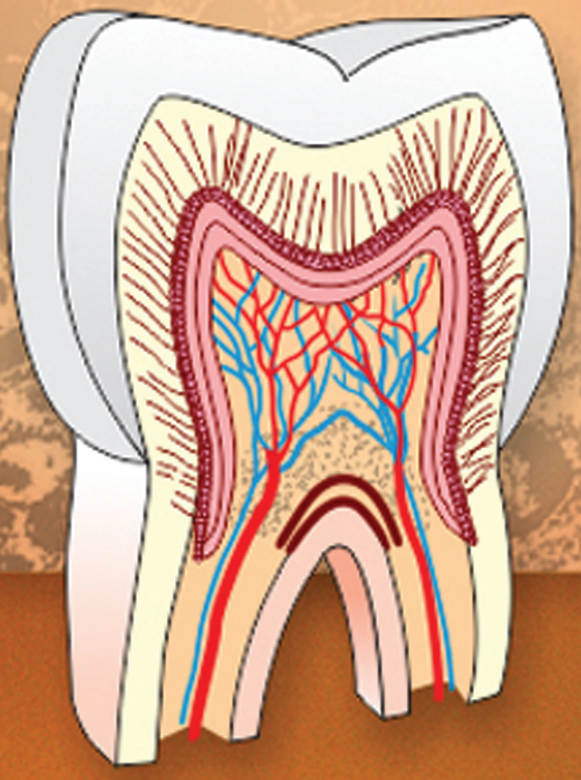
ORBAN's ORAL HISTOLOGY & EMBRYOLOGY



14th Edition

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ORBAN'S ORAL HISTOLOGY AND EMBRYOLOGY

Fourteenth Edition

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To
My Teachers Who Have Guided Me
My Students Who Have Inspired Me
My Family Who Have Encouraged Me
My Associates Who Have Supported Me

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Preface to the Fourteenth Edition

The focus of this edition is to present the subject matter in a way that can be understood easily at the same time without sacrificing details. For this purpose we have incorporated text boxes and flowcharts at appropriate locations. The *Molecular Aspects of Oral Histology* which the undergraduate students need not know have been removed from the printed version of the text but now made available as online supplement (*resources.clinicallearning.com*). Summary is available in this edition with subheadings and it should be read for a quick review before examination. Age Changes in Oral Tissues is a new chapter, but a gist of it is retained in individual chapters. More color and improved quality of illustrations enhances the value of this book. A practical supplement for oral histology with photomicrographs of chosen fields and diagrams made from them with a few identification points should be a welcome addition in the form of ***Atlas of Oral Histology*** book.

The changes that are made in this edition are as a result of positive feedback from our readers. We are very thankful for them and hope to receive more valuable suggestions for the improvement of the book.

G S Kumar

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Preface to the Thirteenth Edition

We, the editorial team, constantly strive to improve this book by incorporating not only additional information that we may have gathered, but also our readers' valuable suggestions. Our contributors are dedicated to this cause and hence, within just three years, we have come up with the next edition of this book.

A salient feature of this edition is the inclusion of Summary and Review Questions at the end of every chapter. 'Appendix' section has been removed and all chapters have been renumbered to give their due identity. The redrawn diagrams and change in the style and format of presentation are bound to be more appealing than before. However, the most important change is the addition of a new chapter 'Lymphoid Tissue and Lymphatics in Orofacial Region'. We have included this chapter because we believe that this topic is not given enough importance in General Histology lectures.

I hope to receive feedback from all our readers to aid further improvement of this book.

G S Kumar

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Brief Contents

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Detailed Contents

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1 An Overview of Oral Tissues

CHAPTER OUTLINE

The oral cavity contains a variety of hard tissues and soft tissues. The hard tissues are the bones of the jaws and the tooth. The soft tissues include the lining mucosa of the mouth and the salivary glands.

The tooth consists of crown and root. That part of the tooth visible in the mouth is called clinical crown; the extent of which increases with age and disease. The root portion of the tooth is not visible in the mouth in health. The tooth is suspended in the sockets of the alveolar bone by the periodontal ligament. The anatomical crown is covered by enamel and the root by the cementum. Periodontium is the term given to supporting tissues of the tooth. They include the cementum, periodontal ligament and the alveolar bone. The innermost portion of the crown and root is occupied by soft tissue, the pulp. The dentin occupies the region between the pulp and enamel in the crown, and between pulp and cementum in the root.

DEVELOPMENT OF TOOTH

The tooth is formed from the ectoderm and ectomesenchyme. The enamel is derived from the enamel organ which is differentiated from the primitive oral epithelium lining the stomodeum (primitive oral cavity). Epithelial mesenchymal interactions take place to determine the shape of the tooth and the differentiation of the formative cells of the tooth and the timing of their secretion. The ectomesenchymal cells which are closer to the inner margins of the enamel organ differentiate into dental papilla and the ectomesenchymal cells closer to the outer margins of the enamel organ become dental follicle. Dentin and pulp are derivatives of dental papilla while cementum, periodontal ligament and alveolar bone, are all derivatives of dental follicle. The cells that form these tissues have their names ending in blast. Thus, ameloblast produces enamel, odontoblast dentin, cementoblast, cementum and osteoblast bone. These synthesizingcells have all the features of a protein secreting cell—well developed ribosomes and a rough endoplasmic reticulum (ER), Golgi apparatus, mitochondria and a vesicular nucleus, which is often polarized. The cells that resorb the tissues have their names ending in 'clast'. Thus, osteoclast resorbs bone, cementoclast, cementum and odontoclast resorbs all the dental tissues. The 'clast' cells have a similar morphology in being multinucleated giant cells. Their ultra structural features include numerous lysosomes and ingested vacuoles.

Dentin is the first hard tissue of the tooth to form. Enamel starts its formation after the first layer of dentin has formed. The enamel formation is from its junction with dentin outwards, first in the cuspal/incisal and later in the cervical regions. Dentin formation is similar, but from the dentinoenamel junction, the formation is pulp ward. Cementum formation occurs after the root form, size, shape and number of roots is outlined by the epithelial root sheath and dentin is laid down in these regions. Formation of enamel, dentin and cementum takes place as a daily event in phases or in increments, and hence they show incremental lines. In dentin and cementum formation, a layer of uncalcified matrix forms first, followed by its mineralization. While in enamel formation enamel matrix is calcified, but its maturation or complete mineralization occurs as a secondary event. Mineralization occurs as a result of supersaturation of calcium and phosphorus in the tissue fluid. The formative cells concentrate the minerals from calcium phosphate (apatite) and secrete them into the organic matrix, in relation to specific substances like collagen, which act as attractants or nucleators for mineralization. The mechanism of mineralization is quite similar in all the hard tissues of tooth and in bone (Fig. 1.1).

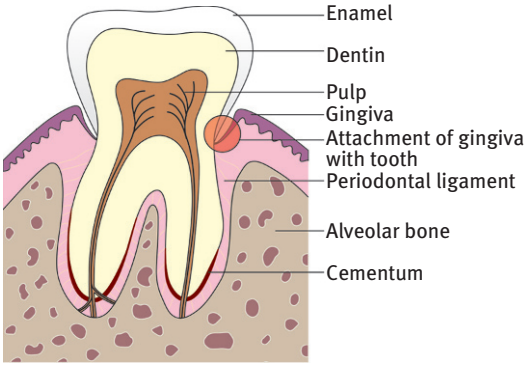

Figure 1.1 Diagrammatic representation of tooth in situ.

ENAMEL

The enamel is the hardest tissue in the human body. It is the only ectodermal derivative of the tooth. Inorganic constituents account for 96% by weight and they are mainly calcium phosphate in the form of hydroxyapatite crystals. These apatite crystals are arranged in the form of rods. All other hard tissues of the body, dentin, cementum and bone also have hydroxyapatite as the principal inorganic constituent. Hydroxyapatite crystals differ in size and shape; those of the enamel are hexagonal and longest. Enamel is the only hard tissue, which does not have collagen in its organic matrix. The enamel present in the fully formed crown has no viable cells, as the cells forming it—the ameloblast degenerates, once enamel formation is over. Therefore, all the enamel is formed before eruption. This is of clinical importance as enamel lost, after tooth has erupted, due to wear and tear or due to dental caries, cannot be formed again. Enamel lacks not only formative cells but also vessels and nerves. This makes the tooth painless and no blood oozes out when enamel is drilled while making a cavity for filling.

