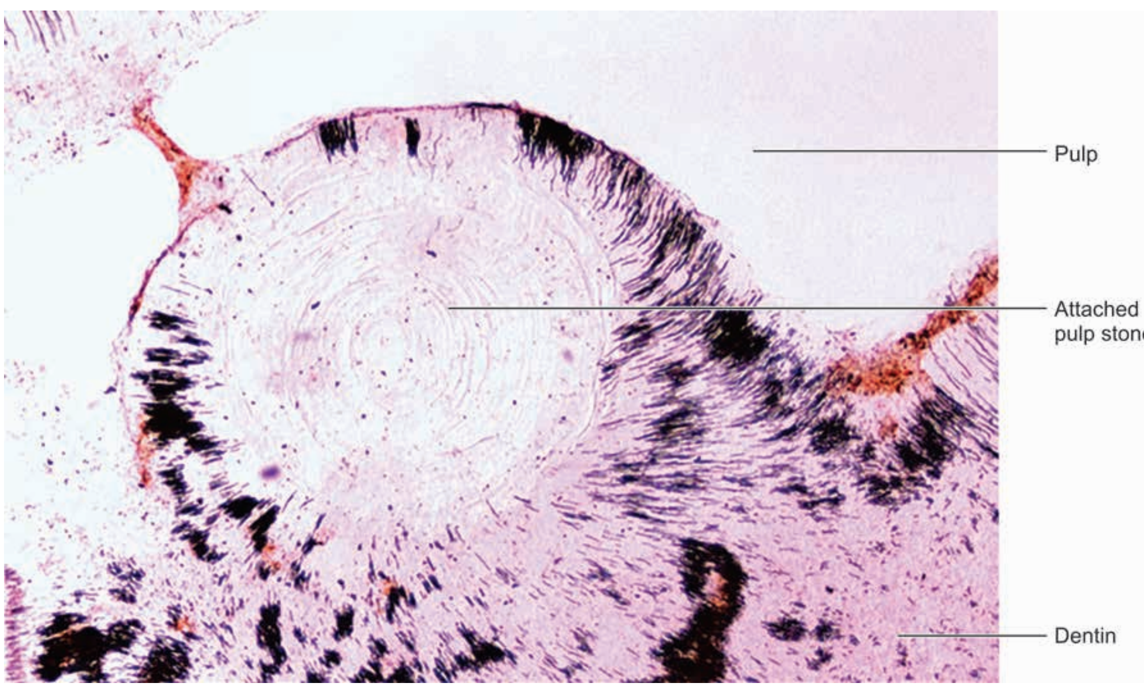
Pulp stones are calcifications seen in the pulp. They can be true or false depending on structure. True pulp stones have a tubular structure resembling dentin while false pulp stones are concentric rings of calcified material not resembling dentin. Pulp stones are also classified into:

1. **Free pulp stones:** Lying free in the pulp without being attached to the dentin (Fig. 6.2).
2. **Attached pulp stones:** These are attached to the dentin.
3. **Embedded pulp stones:** When pulp stones are completely surrounded by dentin.

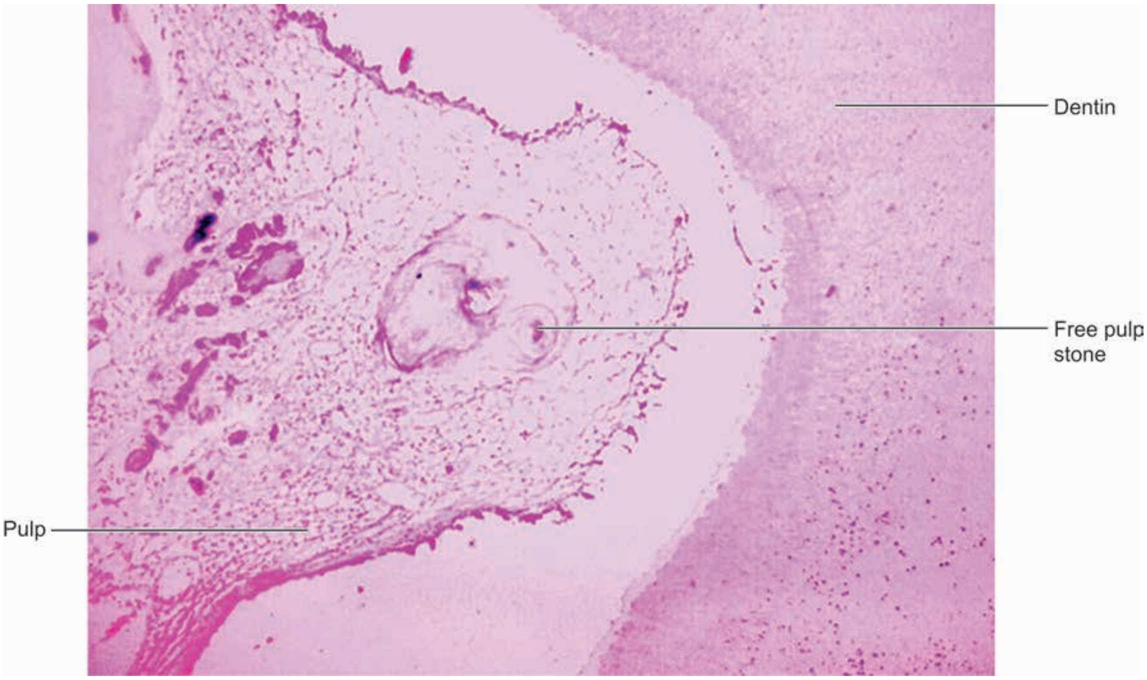
Identification Points (Fig. 6.2)

Pulp Stones

- Calcifications seen in pulp.
- May be true or false pulp stones
- Can be free, attached or embedded.



Pulp stone-attached (photomicrograph of ground section 10X)



Pulp stone-free (photomicrograph of decalcified section 10X)

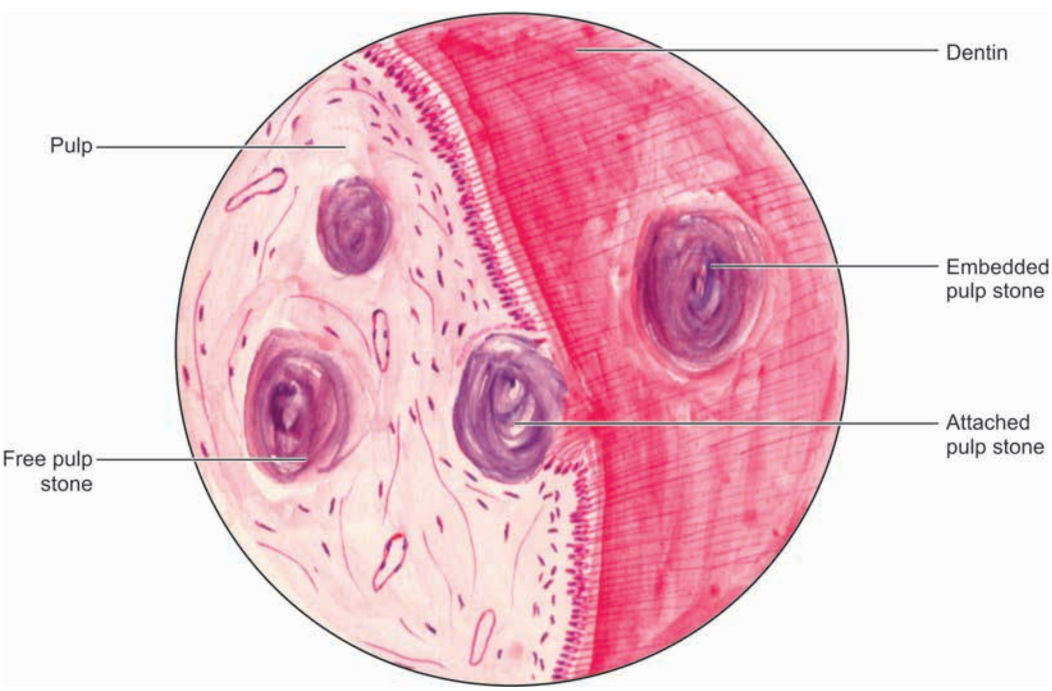


Fig. 6.2: Pulp stones

CHAPTER

7

Cementum

- Structure of cementum
  - Cellular cementum
  - Acellular cementum
- Cementoenamel junctions

Cementum is a calcified structure that forms the outer covering of the root of the tooth. Cementum forms a part of attachment apparatus into which periodontal ligament is inserted to hold the tooth in the alveolar socket. The structure of cementum can be studied using a ground sections or decalcified sections.

STRUCTURE OF CEMENTUM

Based on histological structure cementum is classified into acellular and cellular cementum.

Cellular Cementum (Fig. 7.1)

Cellular cementum is the cementum in which cementocytes are entrapped. In a ground section of cellular cementum, cementocytes can be easily identified as spider-shaped cells. These cells have a cell body and numerous processes or canaliculi radiating from it. The canaliculi are directed towards the periodontal ligament which is the source of nutrition. Sharpey's fibers are the inserted portion of the periodontal ligament and are seen as black faint lines at an angle to the root surface. Incremental lines of Salter appear as dark lines parallel to root surface. Cellular cementum is located at apical one-third of roots and the furcation areas of multi-rooted teeth.

Identification Points (Fig. 7.1)

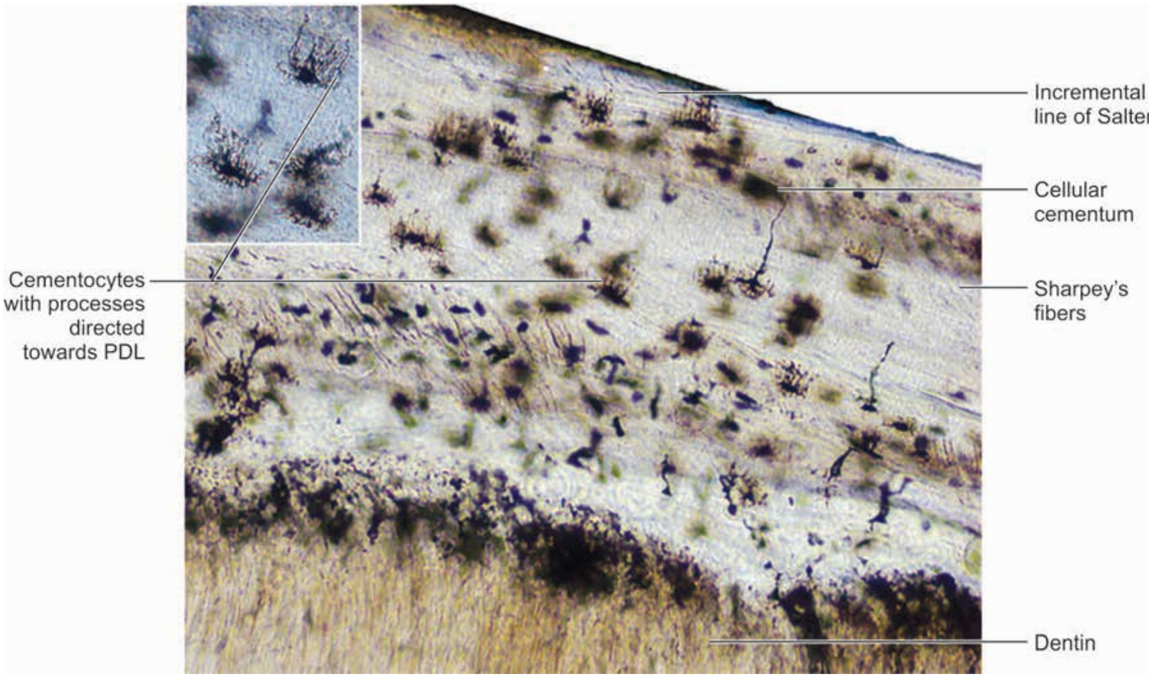
Cellular Cementum
- Entrapped cementocytes are seen
- Cementocytes are spider-shaped with canaliculi directed to the periodontal ligament
- Located at the apical one-third

Acellular Cementum (Fig. 7.2)

Acellular cementum is the type of cementum that does not contain any cells. In a ground section under transmitted light acellular cementum is seen as a structureless layer. Incremental lines of cementum referred to as lines of Salter are seen as dark lines parallel to the root surface representing rhythmic deposition of cementum. Sharpey's fibers are not distinct because they are fully mineralized. Acellular cementum is generally located at the cervical two-thirds of the root.

Identification Points (Fig. 7.2)

Acellular Cementum
- Appears as structureless layer
- No entrapped cells
- Located at the cervical two-thirds



Cellular cementum (photomicrograph 10X)

