MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION

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TRAINING MANUAL FOR PRACTICAL CLASSES IN HISTOLOGY

Microanatomy of Oral Cavity and Orofacial Structures

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Contents

Theme 1. TOOTH DEVELOPMENT AND MICROANATOMY. DENTAL TISSUES

Learning blocks:

• Tooth microanatomy. Dental tissues: enamel, dentin, pulp, and cementum. Periodontal ligament. Periodontium and its components.

• Embryonic sources and stages of tooth development.

• Teething theories.

In humans, the teeth come in two generations: 20 decidous teeth (a.k.a. milk, lacteal, baby, temporary, or primary teeth, 10 in the upper jaw and 10 in the lower jaw) and 32 permanent teeth. Each tooth is anatomically divided into three parts (Figure 1):

1) crown (coated in enamel);

2) cervix:

– anatomical cervix (the boundary between enamel and cementum);

– clinical cervix (the circular site of attachment of the gum epithelium to the tooth);

3) root (coated in cementum).

The tooth consists of hard tissues (enamel, dentin, and cementum) and a soft tissue — dental pulp, which fills the pulpal chamber of the crown and root canals. Tooth tissues develop from two embryonic sources: the multilayer ectodemal epithelium of the oral cavity, which gives rise to enamel; and the mesenchyme (more specifically, neuromesenchyme (ectomesenchyme) composed of cells migrating from the neural crest), which gives rise to the rest of the tooth tissues (dentin, cementum, and the pulp). Periodontal ligament (a.k.a. dental ligament or pericementum) also develops from ectomesenchyme. It connects the root of the tooth with the bony sockets (dental alveoli) and is a part of the supporting

apparatus of the tooth — p e r i o d o n t i u m . Apart from the periodontal ligament, periodontium includes gums, cementum, and alveolar processes.

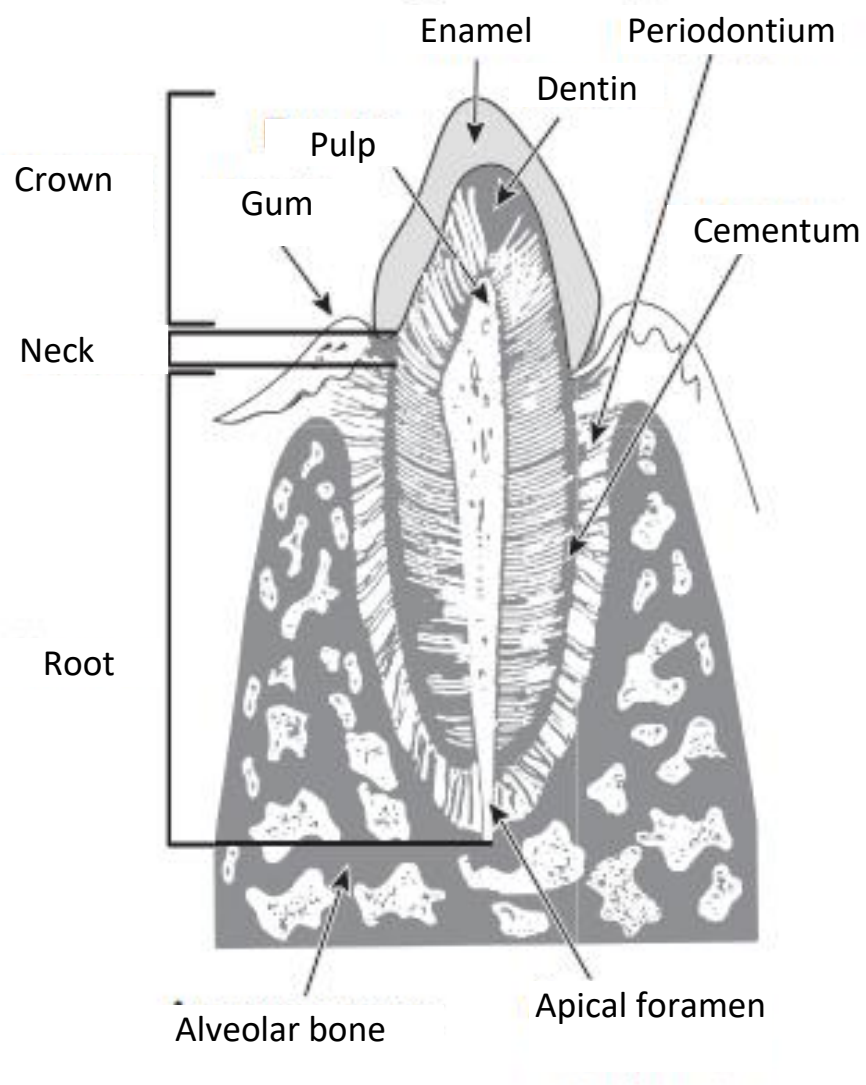


Figure 1. Tooth anatomy (adapted from [1]).

Tooth development (odontogenesis) includes:

1) early stages:

– initial (bud stage);

– morphogenesis (cap stage);

– differentiation (bell stage);

2) advanced stages (histogenesis).

Early stages of tooth development

Tooth germ initiation

On week 6 of embryogenesis, along the upper and lower edges of the primitive oral pit (stomodeum), the oral epithelium forms arcuate epithelial plates accommodated to the shape of the jaws and growing into the underlying mesenchyme. Subsequently, the epithelial plates are divided into the anterior buccal-labial or vestibular plate and the dental plate. When splitting, vestibular plate forms the vestibule of the oral cavity, which will separate the prospective lips and cheeks from the gums (Fig. 2).

On week 8 of embryogenesis, increased proliferation of epithelial cells leads to formation of 10+10 rounded thickenings in the upper and lower dental plates. These primary areas, termed tooth buds (or dental buds), will develop into enamel organs (Fig. 3). Each tooth bud is surrounded by aggregations of mesenchymal cells.

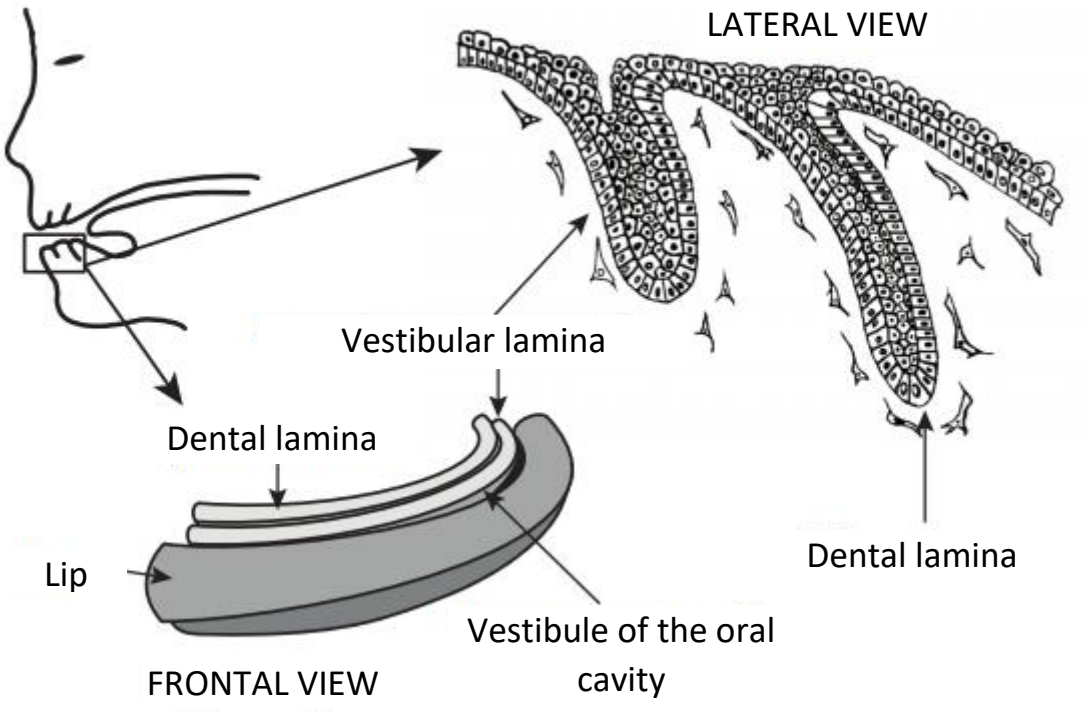


Figure 2. Formation of vestibular lamina, dental lamina, lips, and vestibule of the oral cavity (adapted from [1]).

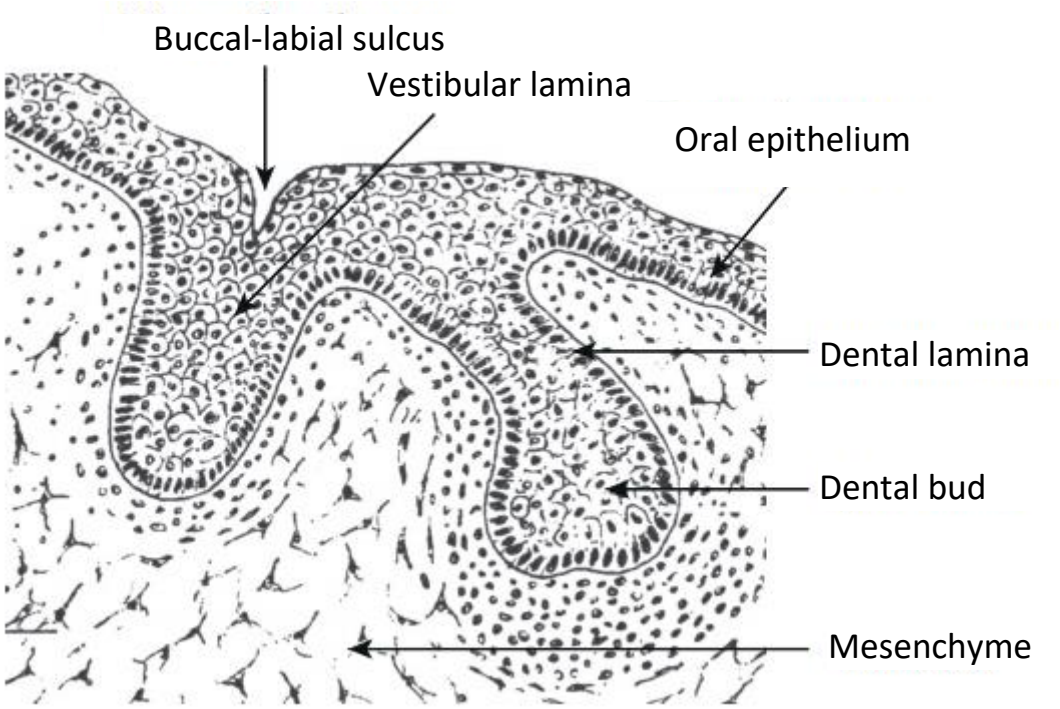


Figure 3. Formation of dental bud in an embryo, week 8 (adapted from [2]).

Tooth germ morphogenesis

On weeks 8–10 of embryogenesis, dental buds progressively submerge into the underlying mesenchyme and eventually form the cap-shaped epithelial enamel organs, which correspond to prospective crowns of milk teeth. The bowl of enamel organ contains mesenchymal cells (dental papilla). Around the enamel organ, ectomesenchyme forms a capsule (dental sac a.k.a. dental follicle). The tooth germ now consists of dental papilla, enamel organ, and dental follicle (Figure 4).

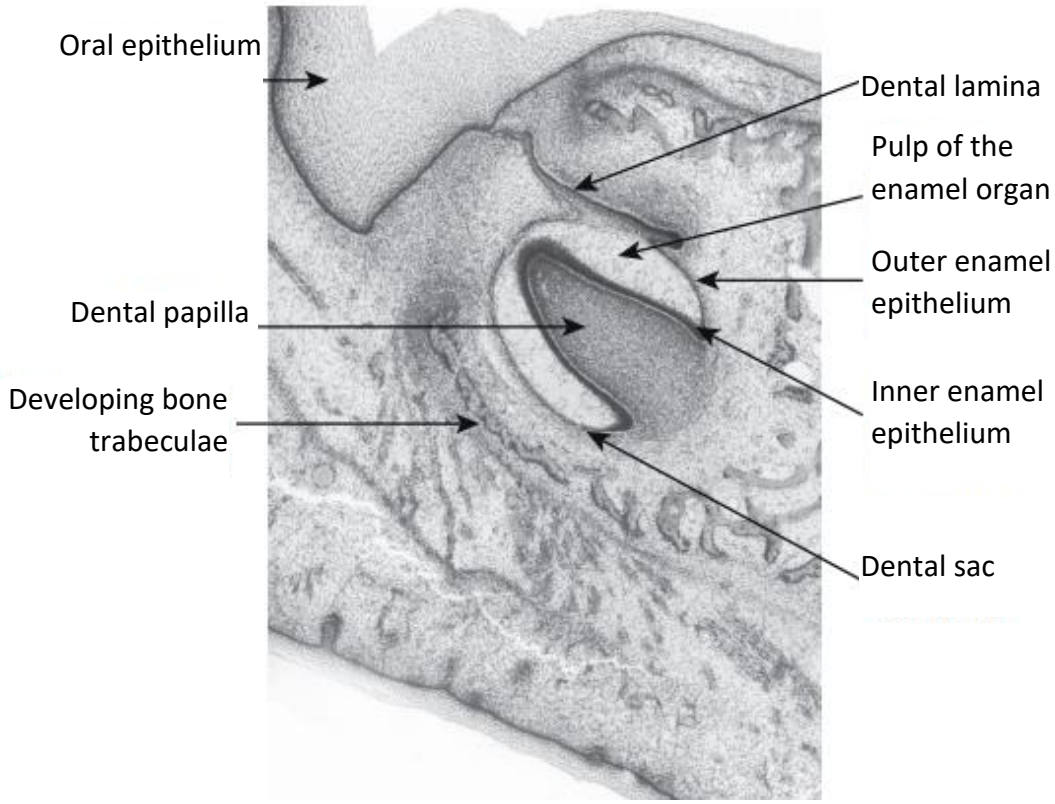


Figure 4. Tooth germ at the stage of morphogenesis (histological image).

Tooth germ differentiation

Eventual lengthening of the ingrowing dental papilla reshapes the enamel organ into a more advanced 'bell' structure (weeks 11–12 of embryogenesis). The cells start to differentiate into progenitors of particular dental tissues. Also at this stage, the residual dental plate splits into discrete clusters of epithelial cells known as the *epithelial rests of Malassez*. The differentiation stage is complete by the end of month 4 of intrauterine development.

Differentiation of the enamel organ proceeds as follows:

The ***outer enamel epithelium***, a layer of cuboidal cells at the convex surface of the enamel organ, are mostly atrophied; they are also thought to protect the enamel during eruption and to play a role in root formation.

The ***inner enamel epithelium*** is a layer of columnar cells at the concave surface of the enamel organ. These cells differentiate into pre-ameloblasts —

precursors of enameloblasts (a.k.a. ameloblasts or adamantoblasts) that will produce enamel. Pre-ameloblasts also promote (induce) differentiation of mesenchymal cells in the dental papilla, which differentiate into pre-odontoblasts.

The ***enamel pulp*** (stellate reticulum) is a network of active cells in the central part of the enamel organ. These cells secrete proteins and glycosaminoglycans, which hold water. The abundant intercellular fluid serves as a reserve of salts and nutrients for differentiating ameloblasts. It also helps to maintain the shape of the anlage and prevents compression and collapse of the inner epithelium and dental papilla.

The ***intermediate layer*** contains several strata of squamous cells with poorly defined organelles located between the inner enamel epithelium and the enamel pulp (Figure 5). The intermediate layer probably participates in calcification of the enamel and formation of the tooth cuticle (worn away soon after eruption). It is also considered a cambial layer for pre-ameloblasts and the enamel pulp.

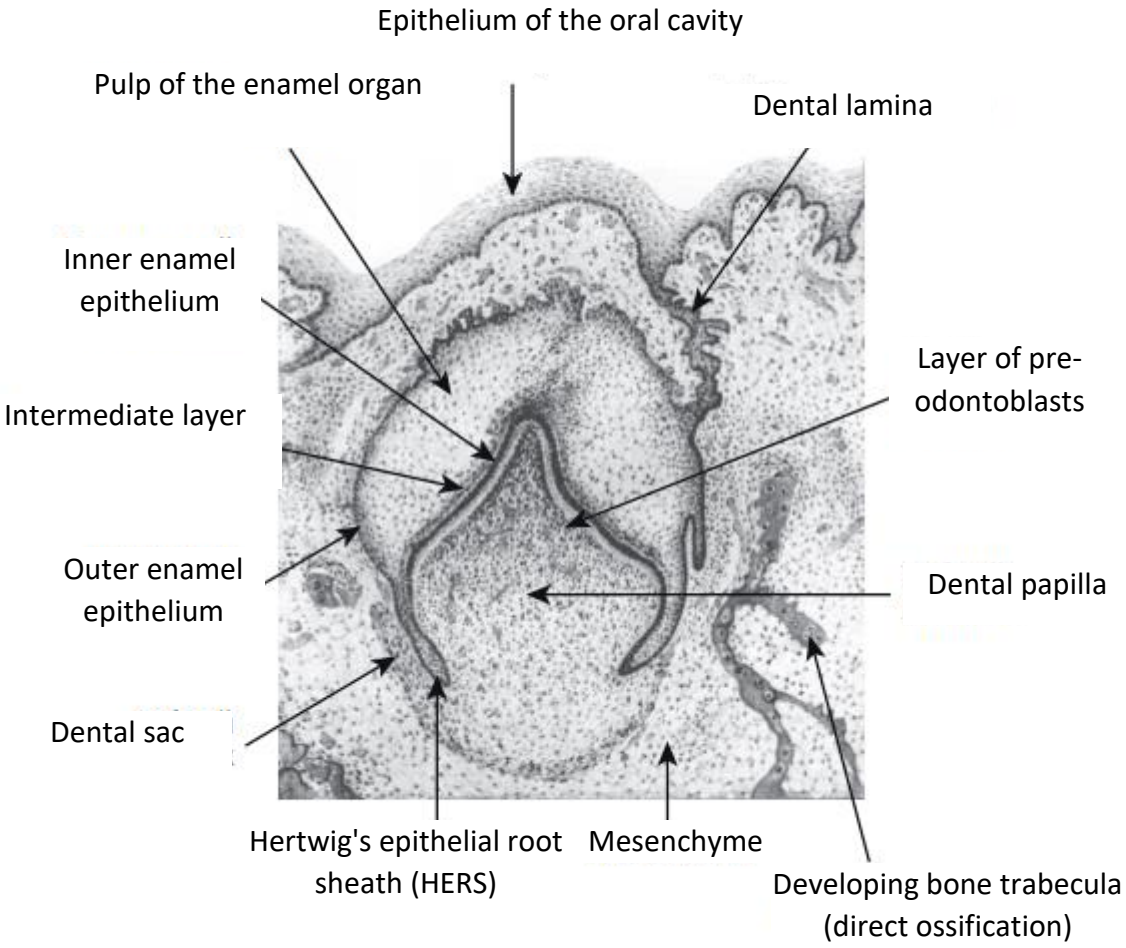


Figure 5. Differentiating tooth germ (histological image, adapted from [3]).

Differentiation of the mesenchymal dental papilla occurs simultaneously with the differentiation of the epithelial enamel organ and involves delamination (Figure 5):

The ***peripheral layer*** is most distinguished at the apex of the dental papilla, where it contacts the basement membrane of the inner enamel epithelium. This layer has several strata of closely spaced basophilic pre-odontoblasts that will soon differentiate into dentin-producing odontoblasts (Figure 6).