DENTIN

The dentin forms the bulk of the tooth. It consists of dentinal tubules, which contains the cytoplasmic process of the odontoblasts. The tubules are laid in the calcified matrix—the walls of the tubules are more calcified than the region between the tubules. The apatite crystals in the matrix are plate like and shorter, when compared to enamel. The numbers of tubules near the pulp are broader and closer and they usually have a sinusoidal course, with branches, all along and at their terminus at the dentinoenamel or cementodentinal junction. The junction between enamel and dentin is scalloped to give mechanical retention to the enamel. Dentin is avascular. Nerves are present in the inner dentin only. Therefore, when dentin is exposed, by loss of enamel and stimulated, a pain-like sensation called sensitivity is experienced. The dentin forms throughout life without any stimulation or as a reaction to an irritant. The cells that form the dentin—the odontoblast lies in the pulp, near its border with dentin. Thus, dentin protects the pulp and the pulp nourishes the dentin. Though dentin and pulp are different tissues they function as one unit.

PULP

The pulp, the only soft tissue of the tooth, is a loose connective tissue enclosed by the dentin. The pulp responds to any stimuli by pain. Pulp contains the odontoblast. Odontoblasts are terminally differentiated cells, and in the event of their injury and death, they are replaced from the pool of undifferentiated ectomesenchymal cells in the pulp. The pulp is continuous with the periodontal ligament through the apical foramen or through the lateral canals in the root. Pulp also contains defense cells. The average volume of the pulp is about 0.02 cm^3.

CEMENTUM

The cementum is comparable to bone in its proportion of inorganic to organic constituents and to similarities in its structure. The cementum is thinnest at its junction with the enamel and thickest at the apex. The cementum gives attachment to the periodontal ligament fibers. Cementum forms throughout life, so as to keep the tooth in functional position. Cementum also forms as a repair tissue and in excessive amounts due to low grade irritants.

The cells that form the cementum; the cementoblast lines the cemental surface. Uncalcified cementum is usually seen, as the most superficial layer of cementum. The cells within the cementum, the cementocytes are enclosed in a lacuna and its process in the canaliculi, similar to that seen in bone, but in a far less complex network. Cementocytes presence is limited to certain regions. The regions of cementum containing cells are called cellular cementum and the regions without it, are known as the acellular cementum. The acellular cementum is concerned with the function of anchorage to the teeth and the cellular cementum is concerned with adaptation, i.e. to keep the tooth in the functional position. Like dentin, cementum forms throughout life, and is also avascular and noninnervated.

PERIODONTAL LIGAMENT

The periodontal ligament is a fibrous connective tissue, which anchors the tooth to the alveolar bone. The collagen fibers of the periodontal ligament penetrate the alveolar bone and cementum. They have a wavy course. The periodontal ligament has the formative cells of bone and cementum, i.e. osteoblast and cementoblast in addition to fibroblast and resorptive cells—the osteoclast. Cementoclasts are very rarely seen as cemental resorption is not seen in health. Fibroblast, also functions as a resorptive cell. Thus, with the presence of both formative and resorptive cells of bone, cementum and connective tissue, and along with the wavy nature of the fibers, the periodontal ligament is able to adjust itself to the constant change in the position of teeth, and also maintains its width. The periodontal fibers connect all the teeth in the arch to keep them together and also attach the gingiva to the tooth. The periodontal ligament nourishes the cementum. The presence of proprioceptive nerve endings provides the tactile sensation to the tooth and

excessive pressure on the tooth is prevented by pain originating from the pain receptors in the periodontal ligament.

ALVEOLAR BONE

Alveolar bone is the alveolar process of the jaws that forms and supports the sockets for the teeth. They develop during the eruption of the teeth and disappear after the tooth is extracted or lost. The basic structure of the alveolar bone is very similar to the bone found elsewhere, except for the presence of immature bundle bone amidst the compact bone lining the sockets for the teeth. The buccal and lingual plates of compact bone enclose the cancellous bone. The arrangement and the density of the cancellous bone varies in the upper and lower jaws and is related to the masticatory load, the tooth receives. The ability of bone, but not cementum, to form under tension and resorb under pressure makes orthodontic treatment possible.

TEMPOROMANDIBULAR JOINT

This only movable bilateral joint of the skull has a movable fibrous articular disk separating the joint cavity. The fibrous layer that lines the articular surface is continuous with the periosteum of the bones. The fibrous capsule, which covers the joint, is lined by the synovial membrane. The joint movement is intimately related to the presence or absence of teeth and to their function.

MAXILLARY SINUS

The maxillary posterior teeth are related to the maxillary sinus in that, they have a common nerve supply and that their roots are often separated by a thin plate of bone. Injuries to the lining and extension of infection from the apex of roots are often encountered in clinical practice. Developing maxillary canine teeth are found close to the sinus. Pseudostratified ciliated columnar epithelium lines the maxillary sinus.

ERUPTION AND SHEDDING OF TEETH

The eruption of teeth is a highly programed event. The teeth developing within the bony crypt initially undergo bodily and eccentric movements and finally by axial movement make its appearance in the oral cavity. At that time, the roots are about half to two thirds complete. Just before the tooth makes its appearance in the oral cavity the epithelium covering it, fuses with the oral epithelium.

The tooth then cuts through the degenerated fused epithelium, so that eruption of teeth is a bloodless event. Root growth, fluid pressure at the apex of the erupting teeth and dental follicle cells contractile force are all shown to be involved in the eruption mechanism. The bony crypt forms and resorbs suitably to adjust to the growing tooth germ and later to its eruptive movements.

The deciduous teeth are replaced by permanent successor teeth as an adaptation to the growth of jaws and due to the increased masticatory force of the masticatory muscles, in the process of shedding. The permanent successor teeth during the eruptive movement cause pressure on the roots of deciduous teeth and induce resorption of the roots. The odontoclast, which has a similar morphology to osteoclast and participates in this event, has the capacity to resorb, all dental hard tissues.

ORAL MUCOSA

The mucosa lining the mouth is continuous anteriorly with the skin of the lip at the vermilion zone and with the pharyngeal mucosa posteriorly. Thus, the oral mucosa and GI tract mucosa are continuous. The integrity of the mucosa is interrupted by the teeth to which it is attached. The oral mucosa is attached to the underlying bone or muscle by a loose connective tissue, called submucosa. The mucosa is firmly attached to the periosteum of hard palate and to the alveolar process (gingiva). The mucosa in these regions is a functional adaptation to mastication, hence, they are referred to as masticatory mucosa. Elsewhere, except in the dorsum of tongue, the mucosa is loosely attached as an adaptation to allow the mucosa to stretch. The mucosa in these regions is referred to as lining mucosa. The stratified squamous epithelium varies in thickness and is either keratinized as in masticatory mucosa or non-keratinized as in lining mucosa. The submucosa is prominent in the lining and is nearly absent in the masticatory mucosa. The cells that have the ability to produce keratin, called keratinocytes, undergo maturational changes and finally desquamate. The non-keratinocytes, do not undergo these changes, and they are concerned either with immune function (Langerhans cells) or melanin production (melanocytes). The mucosa that attaches to the tooth is unique, thin and permeable. The fluid that oozes through this lining into the crevice around the tooth is called gingival fluid. It aids in defense against entry of bacteria, through this epithelium. The mucus of the dorsum of tongue, is called specialized mucosa because it has the taste buds in the papillae.

SALIVARY GLANDS

The major salivary glands (parotid, submandibular and sublingual) and the minor salivary glands present in the submucosa, everywhere in the oral cavity except in gingivae and anterior part of the hard palate; secrete serous, mucous or mixed salivary secretion, into the oral cavity by a system of ducts. The acini, which are production centers of salivary secretion, are of two types—the serous and the mucous acini. They vary in size and shape and also in the mode of secretion. The composition and physical properties of saliva differ between mucous and serous secretions. The ducts, act not merely as passageways for saliva, but also modify the salivary secretion with regard to quantity and electrolytes. The ducts, which vary in their structure from having a simple epithelial lining to a stratified squamous epithelial lining, show functional modifications.

LYMPHOID TISSUE AND LYMPHATICS OF OROFACIAL REGION

The tissues of our body are bathed in the tissue fluid. The tissue fluid contains diffusible constituents of blood and waste products discarded by cells. Majority of the tissue fluid returns back to the circulation through veins. About 1/10th is carried by channels called lymphatic vessels. This fluid is called lymph and it passes through the lymph nodes. The lymph nodes are small bean-shaped organs occurring in groups. They function to filter the foreign substances called antigens. The tonsils are similar to lymph nodes and serve to guard the entrance to alimentary and respiratory tracts against antigens that come in contact with them during eating and breathing.

The lymph nodes contain different zones. In it matures the lymphocytes, which are of two types: B and T lymphocytes. The lymph node contains a variety of defense cells. The lymphatic system consists of the primary lymphoid organs, namely the thymus and the bone marrow and the secondary lymphoid organs like the spleen and lymph nodes.