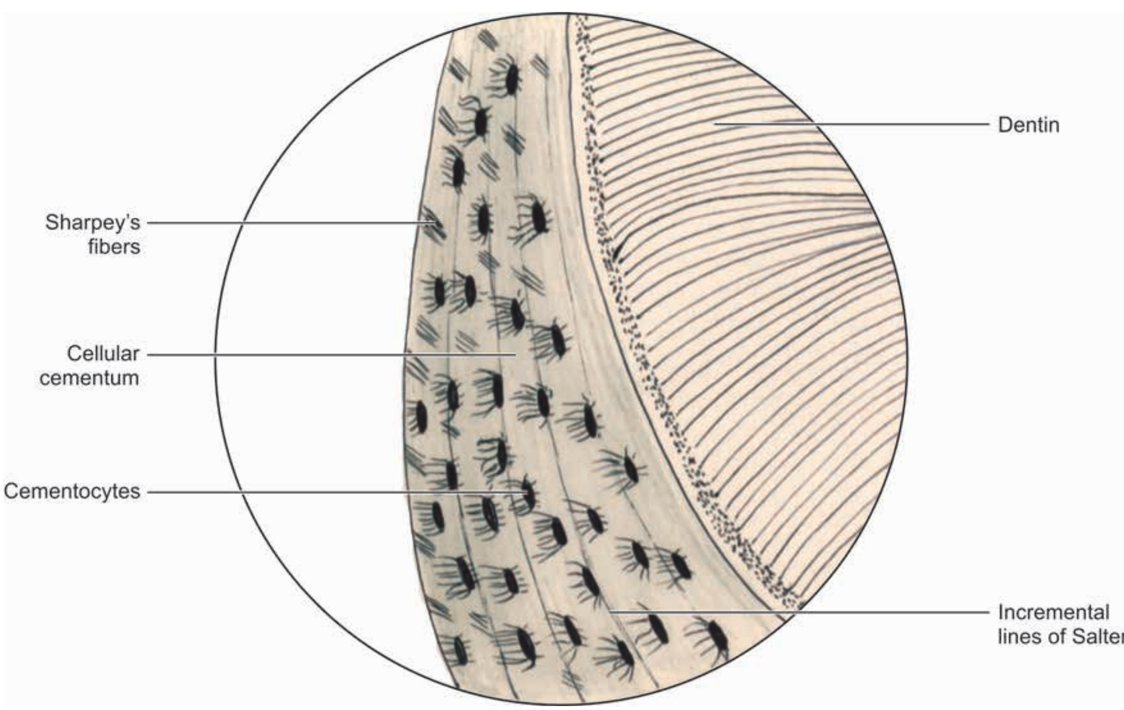
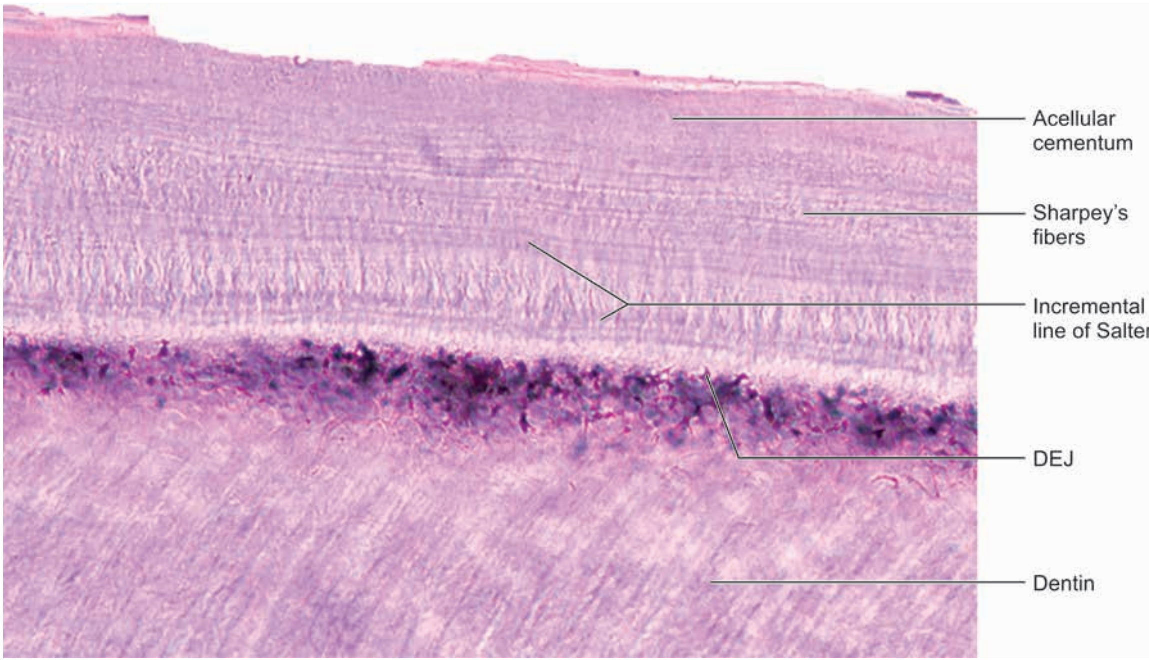


Fig. 7.1: Cellular cementum



Acellular cementum (photomicrograph 10X)

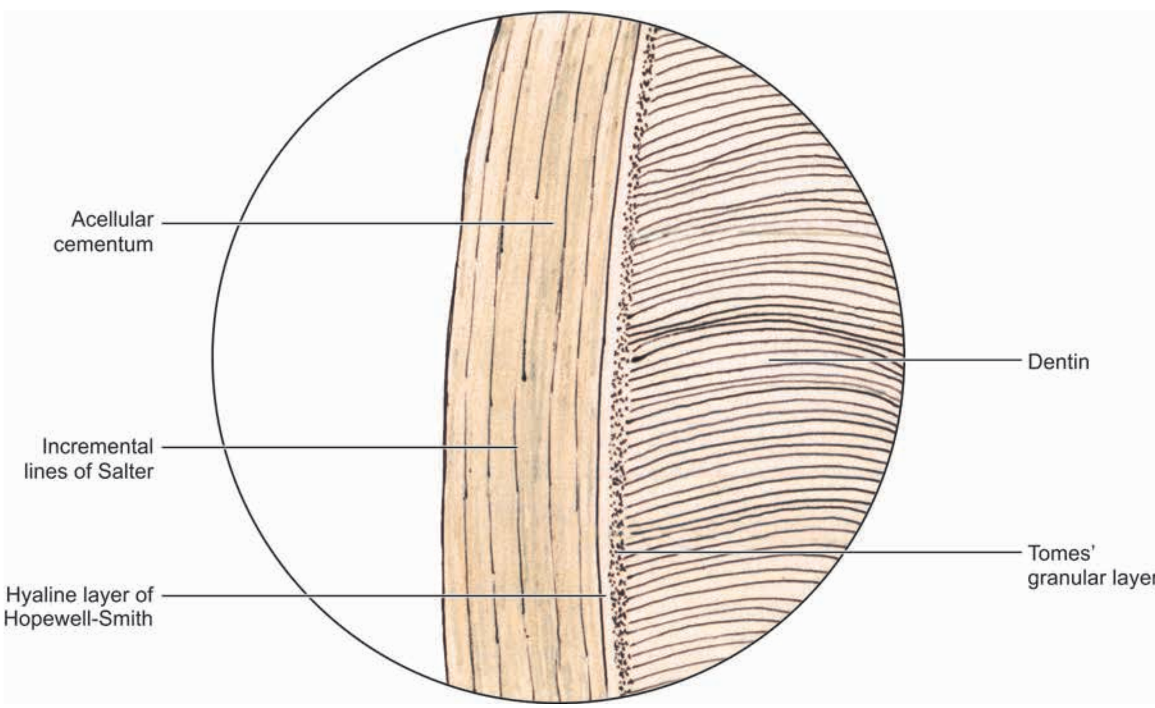


Fig. 7.2: Acellular cementum

CEMENTOENAMEL JUNCTIONS

Cementoenamel junction (CEJ) is the junction between enamel and cementum that occurs at cervical region of tooth.

There are three types of CEJs

Sharp Junction (Fig. 7.3)

Enamel and cementum meet edge to edge. This type of junction is seen in 30% of teeth.

Identification Points (Fig. 7.3)

Cementoenamel Junction—Sharp

- Cementum and enamel meet at sharp point
- Seen in 30% of teeth

Gap Type (Fig. 7.4)

In this type there is no junction, instead a zone of root devoid of cementum is seen. This occurs due to lack of degeneration of Hertwig's epithelial root sheath preventing the differentiation of cementoblasts and therefore cementum formation. In this type of junction, a portion of radicular dentin is exposed causing sensitivity if root is exposed due to gingival recession. This type is seen in 15% of teeth.

Identification Points (Fig. 7.4)

Cementoenamel Junction—Gap

- A gap is present between enamel and cementum
- Occur due to lack of degeneration of HERS
- Seen in 15% of teeth

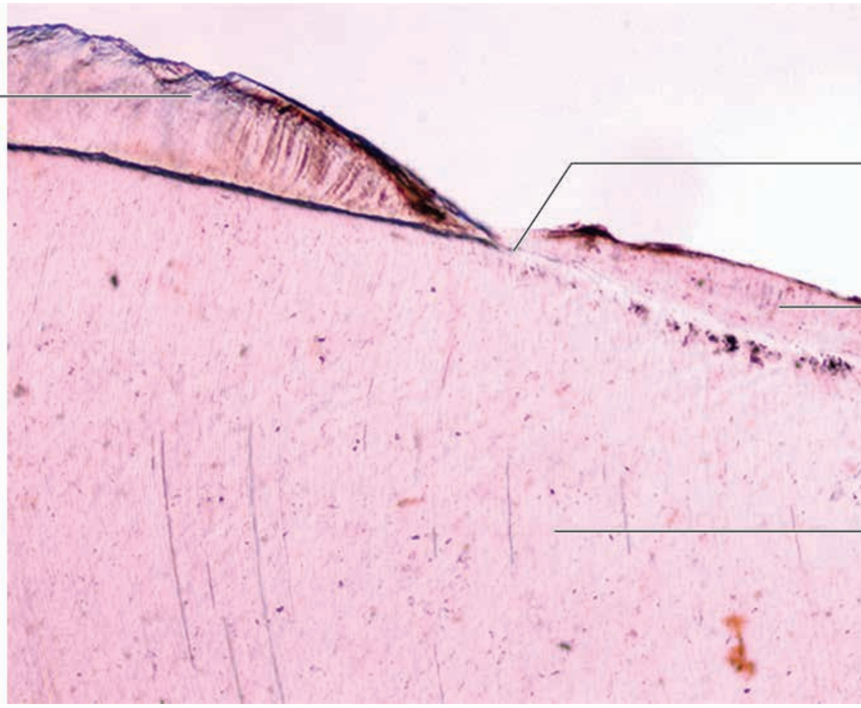
Overlap Type (Fig. 7.5)

Cementum overlaps the cervical region of enamel in this type of cementoenamel junction. The type of cementum seen overlapping enamel is acellular afibrillar cementum. This occurs due to degeneration of reduced enamel epithelium which allows the dental follicle cells to come in contact with newly formed enamel. Then these cells differentiate into cementoblasts and lay down cementum. This type of junction is seen in 60% of teeth.

Identification Points (Fig. 7.5)

Cementoenamel Junction—Overlap

- Cementum overlap cervical portion of enamel
- Acellular afibrillar cementum is seen in the region of overlap
- Seen in 60% of teeth



Cementoenamel junction—sharp junction (photomicrograph 10X)

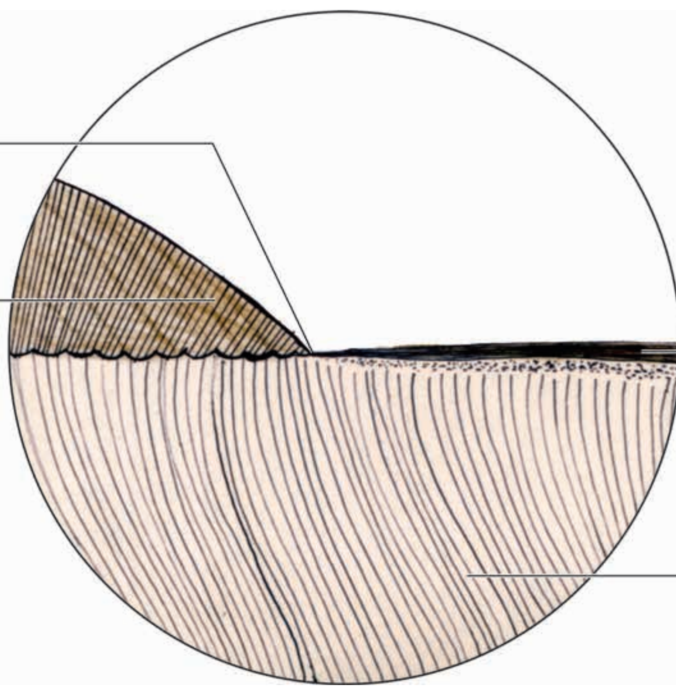
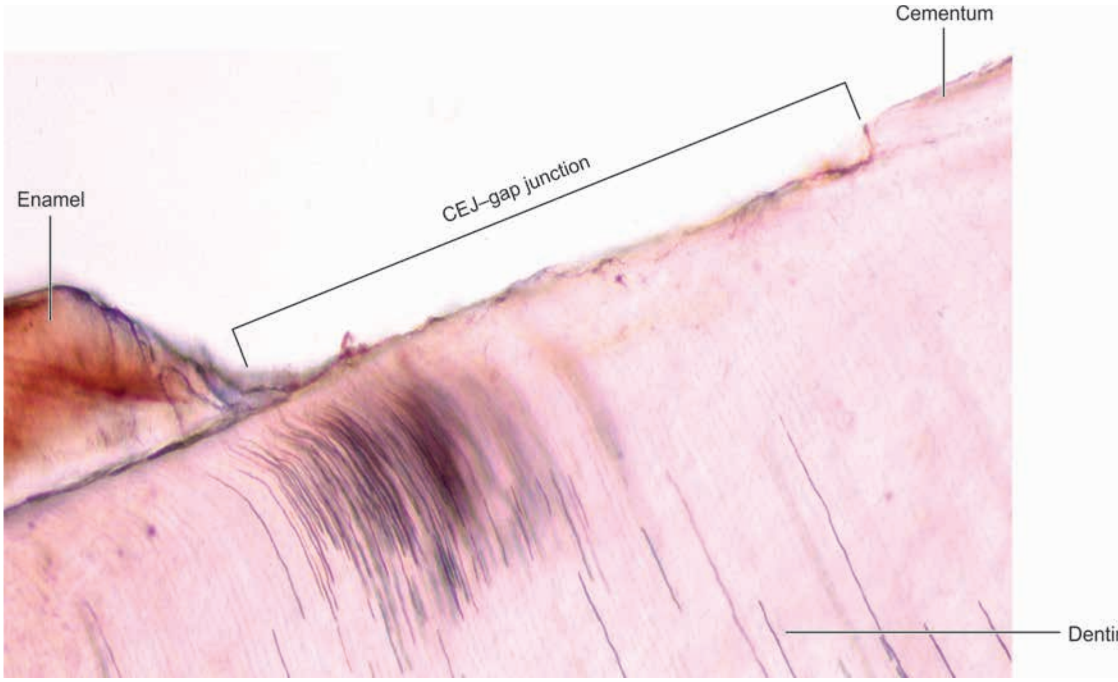


Fig. 7.3: Cementoenamel junction—sharp junction



Cementoenamel junction—gap type (photomicrograph 10X)