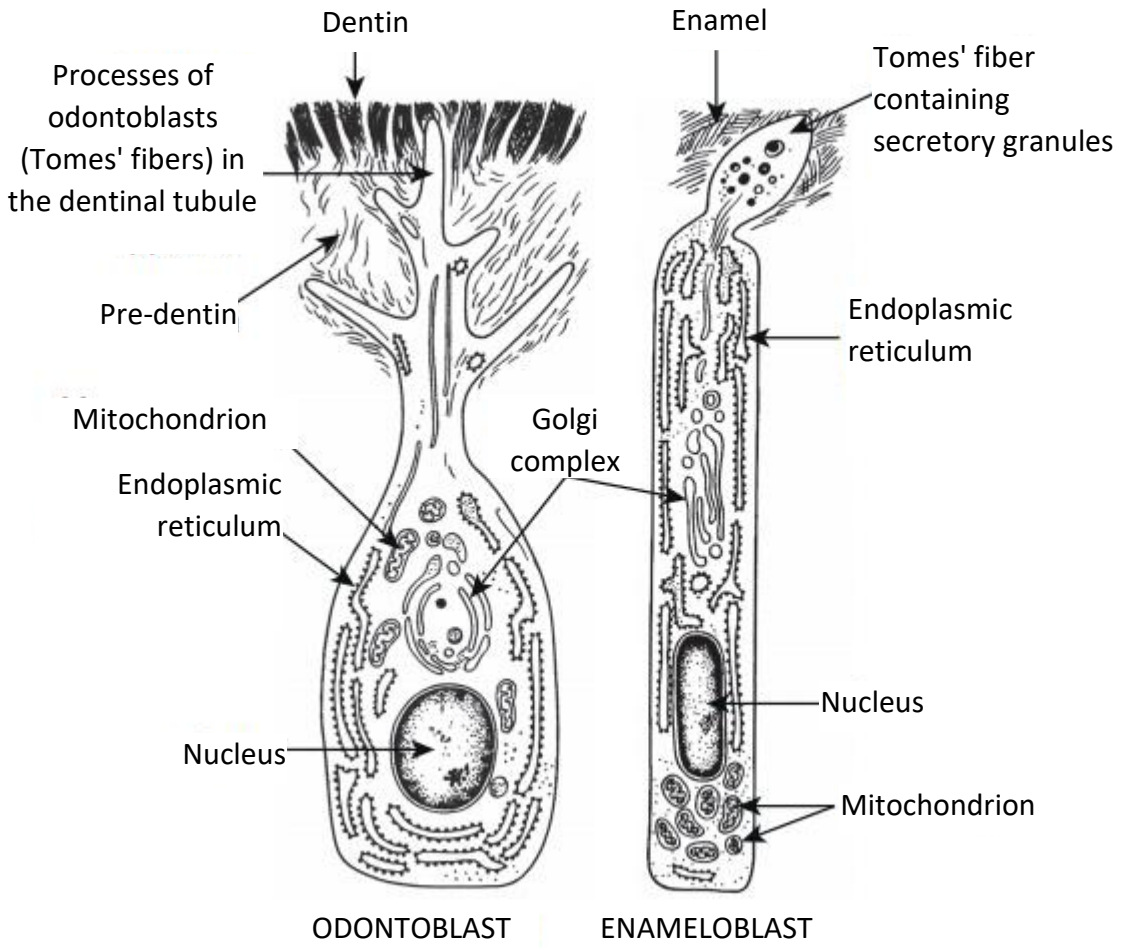


Figure 6. Ultramicroscopic structure of odontoblast and enameloblast (adapted from [4]).

The ***core*** of the dental papilla occupies its major volume and contains undifferentiated mesenchymal cells that will later give rise to the pulp.

Differentiation of the mesenchymal dental sac also involves delamination:

The ***inner layer***, which contacts the dental papilla, differentiates into pre-cementoblasts, which mature into cementoblasts that produce cementum;

The ***outer layer*** differentiates into periodontal ligament cells (fibroblasts) that produce collagen.

Advanced stages of tooth development (histogenesis)

Tooth histogenesis (the formation of dental tissues) begins in the crown (Figure 7), where the nascent dentin, enamel, and pulp become distinguishable by the end of embryonic month 4, whereas the root tissues (dentin, radical pulp, cementum, and periodontal ligaments) emerge by the end of month 5.

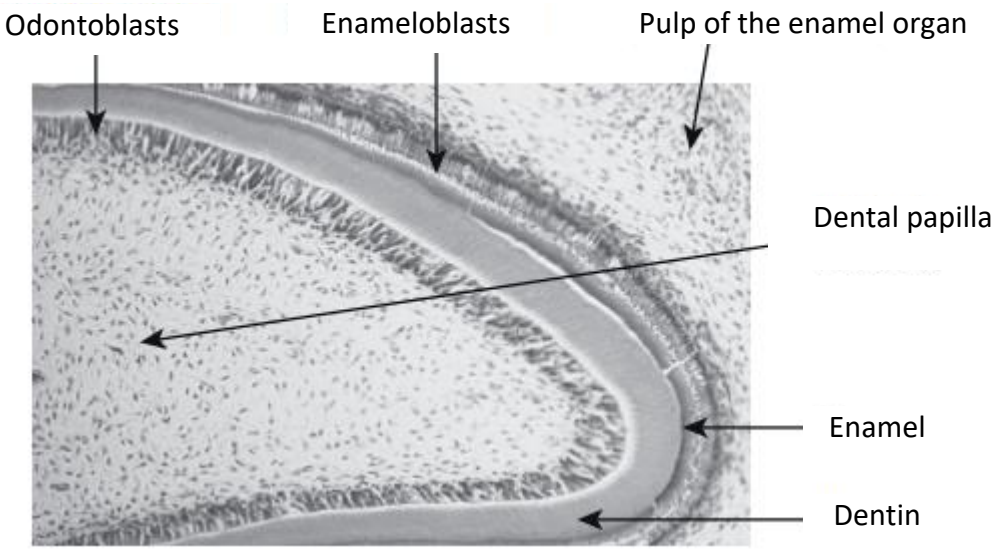


Figure 7. Histogenesis of the enamel and the dentin.

Dentinogenesis (dentin development)

The process of dentin development includes two stages: synthesis of the organic matrix (predentin) and its subsequent calcification (mineralization) into dentin. Odontoblasts are actively involved in both stages.

Predentin formation begins at the apex of the dental papilla.

Odontoblasts start to synthesize procollagen (the nascent fibrous component of extracellular matrix) and the components of ground substance — glycoproteins, proteoglycans, and phosphoproteins (Figure 7). The ground substance macromolecules are enriched with negatively charged chemical groups (sulfates, phosphates) and serve as scaffolds for the binding of calcium ions and formation of hydroxyapatite crystals.

The onset of secretory activity is marked with outgrowth of a process (Tomes' fiber) from the apical portion of each odontoblast.

During dentinogenesis, the peripheral (mantle) dentin with the radially oriented thick collagen fibers (von Korff's fibers) appears first. It is suceeded by the peripulpal dentin with tangential collagen fibers (von Ebner's fibers).

The rapidly increasing thickness of the dentin matrix separates the bodies of odontoblasts further away from the layer of enameloblasts (Figure 8).

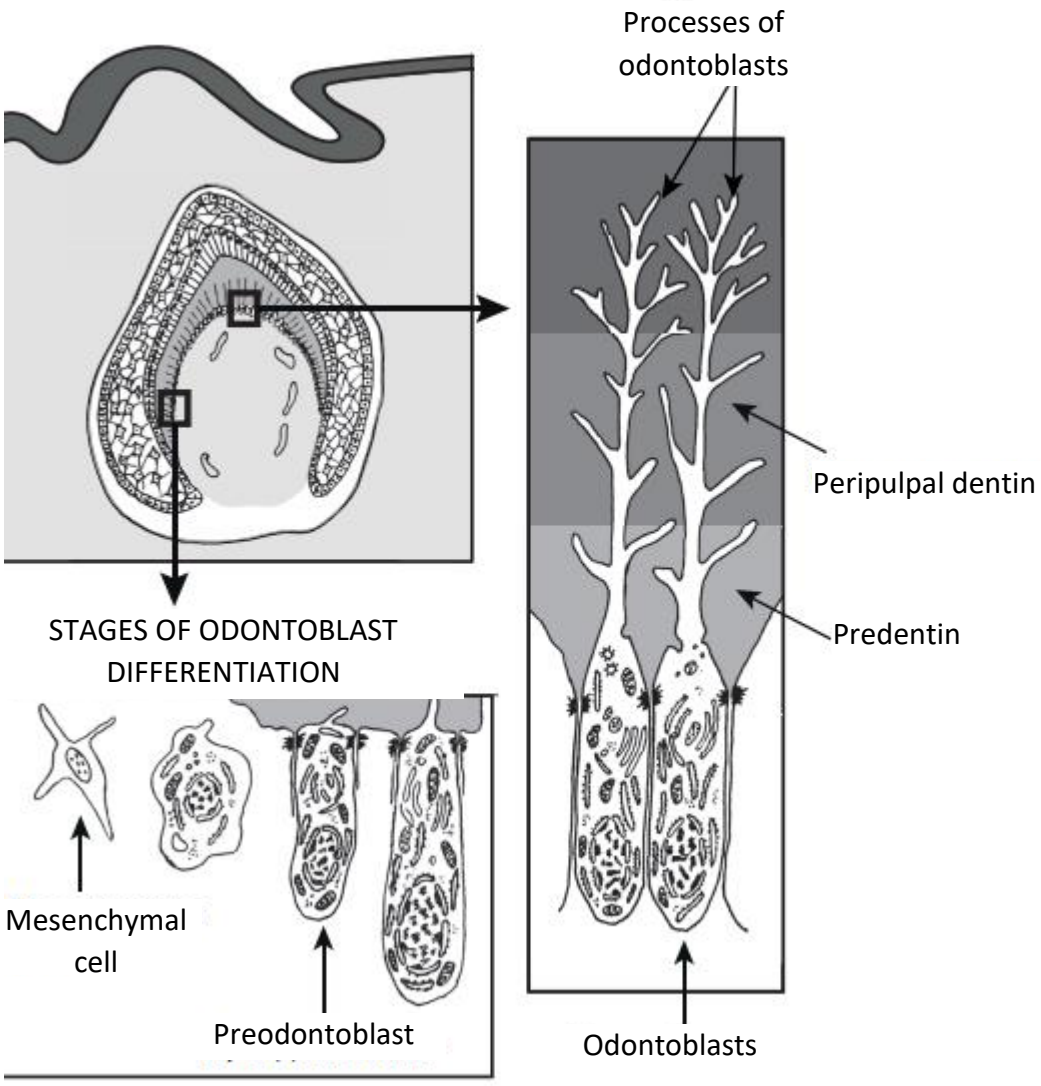


Figure 8. Odontoblast differentiation.

The apical cytoplasmic processes of odontoblasts (Tomes' fibers) elongate and branch. Tunnels in the matrix that contain the branching Tomes' fibers are called *dentinal tubules*. Tomes' fibers continue to deposit the matrix around them, filling the tubules with peritubular (a.k.a. intratubular) dentin. The innermost layer of peritubular dentin is called Neumann's sheath — a thin film of organic substance rich in glycosaminoglycans.

Before the end of embryonic month 5, dentin calcification begins, carried out also by odontoblasts (Figure 9).

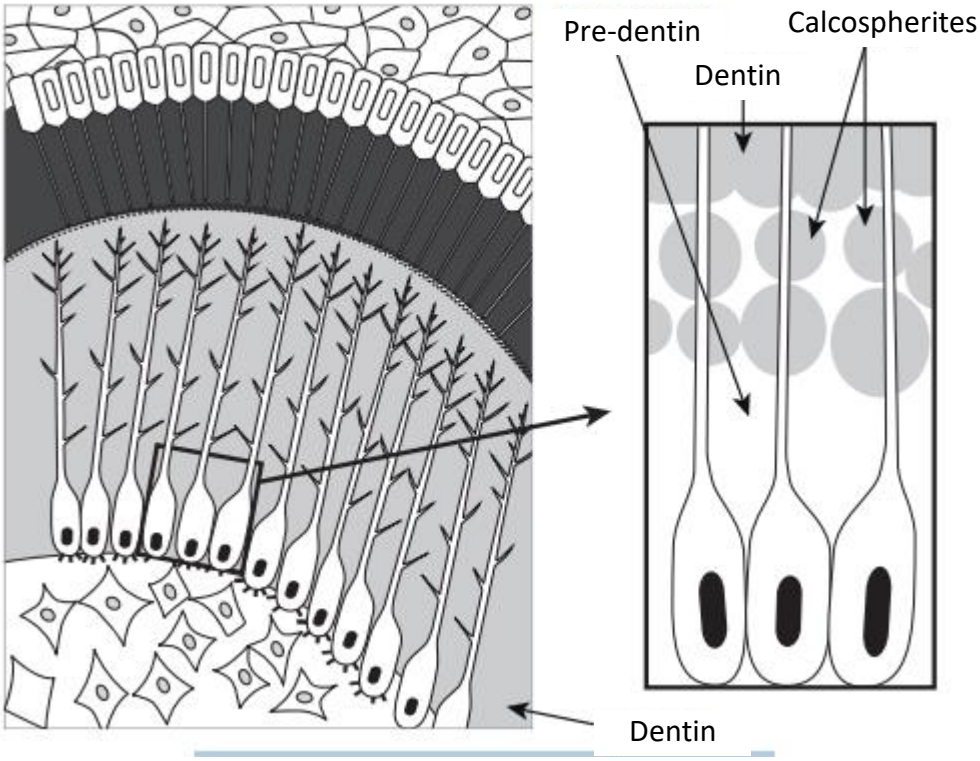


Figure 9. Dentin mineralization.

Mineralization of the mantle dentin occurs through release of matrix vesicles by odontoblast processes to the interspaces of ground substance between collagen fibers. These vesicles contain crystals of hydroxyapatite, which rupture the membranes of the vesicles as they grow and merge into large, homogeneously staining conglomerates. In the peripulpal dentin, odontoblasts deliver ions from blood to the ground substance. Hydroxyapatite crystals are deposited at the cell surface and amid collagen fibers in the form of rounded globules (calcospherites). Growing in volume, the globules merge and form homogeneous calcified structure. Within the mantle dentin of the crown, the merging of large globules is incomplete and leaves hypomineralized areas — interglobular dentin. In the root area, hypomineralized areas of dentin form the Tomes' granular layer.

The mineralized peritubular (intratubular) dentin has minimal content of organic matter and higher content of hydroxyapatite calcium crystals than the intertubular dentin located between the dentinal tubules. For this reason, peritubular dentin is much more rapidly destroyed by caries than intertubular

dentin. Formation of peritubular dentin gradually narrows the lumina of dentinal tubules.

Deposition and mineralization of the dentinal matrix proceed in waves, cyclically, resulting in formation of characteristic markings:

Owen's contour lines, orthogonal to dentinal tubules, correspond to daily rhythms of dentin deposition;

von Ebner's incremental lines are spaced wider than the contour lines and correspond to a slower 5-day cycle of odontoblast activity.

Amelogenesis (enamel development)

Amelogenesis (a.k.a. enamelogenesis) is initiated and spreads from the biting edges of anterior teeth (Figure 7). Microscopic structure of the enamel depends on particular time of its formation. The first and the last layers are aprismatic (prism-free), whereas the intermediate layer shows characteristic patterns of tightly packed rods (prisms).

After mineralization of the underlying dentin, nutrition of the inner enamel epithelium through the basement membrane is disrupted. Under these conditions, nuclei and Golgi complex of the epithelial cells migrate from the basal compartment towards the apex (so-called inversion of the nuclei). The cells stretch vertically and become fully differentiated enameloblasts, which start to produce specific non-collagen enamel matrix proteins — amelogenins and enamelins. Mineralization of the matrix occurs shortly after its secretion, which is typical for enamelogenesis. After the initial aprismatic enamel layer is formed, enameloblasts become separated from the dentin and reveal short cytoplasmic Tomes' processes protruding from their surface through enamel towards the dentin. Enameloblasts continue to produce the organic enamel matrix and deposit it alongside Tomes' processes. Mineralization of the matrix immediately adjacent to the Tomes' processes creates a fine mesh of interprismatic enamel with the embedded Tomes' processes. Mineralization of the matrix filling the cavities

creates enamel prisms — structural and functional units of the enamel. Matrix deposition and calcification proceeds in waves, leading to appearance of Retzius's growth lines. With gradual degeneration of enameloblasts and decline of the Tomes' processes, the deposition is finalized by the last layer of aprismatic enamel.

Mineralization of the enamel occurs through replacement of the decaying organic matrix with the crystals of calcium hydroxyapatite.

Overall, amelogenesis proceeds in 3 stages:

1. secretion of organic matrix as the basis, and its primary mineralization;

2. maturation of the enamel (secondary mineralization);

3. final maturation of the enamel (tertiary mineralization).

Organic matrix that appears at the first stage of enamel development is rich in organic substances (up to 30%). Mature enamel produced at the second stage is 97% mineralized through impregnation with calcium salts (hydroxyapatite crystals). The final maturation begins after the eruption, using saliva as a source of ions, and is most intensive during the first year of life.

Dental pulp development

Differentiation of mesenchymal cells of the dental papilla begins from its apex and spreads to the base. The outer (peripheral) layer of the dental papilla differentiates into odontoblasts, the bodies of which reside in the peripheral layer of the pulp, while processes (Tomes' fibers located within dentinal tubules) perforate the entire thickness of the dentin.

In the middle part of the dental papilla, cells differentiate into fibroblasts that produce components of extracellular matrix (collagen types I and III, and various components of ground substance) and fibrocytes. Populated by macrophages and lymphocytes arriving from the blood, this area gives rise to loose fibrous connective tissue of the dental pulp.

Vascularization of the dental pulp proceeds by ingrowth of arterioles and venules, and their branching into extensive capillary bed. The innervation proceeds concomitantly by ingrowth of abundant receptor and efferent nerve endings.

Dental root development

In the enamel organ, the so-called cervical loop emerges at the circular inner/outer junction of the enamel epithelium. Its cells proliferate and give rise to a two-layer epithelial cord (Hertwig's epithelial root sheath, HERS), which progressively grows into mesenchyme between the dental papilla and the dental sac and participates in the dental root formation. As it grows, the inner layer of HERS promotes differentiation of the nearby dental papilla cells into odontoblasts of the dental root (Figures 10 and 11).

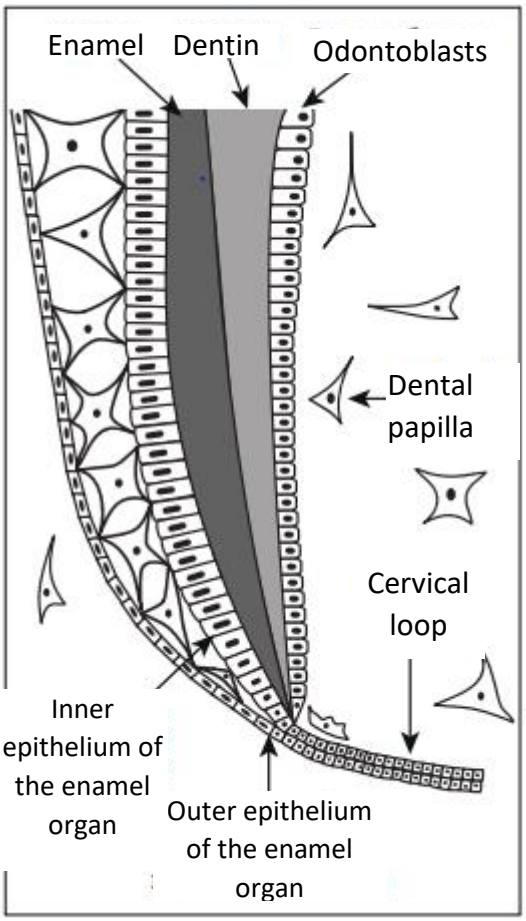
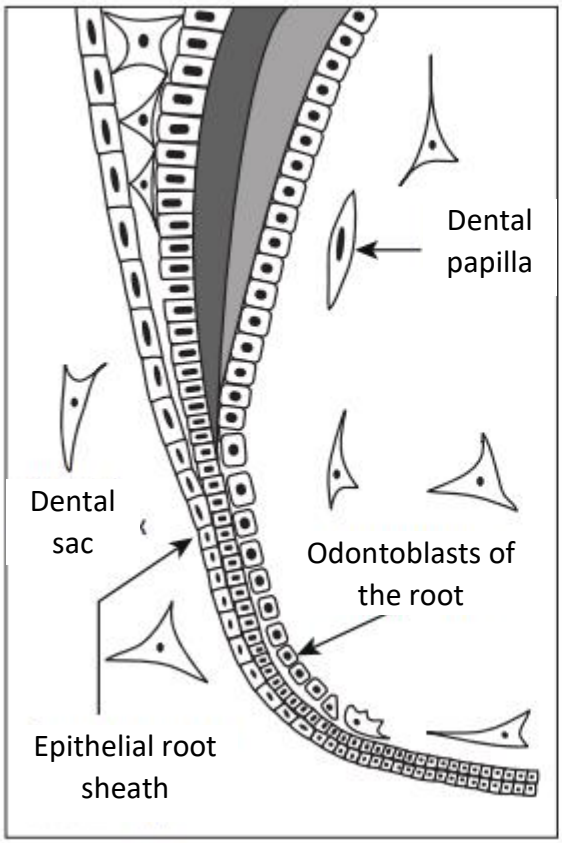


Figure 10. Formation of the cervical loop.