Enlargements of lymph nodes is of clinical significance and occurs as a response to an invading organism or due to tumor cells entering from a draining area or due to tumors arising in the lymph nodes itself.

AGE CHANGES IN ORAL TISSUES

The tissues of the body undergo certain changes in macroscopic and in their microscopic appearance and in their functions with time. These are called age changes. The oral tissues also undergo such changes. Some of the prominent age changes that have a clinical significance are dealt here.

The enamel is lost due to physiological, mechanical or chemical actions resulting in exposure of underlying dentin, causing pain-like sensation, called sensitivity of teeth. Deposition of calcium salts occurs in certain regions of the dentin making the root transparent in these regions. The pulp shows areas of calcified bodies, termed pulp stones. The cementum increases in thickness, especially in the apical third of the root. The periodontal ligament shows a decrease in number of cells and the alveolar bone shows bone loss. The oral mucosa becomes thin, the papillae of the tongue are lost, and mouth becomes dry due to decreased secretion from salivary glands. The person experiences a gradual loss of taste sensation. The attachment of the gingiva to the tooth shifts apically resulting in more exposure of the tooth clinically, i.e. the clinical crown becomes longer with age.

STUDY OF ORAL TISSUES

For light microscopic examination, the tissues have to be made thin and stained, so that the structures can be appreciated. The teeth (and bone) can be ground or can be decalcified before making them into thin slices. In the first method, all hard tissues can be studied. In the second method, all the hard tissues except enamel, pulp and periodontal ligament can be studied. Soft tissues of the mouth require a similar preparation as soft tissues of other parts of the body for microscopic examination.

For traditional light microscopic examination, the tissues have to be made into thin sections and differentially stained by utilizing the variations they exhibit in their biochemical and immunological properties. There are various histochemical, enzyme-histochemical, immunohistochemical, immunofluorescent techniques developed to enhance tissue characteristics. Apart from light microscopy, tissues can be examined using electron microscope, fluorescent microscope, confocal laser scanning microscope and autoradiography techniques for better recognition of cellular details, functions and the series of events that take place within them.

2 Development of Face and Oral Cavity

CHAPTER CONTENTS

This chapter deals primarily with the development of the human face and oral cavity. Consideration is also given to information about underlying mechanisms that is derived from experimental studies conducted on developing subhuman embryos. Much of the experimental work has been conducted on amphibian and avian embryos. Evidence derived from these and more limited studies on other vertebrates including mammals indicates that the early facial development of all vertebrate embryos is similar. Many events occur, including cell migrations, interactions, differential growth, and differentiation, all of which lead to progressively maturing structures (Fig. 2.1). Progress has also been made with respect to abnormal developmental alterations that give rise to some of the most common human malformations. Further information on the topics discussed can be obtained by consulting the references at the end of the chapter.

ORIGIN OF FACIAL TISSUES

After fertilization of the ovum, a series of cell divisions gives rise to an egg cell mass known as the *morula* in mammals (Fig. 2.2). In most vertebrates, including humans, the major portion of the egg cell mass forms the extraembryonic membranes and other supportive structures, such as the placenta. The inner cell mass (Fig. 2.2D) separates into two layers, the *epiblast* and *hypoblast* (Fig. 2.2E). Cell marking studies in chick and mouse embryos have shown that only the epiblast forms the embryo, with the hypoblast and other cells forming supporting tissues, such as the placenta. The anterior (rostral) end of the primitive streak forms the lower germ layer, the *endoderm*, in which are embedded the midline notochordal (and prechordal) plates (Figs. 2.2F and 2.3A). Prospective mesodermal cells migrate from the epiblast through the primitive streak to form the middle germ layer, the *mesoderm*.

Cells remaining in the epiblast form the *ectoderm*, completing formation of the three germ layers. Thus, at this stage, three distinct populations of embryonic cells have arisen largely through division and migration. They follow distinctly separate courses during later development.

Migrations, such as those described above, create new associations between cells, which, in turn, allow unique possibilities for subsequent development through interactions between the cell populations. Such interactions have been studied experimentally by isolating the different cell populations or tissues and recombining them in different ways in culture or in transplants. From these studies it is known, for example, that a median strip of mesoderm cells (the *chorda mesoderm*) extending throughout the length of the embryo induces *neural plate* formation within the overlying ectoderm (Fig. 2.3). The prechordal plate is thought to have a similar role in the anterior neural plate region. The nature of such inductive stimuli is presently unknown. Sometimes cell-to-cell contact appears to be necessary, whereas in other cases (as in neural plate induction) the inductive influences appear to be able to act between cells separated by considerable distances and consist of diffusible substances. It is known that inductive influences need only be present for a short time, after which the responding tissue is capable of independent development. For example, an induced neural plate isolated in culture will roll up into a tube, which then differentiates into the brain, spinal cord, and other structureks.

In addition to inducing neural plate formation, the chorda mesoderm appears to be responsible for developing the organizational plan of the head. As noted previously, the notochord and prechordal plates arise initially

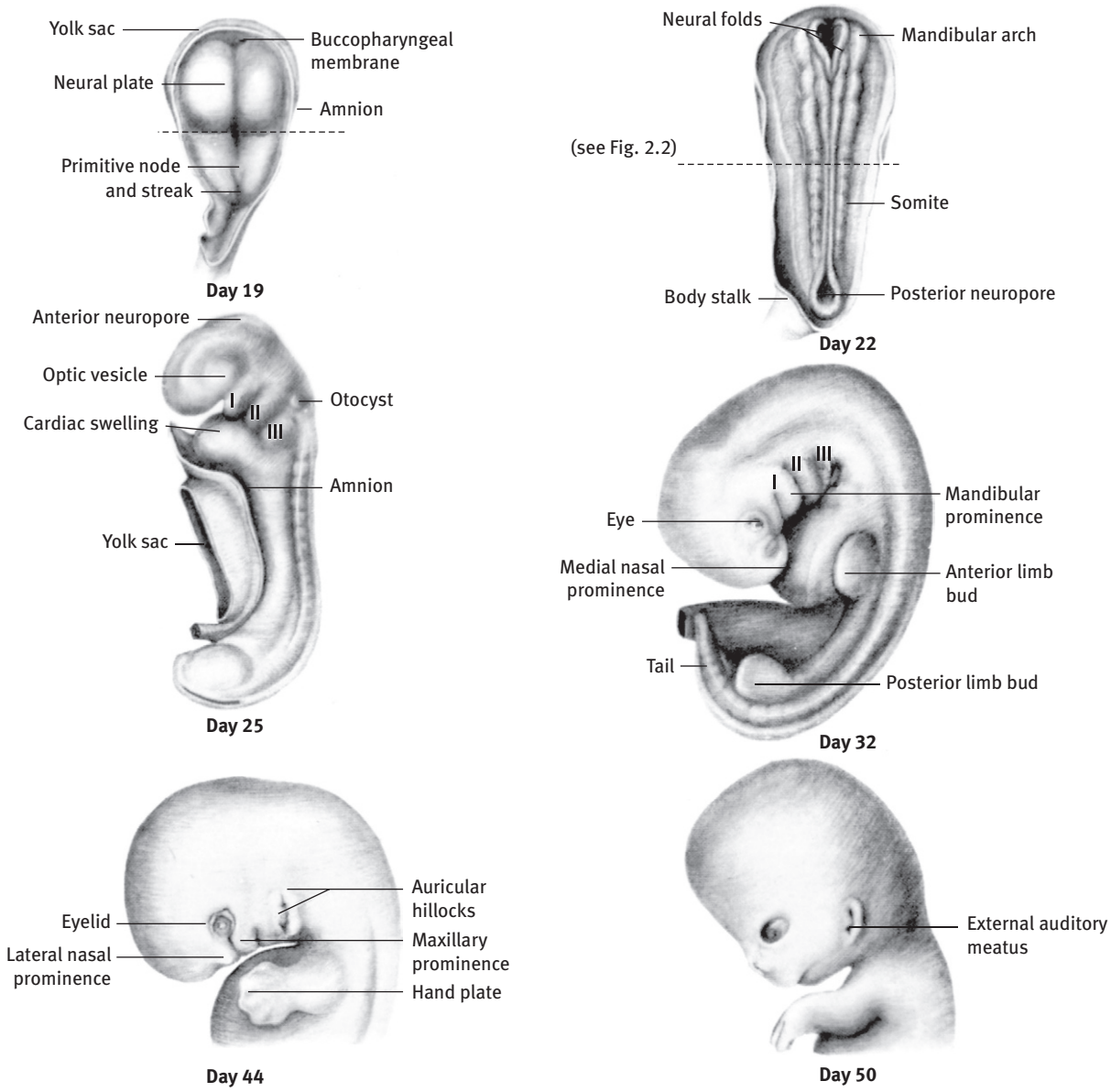

Figure 2.1 Emergence of facial structures during development of human embryos. Dorsal views of gestational day 19 and 22 embryos are depicted, while lateral aspects of older embryos are illustrated. At days 25 and 32, visceral arches are designated by Roman numerals. Embryos become recognizable as 'human' by gestational day 50. Section planes for Figure 2.2 are illustrated in the upper (days 19 and 22) diagrams.

within the endoderm (Fig. 2.3A), from which they eventually separate (Figs. 2.2G and 2.3B). The mesodermal portion differentiates into well-organized blocks of cells, called *somites*, caudal to the developing ear and less-organized somitomeres rostral to the ear (Figs. 2.2 and 2.6). Later these structures form myoblasts and some of the skeletal and connective tissues of the head. Besides inducing the neural plate from overlying ectoderm, the chorda mesoderm organizes the positional relationships of various neural plate components, such as the initial primordium of the eye.