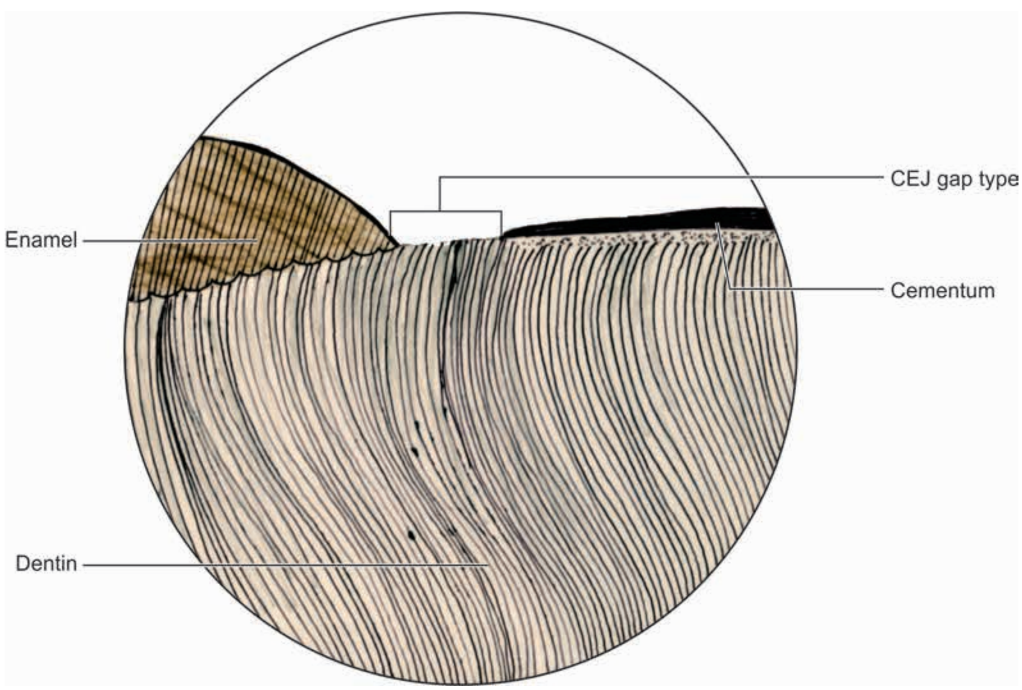
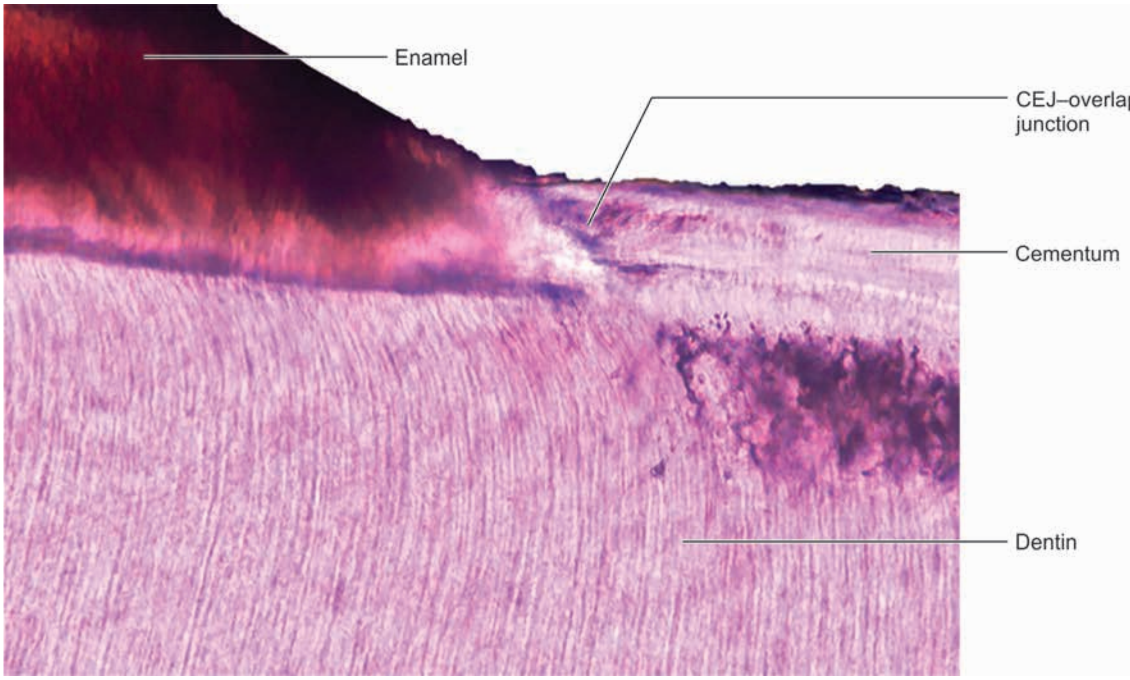


Fig. 7.4: Cementoenamel junction—gap type



Cementoenamel junction—overlap type (photomicrograph 40X)

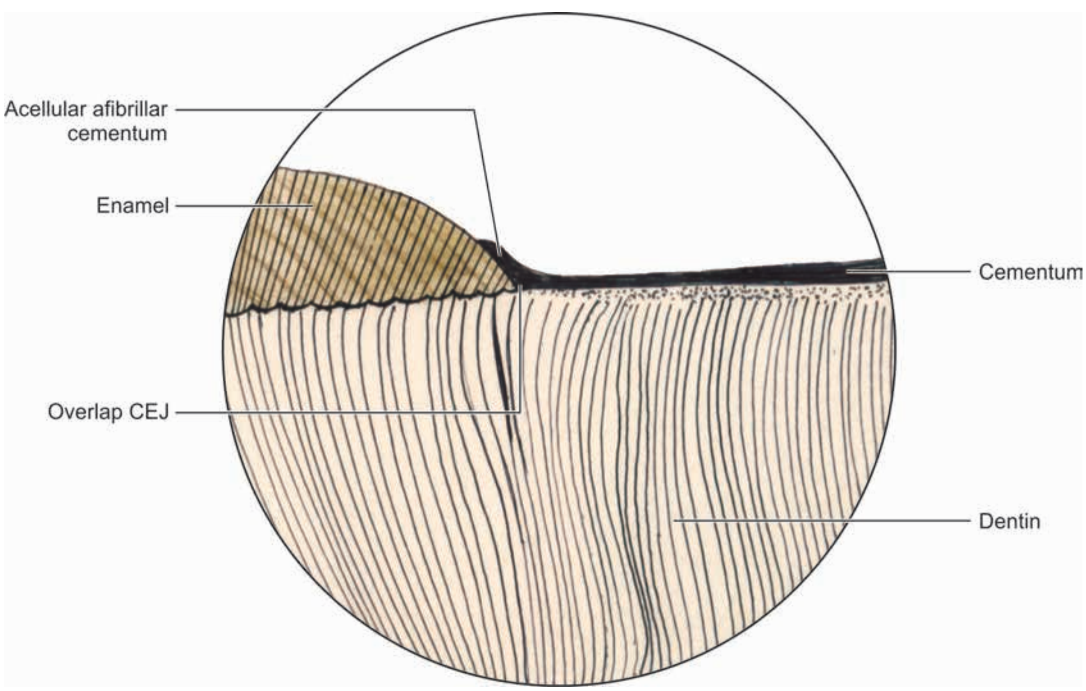


Fig. 7.5: Cementoenamel junction—overlap type

CHAPTER

8

Periodontal Ligament

- Principal fibers of periodontal ligament
- Gingival group of fibers

Periodontal ligament is a connective tissue structure that attaches the tooth to the alveolar bone. It is a part of attachment apparatus of the tooth. The attachment apparatus of the tooth has two hard tissue components, cementum and alveolar bone and two soft tissue components, periodontal ligament and lamina propria of the gingiva. These four components are together called periodontium.

PRINCIPAL FIBERS OF PERIODONTAL LIGAMENT (Fig. 8.1)

Periodontal ligament comprises bundles of collagen fibers attached to cementum on one side and alveolar socket on the other side. The portion of fibers inserted into the bone and cementum are called Sharpey's fibers. These groups of fibers, having specific orientation are called principal fibers of periodontal ligament. They are:

- **Alveolar crest group of fibers:** Extending from the crest of alveolar bone to the cervical part of the cementum.
- **Horizontal group:** Running horizontally between cementum and alveolar bone and arranged perpendicular to the long axis of the tooth.

Identification Points (Figs 8.1 and 8.2)

Principal Fibers

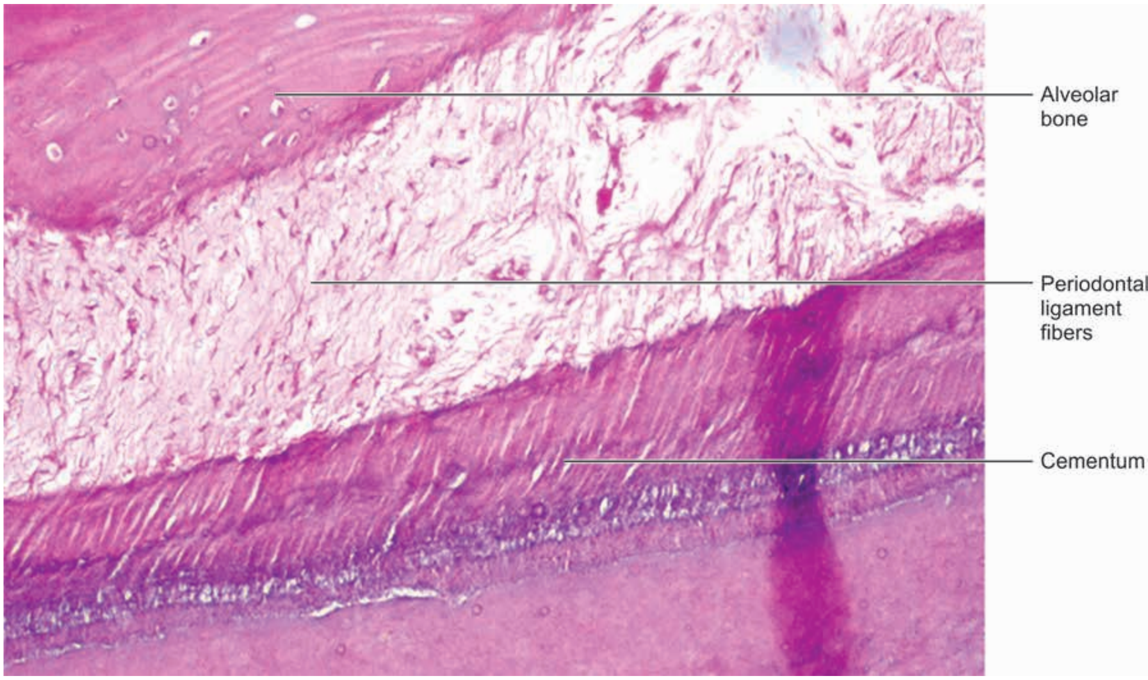
- Fiber groups having specific orientation
- Five groups are seen, helping to attach the tooth to alveolar bone
- Principal fibers are alveolar crest, horizontal, oblique, apical and inter-radicular fibers

- **Oblique group:** These are arranged obliquely from cementum to alveolar bone and insertion in cementum is more apical than insertion in bone. These constitute the major group of fibers.
- **Apical group (Fig. 8.2):** These are located in the apical region of the tooth, radiating from the apex of the root to the base of the alveolar socket.
- **Inter-radicular fibers:** These are seen only in multi-rooted teeth. These fibers radiate from the furcation area to the crest of inter-radicular septum.

GINGIVAL GROUP OF FIBERS (Fig. 8.3)

These are the secondary fibers of periodontal ligament seen in the lamina propria of the gingiva and supplement the principal fibers in maintaining the functional integrity of the teeth. These include:

Dentogingival: Extending from the cervical portion of the cementum to the lamina propria of gingiva.



Principal fibers of periodontal ligament (photomicrograph 10X)

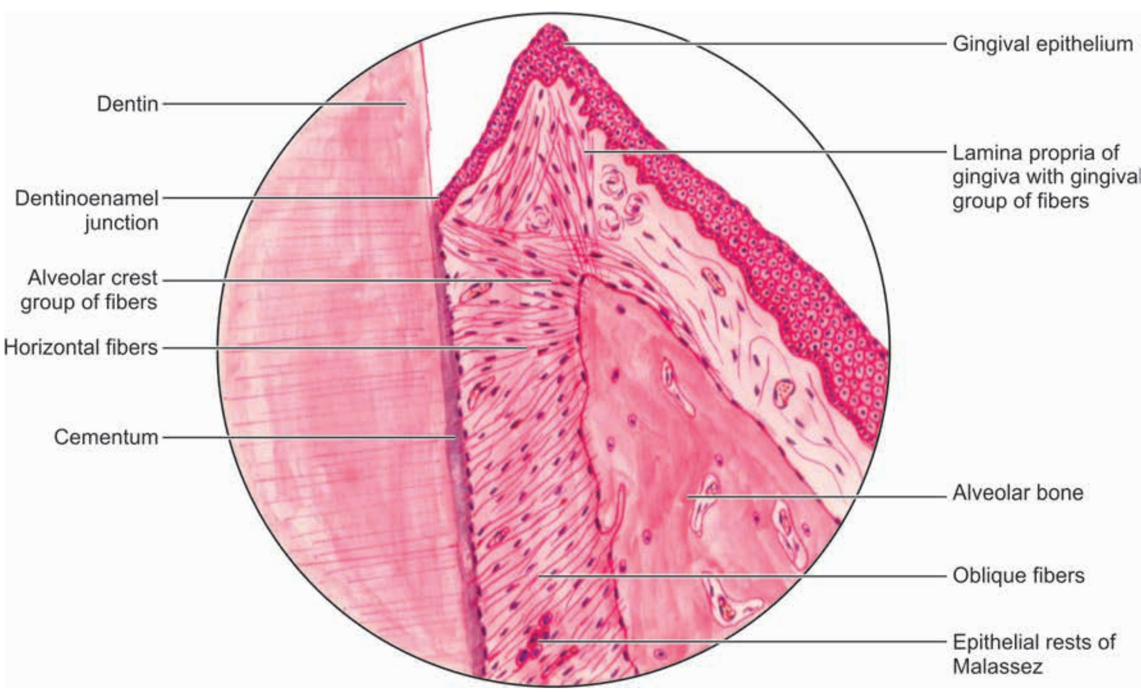
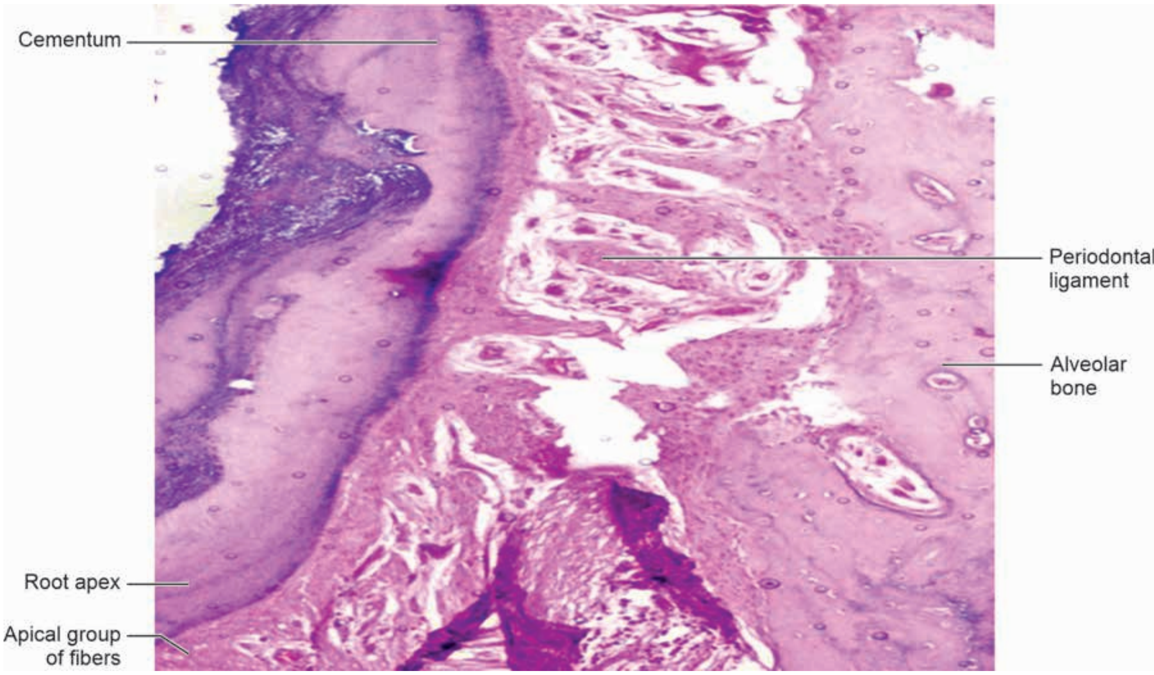