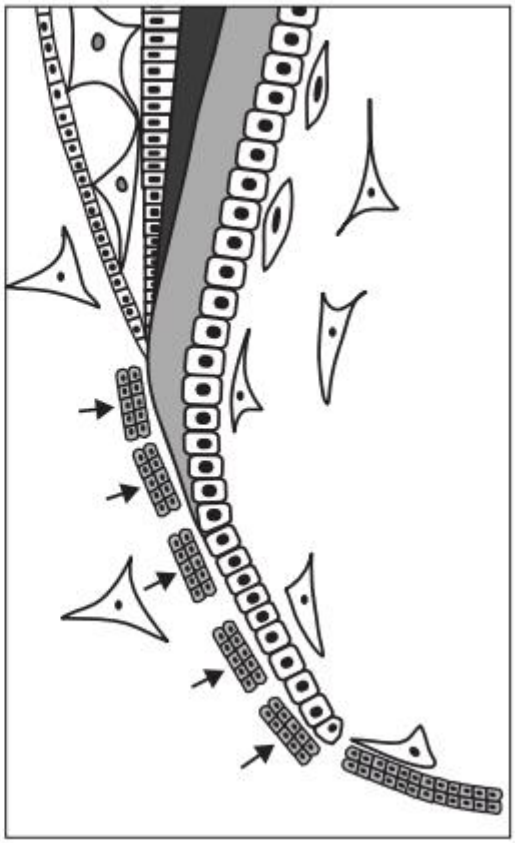


Figure 11. Formation of HERS. **Figure 12.** Formation of ERM.

After formation of dentin in the dental root is finished, HERS disintegrates into separate fragments known as epithelial cell rests of Malassez (ERM), found in the periodontium (Figure 12). Cells of the inner layer of the dental sac, which become exposed to dentin, differentiate into cementoblasts.

Cementogenesis (formation of the cementum)

Formation of cementum, similarly with other hard dental tissues, proceeds in two stages: synthesis of organic matrix — cementoid, or primary cementum, and mineralization of the cementoid into secondary cementum.

Cementoblasts of the dental sac begin to produce organic matrix, cementoid, composed of collagen fibers and amorphous substance. The cementoid is deposited over the surface of highly mineralized, structureless Hopewell-Smith's hyaline layer overlying the root dentin. The Hopewell-Smith layer, apparently produced

by epithelial cells of the root sheath, ensures strong adhesion of the cementum to the adjacent dentin and periodontium. Cementoid mineralization proceeds rhythmically through deposition of calcium hydroxyapatite crystals — deposition of a new cementoid layer coincides with mineralization of the previous layer.

The first to form is ***primary cementum***, which is acellular (does not contain cells). Primary cementum is deposited slowly as the tooth erupts and covers 2/3 of the root surface closer to the crown.

After the eruption of the tooth, a cement containing cells is formed - a cellular ***secondary cementum***, which is cellular (contains cells) and is confined to apical portion of the root.

Development of the periodontal ligament

Periodontal ligament is a derivative of the dental sac, mesenchymal cells of which differentiate into fibroblasts and start to produce the ground substance and procollagen of the nascent extracellular matrix. Periodontal fibers are deposited from both the adjoining cementum and bony alveoli, and accumulate, and mature with the expansion of the root, and become thicker after the eruption.

Teething theories

Teething is the process of eruption and growth of the teeth through the gums, which results in exposure of the crown to oral cavity. Teething proceeds by progressive vertical (axial) motion of the tooth from the site of its development inside the jaw. Complex mechanisms of this process are the subject of several theories:

I. ***Dental root growth theory*** contemplates the pushing-off effect between the growing dental root and the fundus of the bony alveolus. However, this theory does not explain the complex motion of certain teeth within the jaw before the start of their eruption, as well as the eruption of teeth with rudimentary roots.

II. ***Hydrostatic pressure theory*** considers two possibilities:

• A critical increase in the hydrostatic pressure of the tissue fluid in the tooth root area pushes the tooth through the gum. The critical increase in pressure is driven by an increase in vascular permeability and consequent extravascular accumulation of hydrophilic proteins and ground substance molecules, which absorb and retain water. However, surgical resection of the growing root and its surroundings does not prevent the eruption.

• The critical increase in pressure is caused by formation of the dental pulp, which boosts the volume of the dental papilla in its apical portion. According to this theory, eruption of a tooth promotes formation of its root.

III. ***Bone remodeling theory*** considers the eruption a consequence of alternating growth and resorption at the fundi of bony alveoli.

IV. ***Periodontal traction theory*** comes in two versions:

• The propulsive force arises from shortening of the bundles of collagen fibers in the forming periodontium.

• The combined contractile activity/migration of periodontal myofibroblasts is transmitted to collagen fibers, which leads to teething. This explanation is confirmed by the termination of the eruption of the tooth by developmental damage to the periodontium.

Microanatomy of dental zones and tissues

Microanatomy of the enamel

Tooth enamel, the hardest substance in the human body, covers the crown dentin and reaches maximal thickness over the masticatory tubercles (up to 2.5 mm). Mature enamel does not contain cells.

Chemical composition: 96–97% of inorganic substances (mainly crystals of calcium salts: hydroxyapatite, phosphates, carbonate, and fluoride), 3–4% of organic substances (proteins, glycoproteins), and about 2% of water.

Structure: the structural and functional unit of the enamel, enamel prism, is rod-shaped and resembles a keyhole or fish in cross section. Enamel prisms are produced by secretory activity of the Tomes processes of enameloblasts. Bundles

of enamel prisms, orthogonal to the dentino-enamel boundary, pass through the entire thickness of the enamel.

The interprismatic substance, which is less mineralized than the prisms, contains hydroxyapatite crystals oriented orthogonally relative to the crystals within the prisms.

Hunter-Schreger's bands are alternating light and dark bands, about 100 μm wide, visible in tooth sections, running orthogonally to the enamel surface. They correspond to different planes of cutting enamel prisms during the passage of the cut: longitudinal cuts produce light stripes (parazones) and cross cuts produce dark stripes (diazones).

Retzius's lines mark the fronts of enamel growth, which correspond to the diurnal rhythm of enamel deposition (Figure 13). The neonatal growth line is a well-defined dark marking between the enamel layers formed before and after birth. This line corresponds to perinatal period, about 1 week or longer, when amelogenesis is halted. It is histologically distinguishable in all primary teeth and first permanent molars.

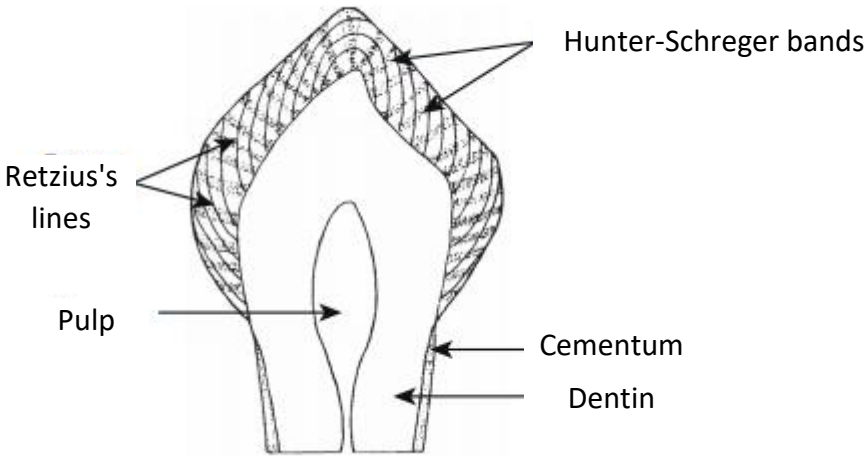


Figure 13. Hunter-Schreger bands and Retzius lines (adapted from [2]).

Enamel tufts and enamel lamellae are areas of insufficiently calcified interprismatic substance, representing potential routes for infections to enter the tooth. Enamel tufts are small conical bodies found at the dentino-enamel boundary. Enamel lamellae are thin sheets passing through the entire thickness of the enamel.

Enamel spindles are fusiform hypomineralized areas of the enamel corresponding to the long processes of odontoblasts (Tomes' fibers) reaching deep into the enamel layer and immured, sandwiched between the enamel prisms. The spindles are found in the inner 1/3 of the enamel thickness.

Enamel cuticle consists of two layers: primary cuticle (Nasmyth's membrane) — the inner thin layer of glycoproteins, which is the last secretory product of enameloblasts, and secondary cuticle — the outer thick layer composed of the remnants of the outer enamel epithelium of the primordial enamel organ. The cuticle is worn out shortly after eruption, except on interdental surfaces.

Enamel pellicle (a.k.a. dental pellicle) is an acquired thin coating of precipitated salivary glycoproteins. It forms on the surface of the enamel and cuticle soon after tooth eruption. The pellicle is destroyed by mechanical cleaning and restored in several hours. The pellicle lubricates the teeth and protects them from demineralizing agents, but at the same time it favors microbial adherence and formation of bacterial plaques.

Microanatomy of the dentin

Dentin is a hard tissue that provides structural basis for the crown, cervix, and root of the tooth. It comprises the calcified extracellular matrix permeated with dentinal tubules, which contain odontoblast processes and minimal volume of extracellular fluid. Odontoblast bodies are located outside the dentin, on its interior side, in the outer layer of the dental pulp.

Chemical composition: 72% of inorganic substances (phosphate salts of calcium and magnesium, calcium fluoride) and 28% of organic substances: pre-collagen and collagen fibers, odontoblast processes, dentin-specific non-collagen calcium-binding proteins (phosphophoryn (dentin phosphoprotein), dentin sialoprotein), nonspecific proteins (osteocalcin, osteonectin), and alkaline phosphatase. Deposition of dentin by odontoblasts continues throughout life and increases with tooth damage. With age, dentin thickness normally increases, while the volume of tooth cavity decreases.

Structure: dentin consists of ground substance, collagen fibers (organized in bundles and running in two directions — radial and tangential) and dentinal tubules (containing odontoblast processes). Three layers of dentin are distinguished:

1) Predentin — zone of young, non-mineralized dentin, adjacent to the pulpal chamber and capable of growth throughout life.

2) Middle layer, which constitutes the main volume of mineralized dentin and contains the tangential von Ebner's fibers.

3) Mantle (surface) layer, fully mineralized and containing von Korff's fibers.

In the developmental perspective, the following types of dentin are distinguished:

• Primary dentin forms during embryonic period and shows strictly ordered arrangement of dentinal tubules.

• Secondary dentin (a.k.a. regular or physiological dentin) forms after the eruption and constitutes the inner layer of peripulpal dentin. Compared to primary dentin, it is less structurally regular and less mineralized.

• Tertiary dentin (a.k.a. reparative, irregular, or traumatic) is formed in response to various sorts of damage (including caries and decay) and protects the pulp from infection. It is characterized by lower mineralization and irregular arrangement of dentinal tubules.

• Transparent (sclerotic) dentin forms through excessive deposition of mineral salts (hypermineralization) causing irreversible occlusion of dentinal tubules.

Microanatomy of the dental pulp

Dental pulp is the soft tissue filling of the pulpal chamber of the crown and the root canals. The pulp consists of non-mineralized loose fibrous connective tissue (cells and prominent extracellular matrix composed of fibers and ground substance) with vessels and nerves. Cells of the pulp include odontoblasts (pulp-specific cells), fibroblasts, as well as smaller numbers of macrophages,

lymphocytes, dendritic cells (antigen-presenting cells), and low-differentiated cells (e.g. pre-odontoblasts). Extracellular matrix of the pulp contains collagen and reticular fibers, but no elastic fibers. The ground substance has composition typical for loose fibrous connective tissue. The pulp contains nerve plexuses, numerous nerve endings, and blood and lymph vessels.

The pulp implements protective, immune, formative, trophic, and sensory **functions**.

The pulp has distinctive layered **structure** comprising *peripheral*, *intermediate*, and *core layers* are distinguished in the pulp (Table 1).

Table 1. Layers and zones of the dental pulp.

Peripheral layer	**Intermediate layer**		**Pulp core**
	Outer zone	**Inner zone**	
- odontoblast cell bodies, - Tomes processes, - capillary loops, - nerve fibers	- cytoplasmic processes of cells of the inner zone, - collagen and reticular fibers, - nerve plexus of Raschkow	- fibroblasts, - fibrocytes, - macrophages, - lymphocytes, - pre-odontoblastsand other low-differentiated cells, - blood and lymph capillaries, - myelinated and non-myelinated nerve fibers	- loose fibrous connective tissue (no elastic fibers), - blood and lymph vessels, - bundles of nerve fibers

Odontoblast cell bodies form *peripheral (odontoblastic) layer*, 1–8 cell-thick and adjacent to the pre-dentin. Odontoblasts are connected by cell junctions and interspersed with tiny amounts of loose fibrous connective tissue containing capillary loops and nerve fibers. The Tomes' processes of odontoblasts run into dentinal tubules.

The next layer, termed *intermediate (subodontoblastic) layer*, comprises the outer cell-free zone (a.k.a. basal layer of Weil) and the inner zone where cell bodies reside. The inner zone has the typical architecture of loose fibrous connective tissue with multiple cell types (fibroblasts, macrophages, etc.), blood capillaries, and nerve fibers. The outer zone, there is an intercellular substance with fibers and processes of cells of the inner layer, blood capillaries and a plexus of nerve fibers (nerve plexus of Raschkow).

Core of the pulp consists of loose fibrous connective tissue with larger vessels and bundles of nerve fibers.

The pulp fillings of the crown chamber and the root canal (respectively, *coronal pulp* and *radicular pulp*) are histologically distinct, the structure of each has its own characteristics (Figure 14):

1) coronal pulp:

- contains loose fibrous connective tissue with diverse cell types;
- contains several strata of prismatic odontoblasts in the peripheral layer;
- is densely vascularized and innervated;

2) radicular pulp:

- contains dense fibrous irregular connective tissue with few fibroblasts/fibrocytes and bundles of thick collagen fibers, the intermediate layer is missing;
- contains 1–2 strata of oblate odontoblasts;
- is poorly vascularized and innervated.

With age, the pulpal chamber volume decreases through deposition of secondary dentin. The declining cellularity of the pulp is accompanied by growing content of collagen fibers and calcified structures — petrification areas and denticles.

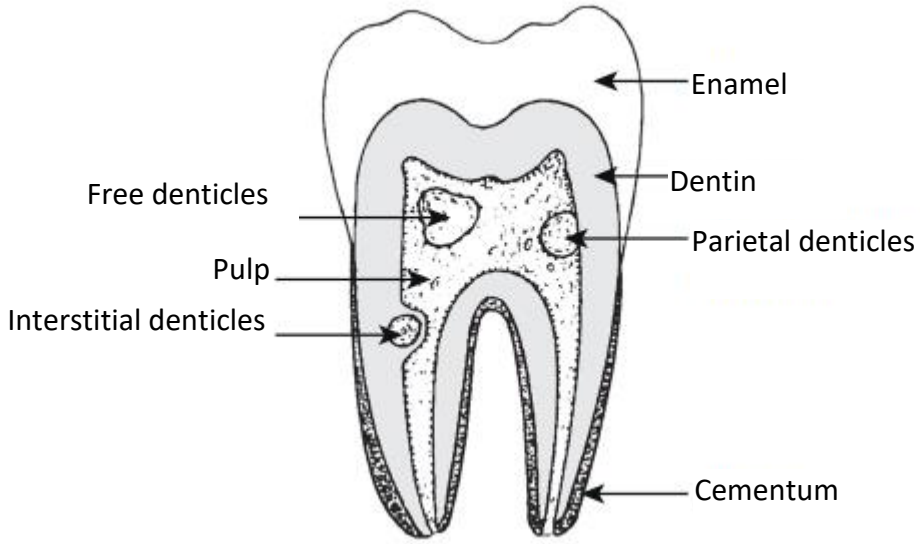


Figure 14. Localization of free, parietal, and interstitial denticles in the dental pulp (adapted from [2]).

Petrification presents as diffuse areas of calcification, mostly found in dental roots along the periphery of nerve fibers and blood vessels, and also in blood vessel walls.

Denticles (a.k.a. pulp stones) are rounded or irregularly shaped calcified bodies. At certain localizations, denticles may compress blood vessels and nerves and cause pain. Denticles are classified as follows:

By location within the pulp, denticles are subdivided into *free* denticles (unattached to any hard tissue), *parietal* (fused to the wall of the pulpal chamber) and *interstitial* (located in the thickness of the dentin) (Figure 14).

By origin and structure, denticles are divided into:

•*true denticles* are produced by odontoblasts and show complex organization typical of mature dentin with dentinal tubules;

•*false denticles* comprise concentric lamellae of calcified material, usually surrounding necrotic or infectious foci, and no dentinal tubules.

Possible causes of denticle formation are:

• disorders (endocrine, autoimmune, inflammatory) and aging;

• nutrient deficiencies (protein, vitamins).

Microanatomy of the cementum

Cementum is another hard dental tissue; it provides coating of the root dentin and the dental cervix (neck of the tooth). Its structure resembles the woven (reticulofibrous) bone tissue, but unlike it, it is devoid of blood vessels and is not subject to constant restructuring.

Chemical composition: 50–60% of minerals (calcium phosphate, calcium carbonate) and 30–40% of organic matter (mainly collagen).

Structure: primary (acellular) and secondary (cellular) types of cementum differ in structure and localization in the dental root (Table 2).

Table 2. Comparison of acellular and cellular cementum.

	Type of cementum	
	Primary (acellular)	**Secondary (cellular)**
Localization	- adherent to dentin, - coats the entire root surface	- located on the outside of acellular cement, - covers the apical 1/3 of the root (in multi-rooted teeth also the furcation area)
Structure	- no cells, - collagen fibers oriented tangentially and radially, - abundant mineralized ground substance, - growth lines are positioned densely	- cementocytes in lacunae, cell processes anastomosing with each other, - collagen fibers arranged irregularly, - abundant mineralized ground substance, - growth lines are positioned loosely

Microanatomy of the periodontal ligament

Periodontal ligament (a.k.a. pericementum) is a dental ligament located in the periodontal fissure, with one end anchored (woven) in the cementum, another end anchored (woven) in the alveolar bone.

Structure: periodontal ligament is composed of dense fibrous regular connective tissue containing bundles of collagen fibers (70%) interspersed with layers of loose fibrous connective tissue (30%).

Major bundles of collagen fibers running in several directions are specified as:

1) *alveolar crest fibers* are found within the dental cervix area, connecting the tooth surface with the alveolar bone crest;
2) *horizontal fibers* are located deeper and run orthogonally to both the tooth and the alveolar bone surfaces, forming a circular ligament;
3) *transseptal fibers* pass over the alveolar crest and connect adjacent teeth;
4) *oblique fibers*, the most abundant, are found in the midportion of periodontal surface running at an angle, with upper ends anchored in the alveolar bone and lower ends anchored in the cementum;
5) *apical fibers* are found in the root apex area, connecting the root with the surrounding bone.

Functions of the periodontium:

• mechanical attachment and fixation of teeth in bony alveoli;

• participation in teething;

• mechanical support and transmission of chewing pressure;

• trophic;

• regenerative;

• sensory (proprioceptive);

• mechanical protection of teeth.