A unique population of cells develops from the ectoderm along the lateral margins of the neural plate. These are the neural crest cells. They undergo extensive migrations, usually beginning at about the time of tube closure (Fig. 2.3), and give rise to a variety of different cells that form components of many tissues. The crest cells that migrate in the trunk region form mostly neural, endocrine, and pigment cells, whereas those that migrate in the head and neck also contribute extensively to skeletal and connective tissues (i.e. cartilage, bone, dentin, dermis, etc.). In the trunk, all skeletal and connective tissues are formed by mesoderm. Of the skeletal or connective tissue of the facial region, it appears that tooth enamel (an acellular skeletal tissue) is the only one not formed by crest cells. The enamel-forming cells are derived from ectoderm lining the oral cavity.

The migration routes that cephalic (head) neural crest cells follow are illustrated in Figure 2.4. They move around the sides of the head beneath the surface ectoderm, en masse, as a sheet of cells. They form all the mesenchyme[1] in the upper facial region, whereas in the lower facial region they surround mesodermal cores

[1]Mesenchyme is defined here as the loosely organized embryonic tissue, in contrast to epithelia, which are compactly arranged.

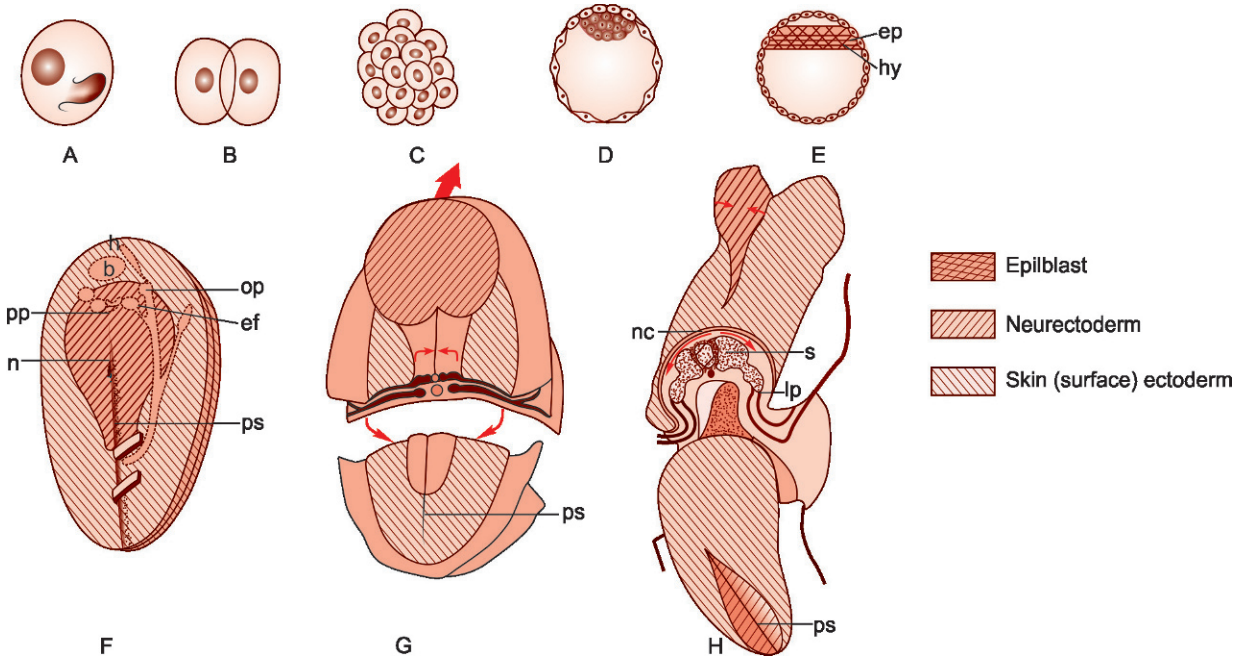

Figure 2.2 Sketches summarizing development of embryos from fertilization through neural tube formation. (**A**) Ovum at the time of fertilization. (**B**) Two-celled embryo. Accumulation of fluid within egg cell mass (morula, **C**) leads to development of blastula (**D**). Inner cell mass (heavily strippled cells in **D**) will form two-layered embryonic disk in **E**. It now appears that only epiblast (ep) will form embryo (see text), with hypoblast (hy) and other cell populations forming support tissues (e.g. placenta) of embryo. In **F**, notochord (n) and its rostral (anterior) extension, prechordal plate (pp), as well as associated pharyngeal endoderm, form as a single layer. Prospective mesodermal cells migrate (arrows in **F**) through primitive streak (ps) and insert themselves between epiblast and endoderm. Epiblast cells remaining on surface become ectoderm. Cells of notochord (and prechordal plate?) and adjacent mesoderm (together termed chorda mesoderm) induce overlying cells to form neural plate (neurectoderm). Only later does notochord separate from neural plate (**G**), while folding movements and differential growth (arrows in **G** and **H**) continue to shape embryo h, heart; b, buccal plate; op, olfactory placode; ef, eye field; nc, neural crest; so, somite; lp, lateral plate. (Modified from Johnston MC and Sulik KK: Embryology of the head and neck. In Serafin D and Georgiade NG, editors: Pediatric plastic surgery, vol. 1, St Louis, 1984, The CV Mosby Co).

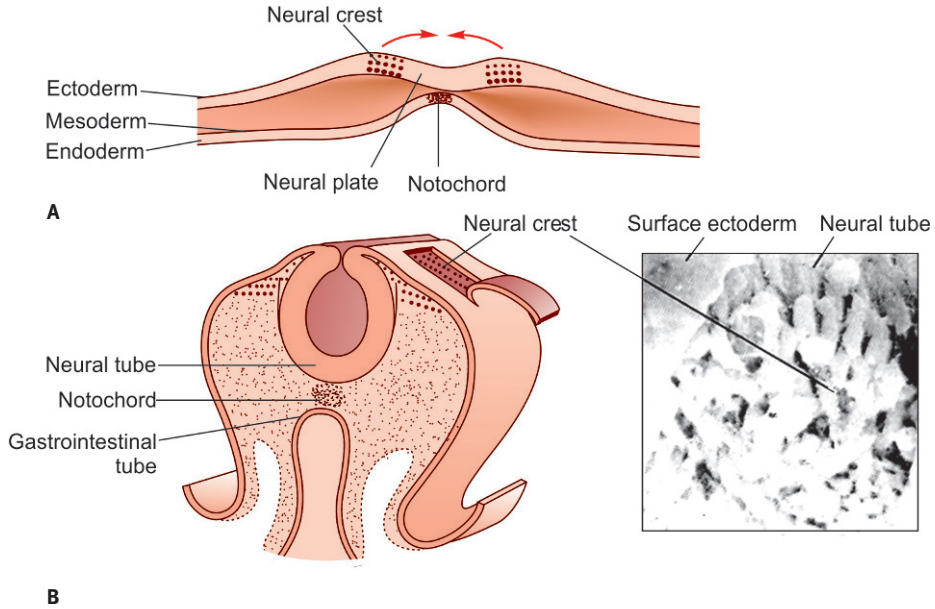

Figure 2.3 Scheme of neural and gastrointestinal tube formation in higher vertebrate embryos (section planes illustrated in Figure 2.1). (**A**) Cross-section through three-germ layer embryo. Similar structures are seen in both head and trunk regions. Neural crest cells (diamond pattern) are initially located between neural plate and surface ectoderm. Arrows indicate directions of folding processes. (**B**) Neural tube, which later forms major components of brain and spinal cord, and gastrointestinal tube will separate from embryo surface after fusions are completed. Arrows indicate directions of migration of crest cells, which are initiated at about fourth week in human embryo. (**C**) Scanning electron micrograph (SEM) of mouse embryo neural crest cells migrating over neural tube and under surface ectoderm near junction of brain and spinal cord following removal of piece of surface ectoderm as indicated in B. Such migrating cells are frequently bipolar (e.g. outlined cell at end of leading end) and oriented in path of migration (arrow).