Fig. 8.1: Principal fibers of periodontal ligament



Apical group of fibers (photomicrograph 4X)

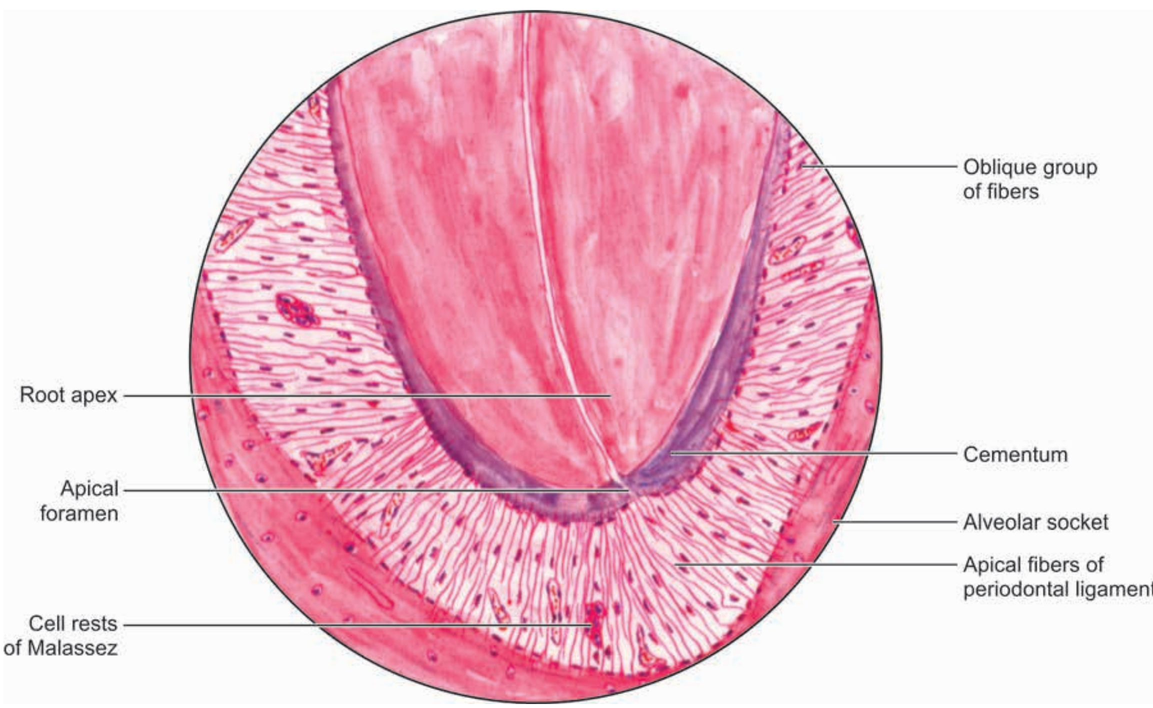


Fig. 8.2: Apical group of fibers

Dentoperiosteal: Extending from cementum to the periosteum of the alveolar crest and of the vestibular and oral surfaces of the alveolar bone.

Alveologingival: Extending from the crest of the alveolar bone to the lamina propria of gingiva.

Circular fibers: These fibers are arranged in the gingival connective tissue, encircling the tooth like a collar.

Identification Points (Fig. 8.3)

Gingival Group of Fibers

- These are supplementary fibers of periodontal ligament
- Major groups are alveologingival, dentogingival, dentoperiosteal and circular fibers
- Assist the principal fibers in function

Transseptal fibers are also accessory fibers extending interproximally between adjacent teeth.

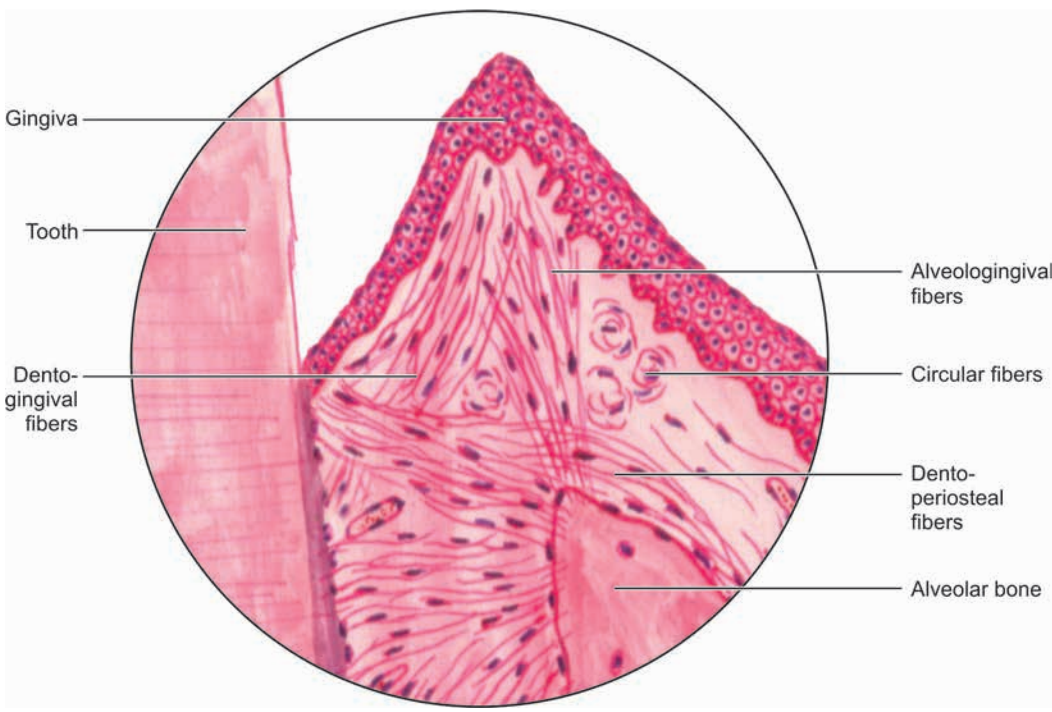


Fig. 8.3: Gingival group of fibers

CHAPTER

9

Maxillary Sinus

- Microscopic features

Maxillary sinus is a paranasal sinus located in the body of the maxilla. Maxillary sinus is also called antrum of Highmore.

MICROSCOPIC FEATURES (Fig. 9.1)

Maxillary sinus is lined by pseudostratified ciliated columnar epithelium. This epithelial layer is mainly composed of ciliated columnar cells. Along with these, there are non-ciliated columnar cells, basal cells and goblet cells. Goblet cells are unicellular secretory organ which are goblet-shaped with a basally placed nucleus and apical cytoplasm filled with secretory products. In a hematoxylin and eosin stained section, cytoplasm of goblet cells appears empty. Cilia of the lining epithelium help to move the secretions.

This epithelium is separated from subepithelial connective tissue by a basal lamina. Subepithelial connective tissue layer has collagen fibers and fibroblasts and also minor salivary glands which include both serous and mucous glands. This layer is attached to the periosteum of the bone of the maxilla.

Identification Points (Fig. 9.1)

Maxillary Sinus

- Lined by pseudostratified ciliated columnar epithelium
- Goblet cells are present
- Connective tissue contains mixed salivary gland

Maxillary sinus (photomicrograph 4X)

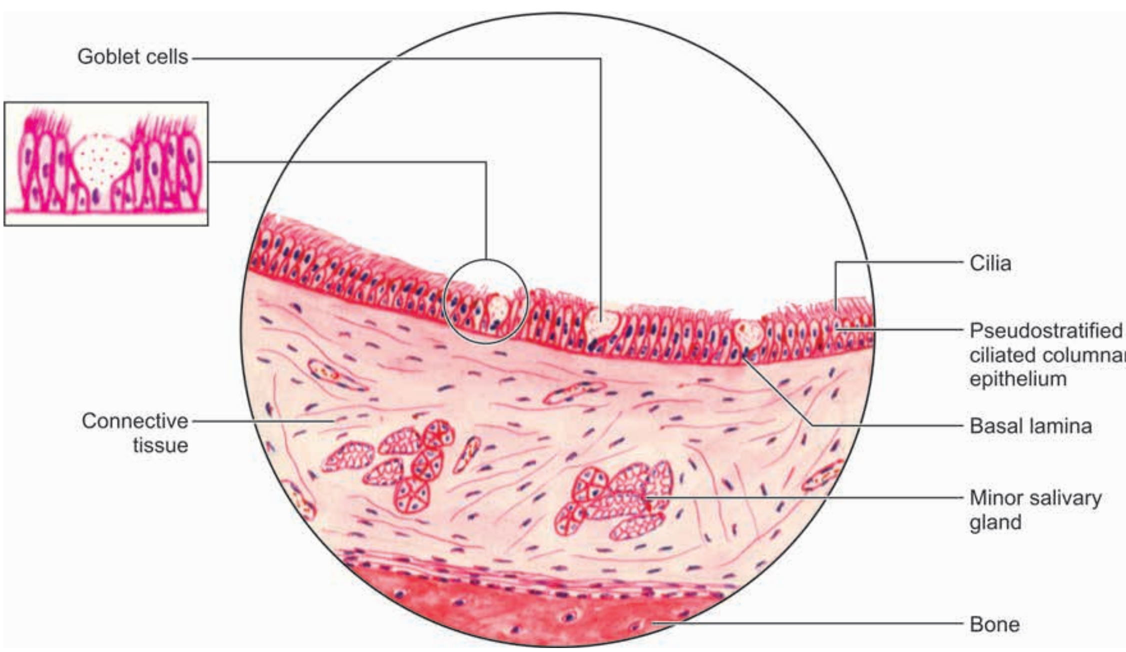


Fig. 9.1: Histology of maxillary sinus

CHAPTER

10

Oral Mucosa

STRUCTURE OF ORAL MUCOSA

Oral mucosa is the moist lining of the oral cavity, which is lined by stratified squamous epithelium. The connective tissue beneath is called lamina propria and is composed of collagen fibers, along with the various cells of connective tissue in a ground substance. The interface between epithelium and connective tissue is irregular with projections of epithelium interdigitating with those of connective tissue. The projections of epithelium are called epithelial ridges or rete ridges and of connective tissue are called connective tissue papillae. Epithelium is separated from lamina propria by a distinct basement membrane. Below the mucosa is submucosa containing large vessels and nerves, fat tissue, minor salivary gland and muscle in certain regions of oral mucosa. Based on the histological structure oral mucosa is classified into keratinized and nonkeratinized mucosa. Nonkeratinized mucosa lines the cheek, lip, soft palate, alveolar mucosa, floor of the mouth, ventral aspect of the tongue, etc. Keratinized mucosa is seen in gingiva and hard palate.

Buccal Mucosa (Nonkeratinized Mucosa) (Fig. 10.1)

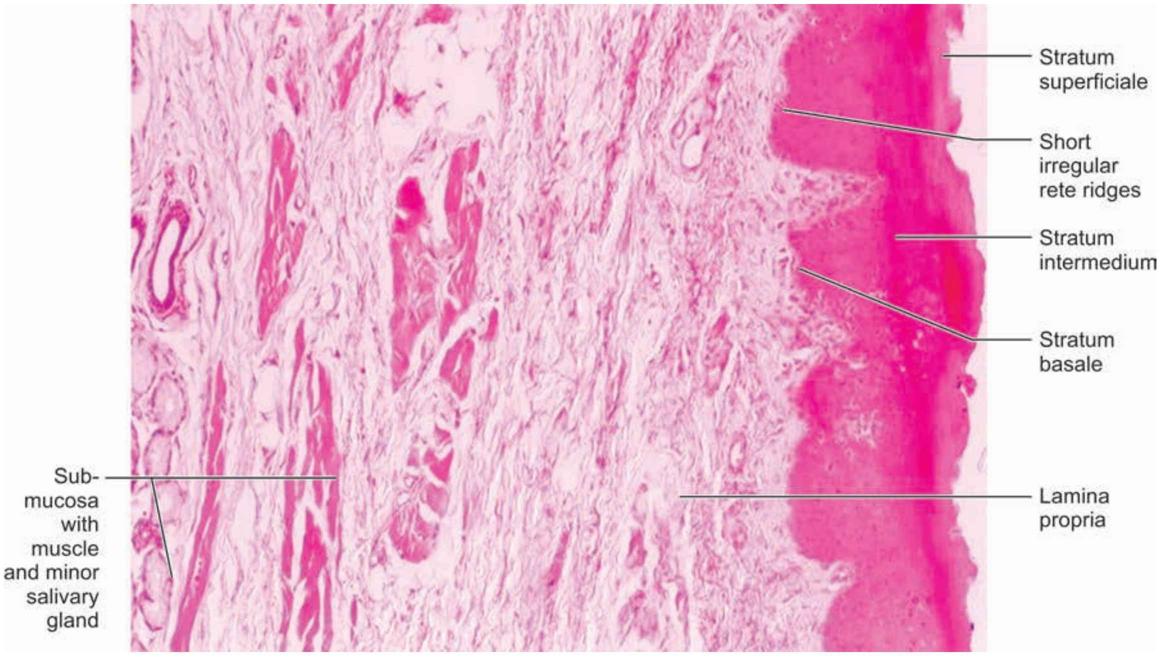
Histologically buccal mucosa shows two parts, overlying epithelium and lamina propria. The epithelium is thick and is nonkeratinized stratified squamous. Epithelium has three distinct layers, **stratum basale, stratum intermedium and stratum superficiale.**

Stratum basale is composed of a single layer of cuboidal cells arranged on basement membrane. Above this a suprabasal layer resembling basal layer may be seen. Above the basal layer is stratum intermedium made up of a few layers of polyhedral cells with centrally placed nucleus. The stratum superficiale is made up of a few layers of flattened cells with flattened nuclei. Epithelial ridges of nonkeratinized epithelium are short

Identification Points (Fig. 10.1)

Nonkeratinized Mucosa

- Lined by stratified squamous epithelium
- Epithelium has three layers—stratum basale, stratum intermedium and stratum superficiale
- Lamina propria merges with submucosa



Buccal mucosa (photomicrograph 4X)