Learning tasks and tests

Answer the questions:

1. Name the embryonic sources of dental tissues.

2. Define and describe the enamel organ.

3. What are the dental sac and the dental papilla? At what stage of odontogenesis are they formed?

4. Define and describe the basic events of dental germ differentiation.

5. Describe dentinogenesis.

6. Describe enamelogenesis.

7. Describe development of cementum and periodontal ligament.

8. Describe development of the pulp.

9. Discuss the teething theories.

10. What is the Hertwig's epithelial sheath? Describe its role in odontogenesis.

11. What are the epithelial remnants of Malassez? What embryonic structures are they derived from?

12. What are the Hunter-Schreger's bands and Retzius's lines?

13. Describe the structure of dentin; specify the layers and describe their histogenesis.

14. Specify the difference between primary and secondary dentin.

15. What are distinctive structural features of coronal and radicular (root) pulp?

16. Specify localization and structure of the basal layer of Weil.

17. What are tooth denticles? What causes them? Are they harmful?

18. Describe and characterize primary and secondary cementum (morphologically and functionally).

19. Describe the periodontal ligament and specify its functional significance.

20. What are the enamel beams, enamel plates, and enamel spindles?

21. What are von Korff's fibers and von Ebner's fibers?

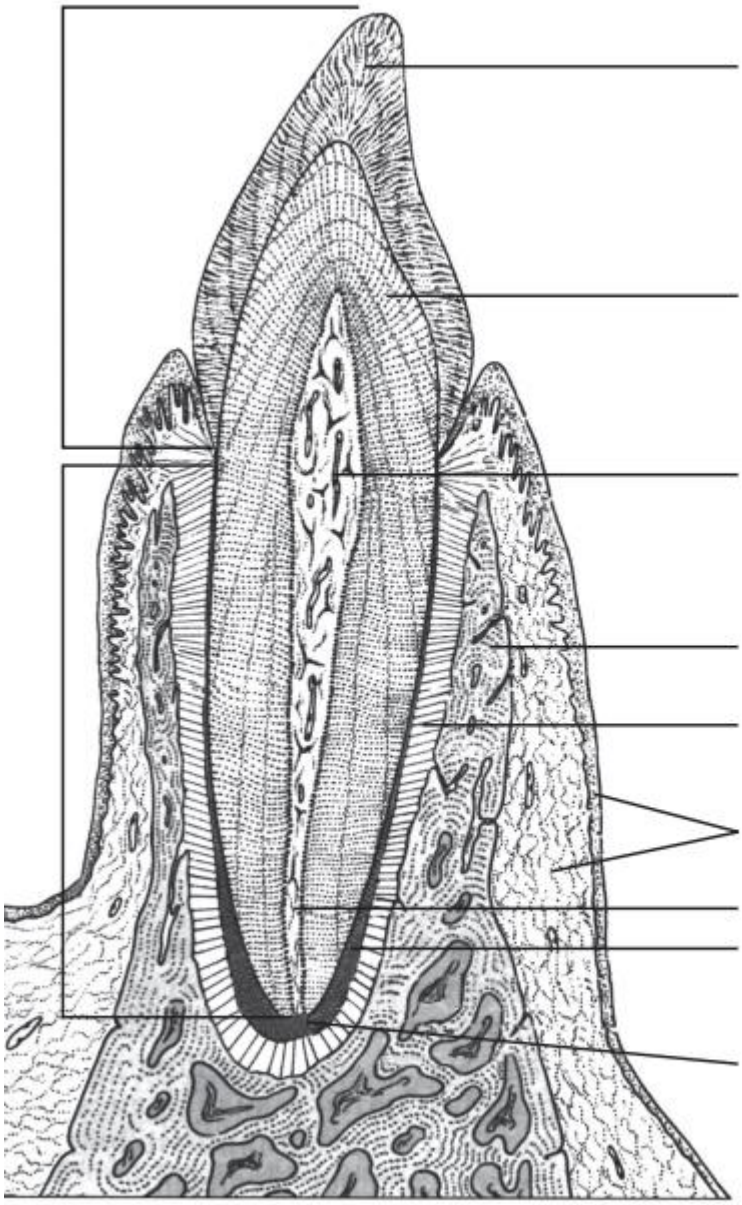
22. What are Tomes' fibers? Describe their formation.

23. Describe histophysiological distinctions between odontoblasts and enameloblasts.

24. What is the Nasmyth's membrane (primary enamel cuticle)? Specify its developmental sources.

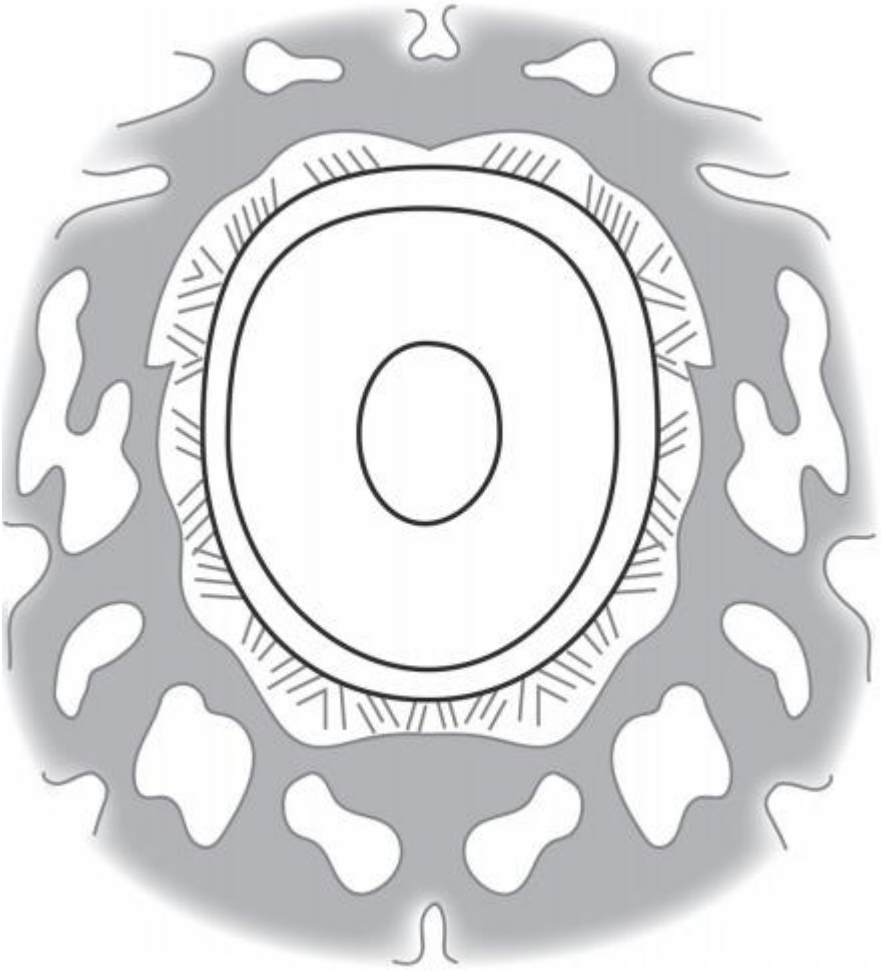
25. Describe sclerotic dentin and specify the mechanism of its formation.

Task 1. Tooth (longitudinal section) [5].



Study the diagram, label structural elements and zones: 1 – enamel, 2 – dentin, 3 – pulp, 4 – cementum, 5 – periodontal ligament, 6 – bony alveolus, 7 – gum, 8 – root canal, 9 – apical foramen, 10 – crown, 11 – root.

Task 2. Tooth (cross section through the root).



Study the diagram, indicate and label the structures: I – pulp (a – core, b – odontoblastic layer, c – blood vessels), II – dentin, III – cementum, IV – periodontal ligament fibers, V – bony alveolus.

Task 3. *Slide № 122* — Enamel organ

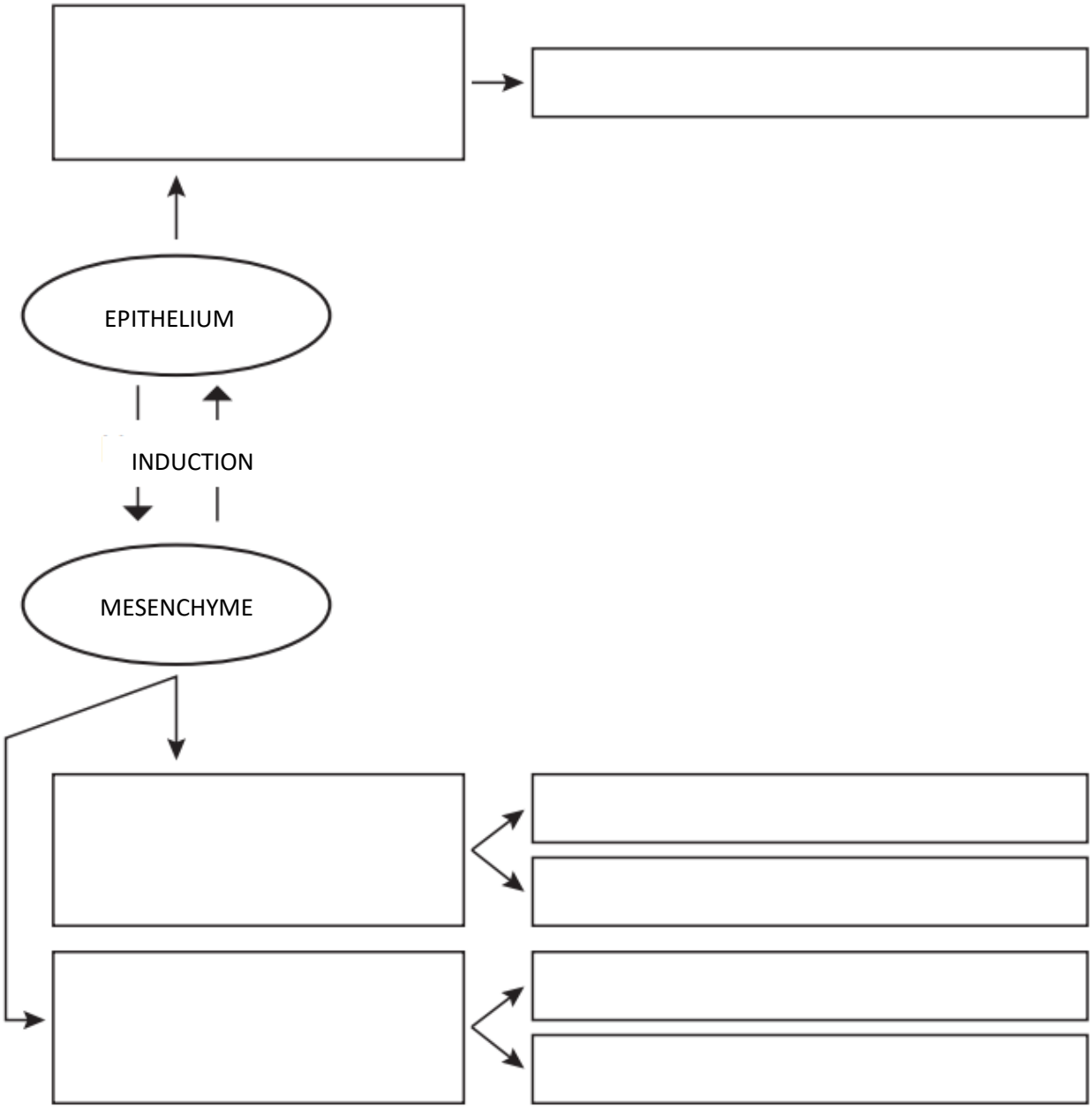
Sketch the microscopic view and label the structures: 1 – oral epithelium, 2 – dental furrow, 3 – dental lamina, 4 – enamel organ, 5 – dental papilla, 6 – dental sac, 7 – bony alveolus.

Task 4. *Slide № 123* — Dental histogenesis (development of the dentin and the enamel).

Sketch the microscopic view and label the structures: 1 – oral epithelium, 2 – bony alveolus, 3 – bone trabecula, 4 – osteocytes, 5 – osteoblasts, 6 – osteoclast, 7 – loose connective tissue, 8 – epithelial cord (remnants of the dental lamina), 9 – outer enamel epithelium, 10 - pulp of the enamel organ, 11 – enameloblasts, 12 – enamel, 13 – odontoblasts, 14 – dentin, 15 – pre-dentin, 16 – dental papilla, 17 – blood vessels.

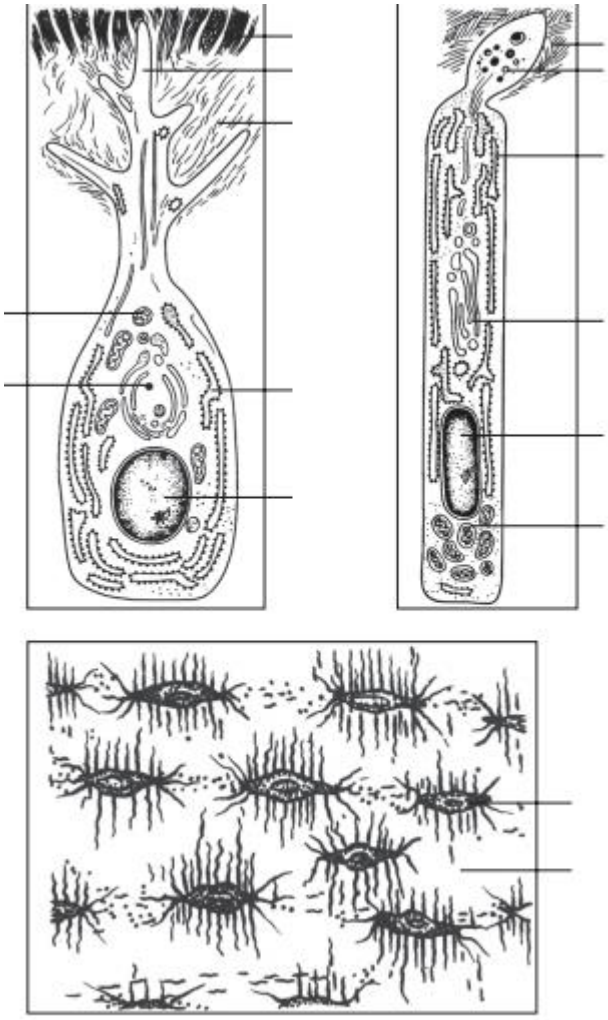
Task 5. Embryonic sources of dental tissues.

Fill in the names of dental tissues with regard to embryonic sources of their development.



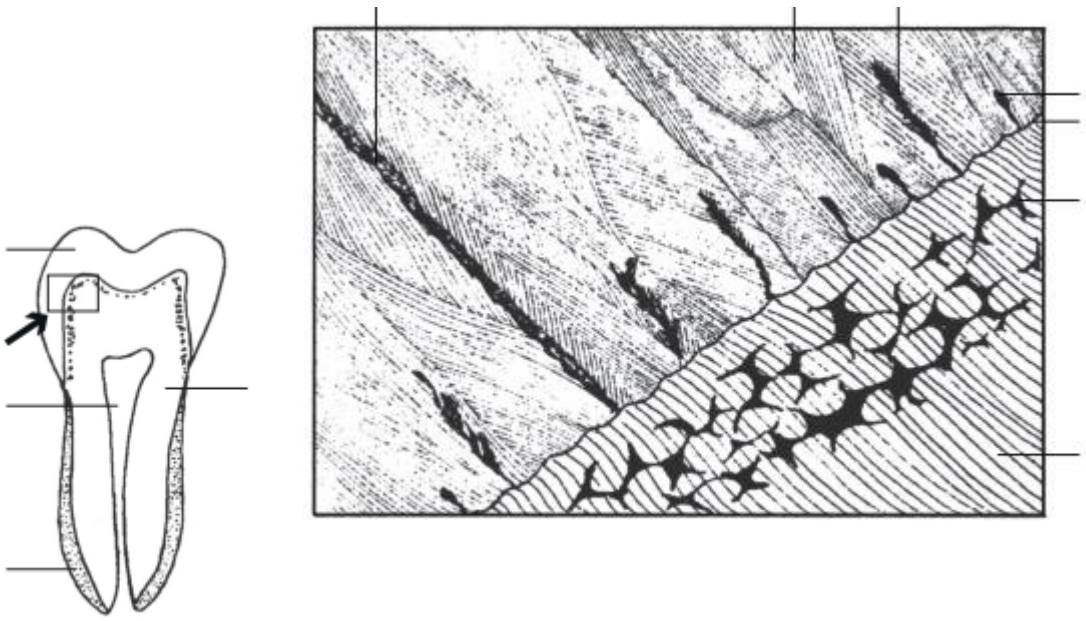
Task 6. Ultramicroscopic structure of odontoblasts, enameloblasts, and cementocytes [4].

Specify cell types (I – odontoblast; II – enameloblast, and III – cementocytes in the cementum) **and label characteristic elements:** 1 – granules in the Tomes processes of enameloblast, 2 – granular endoplasmic reticulum, 3 – Golgi complex, 4 – cell nucleus, 5 – mitochondria; 6 – enamel; 7 – branching Tomes fiber of odontoblast, 9 – pre-dentin, 10 – dentin; 11 – cementocyte, 12 – mineralized ground substance.



Task 7. Dentino-enamel boundary [2].

Zoomed inset showing a histological image of dentino-enamel boundary (marked with arrow in the diagram). Label the major dental structures in the diagram and corresponding histological features in the image: I – the enamel with 1 – enamel lamella, 2 – enamel spindles, 3 – enamel prisms, and 4 – enamel tufts; II – dentino-enamel boundary; III – the dentin with 6 – dentinal tubules and 7 – interglobular dentin; IV – the pulp; V – the cementum.

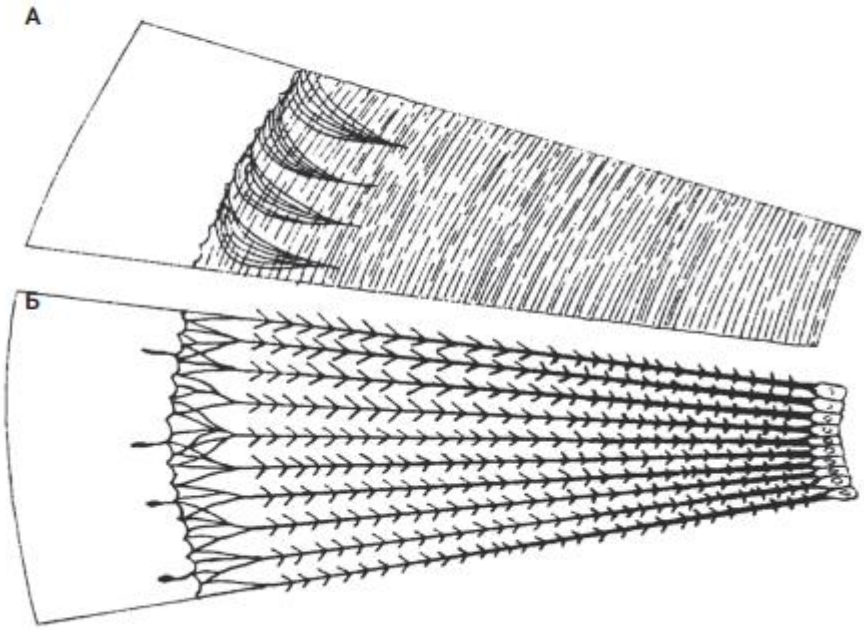


Task 8. Structure of dentin [2].

Name the diagrams:

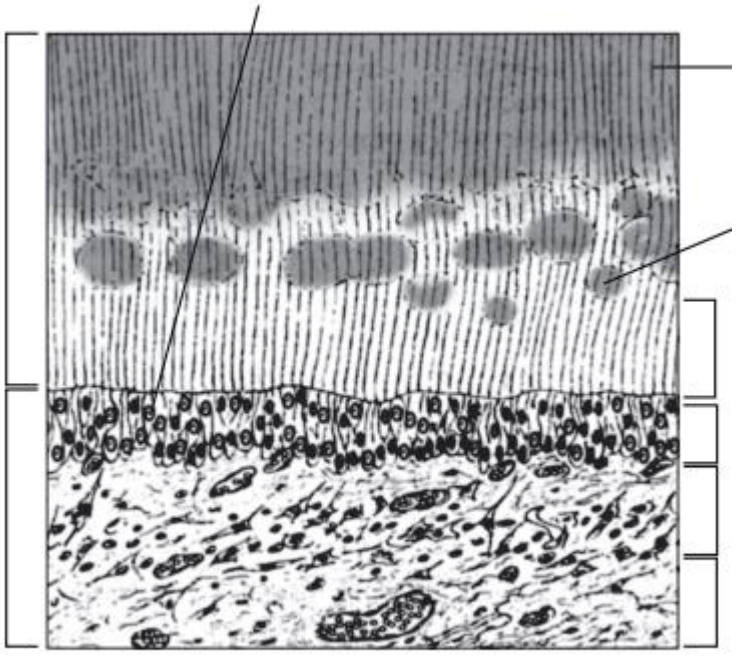
А: _____

Б: _____



After naming the diagrams, **indicate and label the structures**: I – enamel including 1 – enamel spindles; II – dentino-enamel boundary; III – dentin including 2 – peripulpal dentin, 3 – mantle dentin, 4 – pre-dentin, 5 – radial fibers (von Korff's fibers), 6 – tangential fibers (von Ebner's fibers), 7 – dentinal tubules; and IV – dental pulp including 8 – cell bodies of odontoblasts.

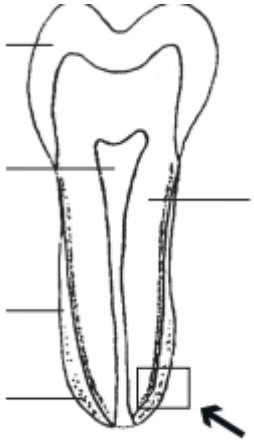
Task 9. Dentino-pulpal boundary [2].