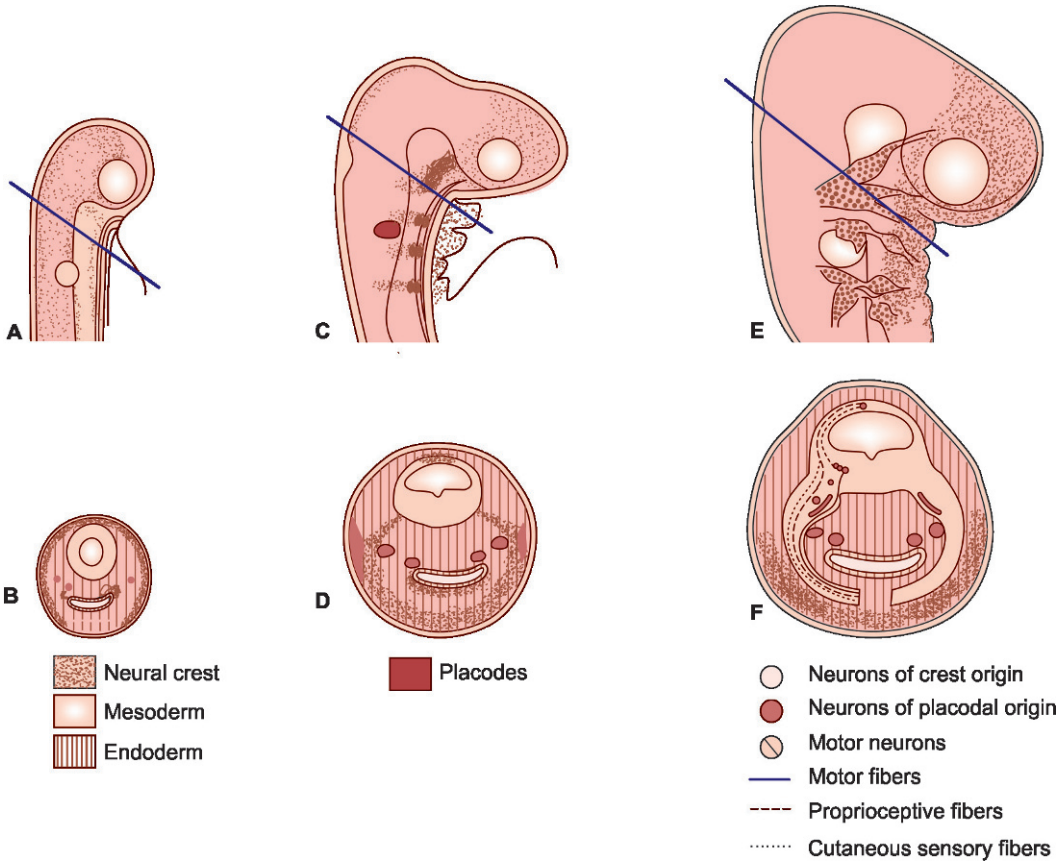

Figure 2.4 **A** and **B**, Migratory and **C** and **D**, postmigratory distributions of crest cells (stipple) and origins of cranial sensory ganglia. Initial ganglionic primordia (**C** and **D**) are formed by cords of neural crest cells that remain in contact with neural tube. Section planes in **C** and **E**, pass through primordium of trigeminal ganglion. Ectodermal 'thickenings,' termed placodes, form adjacent to distal ends of ganglionic primordia—for trigeminal (V) nerve as well as for cranial nerves VII, IX, and X. They contribute presumptive neuroblasts that migrate into previously purely crest cell ganglionic primordia. Distribution of crest and placodal neurons is illustrated in **E** and **F** (Adapted from Johnston MC and Hazelton RD: Embryonic origins of facial structures related to oral sensory and motor functions. From Bosma JB, editor: Third symposium on oral sensation and perception, Springfield, IL, 1972, Charles C Thomas Publisher).

already present in the visceral arches. The pharyngeal region is then characterized by grooves (clefts and pouches) in the lateral pharyngeal wall endoderm and ectoderm that approach each other and appear to effectively segment the mesoderm into a number of bars that become surrounded by crest mesenchyme (Figs. 2.4C, D and 2.7A).

Toward the completion of migration, the trailing edge of the crest cell mass appears to attach itself to the neural tube at locations where sensory ganglia of the fifth, seventh, ninth, and tenth cranial nerves will form (Fig. 2.4C and D). In the trunk sensory ganglia, supporting (e.g. Schwann) cells and all neurons are derived from neural crest cells. On the other hand, many of the sensory neurons of the cranial sensory ganglia originate from placodes in the surface ectoderm (Fig. 2.4C and F).

Eventually, capillary endothelial cells derived from mesoderm cells invade the crest cell mesenchyme, and it is from this mesenchyme that the supporting cells of the developing blood vessels are derived. Initially, these supporting cells include only pericytes, which are closely apposed to the outer surfaces of endothelial cells. Later, additional crest cells differentiate into the fibroblasts and smooth muscle cells that will form the vessel wall. The developing blood vessels become interconnected to form vascular networks. These networks undergo a series of modifications, examples of which are illustrated in Figure 2.5, before they eventually form the mature vascular system. The underlying mechanisms are not clearly understood.

Almost all the myoblasts that subsequently fuse with each other to form the multinucleated striated muscle fibers are derived from mesoderm. The myoblasts that form the hypoglossal (tongue) muscles are derived from somites located beside the developing hindbrain. Somites are condensed masses of cells derived from mesoderm located adjacent to the neural tube. The myoblasts of the extrinsic ocular muscles originate from the prechordal plate (Fig. 2.2F). They first migrate to poorly condensed blocks of mesoderm (somitomeres) located rostral to (in front of) the otocyst, from which they migrate to their final locations (Fig. 2.6). The supporting connective tissue found in facial muscles is derived from neural crest cells. Much of the development of the masticatory and other facial musculature is closely related to the final stages of visceral arch development and will be described later.

A number of other structures in the facial region, such as the epithelial components or glands and the enamel organ of the tooth bud, are derived from epithelium that grows (invaginates) into underlying mesenchyme. Again, the connective tissue components in these structures (e.g. fibroblasts, odontoblasts, and the cells of tooth-supporting tissues) are derived from neural crest cells.

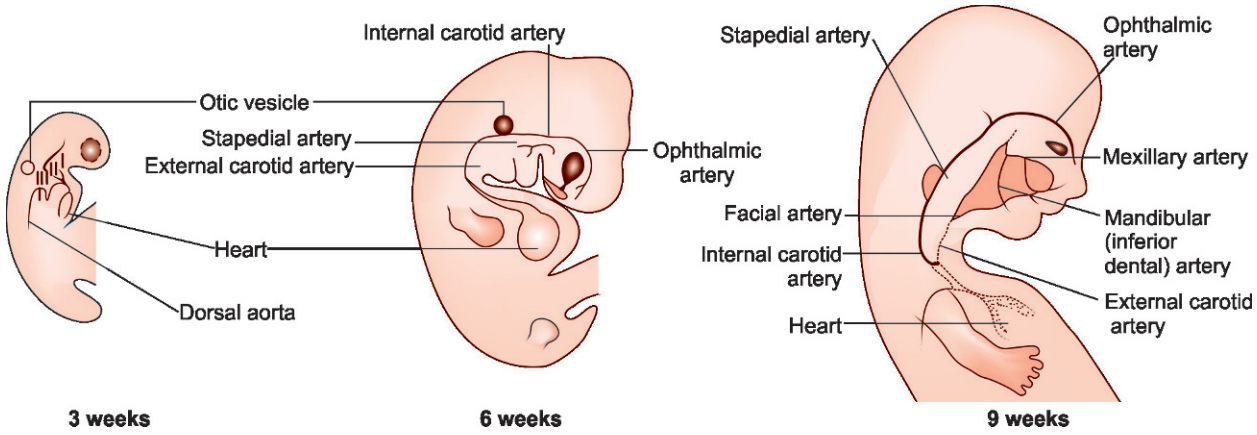

Figure 2.5 Development of arterial system serving facial region with emphasis on its relation to visceral arches. In 3-week human embryo visceral arches are little more than conduits for blood traveling through aortic arch vessels (indicated by Roman numerals according to the visceral arch containing them) from heart to dorsal aorta. Other structures indicated are eye (broken circle) and ophthalmic artery. In 6-week embryo first two aortic arch vessels have regressed almost entirely, and distal portions of arches have separated from heart. Portion of third aortic arch vessel adjacent to dorsal aorta persists and eventually forms stem of external carotid artery by fusing with stapedial artery. Stapedial artery, which develops from second aortic arch vessel, temporarily (in humans) provides arterial supply for embryonic face. After fusion with external carotid artery proximal portion of stapedial artery regresses. Aortic arch vessel of fourth visceral arch persists as arch of aorta. By 9 weeks primordium of definitive vascular system of face has been laid down (From Ross RB, and Johnston MC: Cleft lip and palate, Baltimore, 1972, The Williams & Wilkins Co).