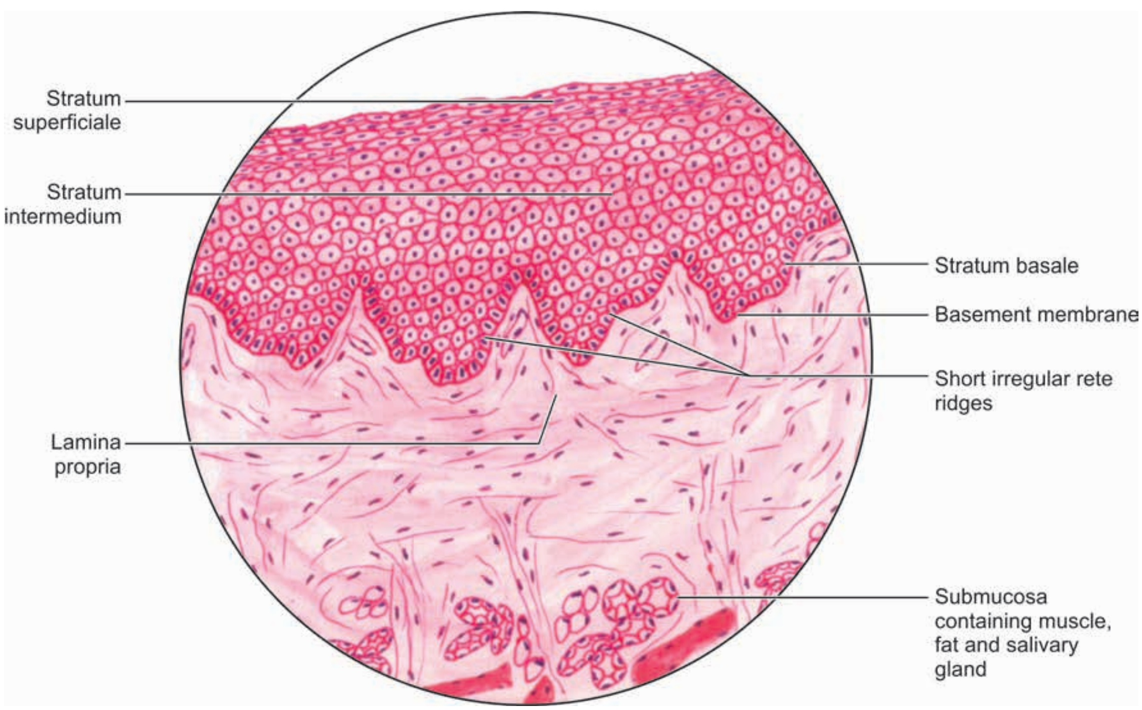


Fig.10.1: Buccal mucosa

and irregular, interdigitating with connective tissue papillae.

- Lamina propria is less dense compared to keratinized mucosa, and shows collagen fibers fibroblasts and blood vessels.
- The lamina propria of the buccal mucosa merges with underlying submucosa which contain large blood vessels, nerves, fat cells, muscle and minor salivary gland. This submucosa attaches the mucosa to the underlying muscle tissue.

Generally all the nonkeratinized mucosa histologically appear similar to the buccal mucosa except for slight structural variations.

Soft palate is lined by thin nonkeratinized stratified squamous epithelium which may show taste buds. Lamina propria is thick with numerous short papillae. Submucosa contains many minor salivary glands.

Ventral surface of the tongue has a thin nonkeratinized epithelial lining. Lamina propria is thin having numerous short papillae. Submucosa is thin and irregular with abundant blood vessels. In areas where submucosa is absent, mucosa is directly attached to the muscles of the tongue.

Floor of the mouth is lined by very thin nonkeratinized epithelium. Lamina propria shows short papillae and is rich in vascular supply. Submucosa is loose fibrous tissue with fat and minor salivary glands.

In **alveolar mucosa** lining epithelium is thin and nonkeratinized with short rete ridges. Lamina propria has many capillary loops. Submucosa comprises loose connective tissue containing minor salivary glands. The submucosa attaches alveolar mucosa to the periosteum of alveolar bone.

Keratinized Mucosa—Gingiva (Fig. 10.2)

In a histological section gingiva shows two portions, epithelium and lamina propria.

Identification Points (Fig. 10.2)

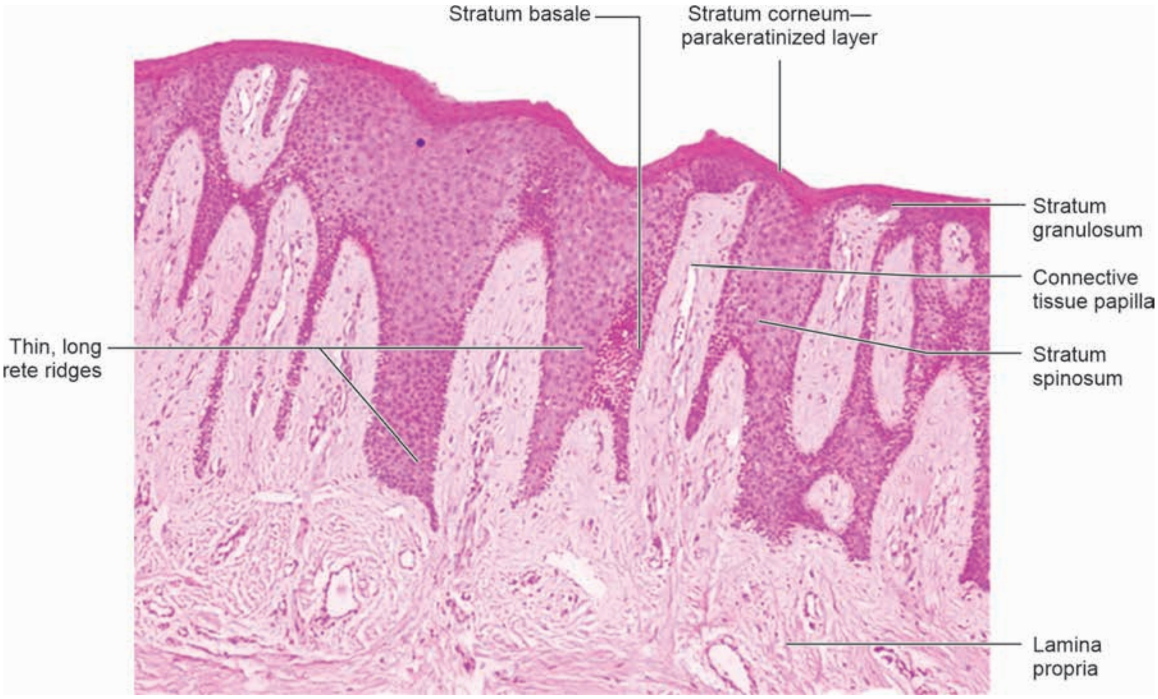
Gingiva

- Lined by keratinized stratified squamous epithelium
- Epithelium has four layers—stratum basale, spinosum, granulosum and corneum
- Dense lamina propria is directly attached to the periosteum of alveolar bone

The overlying epithelium is keratinized and stratified squamous with four distinct layers. The first layer adjacent to the basement membrane is stratum basale composed of a single layer of cuboidal cells with nucleus arranged perpendicular to the basement membrane. The layer above is stratum spinosum or prickle cell layer composed of a few layers of polyhedral cells. Cells have a prickly appearance because they shrink while tissue processing retaining the intercellular attachment. The next layer is stratum granulosum made up of a few layers of flattened cells, cytoplasm of which contains hematoxyphilic keratohyaline granules. The most superficial layer is stratum corneum composed of many layers of flattened cells filled with keratin. These cells are devoid of nucleus and cytoplasmic organelles. Gingival epithelium predominantly shows parakeratinized surface layer which is characterized by the presence of retained pyknotic nucleus in the corneal layer (in case of orthokeratinization nuclei are absent).

The interface between epithelium and connective tissue is irregular with long irregular epithelial ridges. Lamina propria shows dense bundles of collagen fibers.

Submucosa is absent and lamina propria is directly attached to periosteum of alveolar bone. This type of attachment is called mucoperiosteal attachment which makes the attachment between epithelium and connective tissue firm and immobile.



Gingiva (photomicrograph 10X)

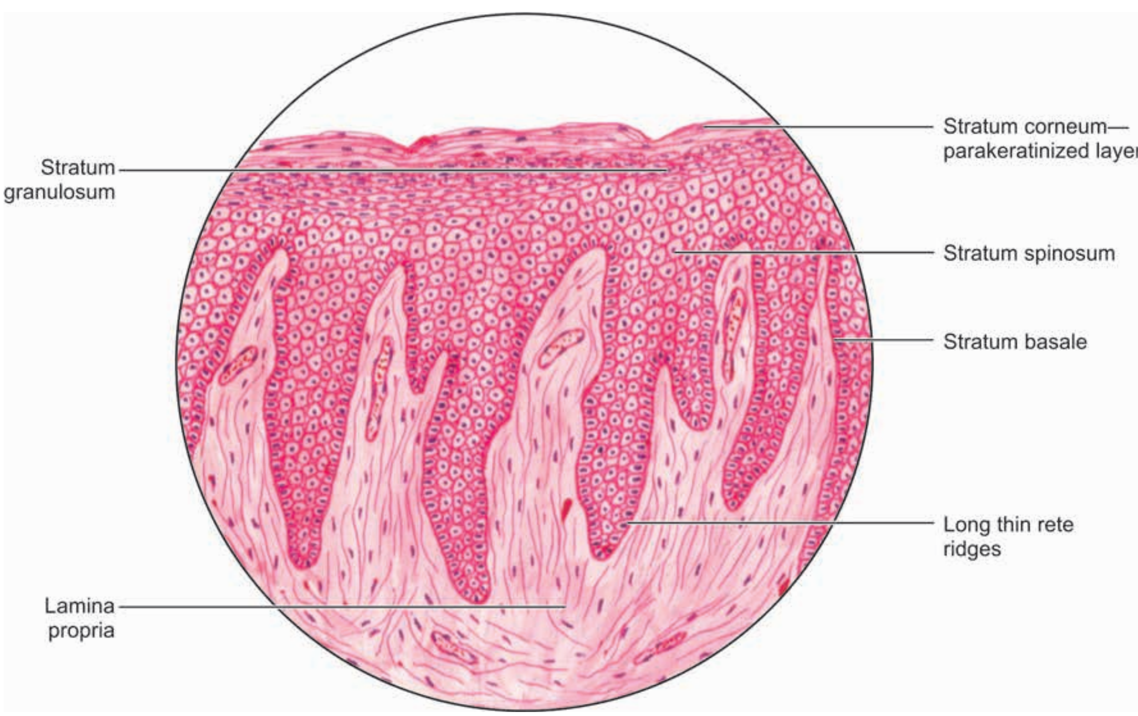


Fig. 10.2: Gingiva

Hard Palate—Anterolateral Region (Fatty Zone) (Fig. 10.3)

Hard palate is lined by masticatory mucosa and therefore structurally somewhat similar to gingiva. The anterolateral region of the palate is lined by keratinized stratified squamous epithelium with four distinct layers, i.e. stratum basale, stratum spinosum, stratum granulosum, stratum corneum. The epithelial ridges are long and regular interdigitating with connective tissue papillae. Lamina propria is dense and is more thicker than in the posterior region of hard palate. In contrast to gingiva this region has submucosa filled with fat tissue. The zone of fat tissue is divided into compartments by vertical band of dense connective tissue which attaches mucosa firmly to the periosteum of palatal bone.

Hard Palate—Posterolateral Region (Glandular Zone) (Fig. 10.4)

Glandular zone is structurally similar to fatty zone with overlying keratinized stratified squamous epithelium having four distinct layers, i.e. stratum basale, stratum spinosum, stratum granulosum and stratum corneum. The rete ridges are long and regular interdigitating with connective tissue papilla. Lamina

Identification Points (Fig. 10.3)

Fatty Zone of Hard Palate

- Lined by keratinized stratified squamous epithelium
- Epithelium has four layers—stratum basale, spinosum, granulosum and corneum
- Submucosa is present containing fat tissue

Identification Points (Fig. 10.4)

Glandular Zone of Hard Palate

- Lined by keratinized stratified squamous epithelium
- Epithelium has four layers—stratum basale, spinosum, granulosum and corneum
- Submucosa is present containing glandular tissue

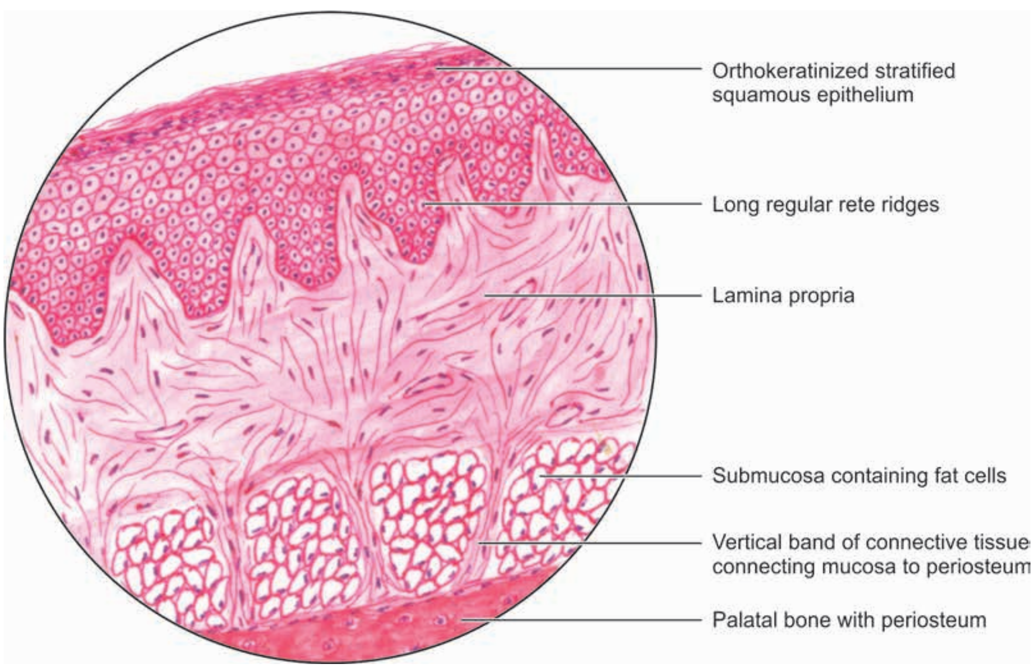
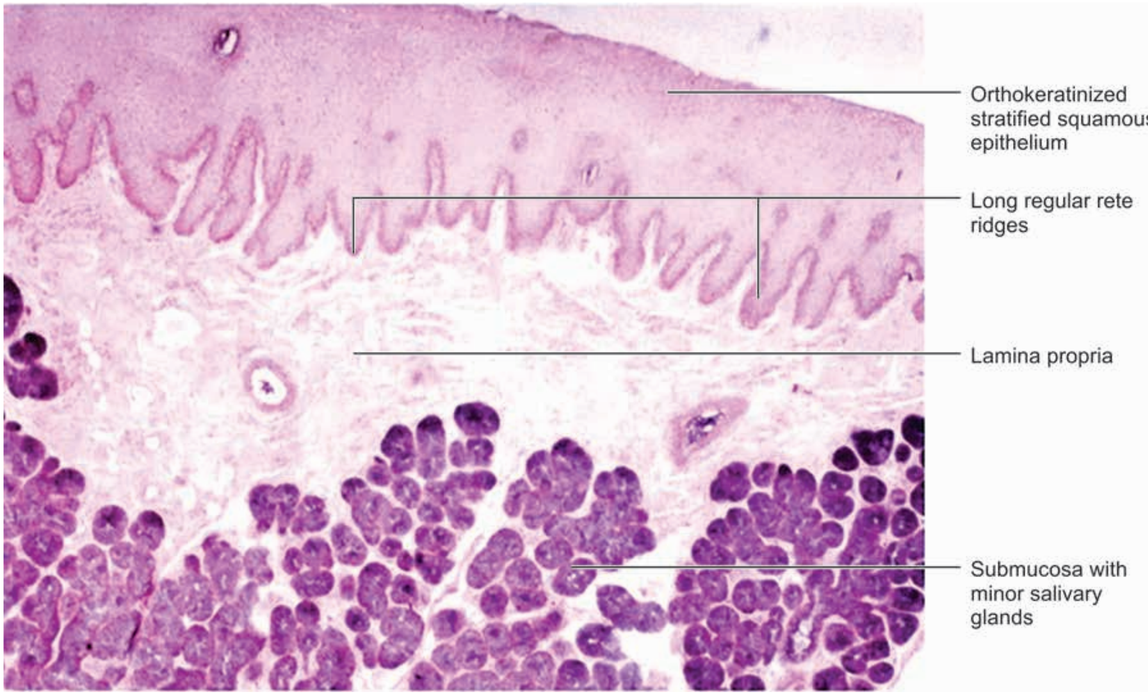