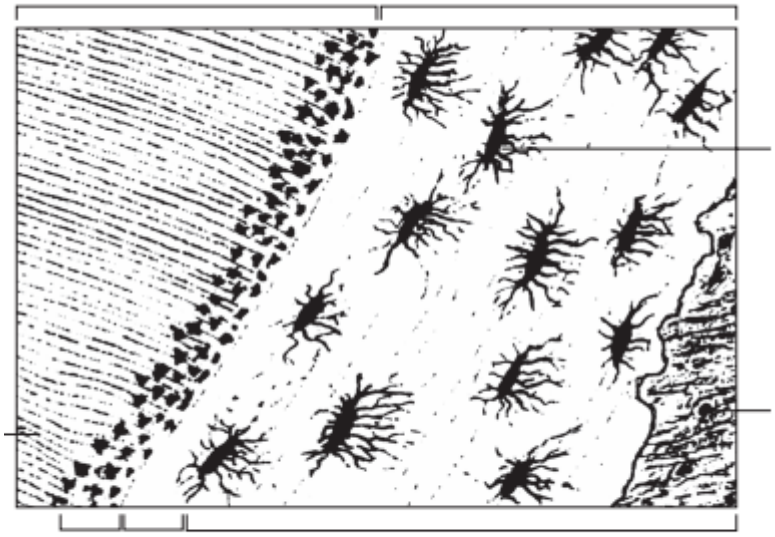


Study the diagram and label the structures: I – dentin including 1 – predentin, 2 – dentinal tubules, 3 – calcospherites; and II – dental pulp including 4 – peripheral layer, 5 – intermediate layer, 6 – core of the pulp, 7 – bodies of odontoblasts.

Task 10. Cementum [2].

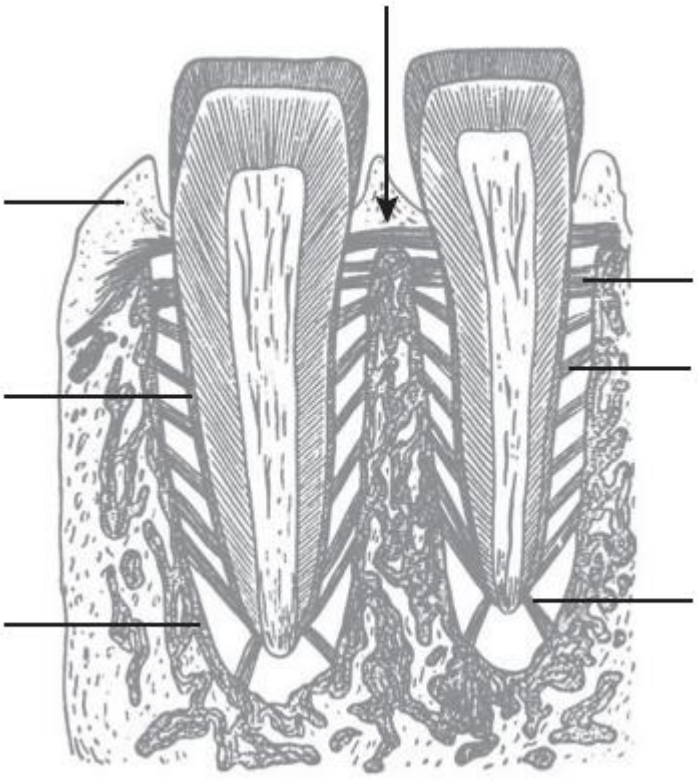
Zoomed inset showing a histological image of dentino-cemental boundary (marked with arrow in the diagram). **Label the structures:** I – enamel; II – dentin, including 1 – dentinal tubules, 2 – Tomes granular layer; and III – cementum, including 3 – cementocytes, 4 – Sharpey's fibers (perforating ends of collagen bundles) of the periodontal ligament, 5 – acellular cementum, 6 – cellular cementum.





Task 11. Periodontal ligament (PDL) [6].

Study the diagram and label the structures: 1 – alveolar bone, 2 – cementum, 3 – gums, 4 – oblique PDL fibers, 5 – apical PDL fibers, 6 – horizontal PDL fibers, 7 – transseptal PDL fibers.



Lesson 1 mastered:

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Theme 2. MICROANATOMY OF ORAL CAVITY AND ITS STRUCTURES. OROFACIAL DEVELOPMENT. BRANCHIAL APPARATUS AND ITS DERIVATIVES

Learning blocks:

• Organs and structures of oral cavity, morphological and functional characterization of different compartments of oral mucosa.

• Comparative microanatomy of lips, cheeks, hard palate, soft palate, and gums.

• Microanatomy of the tongue. Lingual papillae: filiform, foliate, grooved, and fungiform.

• Branchial apparatus and its derivatives.

• Development of facial structures.

• Formation of the palate. Primary and secondary palates.

• Development of the tongue.

• Congenital orofacial defects.

Oral cavity (a.k.a. buccal cavity or mouth) should be considered as the anterior portion of the gastrointestinal tract. The walls of gastrointestinal tract comprise four histologically distinct layers (tunics):

1) tunica mucosa, the innermost layer, consists of three sublayers (laminae):

- epithelium;
- lamina propria mucosae represented by loose fibrous connective tissue with blood and lymph vessels, nerve fibers, and lymphoid aggregations;
- lamina muscularis mucosae (a.k.a. muscularis mucosae) represented by thin layers or bundles of smooth muscle cells;

2) tunica submucosa composed of loose fibrous connective tissue with blood and lymph vessels, nerve plexuses, and lymphoid aggregations;

3) muscular layer (a.k.a. muscularis propria);

4) tunica externa (serosa or adventitia) composed of loose fibrous connective tissue with blood vessels and nerves.

As a true portion of gastrointestinal tract, oral cavity is lined with tunica mucosa supported from beneath by tunica submucosa, though in certain areas of the oral cavity the submucosa is missing.

Apart from the walls of oral cavity, its formation gives rise to multiple accessory structures including lips, cheeks, gums, hard palate, and soft palate with uvula, as well as tongue, tonsils, salivary glands, and teeth.

Oral cavity is subdivided into two anatomical compartments: (1) the vestibule, externally bounded by the cheeks and the lips and internally bounded by alveolar processes of the jaws with teeth; and (2) the oral cavity proper located behind/between the alveolar processes, bounded by the hard and soft palate from

above and by the root of the tongue and the muscular diaphragm of the mouth floor from below.

Oral mucosa

Functions of oral mucosa

1) protective — oral mucosa protects the underlying tissues from mechanical and chemical damage and infectious agents (microorganisms);

2) sensory — oral mucosa harbors a variety of receptors activated by gustatory, thermal, tactile, and noxious stimuli;

3) secretory — oral mucosa contains glandular derivatives responsible for the production of saliva;

4) excretory — oral mucosa participates in the efflux of uric acid and other metabolites, especially in patients with kidney dysfunction;

5) immune — oral mucosa provides a major protective barrier and the site of local immunity reactions implemented by multiple cell types in lymphoid aggregations or dispersed in loose connective tissue and the epithelium (Langerhans cells, macrophages, lymphocytes, and plasma cells);

6) absorptive — the surface is permeable to a number of chemical substances including salts (iodine, potassium, sodium, etc.) and free amino acids.

The ducts of salivary glands open into oral cavity. Saliva performs a number of functions enabled by its unique content of active substances: antibacterial (lysozyme, lactoferrin), regenerative (epithelial growth factors), anti-mechanical damage protection (moisturizing and softening food, and acting as a lubricant: water and mucin). Importantly, the saliva ensures the initial stage of carbohydrate digestion (ensured by the presence of amylase and maltase enzymes).

Structural features of oral mucosa

1) Oral cavity is lined with non-keratinized stratified squamous epithelium, subject to partial keratinization in certain areas with strong exposure to friction and abrasion during masticatory process (gingivae, hard palate, midportion of the cheek, and filiform papillae of the tongue). These specific areas (collectively termed 'masticatory mucosa') may show varying degree of keratinization (keratosis) classified as follows:

- Orthokeratosis — average physiological degree of keratinization of the oral epithelium over the hard palate and gingivae. The epithelium contains up to 20 layers of dead keratinized cells (squamous scales), which ensure its high mechanical strength and resistance to the action of chemicals. The scales have homogeneous filling with poorly distinguishable keratin fibrils (in contrast to epidermal scales). Physiological desquamation of this oral equivalent of stratum corneum occurs through degradation of desmosomes (a.k.a. desmosomal dissociation).
- Hyperkeratosis — excessive keratinization accompanied by formation of thickened, observably whitish areas.
- Parakeratosis — lower-than-average degree of keratinization (considered a normal variation). On the surface of the epithelial layer are flat horny cells containing keratin, remaines of organelles, and pyknosized nuclei. Keratohyalin granules are present in the granular layer, but in a smaller amount.

2) The structure of lamina propria mucosae is similar to skin dermis, comprising the papillary layer of loose fibrous connective tissue and the reticular layer of dense fibrous connective tissue. It contains secretory portions of small salivary glands. In the anterior areas (labial and buccal surfaces, floor of the mouth) these glands have mixed secretory profiles (mucous/serous), small salivary glands next to the vallate papillae of the tongue are predominantly serous. In the posterior areas including the tongue, posterior hard palate and soft palate, and root of the tongue), small mucous glands predominate.

3) The muscular lamina (muscularis mucosae) in the mouth is missing. Sparse bundles of smooth muscle cells are preserved in the vallate papillae of the tongue.

Tunica submucosa effectively separates the mucosa from the underlying muscles thus rendering the mucosa soft and mobile. Oral submucosa consists of loose fibrous connective tissue containing fat tissue lobules and secretory portions of small salivary glands (predominantly mixed mucous/serous). Because of the prominent submucosa the mucosa of the cheeks, lips, ventral surface of the tongue, and soft palate, their surface is flexible and easily folds. The absence of submucosa in gingivae, dorsolateral sides of the tongue, and certain areas of hard palate makes their surface firm and stiff (as the mucosa adheres directly to bones or muscles).

Blood supply and innervation of oral mucosa

Oral mucosa is densely vascularized and getting richer blood supply than the skin. The extensive microcirculatory bed of oral mucosa receives blood from prominent submucosal arteries branching into arterioles and capillaries of the reticular and papillary layers of lamina propria. The capillaries are somatic and in certain areas (e.g. gingivae) also visceral. Arterioles and venules of lamina propria form numerous anastomoses.

Oral mucosa is richly innervated to ensure specific perception of various stimuli and reflexes necessary for chewing, salivation, swallowing, and speech. The innervation comes from trigeminal nerve, with afferent fibers also from facial, glossopharyngeal, and vagus nerves. Sensory nerves reach lamina propria, and many free nerve endings (responding to noxious and thermal stimuli) are found in lamina propria and the epithelium.

Clinical implications

1) The color of oral mucosa reflects:

- Condition of vasculature in lamina propria mucosae, beneath the fairly transparent epithelium, in terms of vascular density and perfusion;

• Erythrocyte counts and hemoglobin content: normal — pink, in anemia — pale pink;

• Inflammatory reactions to pathogens, allergens, etc. manifest as redness and swelling;

• The color also reflects epithelial thickness, transparency, and degree of keratinization: non-keratinized areas are brighter (pinker) than keratinized. With the increasing thickness of keratinized layer of dead cells (scales) the surface looks paler — hence the 'whitish' areas of hyperkeratosis.

• Metal poisonings result in abnormal pigmentation, e.g. chronic lead or bismuth poisoning presents with dark band along gum margins, whereas exposure to amalgam results in grayish-blue patterns on the gums ("amalgam tattoo").

2) Cytological assessment of the oral epithelium differentiation status provides important clinical indicators for a number of disorders including inflammatory, dystrophic, and neoplastic conditions, including those with genetic component.

3) Areas comprising submucosa are preferable sites for injections of local anesthetics and collection of biopsies.

4) Obstruction of excretory ducts in small salivary glands may turn them into mucus-filled cystic lesions (mucocele). Another important clinical concern is the role of salivary glands as primary sites of tumorigenesis.

Oral mucosa has distinct **morphological and functional compartments** including

1) masticatory mucosa (gum, hard palate) — partially keratinized on the exterior, is firmly attached to the underlying bone, shows high mechanical strength and low permeability, and participates in the mechanical processing of food;

2) lining mucosa (cheek, lip, bottom of the oral cavity, alveolar processes, soft palate, and ventral surface of the tongue) — non-keratinized, mobile, elastic and flexible, attached to submucosa with underlying muscles (mostly) or bone, and more permeable to water and chemicals than other mucosal areas;
3) specialized mucosa (dorsolateral surface of the tongue with papillae).

Oral structures

Gums

Gums (*Lat.* gingivae) are masses of oral mucosa around the teeth. Each gum consists of three portions: free gingiva, attached gingiva, and the gingival interdental papilla (Figure 15).

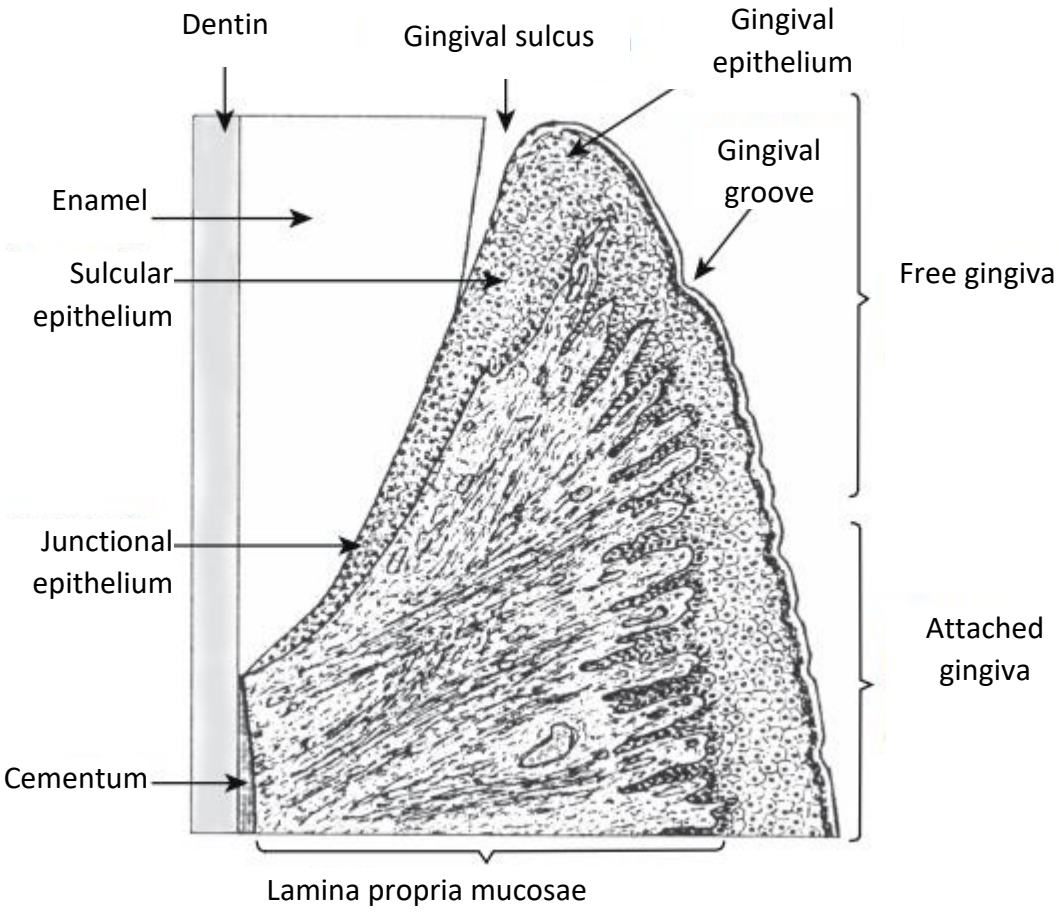


Figure 15. Gum microanatomy (adapted from [2]).

The attached portions firmly adhere to the periosteum of alveolar processes of jaws, which makes the gums endurant to the mechanical stress of mastication. The unattached margin of the gum is separated from the tooth by narrow gingival sulcus. The gingival interdental papillae are triangular areas of mucosa covering the fissures between adjacent teeth.

Gingival mucosa has partially keratinized stratified squamous epithelium. In the papillary layer of lamina propria, loose fibrous connective tissue forms deep papillae rich in blood vessels and nerve fibers with numerous nerve endings. In the reticular layer of lamina propria, thick vertical bundles of collagen fibers firmly attach the gum to periosteum.

The gums have no glands, nor submucosa.

Hard palate

Hard palate is covered with mucosa, supported by submucosa except in certain areas, firmly attached to the periosteum of palatine bones. The mucosa is very thin in the suture area and thicker in other portions. The stratified squamous epithelium is partially keratinized. The lamina propria consists of connective tissue with high content of collagen fibers, sometimes referred to as dense fibrous connective tissue.

Mucosa of the hard palate is divided into four zones: *fatty*, *glandular*, *palatine raphe* (median) and *marginal* (lateral) (Figure 16).

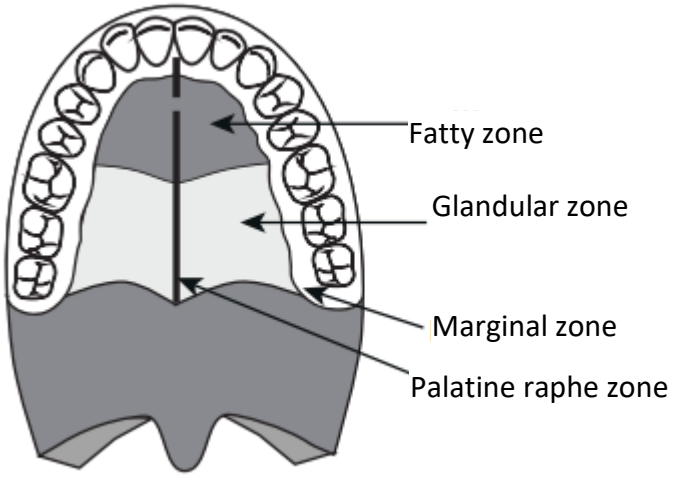


Figure 16. Zones of the hard palate.

Fatty zone (named so because of the presence of adipose tissue in the submucosa) corresponds to anterior 1/3 of the hard palate with characteristic ridges — palatal rugae. *Glandular zone* corresponds to posterior 2/3 of the hard palate and its submucosa contains secretory portions of palatine mucous salivary glands. The *palatine raphe zone* is a narrow, slightly elevated strip along the midline of the hard palate, behind the incisive papilla and backwards. Palatine raphe has no submucosa. *Marginal zone* represents a transition between the hard palate and the gums. Similarly with the palatine raphe zone, it has no submucosa.

Lips

Lips (*Lat.* labia) represent a boundary between external surface of the body and the inside of digestive tract, with the corresponding gradual transition of the skin into mucous membrane. Structural basis of the lips is formed by skeletal muscle tissue (orbicularis oris muscle). The lips consist of three microanatomical parts (zones): *cutaneous*, *intermediate*, and *mucous* (Figure 17).