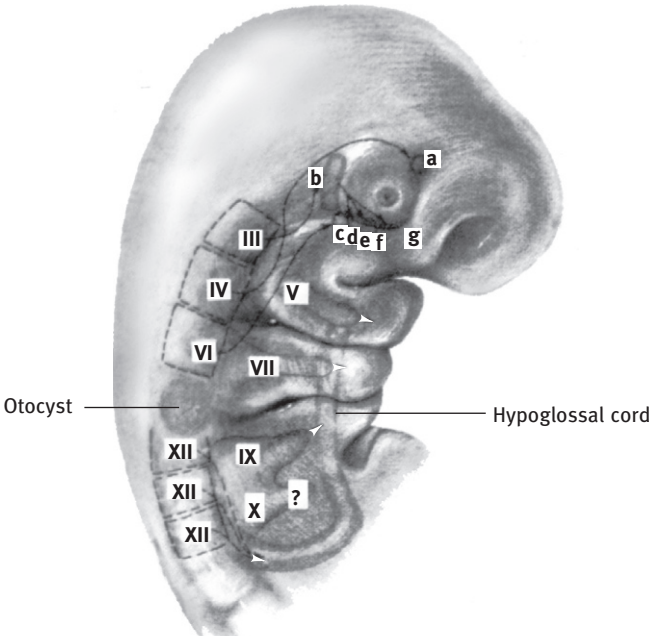

Figure 2.6 Migration paths followed by prospective skeletal muscle cells.Somites, or comparable structures from which muscle cells are derived, give rise to most skeletal (voluntary) myoblasts (differentiating muscle cells). Condensed somites tend not to form in head region of higher vertebrates, and their position in lower forms is indicated by broken lines. It is from these locations that extrinsic ocular and 'tongue' (hypoglossal cord) muscle contractile cells are derived from postoptic somites. Recent studies indicate that myoblasts which contribute to visceral arch musculature have similar origins and originate as indicated by Roman numerals according to their nerves of innervation. At this stage of development (approximately day 34) they are still migrating (arrowheads) into cores of each visceral arch. Information about fourth visceral arch is still inadequate, as indicated by question mark (?). Origin of extrinsic ocular myoblasts is complex (see text).

DEVELOPMENT OF FACIAL PROMINENCES

On the completion of the initial crest cell migration and the vascularization of the derived mesenchyme, a series of outgrowths or swellings termed 'facial prominences' initiates the next stages of facial development (Figs. 2.7 and 2.8). The growth and fusion of upper facial prominences produce the primary and secondary palates. As will be described below, other prominences developing from the first two visceral arches considerably alter the nature of these arches.

Development of the Frontonasal Region: Olfactory Placode, Primary Palate, and Nose

After the crest cells arrive in the future location of the upper face and midface, this area often is referred to as the frontonasal region. The first structures to become evident are the olfactory placodes. These are thickenings of the ectoderm that appear to be derived at least partly from the anterior rim of the neural plate (Fig. 2.2F). Experimental evidence indicates that the lateral edges of the placodes actively curl forward, which enhance the initial development of the lateral nasal prominence (LNP, sometimes called the nasal wing—see Fig. 2.7A). This morphogenetic movement combined with persisting high rates of cell proliferation rapidly brings the LNP forward so that it catches up with the medial nasal prominence (MNP), which was situated in a more forward position at the beginning of its development (Fig. 2.7A and C). However, before that contact is made, the maxillary prominence (MxP) has already grown forward from its origin at the proximal end of the first visceral arch (Figs. 2.7A and 2.13) to merge with the LNP and make early contact with the MNP (Fig. 2.7G). With development of the lateral nasal prominence—medial nasal prominence contact,

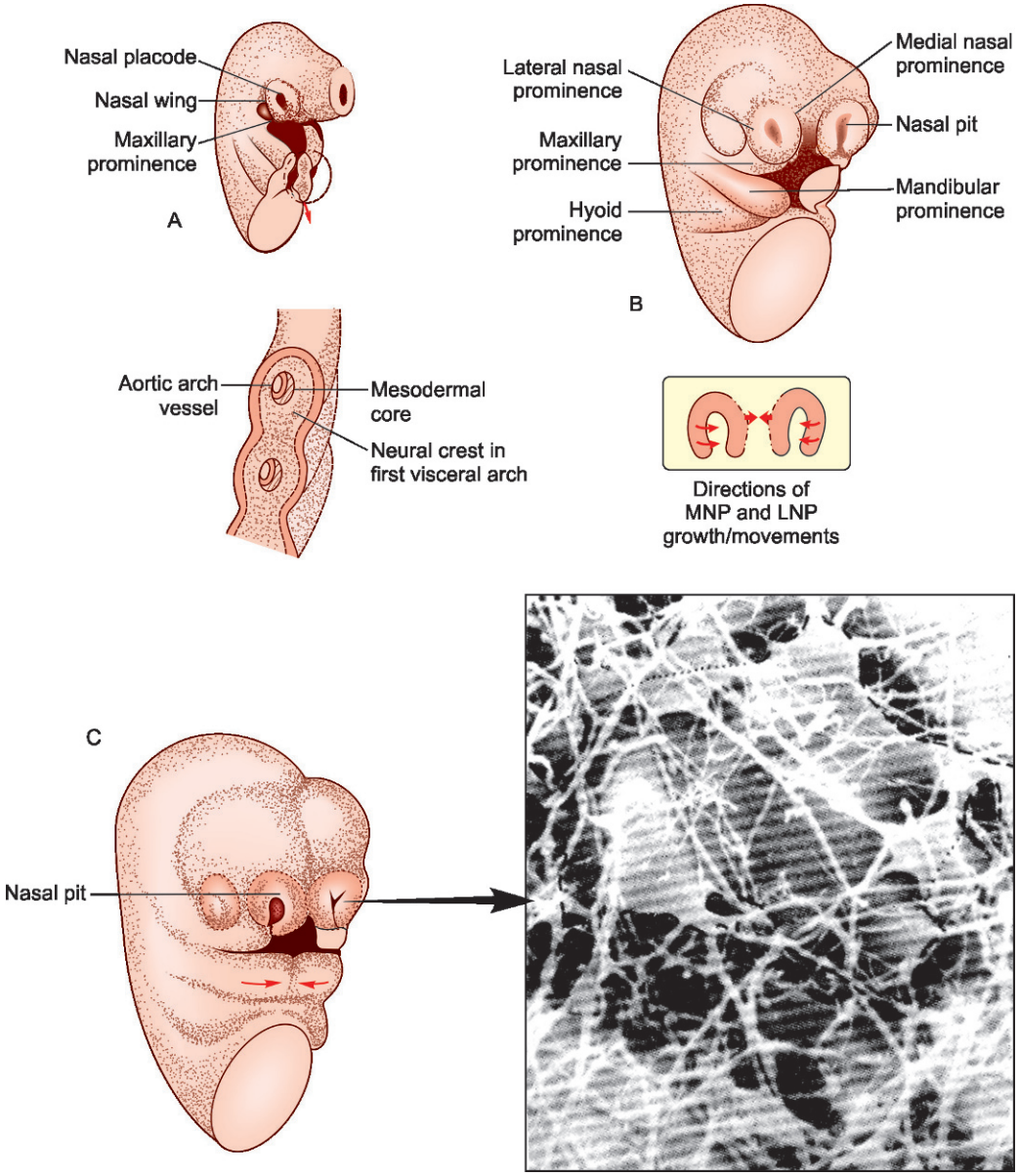

Figure 2.7 Scheme of development of facial prominences.After completion of crest cell migration. (**A**) Facial prominence development begins, with curling forward, lateral portion of nasal placode and is completed after fusion of prominences with each other or with other structures, **C**. (Details are given in text). Heart and adjacent portions of visceral arches have been removed in **A**, and most of heart has been removed in **B**, and **C**. Arrows indicate direction of growth and/or movement. Mesenchymal cell process meshwork (CPM) is exposed after removal of epithelium (**C**) and is illustrated to right side of **C**. Single mesenchymal cell body is outlined by broken line.

all three prominences contribute to the initial separation of the developing oral cavity and nasal pit (Fig. 2.7C). This separation is usually called the *primary palate* (Fig. 2.9A to C). The combined right and left maxillary prominences are sometimes called the intermaxillary segment.