Fig. 10.3: Anterolateral area of palate (fatty zone)



Posterolateral region of palate (glandular zone) (photomicrograph 10X)

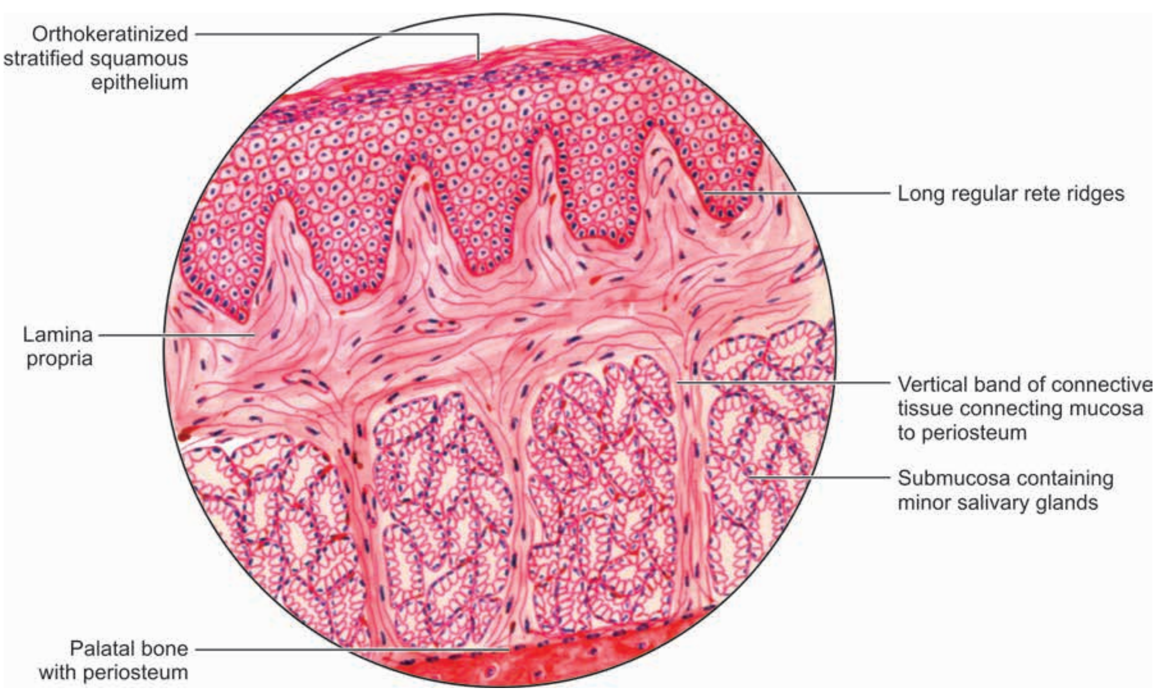
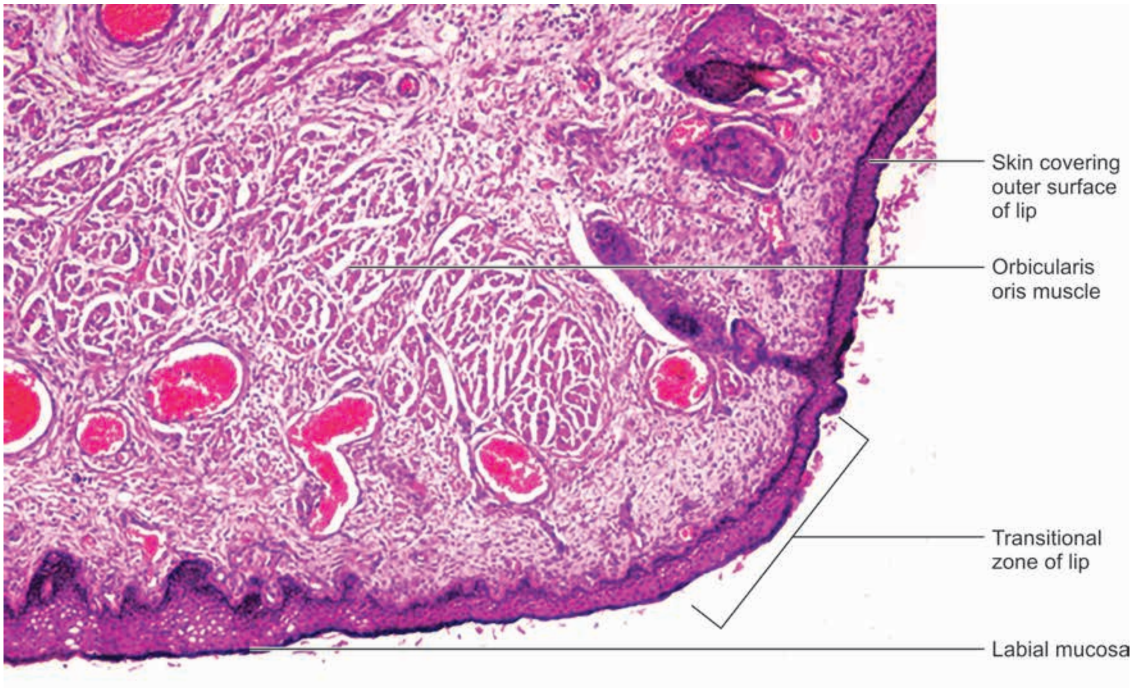


Fig. 10.4: Posterolateral region of palate (glandular zone)



Vermilion zone of lip (photomicrograph 4X)

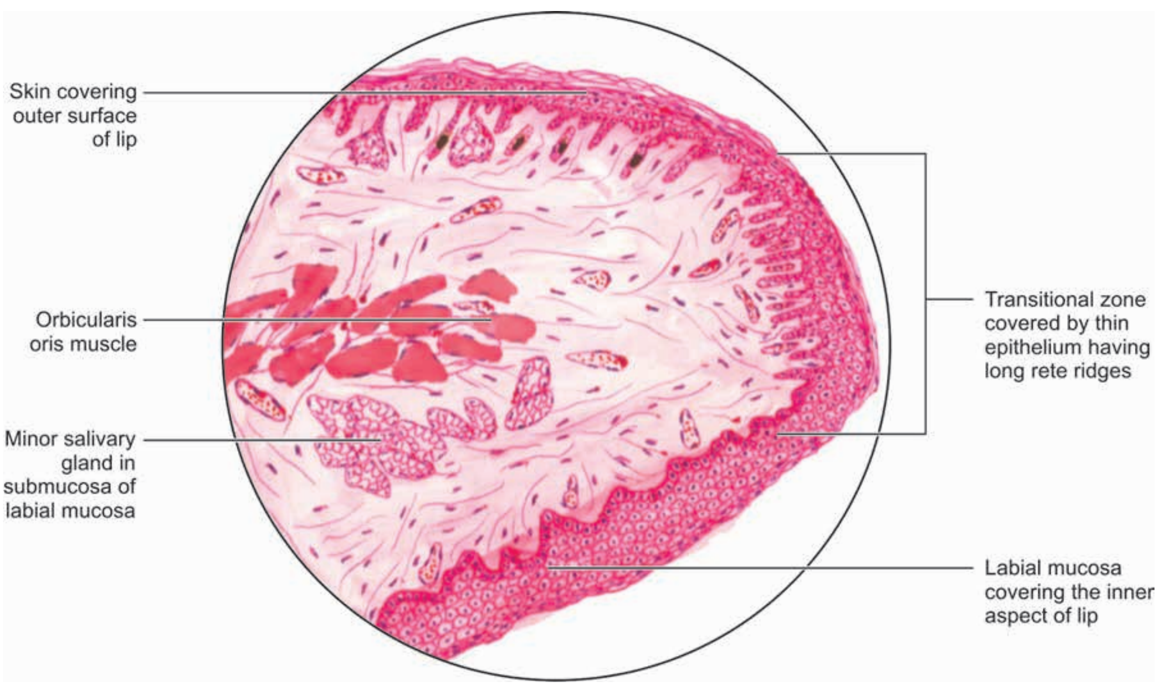


Fig. 10.5: Vermilion zone of lip

propria is dense and is less thicker than in the anterior region of palate. In contrast to antero-lateral region submucosa contains minor salivary gland tissue. The zone of submucosa is divided into compartments by vertical band of dense connective tissue which attaches mucosa firmly to the periosteum of palatal bone.

Vermilion Border of Lip (Transitional Zone) (Fig. 10.5)

This zone is the transitional zone between the skin lining the outer surface of the lip and the labial mucosa lining the inner aspect. The skin is composed of keratinized stratified squamous epithelium with all appendages like hair follicles, sweat glands and sebaceous glands. The labial mucosa is lined by nonkeratinized stratified squamous epithelium. The connective tissue beneath shows minor salivary gland tissue. The central portion of lip shows orbicularis oris muscle. The transitional zone has a thin lining epithelium with thin keratinization on the surface. There are many long papillae reaching high into epithelium carrying many capillary loops. This makes it more red compared to labial mucosa. Underlying connective tissue is characteristically devoid of glands.

Identification Points (Fig. 10.5)

Vermilion Border of Lip

- Transitional zone of lip
- Lined by thin parakeratinized epithelium
- Long thin connective tissue papillae containing many capillary loops

Papillae of the Tongue

Filiform Papillae (Fig. 10.6)

Filiform (hair-like) papillae are seen as hair-like or thread-like projection on the dorsal aspect of the tongue. Filiform papilla in a histological section is seen as cone-shaped structure lined by stratified squamous epithelium with thick keratin on the surface. Central core of connective tissue supports the blood vessels. *Taste buds are not seen* in these papillae.

Identification Points (Fig. 10.6)

Filiform Papillae

- Seen as hair or finger-like projections
- Lined by keratinized stratified squamous epithelium
- Do not contain taste buds

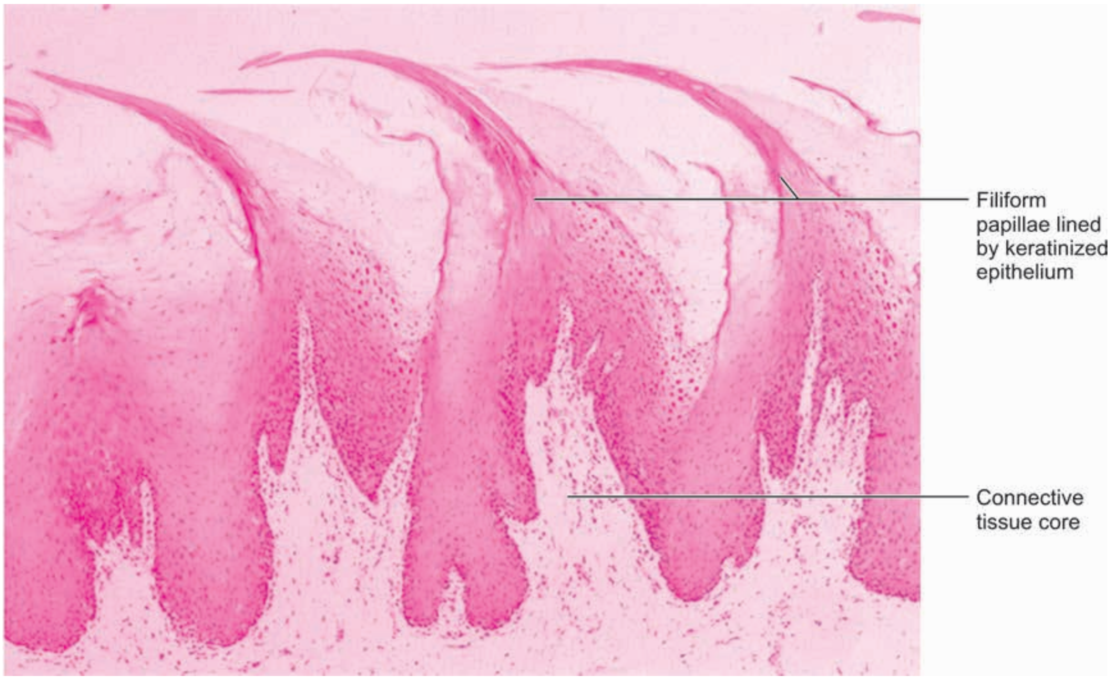
Circumvallate Papillae (Fig. 10.7)

The circumvallate (walled) papillae are seen in the anterior two-thirds of the tongue just anterior to sulcus terminalis. These are 10–12 in number. The superficial surface of these papillae is at the level of surface of tongue and a V-shaped sulcus is present all around the papillae separating them from the adjacent portion of tongue. The lining epithelium is keratinized stratified squamous epithelium at the superficial surface and nonkeratinized on the lateral surface of circumvallate papillae. Taste buds are seen only on the lateral surface. Central portion is occupied by the connective tissue. The characteristic feature of this papilla is presence of serous minor salivary glands (von Ebner's gland) in the connective tissue beneath it. These glands secretes watery saliva into the V-shaped trough around the papillae to flush out the food debris.