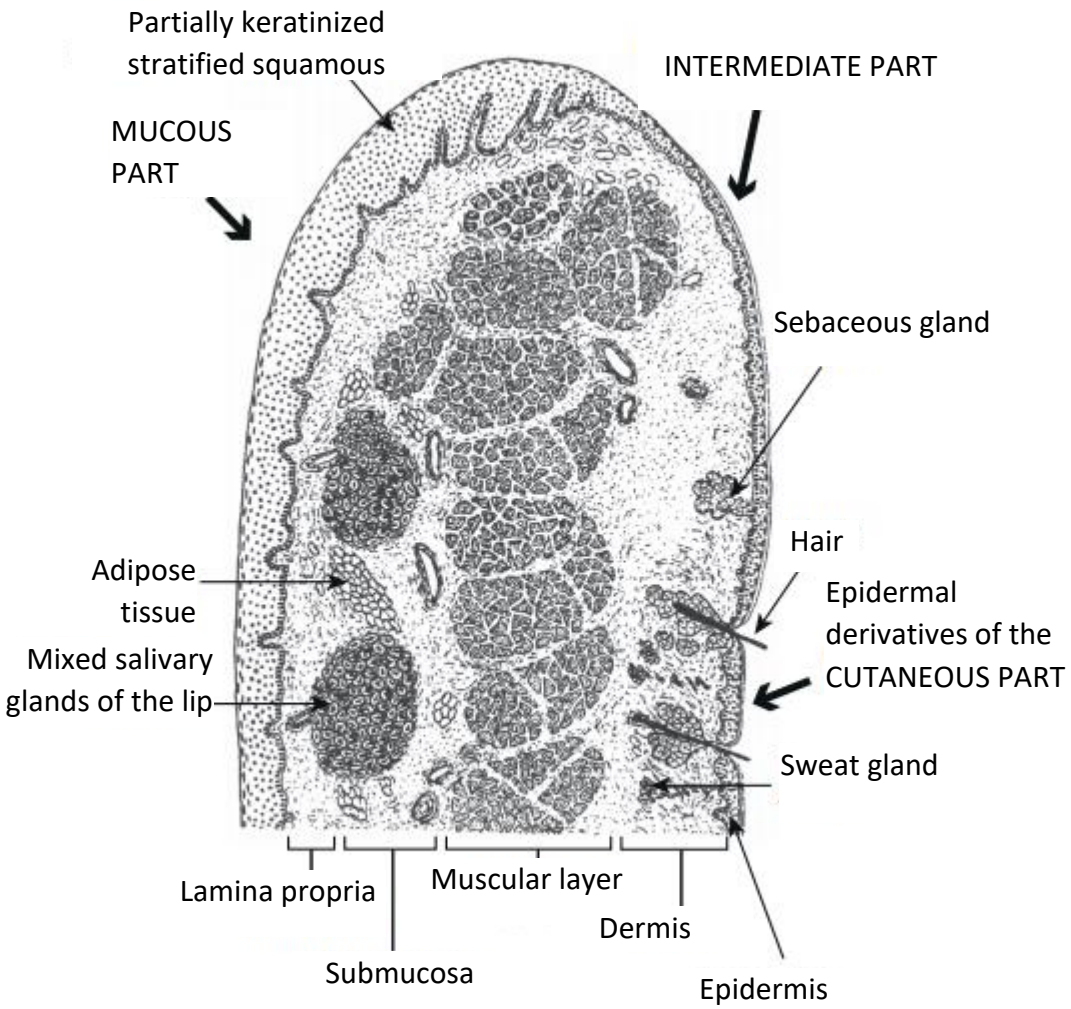


Figure 17. Lip microanatomy (adapted from [2]).

Cutaneous part shows the typical thin skin microanatomy with properly keratinized stratified squamous epithelium (epidermis) and its derivatives (hairs, sweat and sebaceous glands). Lip movements are ensured by skeletal muscle fibers anchored in the dermis.

Transition to the *intermediate part* ('vermilion border') is marked with sharp thickening of the epithelium, whereas its stratum corneum becomes thin and transparent. The vermilion red appearance is a combined effect of rich vascularization and epithelial transparency. Hairs and sweat glands disappear, sebaceous glands remain (especially in the corners of the mouth and on the upper lip). Lamina propria forms prominent papillae with abundant capillary loops approaching the basement of the epithelium. High density of nerve endings in the papillae ensures high sensitivity of this area.

Mucous part of the lip has typical oral mucosa lined with non-keratinized (partially keratinized) stratified squamous epithelium. Its cells are polygonal and large (except the top squamous layers). Lamina propria mucosae makes a gradual transition into submucosa, adjacent to the muscles and rich in blood vessels, fat lobules, and secretory portions of the mixed labial salivary glands —complex branched alveolar-tubular glands, predominantly mucous, decreasing in density in lateral directions, with excretory ducts opening into the vestibule of the oral cavity.

Cheeks

The cheeks (*Lat.* buccae) constitute lateral walls of the mouth, supported mechanically and locomotively by skeletal muscle tissue of the buccinator muscles. The cheeks have two surfaces — *skin* and *mucous*.

At the skin surface, the cheeks have the typical thin skin microanatomy with the epidermis, epidermal derivatives, and the dermis rich in adipose tissue and elastic fibers (Figure 18).

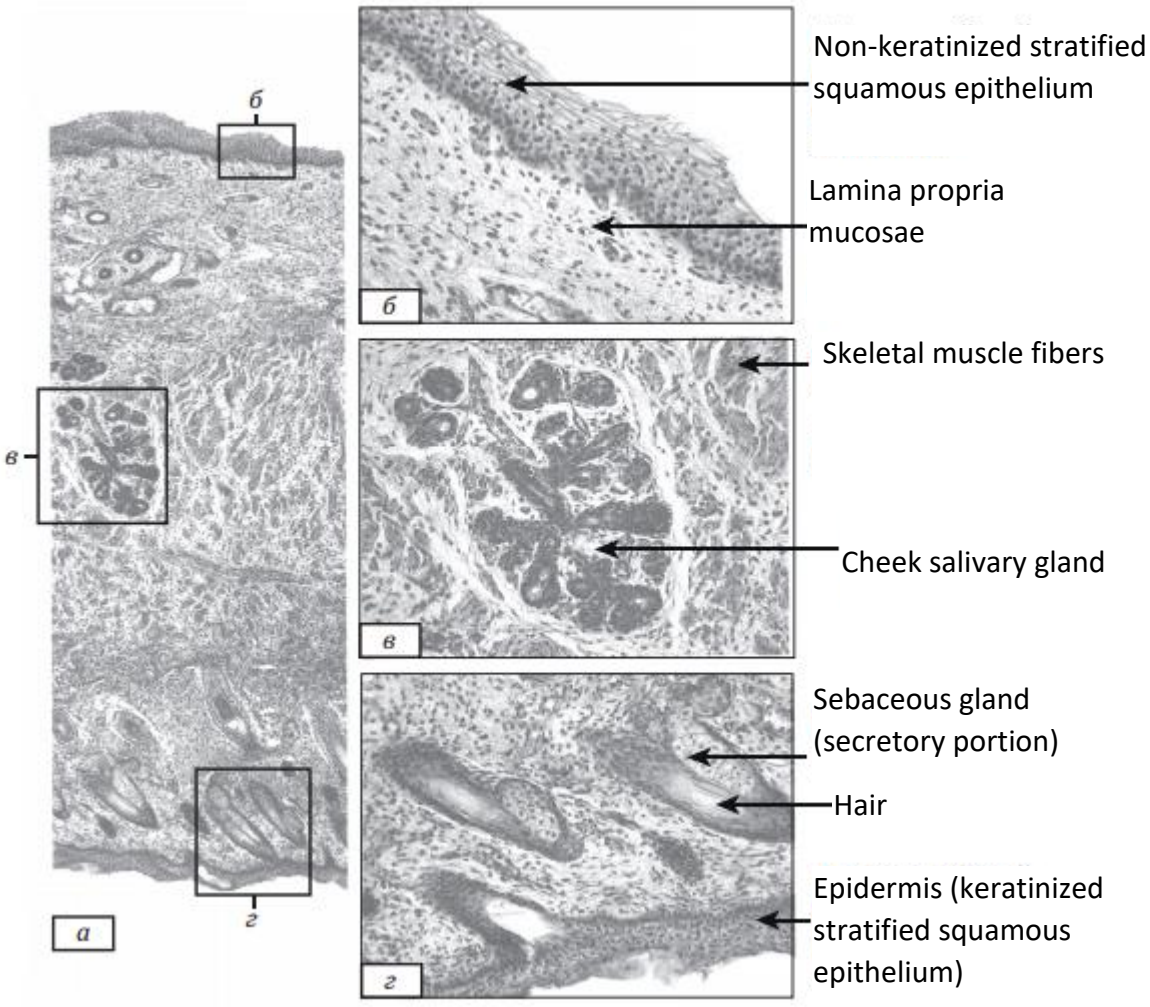


Figure 18. Histological image of human fetal cheek (a), with the magnified insets representing mucous portion (b), maxillary portion (c) and skin portion (d) (adapted from [1]).

At the mucous surface, cheek microanatomy is location-dependent. Three zones of the buccal mucosa are distinguished: the *upper (maxillary) zone, the lower (mandibular) zone, and the intermediate* zone running parallel to the dental occlusion area. The mucous surface is covered with prominent non-keratinized stratified squamous epithelium with the signs of partial keratinization in the intermediate zone, which occasionally presents with characteristic pale horizontal streak termed linea alba (Latin for 'white line'). In the intermediate zone, salivary glands are often missing, however, small superficially located sebaceous glands can be observed.

In the buccal area, lamina propria mucosa contains thick bundles of collagen fibers, more characteristic of dense than loose fibrous connective tissue, running deeper into submucosa and anchoring in the underlying skeletal muscles.

The prominent submucosa of the buccal area contains abundant secretory portions of buccal salivary glands (mixed, chiefly mucous) and the buccal fat pad of adipose tissue. The glands are embedded in cheek muscles, with the density and volume of secretory portions increasing towards the posterior.

Soft palate

Soft palate is the anatomical continuation of the hard palate, a fold of oral mucosa separating the mouth from the pharynx. It has flexible muscular-fibrous consistency and brighter pink-reddish coloration (compared with the hard palate) due to its higher vascular density. Soft palate has two surfaces —anterior (oral a.k.a. oropharyngeal) and posterior (nasal a.k.a. nasopharyngeal) (Figure 19).

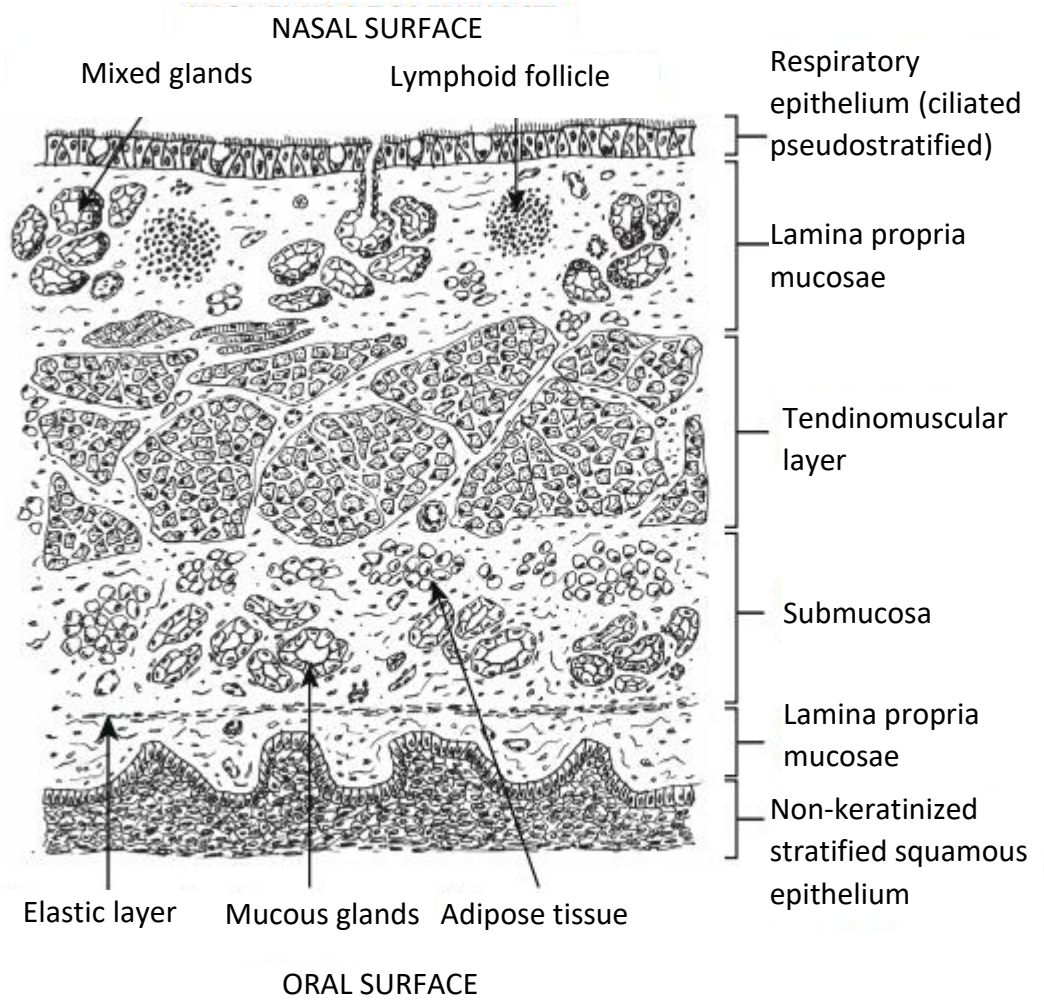


Figure 19. Soft palate microanatomy (adapted from [2]).

Oropharyngeal surface of the soft palate and uvula are lined with non-keratinized stratified squamous epithelium comprising occasional taste buds. Lamina propria contains interwoven elastic fibers. Submucosa of the anterior soft palate contains secretory portions of mucous salivary glands and lobules of adipose tissue.

Nasopharyngeal surface of the soft palate is lined with pseudostratified simple columnar ciliated epithelium. Lamina propria contains secretory portions of mixed or mucous glands and occasional lymphoid follicles. Nasopharyngeal surface of the soft palate has no submucosa.

Bundles of skeletal muscle fibers and their tendinous fascia (tendinomuscular layer) provide structural and locomotive support for the soft palate. Secretory portions of salivary glands can be observed in this layer (deeper than submucosa) submerged between the muscle bundles.

Tongue

The tongue (*Lat.* lingua) is a muscular organ within the oral cavity. The tongue participates in mechanical processing of food, swallowing, taste perception, and speech production. The tongue is coated in mucosa and has structural core of skeletal muscles (muscular body) comprising layers of skeletal muscle fibers arranged in bundles with specific orientation to provide complex multidirectional motions. The bundles of skeletal muscle fibers are interspersed with layers of loose fibrous connective tissue containing blood vessels, nerves, and fat lobules (Figure 20). The tongue is divided into two symmetrical halves by longitudinal septum of dense connective tissue. It also has two anatomically distinct portions termed the body and the root (a.k.a. the body and the base) of the tongue (Figure 21). Topography and structure of the lingual mucosa on the dorsal, ventral and lateral sides differ.

The upper (dorsal) and the lateral surfaces of the tongue bear characteristic lingual papillae, with a core of loose fibrous connective tissue (primary and secondary connective tissue papillae) coated in epithelium. These areas

have no submucosa, with lamina propria mucosae firmly attached to the underlying muscle tissue (Figure 20).

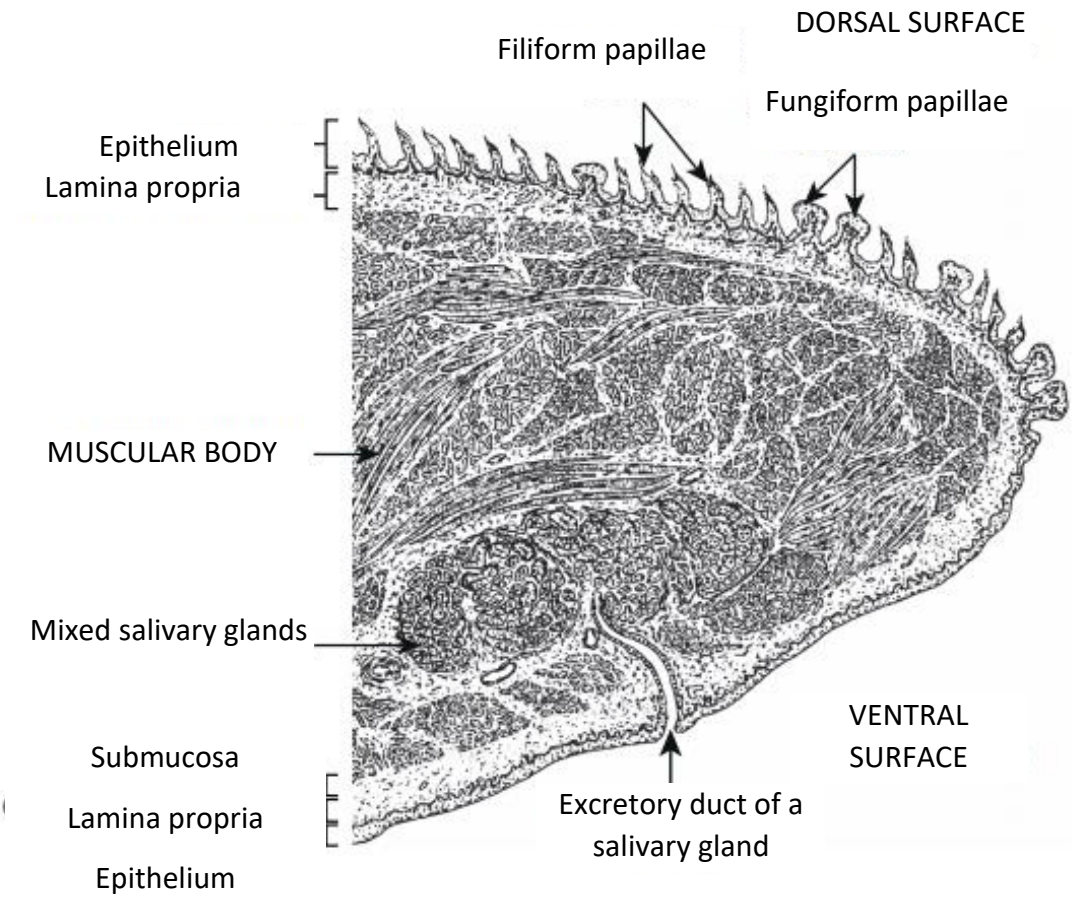


Figure 20. Tip of the tongue (adapted from [2]).

Lingual papillae are morphologically subdivided into filiform, foliate, fungiform, and vallate (a.k.a. circumvallate, 'surrounded by a valley'). The tongue has non-keratinized stratified squamous epithelium all over its surface, with the exception of filiform papillae covered with keratinized stratified squamous epithelium. Of note, the cone-shaped filiform papillae harbor no taste buds thus playing no role in gustatory sensation; instead, they provide the rough texture of the tongue that facilitates sensation of touch, food processing, and speaking. Other types of papillae (foliate, fungiform, and vallate) have taste buds (a.k.a. taste bulbs) in the epithelium. In vallate papillae, the connective tissue core contains bundles of smooth muscle cells. Lingual papillae occupy specific domains of the tongue surface (Figure 21).

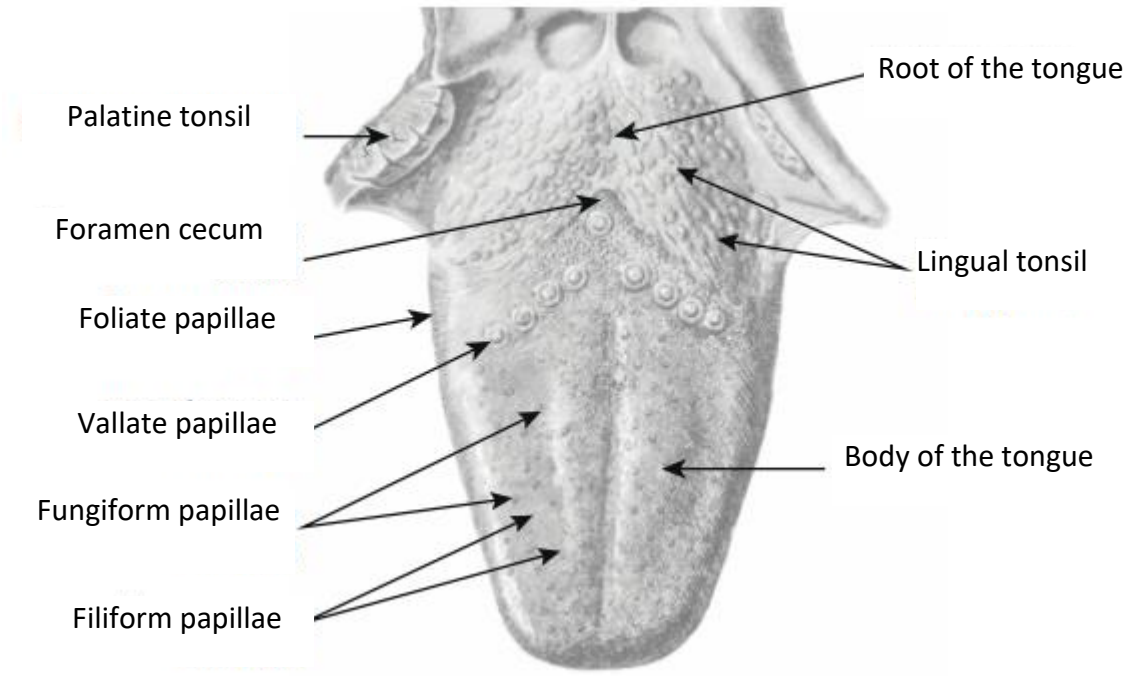


Figure 21. Topography of the dorsolateral surface of the tongue (adapted from [1]).