The contacting epithelia form the epithelial seam. Before contact many of the surface epithelial (peridermal) cells are lost, and the underlying basal epithelial cells appear to actively participate in the contact phenomenon by forming processes that span the space between the contacting epithelia. During the fifth week of human embryonic development, a portion of the epithelial seam breaks down and the mesenchyme of the three prominences becomes confluent. Fluid accumulates between the cells of the persisting epithelium behind the point of epithelial breakdown. Eventually, these fluid-filled spaces coalesce to form the initial nasal passageway connecting the olfactory pit with the roof of the primitive oral cavity (Fig. 2.9). The tissue resulting from development and fusion of these prominences is termed the *primary palate* (outlined by broken lines in Fig. 2.9). It forms the roof of the anterior portion of the primitive oral cavity, as well as forming the initial separation between the oral and nasal cavities. In later development, derivatives of the primary palate form portions of the upper lip, anterior maxilla, and upper incisor teeth.

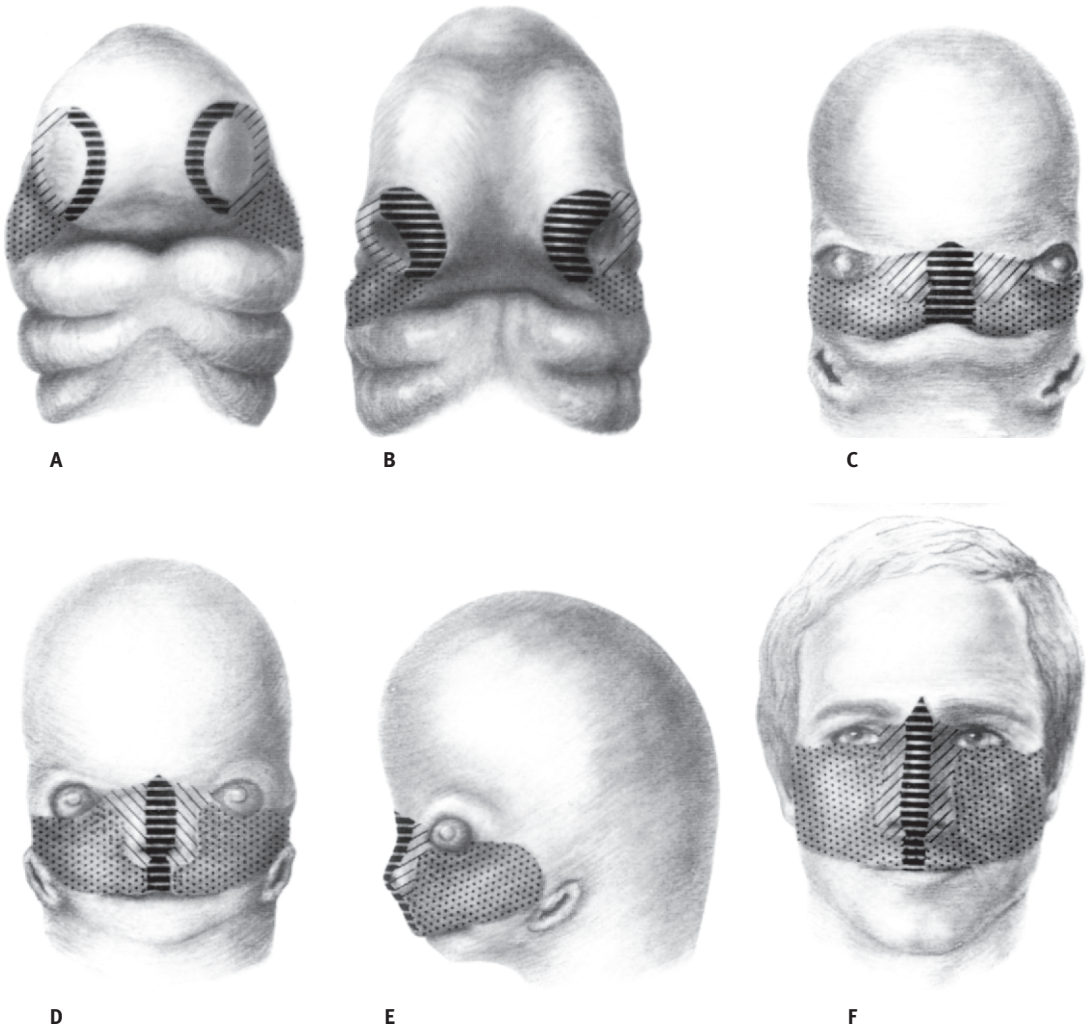

Figure 2.8 Schematic development of human face: maxillary prominence (stipple), lateral nasal prominence (oblique hatching), and medial nasal prominence (dark). (**A**) Embryo 4 to 6 mm in length, approximately 28 days. Prospective nasal and lateral nasal prominences are just beginning to form from mesenchyme surrounding olfactory placode. Maxillary prominence forming at proximal end of first (mandibular) arch under eye (compare to Fig. 2.3). (**B**) Embryo 8 to 11 mm in length, approximately 37 days. Medial nasal prominence is beginning to make contact with lateral nasal and maxillary prominences. (**C**) Embryo 16 to 18 mm in length, approximately 47 days. (**D**) and (**E**) Embryo 23 to 28 mm in length, approximately 54 days. (**F**) Adult face. Approximate derivatives of medial nasal prominence, lateral nasal prominence, and maxillary prominence are indicated.

The outlines of the developing external nose can be seen in Figure Although the nose is disproportionately large, the basic form is easily recognizable. Subsequent alterations in form lead to progressively more mature structure (Fig. 2.1, day 50 specimen). Figure 2.8 is a schematic illustration of the contribution of various facial prominences to the development of the external face.

Development of Maxillary Prominences and Secondary Palate

New outgrowths from the medial edges of the maxillary prominences form the shelves of the secondary palate. These palatal shelves grow downward beside the tongue (Fig. 2.10), at which time the tongue partially fills the nasal cavities. At about the ninth gestational week, the shelves elevate, make contact, and fuse with each other above the tongue (Fig. 2.11). In the anterior region, the shelves are brought to the horizontal position by a rotational (hinge-like) movement. In the more posterior regions, the shelves appear to alter their position by changing shape (remodeling) as well as by rotation. Available evidence indicates that the shelves are incapable of elevation until the tongue is first withdrawn from between them. Although the motivating force for shelf elevation is not clearly defined, contractile elements may be involved.

Fusion of palatal shelves requires alterations in the epithelium of the medial edges that begin prior to elevation. These alterations consist of cessation of cell division, which appears to be mediated through distinct underlying biochemical pathways, including a rise in cyclic AMP levels. There is also loss of some surface epithelial (peridermal) cells (Fig. 2.12) and production of extracellular surface substances, particularly glycoproteins, that appear to enhance adhesion between the shelf

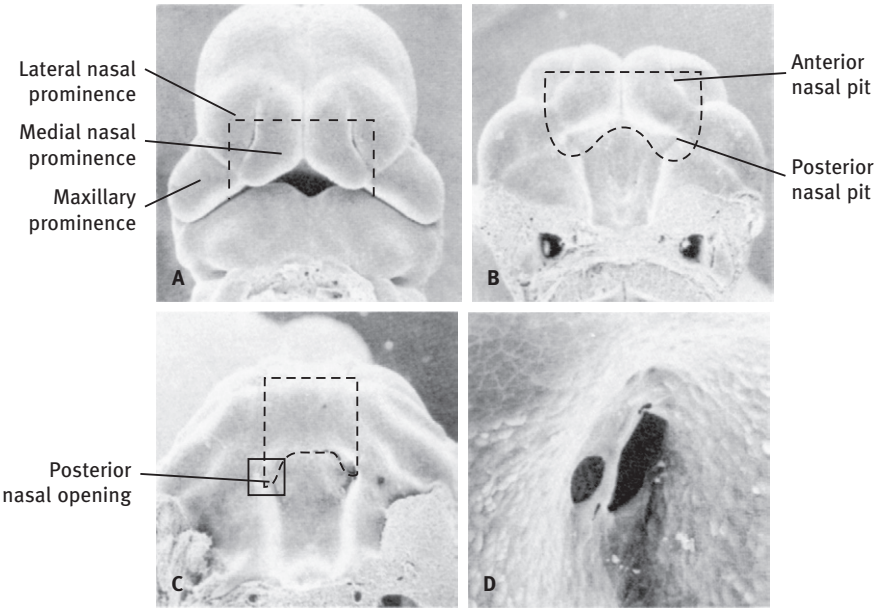

Figure 2.9 Some details of primary palate formation, here shown in mouse, are conveniently demonstrated by scanning electron micrographs (SEMs). Area encompassed by developing primary palate is outlined by broken lines. (**A**) and (**B**) Frontal and palatal views showing moderately advanced stage of primary palate formation. (**C**) and (**D**) In this more advanced stage, elimination of epithelial connection between anterior and posterior nasal pits is nearing completion. Area outlined by solid lines in **C** is given in **D**, showing that last epithelial elements are regressing as nasal passage is now almost completely opened.