Identification Points (Fig. 10.7)

Circumvallate Papillae

- Lined by keratinized epithelium on superficial surface and nonkeratinized epithelium on the lateral surface
- Taste buds are seen on the lateral surface
- von Ebner's glands are present in the connective tissue



Filiform papillae of tongue (photomicrograph 4X)

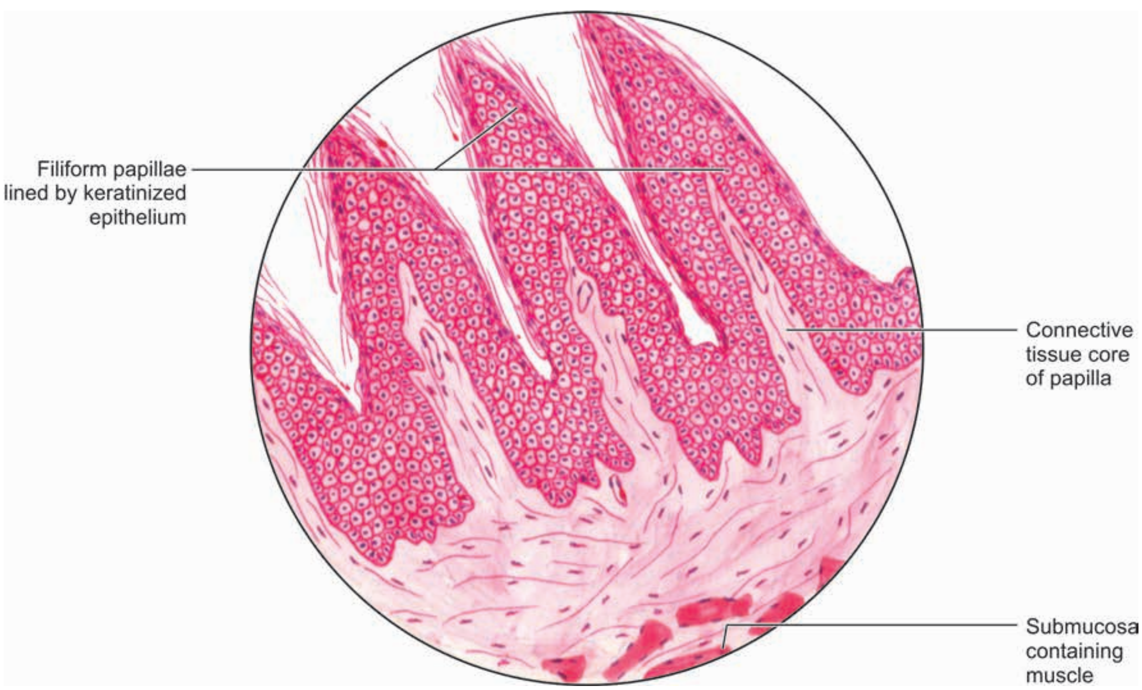
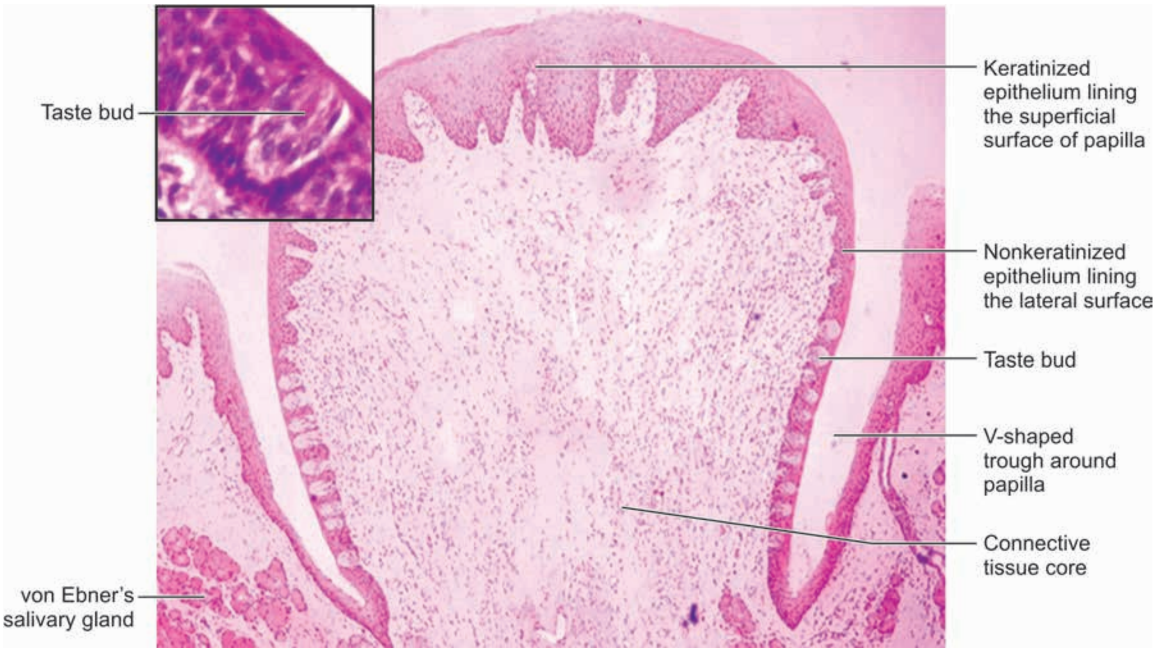


Fig. 10.6: Filiform papillae of tongue



Circumvallate papilla of tongue (photomicrograph 4X)

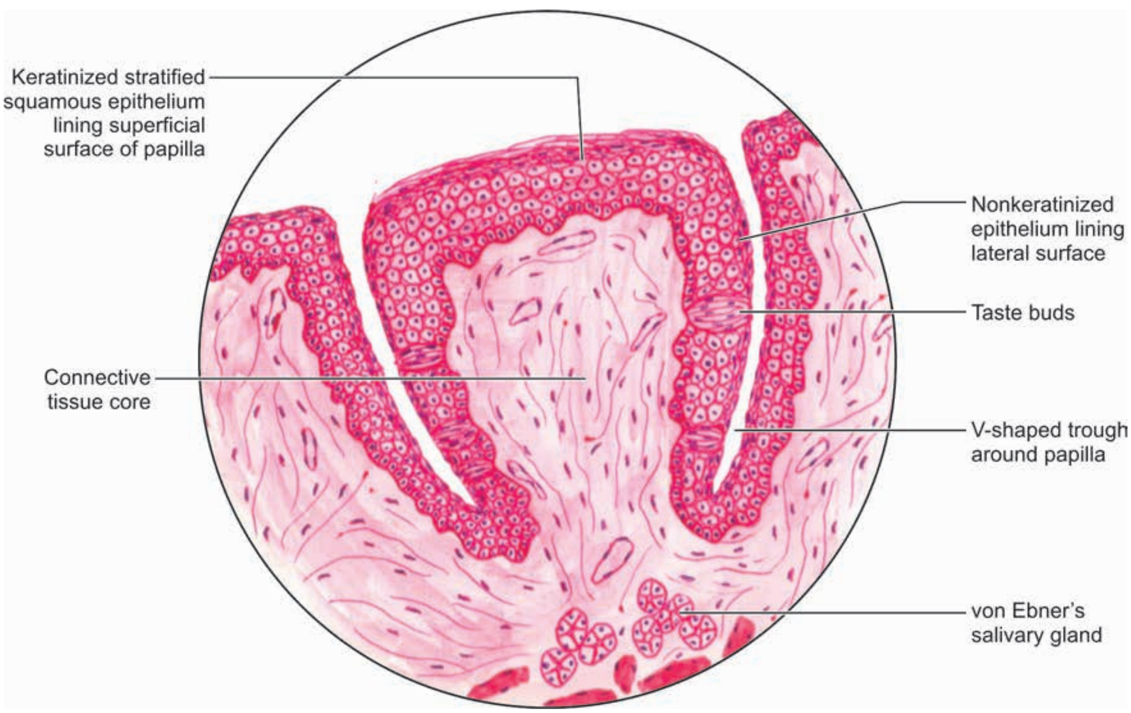


Fig. 10.7: Circumvallate papilla of tongue

Fungiform Papillae (Fig. 10.8)

Fungiform (fungus-like) papillae are dome-shaped structures projecting above the surface of the tongue and located between the filiform

Fungiform papillae of tongue (photomicrograph 4X)

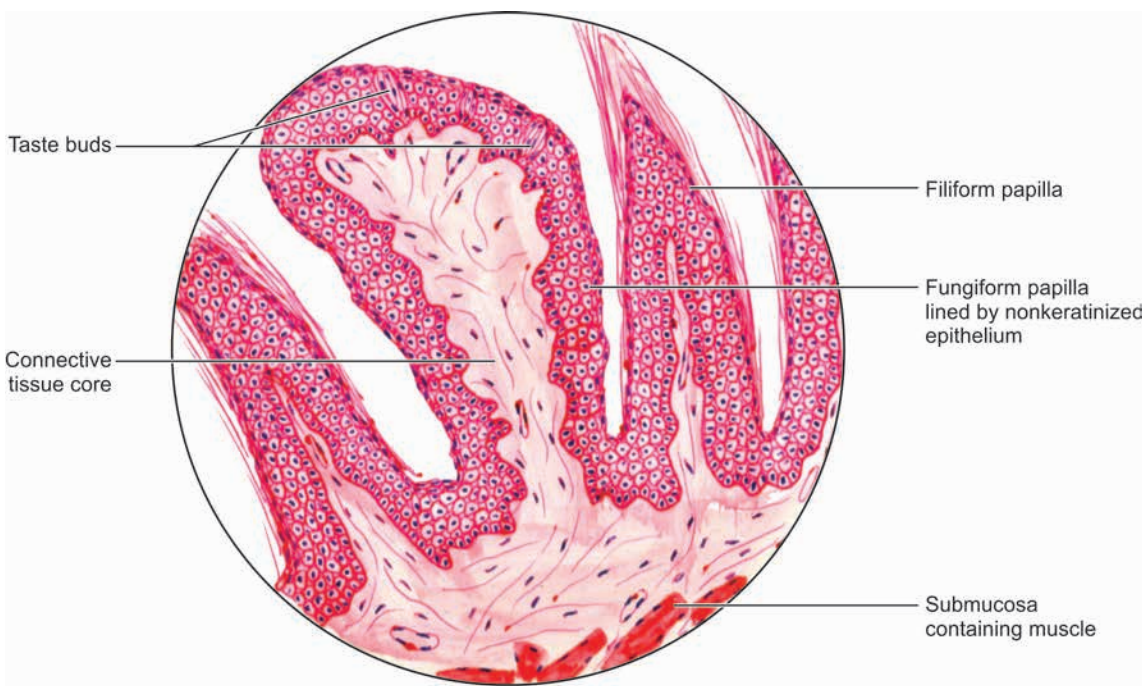


Fig. 10.8: Fungiform papillae of tongue

Identification Points (Fig. 10.8)

Fungiform Papillae

- Dome-shaped projections
- Lined by nonkeratinized epithelium
- Taste buds are seen on the superficial surface

papillae. The epithelium covering the fungiform papillae is thin nonkeratinized stratified squamous epithelium making this papilla reddish in color. The superficial surface of the papillae contains a few taste buds. The supporting connective tissue shows collagen fibers, fibroblasts and blood vessels.

CHAPTER

11

Salivary Gland

- Structure of salivary glands
  - Serous gland
  - Mucous gland
  - Mixed gland

Salivary glands are exocrine glands which secrete saliva that reaches the oral cavity through their ducts. There are three pairs of major salivary glands located extra orally and numerous minor salivary glands situated intraorally. Based on the type of secretion, salivary glands are divided into:

- **Serous gland:** Parotid and von Ebner's gland.
- **Mucous glands:** Sublingual gland and all the minor salivary glands except for von Ebner's glands.
- **Mixed gland:** Submandibular salivary gland.

Both major and minor salivary glands are composed of parenchymal components supported by the connective tissue. Parenchymal component includes secretory acini and ductal system. Connective tissue forms a capsule around the gland and extends in between the acini to divide the gland into lobes and lobules. Connective tissue carries the blood vessels and nerves.

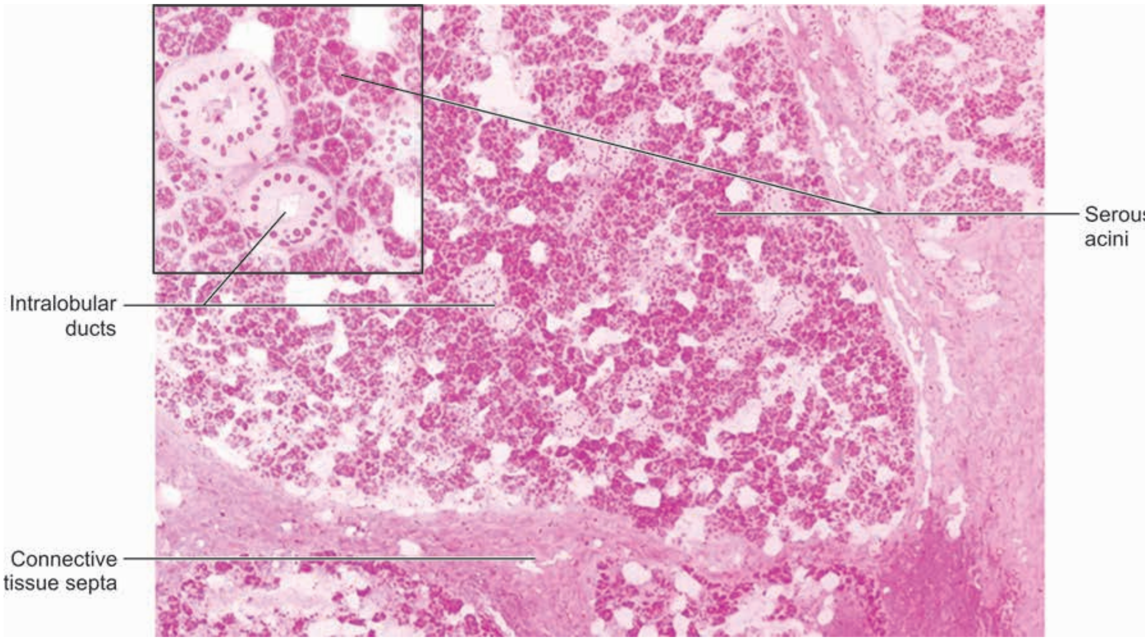
SEROUS SALIVARY GLAND—PAROTID GLAND (Fig. 11.1)

Serous salivary gland is composed of numerous serous secretory units called serous acini. Serous acinus is a collection of many serous cells. Serous cells are the secretory cells and are pyramidal in shape with a broad base resting on a basement membrane and a narrow apex facing towards the lumen. Nucleus is round and located at the basal one-third of the cell. Apical part of the cytoplasm is filled with zymogen granules and appears eosinophilic in a hematoxylin and eosin stained section. Serous cells are arranged to form an acinus. Serous acinus is round or circular in shape, has fewer cells and a small lumen. Salivary gland is divided into lobes by connective tissue septa that carry blood vessels. Intralobular and interlobular ducts are seen.