Filiform papillae are the smallest and the most numerous. The cone-shaped filiform papillae are ubiquitously distributed across the dorsal surface of the tongue. The epithelium over them forms pointed, heavily keratinized projections. The connective tissue core of filiform papillae is rich in collagen, blood vessels, and nerve fibers. Their function is mechanical and consists in pressing the pieces of masticated food to the hard palate and directing the bolus towards the esophagus.

Fungiform papillae are found in the anterior portion of the tongue, placed solitary or in small groups among the numerous petite filiform papillae. The taste buds are found in apical portions of the papillae.

Foliate papillae are present in young children and absent in adults. These papillae can be described as mucosal folds, running in parallel, with excretory ducts of small serous salivary glands opening in between. Foliate papillae are located bilaterally, 3–8 papillae on each side, close to the V-shaped boundary between the body and the base of the tongue (terminal sulcus). Taste buds occupy lateral surfaces of the papillae.

Vallate (or circumvallate) *papillae* are the largest and the fewest of the lingual papillae. The total of 6-15 vallate papillae are distributed over the terminal sulcus area. By contrast with other types of lingual papillae, they are level with the lingual surface (not elevated). Each vallate papilla is located within a circular crater in the lingual surface, separated by deep valley running along its circumference, containing excretory openings of the associated serous salivary glands (von Ebner's glands). Secretions of von Ebner's glands rinse and cleanse the valleys (incidentally, these secretions contain enzyme lipase which destroys lipids). Secretory portions of von Ebner's glands are located deep beneath the lingual surface, among bundles of skeletal muscle fibers.

Taste buds (or taste bulbs) are ellipsoidal chemoreceptors located in the epithelium of the foliate, fungiform, and vallate papillae. Each bud connects with oral cavity via small opening in the epithelium (taste pore) (Figure 22). Taste buds can be described as 40–60 cell clusters representing three cell types: sensory epithelial cells (taste receptor cells), supporting cells, and basal cells.

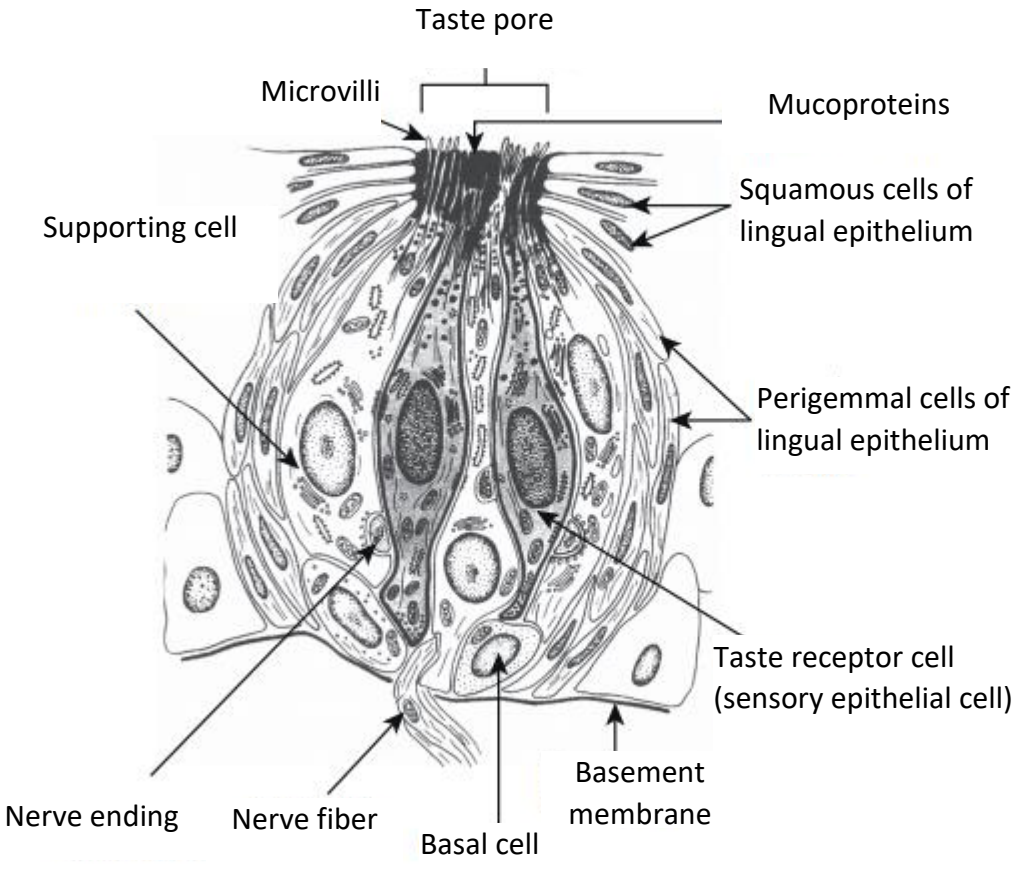


Figure 22. Taste bud structure (adapted from [1]).

Ventral surface of the tongue is soft and flexible (tangentially movable) due to the presence of submucosa, which separates the mucosa from the muscular body of the tongue. Anterior portion of the ventral lingual surface contains small mixed salivary glands, secretory portions of which are found in lamina propria mucosae, submucosa, or deeper in the connective tissue layers interspersing the bundles of skeletal muscle tissue.

Development of the oral cavity and orofacial structures

Embryonic sources of the oral cavity and orofacial structures

The formation of cephalocaudal and lateral folds and the sagittal flexion of the embryo result in the gut closure. The primitive gut is derived from endoderm and wrapped in splanchnopleure (a.k.a. visceral layer of the lateral plate mesoderm). The cranial (anterior) portion of the gut endoderm contains the prechordal plate area. Invaginations of the ectoderm at the poles of the embryo form mouth and anal bays which come into contact with the corresponding blind termini of the gut. The mouth bay (a.k.a. primitive oral pit, or stomodeum) remains separated from the endodermal oropharyngeal intestine by a septum — oral membrane.

On week 4 of embryogenesis the oral membrane is dismissed, as well as its anal counterpart — cloacal membrane, which opens the gut termini. The ectoderm of the stomodeum subsequently gives rise to the oral and lingual epithelia, tooth enamel, and parenchyma of salivary glands and their ducts. The (voluntary) skeletal muscles of the craniofacial region, as well as skin dermis and connective tissue of the dorsal (occipital) region of the head develop from anterior somites and lateral plate mesoderm (mesenchyme of mesodermal origin), whereas mesodermal structures of the oral cavity, face, and pharynx (orofacial and oropharyngeal mesodermal derivatives, including bones, cartilage, tendons, muscles, and dermis), develop from neuromesenchyme (a.k.a. 'ectodermal mesenchyme', ectomesenchyme), which is a neural crest derivative. Neuromesenchyme is also the developmental source of dentin, dental pulp, and cementum.

Branchial apparatus and its derivatives

Branchial apparatus (a.k.a. pharyngeal apparatus) is a transient anatomical module that makes a major contribution to the orofacial development (Figure 23). It includes five pairs of branchial pouches, five pairs of branchial arches and five pairs of branchial clefts, designated I–V, although the fifth pair is rudimentary.

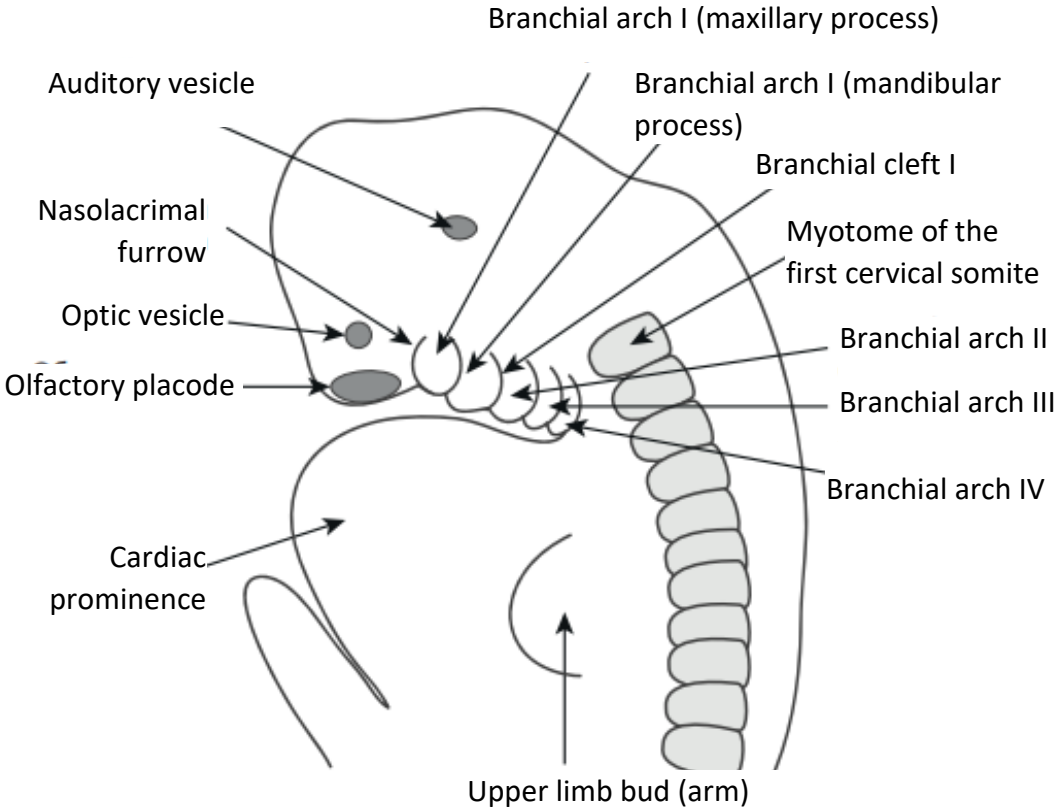


Figure 23. Branchial apparatus of a 5–6 week embryo.

Branchial pouches (a.k.a. pharyngeal pouches) are bilateral pits in the pharyngeal portion of the gut, whereas branchial clefts are ectodermal invaginations that oppose the branchial pouches from the outside. Branchial arches are thick ridges of mesenchyme separating the pouch/cleft regions (Figures 23 and 24). Every branchial arch has a core of mesodermal mesenchyme, surrounded by neuromesenchyme at the periphery. Formation of the branchial apparatus starts on week 4 of embryogenesis; its multiple derivatives are listed in Table 3.

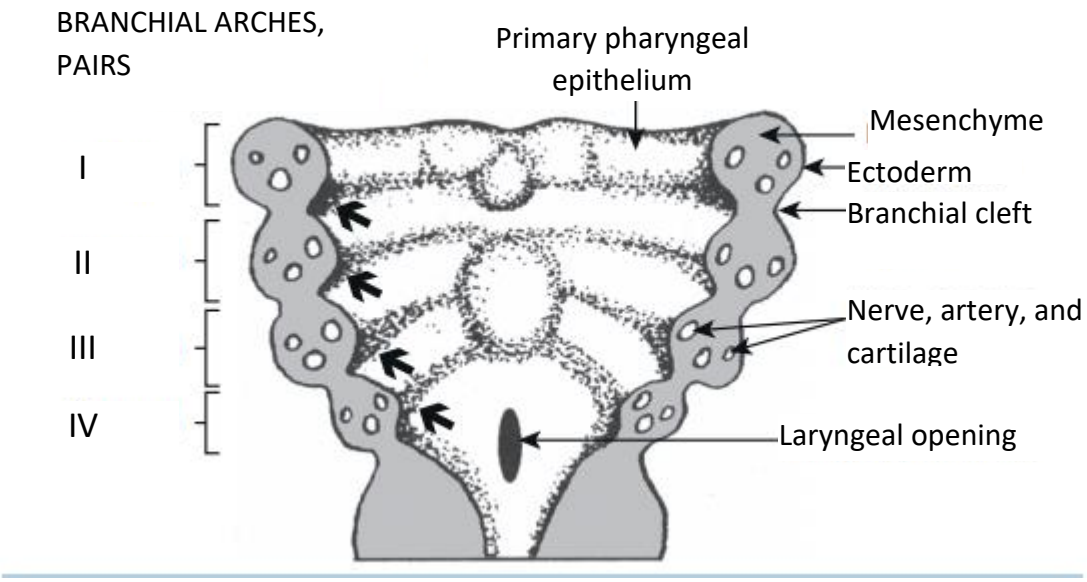


Figure 24. Pharynx of a 5 week human embryo, shown in longitudinal section with bold arrows indicating branchial pouches (adapted from [2]).

Table 3. Derivatives of the branchial apparatus.

Branchial pouches	
I	middle ear cavity and Eustachian tube
II	palatine tonsils
III–IV	parathyroid glands and the thymus
V	rudimentary
Branchial arches	
I (mandibular)	maxilla and mandible, malleus and incus, chewing muscles, body of the tongue
II (hyoid)	hyoid bone, stapes, facial muscles
III	thyroid cartilage, root of the tongue
IV	epiglottis
V	rudimentary
Branchial clefts	
I	external auditory canal; the auricle develops from the fold surrounding the external auditory orifice
II–V	rudimentary

Development of anterior portion of the oral cavity and the nasal cavity is primed by the primitive oral pit (stomodeum). On week 4, the primary oral fossa is a cleft formed by five protrusions: the unpaired frontal process and the paired upper jaw (maxillary) and lower jaw (mandibular) processes. By embryonic week 10, the early stage of orofacial development is complete (Figure 25).

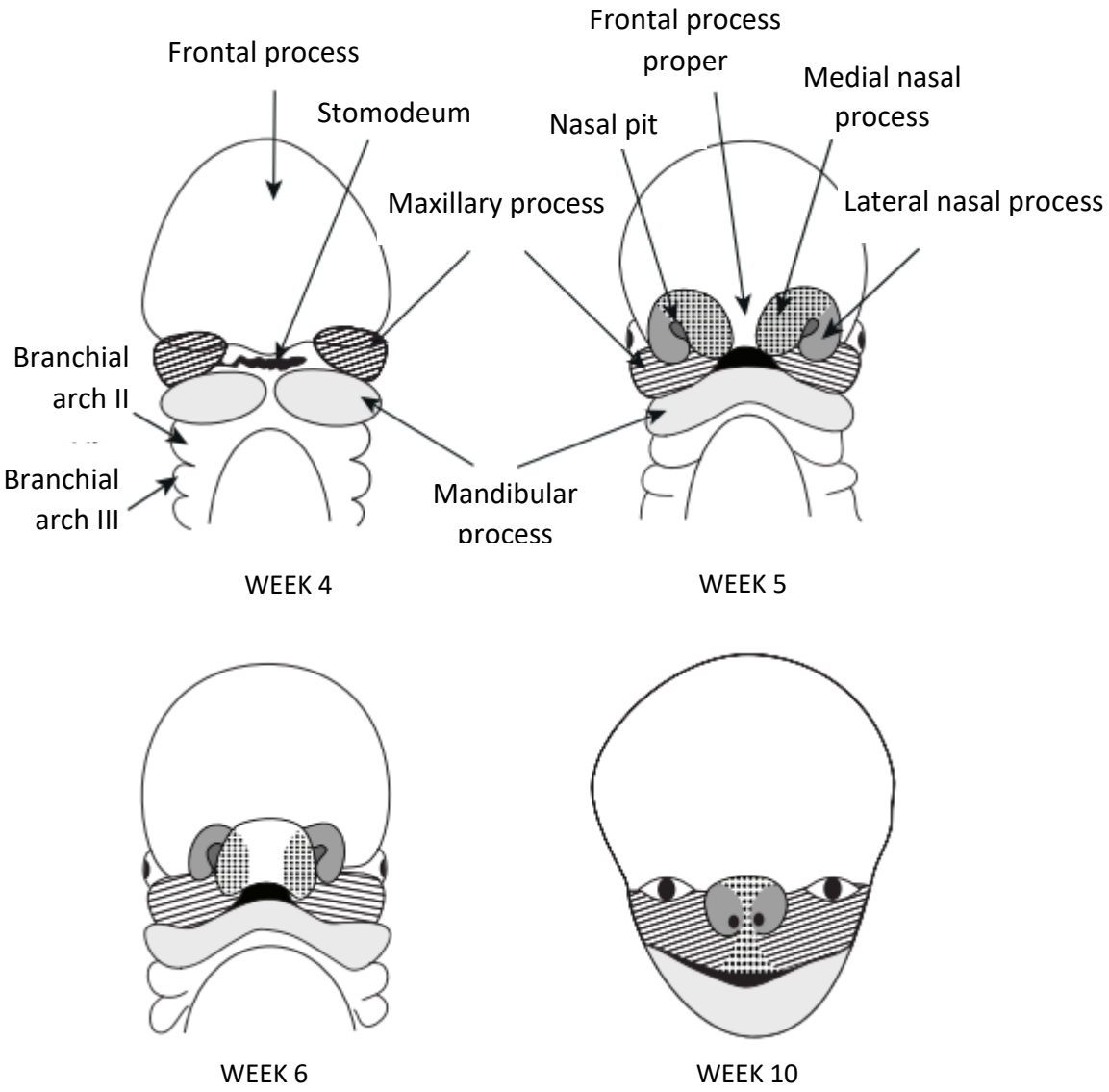


Figure 25. Development of orofacial structures in human embryo, weeks 4–10.

The maxillary and mandibular processes are derivatives of the first pair of branchial arches. On embryonic week 5, the frontal process is subdivided into:

1) frontal process proper ('itself');

2) paired medial nasal processes;

3) paired lateral nasal processes.

The medial and lateral nasal processes are separated by paired ectodermal invaginations — olfactory pits, which will give rise to the paired nasal passages and choanas.

Development of the lower jaw and lower lip occurs on embryonic week 5 by fusion of the two mandibular processes.

Bone tissue of the lower jaw forms by direct (intramembranous) osteogenesis near the paired bows of hyaline cartilage (Meckel's cartilage) formed from mesenchyme of the mandibular processes.

The upper jaw and upper lip are formed on week 5.5–6 by fusions of the maxillary processes and the medial nasal processes (Figure 25), giving rise to midportions of the upper jaw and the upper lip, as well as the primary palate — a horseshoe area that separates the nasal passages from the oral cavity.

Another pair of ectodermal protrusions is formed between the maxillary processes and the lateral nasal processes. These protrusions, directed towards developing eyes, give rise to the lacrimal sac with the nasolacrimal canal connecting the medial angle of the eye and the nasal cavity.

The medial and lateral nasal processes are also involved in formation of the nose. The fusion of the medial nasal processes produce the nasal dorsum, and the wings of the nose develop from the lateral nasal processes (Figure 25).

Development of the palate begins on embryonic week 6–7. The growing palatine (or palatal) processes of the maxilla divide the primary oral cavity into the definitive oral and nasal cavities. Initially, the palatine processes are positioned at the sides of the tongue, the same level with the floor of the mouth (Figure 26). By the end of embryonic month 2, the rapid growth of the lower jaw pulls down the floor of the mouth with the tongue, as the volume of the primary oral cavity increases. The palatine processes stay at the same level, eventually fuse with each other along the middle line (the palatine raphe) thus forming the secondary palate (Figure 26). Frontal ends of the palatine processes fuse with the

primary palate. The primary palate and anterior portion of the secondary palate eventually harbor ossification foci and develop into the hard palate. Posterior portion of the secondary palate develops into the soft palate and uvula.

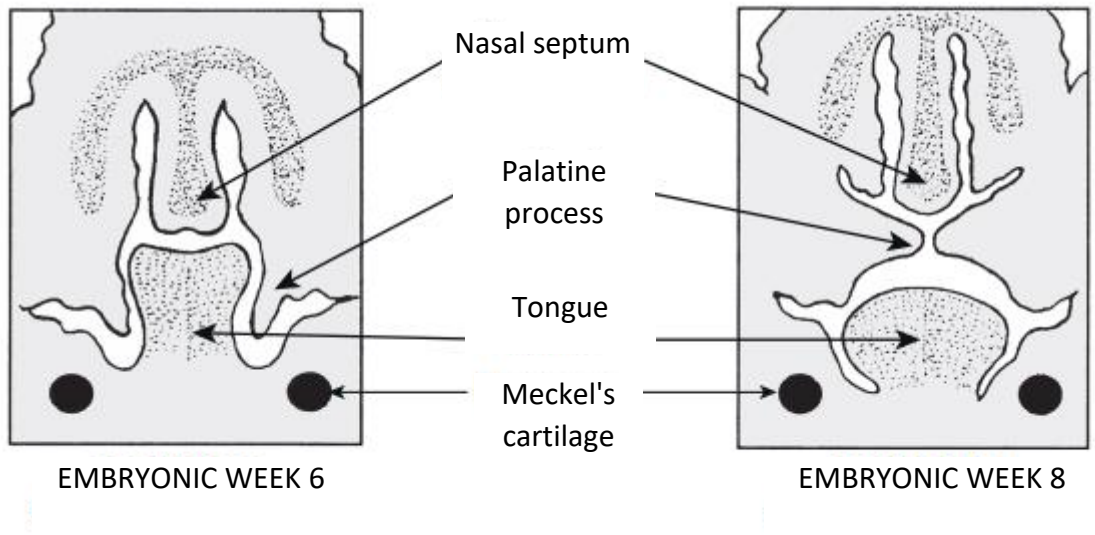


Figure 26. Development of the palate and separation of the oral cavity from the nasal cavity (adapted from [2]).