Identification Points (Fig. 11.1)

Serous Salivary Gland

- Composed of round acini with small lumen
- Nucleus of cells is ovoid and present at basal one-third
- Apical cytoplasm stain eosinophilic because of secretory granules



Serous salivary gland (photomicrograph 10X)

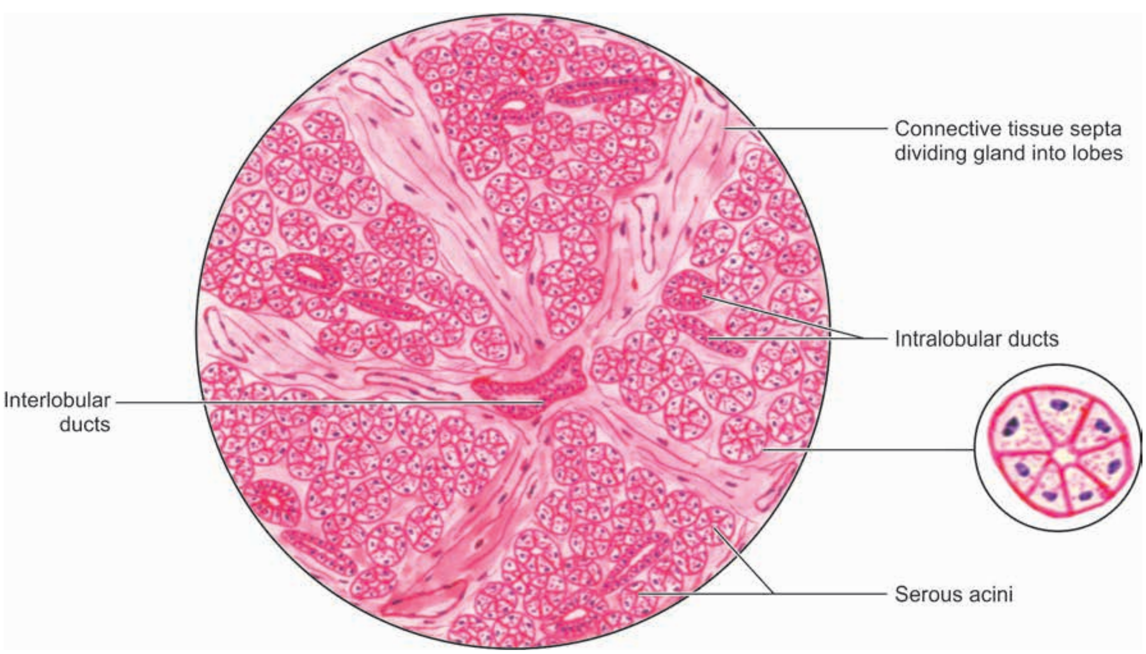


Fig. 11.1: Serous salivary gland

MUCOUS SALIVARY GLAND—SUBLINGUAL GLAND (Fig. 11.2)

Mucous salivary glands have numerous secretory units called mucous acini. These acini are collection of mucous cells. Mucous cells are columnar in shape with nucleus flattened and pressed against the basement membrane. Cytoplasm is restricted to basal region. Apical portion of the cell is filled with mucous secretory droplets and appear empty in H & E stained sections. Mucin can be stained positively using periodic acid–Schiff stain. Mucous cells are arranged to form mucous acini that are ovoid or tubular with large lumen. In a histologic section along with acini intralobular ducts are also seen. Connective tissue septum is present between the acini that shows blood vessels and interlobular ducts.

Identification Points (Fig. 11.2)

Mucous Salivary Gland

- Composed of tubular acini with large lumen
- Nucleus of cells is flat and pressed to basal plasma membrane
- Apical cytoplasm appears empty

MIXED SALIVARY GLAND—SUBMANDIBULAR GLAND (Fig. 11.3)

In a mixed salivary gland both serous and mucous acini are seen. The number can vary depending on predominantly serous or mucous. Along with these acini, mixed acini are also seen having both serous and mucous cells. In a mixed acinus the basic secretory unit is a typical mucous acinus in a tubular shape. The blind end of this tubular structure is capped by a group of serous cells that form a crescent-shaped structure. This crescent-shaped structure is called 'demilune of Gianuzzi'. Intralobular and interlobular ducts, connective tissue septa are also seen.

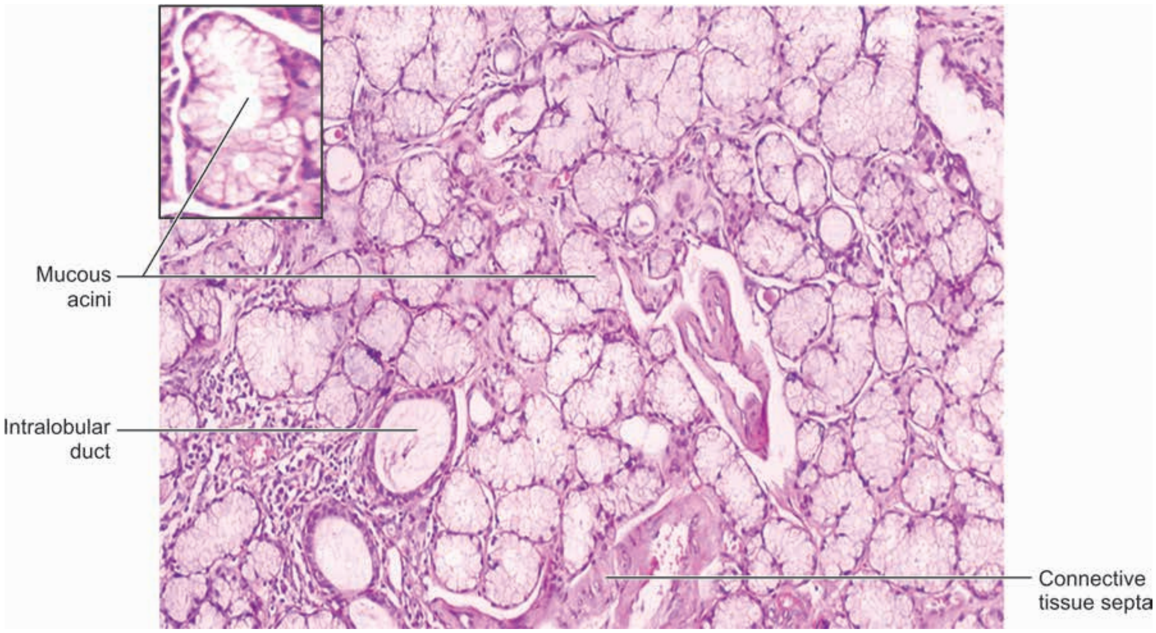
Identification Points (Fig. 11.3)

Mixed Salivary Gland

- Contains both serous and mucous acini
- The blind end of mucous acini has serous demilunes
- Demilunes are crescent-shaped and named 'demilune of Gianuzzi'

Table 11.1: Differences between serous and mucous acini

Serous acini	*Mucous acini*
• Circular or round in shape	• Ovoid or tubular in shape
• Smaller in size	• Larger
• Composed of less number of cells	• More number of cells
• Small lumen	• Wider lumen
• Cells are pyramidal in shape	• Cells are columnar in shape
• Nucleus is round and placed at basal one-third of the cell	• Nucleus is flattened and pressed against basal plasma membrane of the cell
• Apical cytoplasm appears eosinophilic because of zymogen granules	• Apical cytoplasm appears empty in H & E stained sections



Mucous salivary gland (photomicrograph 10X)

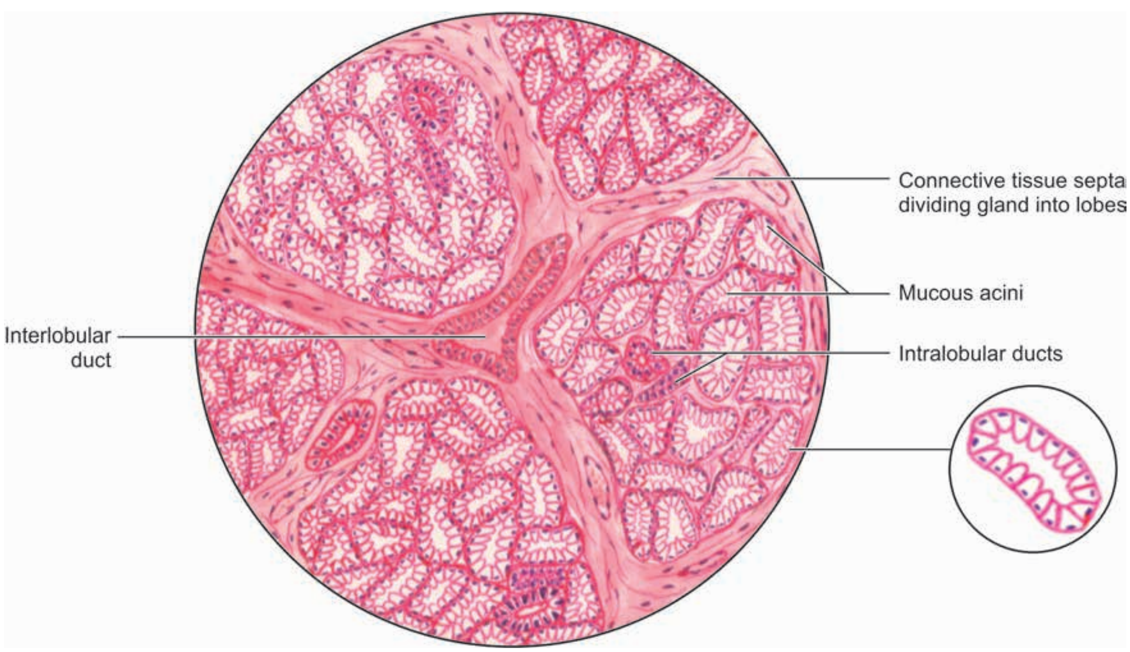
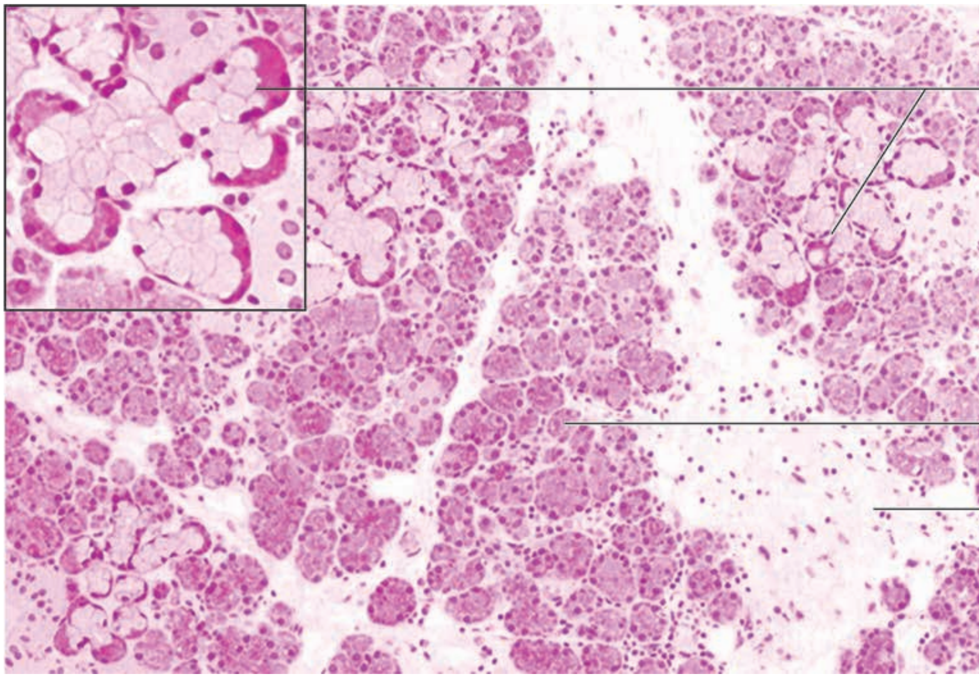


Fig. 11.2: Mucous salivary gland



Mixed salivary gland (photomicrograph 10X)

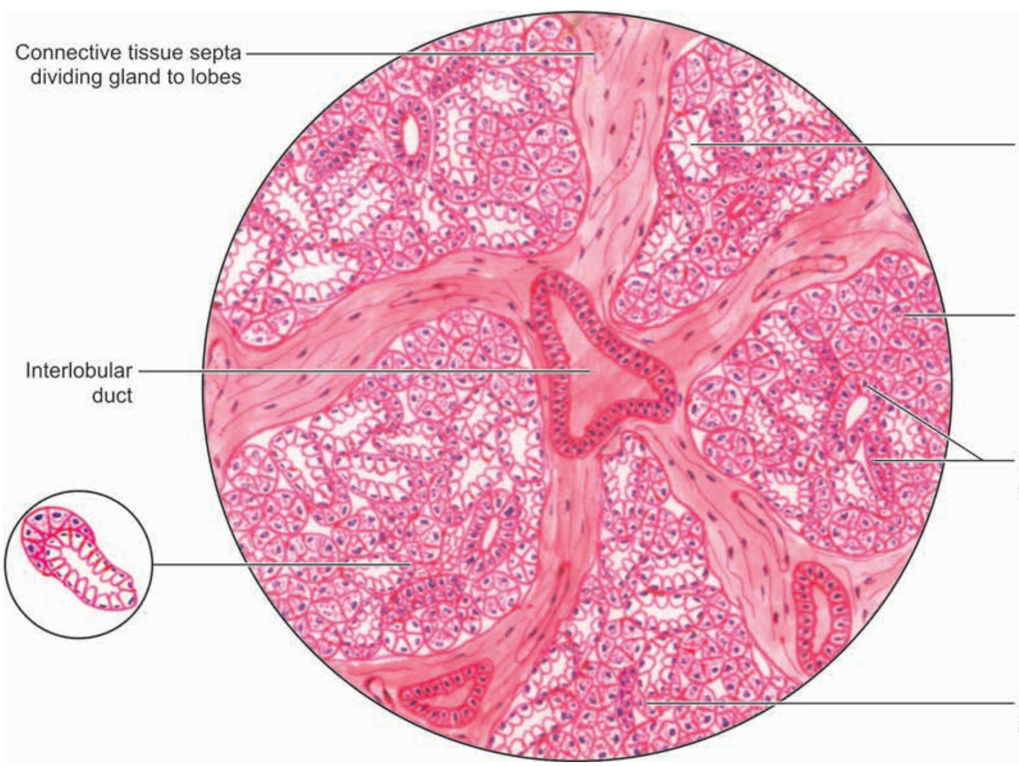


Fig. 11.3: Mixed salivary gland