Development of the tongue starts on embryonic week 4. Its embryonic sources include:

1) *tuberculum impar* (a.k.a. median lingual tubercle, an unpaired medial swelling of the branchial arches I);

2) two lateral lingual tubercles from the same pair of branchial arches;

3) *copula linguae* (a.k.a. hypobranchial eminence) — median swelling of branchial arches III;

4) myotomes of the occipital somites.

On week 4, the unpaired tuberculum impar appears at the floor of the primary oral cavity, corresponding to the I–II branchial arches boundary. The adjacent foramen cecum gives rise to a small area of the lingual dorsum, corresponding to the remnants of thyroid diverticulum (Figure 27).

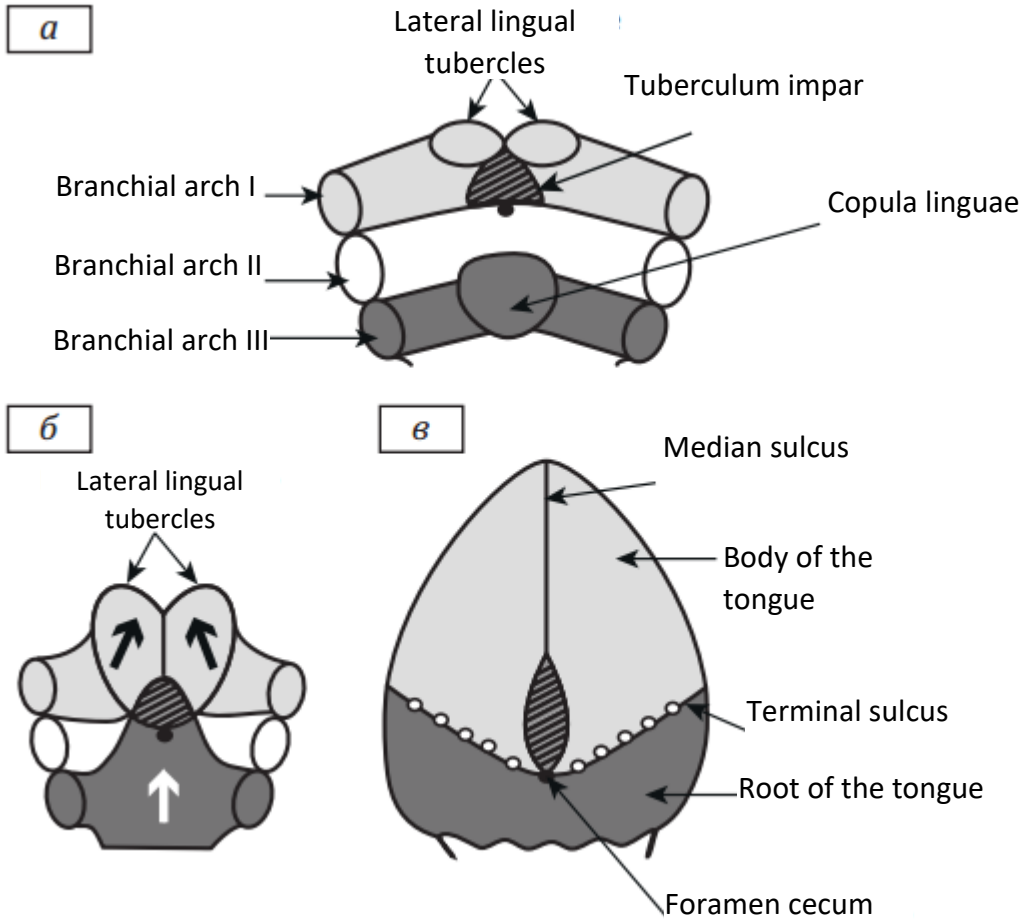


Figure 28. Development of the tongue: embryonic week 4 (a), embryonic week 5 (b), final stage (c).

Anterior to the unpaired median lingual tubercle, two paired thickenings are formed - the lateral lingual tubercles. When they merge, most of the body of the tongue is formed. The root of the tongue arises from a copula, located between the ventral ends of the II and III branchial arches. The muscles of the tongue develop from the myotomes of the occipital somites.

The lingual tonsil is formed at the 9th week of embryogenesis by the migration of lymphocytes into the mucosa of the root of the developing tongue.

Congenital orofacial malformations

• Cleft palate results from failed fusion of the palatine processes, leading to an opening in the roof of the mouth.

• Cleft lip results from failed fusion of the medial nasal and maxillary processes. The variants include median cleft, which results from failed fusion of the medial nasal processes, and bilateral clefts, which result from failed fusions between the medial nasal and maxillary processes.

• Oblique facial cleft results from failed fusion between the maxillary and lateral nasal processes.

• Transverse facial cleft (macrostomia) results from failed fusion of the maxillary and mandibular processes in their lateral areas, leading to formation of unusually large mouth gap.

• Microstomia results from excessive fusion of the lateral zones of the maxillary and mandibular processes.

• Ankyloglossia is caused by shortened frenulum of the tongue, tethering the tongue to the mouth floor with the resulting decrease in its mobility.

• Split tip of the tongue (fissured tongue) results from incomplete fusion of the lateral lingual tubercles.

• Accessory tongue is an extremely rare malformation resulting from abnormal morphogenesis of the unpaired lingual tubercle.

• Macroglossia refers to excessively large tongue due to increased amount (overgrowth) of muscle tissue.

• Microglossia refers to small, underdeveloped tongue.

The causes of congenital orofacial malformations include:

- genetic mutations;
- infectious diseases;
- toxic exposure (teratogens, irradiation);
- nutritional and hormonal imbalances.

The critical period of harmful exposure falls on 4–8 weeks of embryonic development. The congenital orofacial malformations lead to breathing disorders and malnutrition in infants and require surgical treatment.

Learning tasks and tests

Answer the questions:

1. Name microanatomical parts (zones) of the lip and specify their structural features.

2. Provide comparative histological characterization of the soft palate and the hard palate (specify distinctive features).

3. Specify the morphological and functional distinctions for maxillary, mandibular, and intermediate zones of the cheek.

4. What zones can be identified in the hard palate? Describe their histophysiology.

5. What are characteristic features of gum microanatomy?

6. Specify the morphological features distinguishing the gums from other elements of the oral cavity.

7. What are lingual papillae? Provide their classification.

8. Define taste buds. Specify the localization of taste buds in different types of lingual papillae.

9. Describe the branchial apparatus and name its derivatives.

10. Describe the formation of facial structures in embryo.

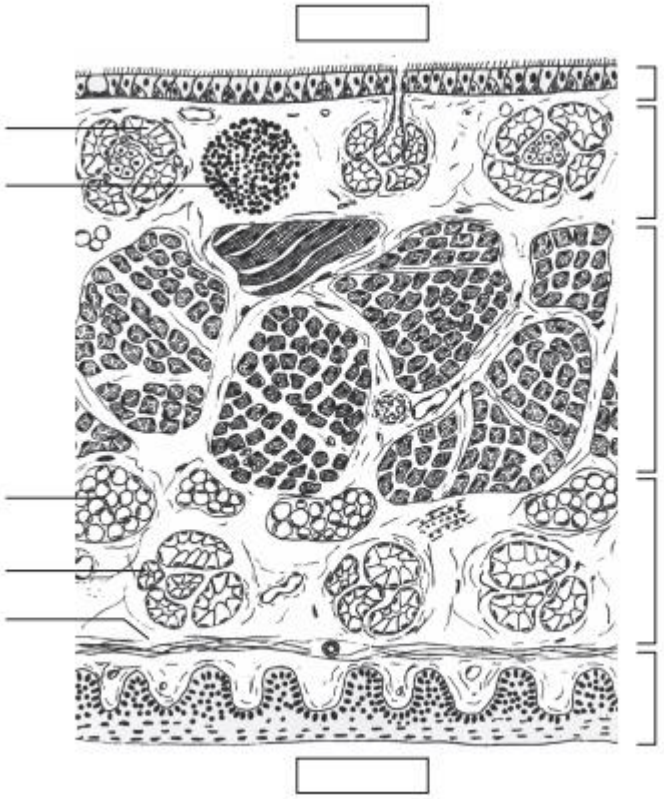
11. Describe formation of the palate in embryo.

12. Specify the embryonic sources of tongue development.

13. Provide examples of congenital malformations of the maxillofacial area.

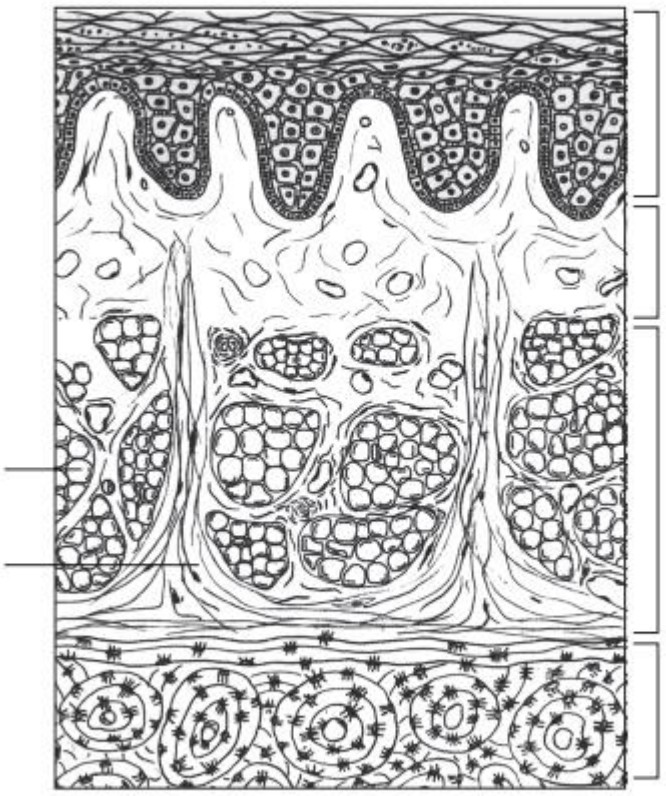
Task 1. Microanatomy of the soft palate [1].

Study the scheme and label structural elements of the soft palate: box I, nasopharyngeal (posterior) surface; box II, oropharyngeal (anterior) surface. 1 – pseudostratified simple columnar ciliated epithelium, 2 – non-keratinized stratified squamous epithelium, 3 – lamina propria mucosae, 4 – tendinomuscular layer, 5 – submucosa, 6 – mixed glands, 7 – lymphoid follicle, 8 – adipose tissue, 9 – mucous glands, 10 – elastic fibers.



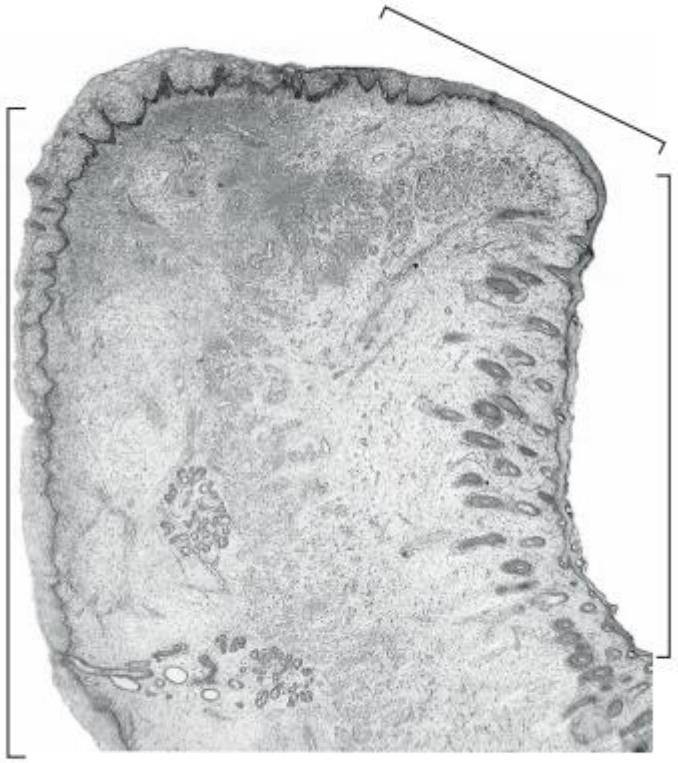
Task 2. Microanatomy of the hard palate (fatty zone) [1].

Study the scheme and label structural elements of the fatty zone of the hard palate: 1 – partially keratinized stratified squamous epithelium, 2 – lamina propria mucosae, 3 – submucosa, 4 – adipose tissue, 5 – bundles of collagen fibers, 6 – palatine bone.



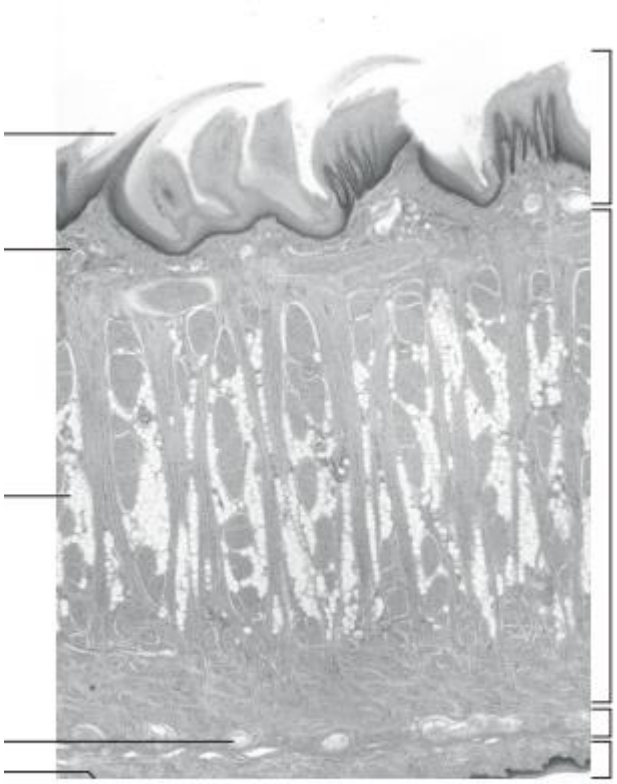
Task 3. Microanatomy of the lip [1].

Study the image and indicate microanatomical parts (zones) of the lip: 1 – skin part, 2 – intermediate part (vermilion border), 3 – mucous part.



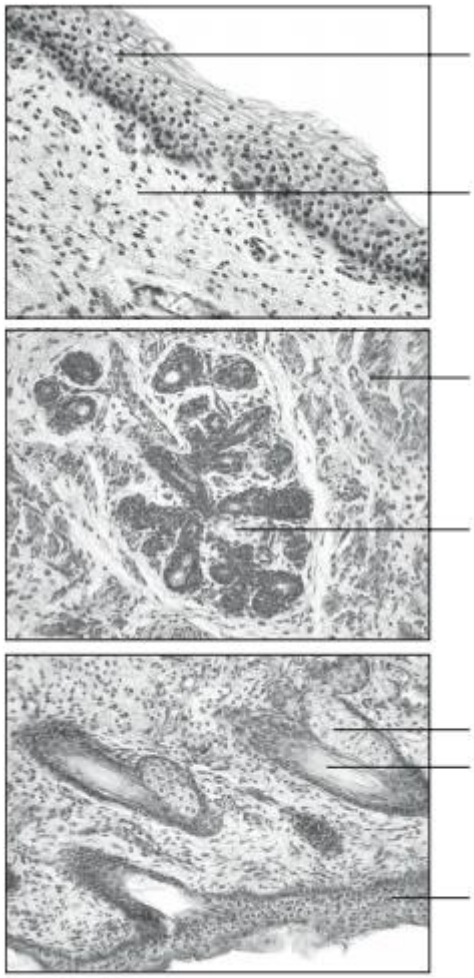
Task 4. Microanatomy of the tongue [1].

Study the image and label structural elements of the tongue: 1 – mucosa, 2 – submucosa, 3 – muscular body, 4 – partially keratinized stratified squamous epithelium, 5 – non-keratinized stratified squamous epithelium, 6 – adipose tissue, 7 – blood vessel.



Task 5. Microanatomy of the cheek [1].

Study the images and label zones structural elements of the cheek: I, mucous surface zone: 1 – non-keratinized stratified squamous epithelium, 2 –lamina propria mucosae; II, maxillary zone: 3 – skeletal muscle fibers, 4 – buccal salivary gland; III, skin surface zone: 5 – keratinized stratified squamous epithelium, 6 – hair, 7 – secretory portion of sebaceous gland.



Task 6. Comparative morphological characterization of hard and soft palate. Complete the table:

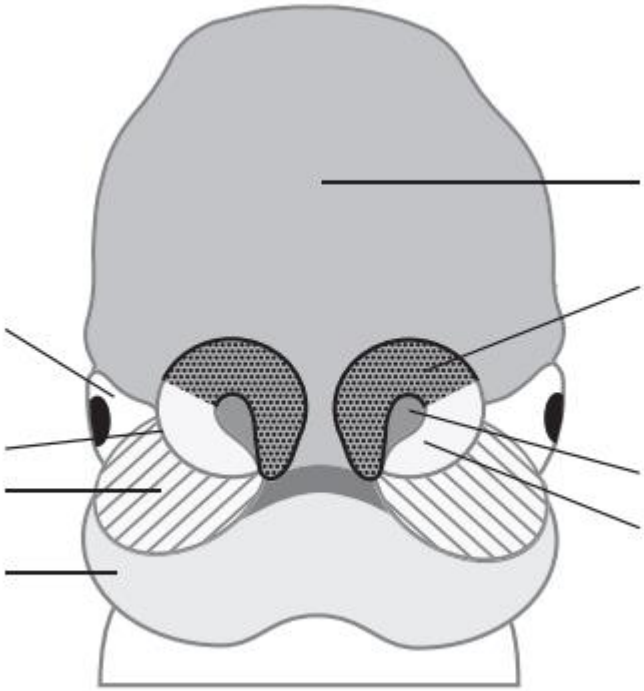
Soft palate						
Tunicae	**Hard palate zones**	marginal				
		glandular				
		fatty				
		palatine raphe				
			Mucosa	epithelium	**Submucosa**	**Muscularis**
				lamina propria		

Task 7. Papillae of the tongue. Complete the table, specifying distinctive morphological and functional features of the lingual papillae.

Filiform papillae	Localization	
	Function	
	Epithelium	
	Lamina propria	
	Taste buds	
	Glands	
Foliate papillae	Localization	
	Function	
	Epithelium	
	Lamina propria	
	Taste buds	
	Glands	
Vallate papillae	Localization	
	Function	
	Epithelium	
	Lamina propria	
	Taste buds	
	Glands	
Fungiform papillae	Localization	
	Function	
	Epithelium	
	Lamina propria	
	Taste buds	
	Glands	

Task 8. Development of the face.

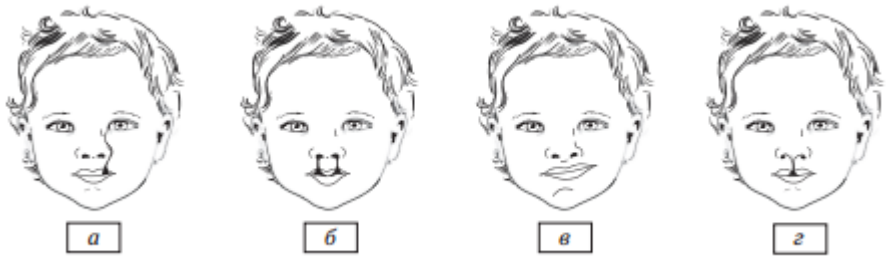
Label the developing facial elements: 1 – medial nasal process, 2 – lateral nasal process, 3 – olfactory pit, 4 – frontal process, 5 – eye, 6 – nasolacrimal furrow, 7 – maxillary process, 8 – mandibular process.



Task 9. Orofacial malformations.

Indicate the malformation for each drawing and complete the table.

	Malformation	No fusion between:
a		
b		
c		
d		



Lesson 2 mastered:

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