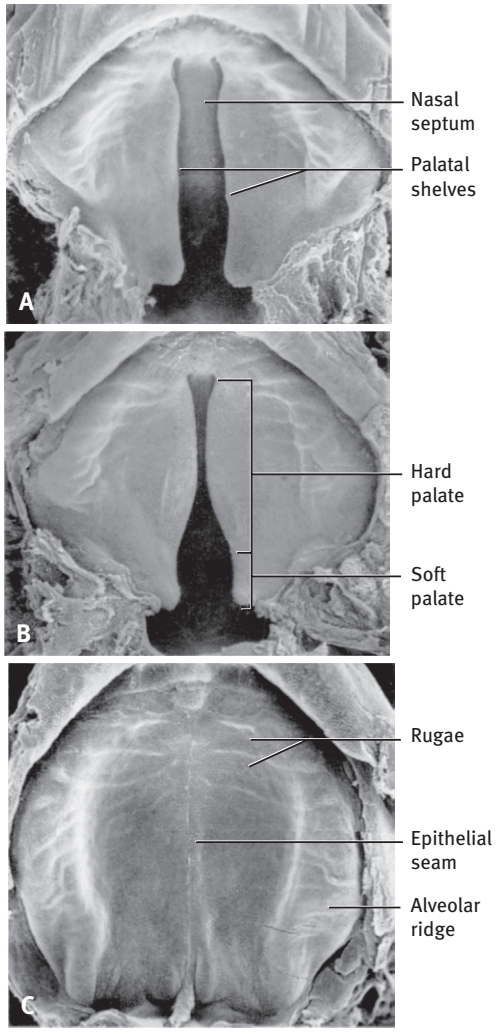

Figure 2.10 Scanning electron micrographs of developing human secondary palate. (**A**) Near completion of shelf elevation; (**B**) palatal shelves almost in contact; (**C**) contact between shelf edges has been made almost throughout entire length of hard and soft palate. Contacting epithelial seam rapidly disappears (see text) (From Russell MM: Comparative Morphogenesis of the Secondary Palate in Murine and Human Embryos, PhD thesis, University of North Carolina, 1986).

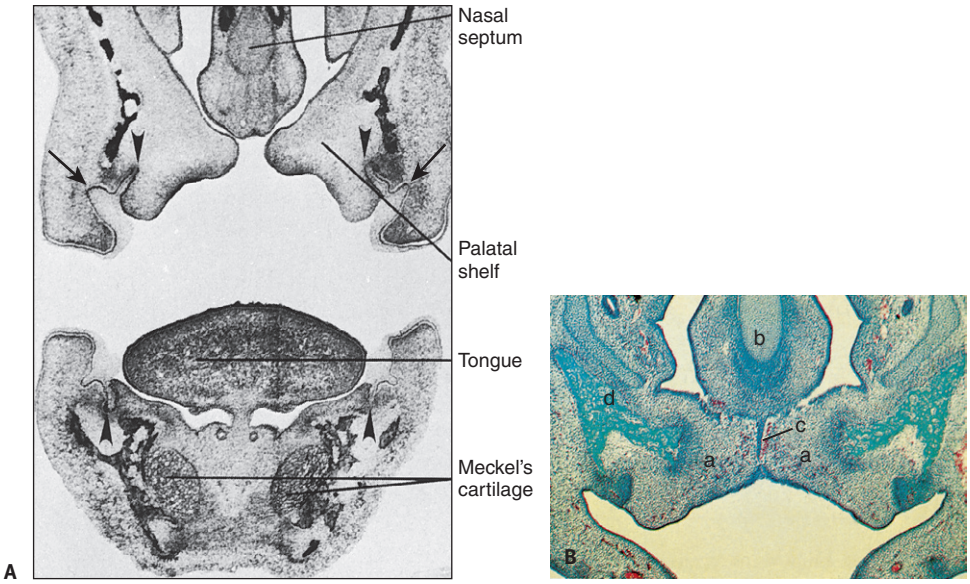

Figure 2.11 (**A**) Coronal section through secondary palates of 6 week old human embryo with arrowheads denoting the dental lamina and arrows denoting the vestibular lamina. (**B**) Coronal section through 8 week old human embryo showing contact of palatal shelves (a) and secondary nasal septum (b). Midline epitheliail seam (c) and developing Maxilla (d) are also seen (Masson trichrome X30).

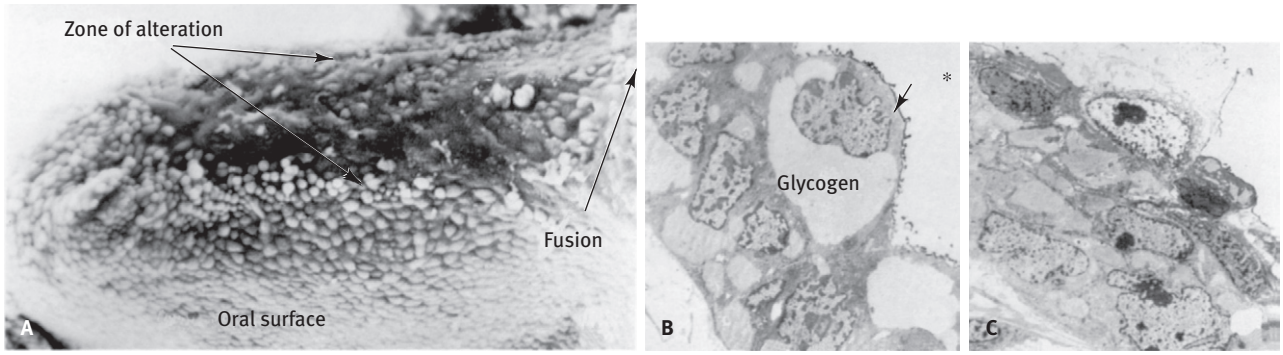

Figure 2.12 Scanning and transmission electron micrographs of palatal shelf of human embryo at same stage of development as reconstruction in Figure 2.9B. (**A**) Posterior region of palatal shelf viewed from below and from opposite side. Fusion will occur in 'zone of alteration,' where surface epithelial (peridermal) cells have been lost (see text). Transmission electron micrographs of specimen in **A**. Surface cells of oral epithelium in **B** contain large amounts of glycogen, whereas those of zone of alteration in **C** are undergoing degenerative changes and many of them are presumably desquamated into oral cavity fluids. Asterisk in B indicates heavy metal deposited on embryo surfaces for scanning electron microscope (**A** to **C** from Waterman RE and Meller SM: Anat Rec 180:11, 1974).

edges as well as between the shelves and inferior margin of the nasal septum (Fig. 2.11).

The ultimate fate of these remaining epithelial cells is controversial. Some of them appear to undergo cell death and eventually are phagocytized, but recent studies indicate that many undergo direct transformation in mesenchymal cells. The fate of cells in the epithelial seam of the primary palate described previously also is questionable. Some of the epithelial cells remain indefinitely in clusters (cell rests) along the fusion line. Eventually, most of the hard palate and all of the soft palate form from the secondary palate (see Chapter 8).

Development of Visceral Arches and Tongue

The pituitary gland develops as a result of inductive interactions between the ventral forebrain and oral ectoderm and is derived in part from both tissues (Fig. 2.13). Following initial crest cell migration (Fig. 2.7A), these cells invade the area of the developing pituitary gland and are continuous with cells that will later form the maxillary prominence. Eventually, crest cells form the connective tissue components of the gland.

In humans there is a total of six visceral arches, of which the fifth is rudimentary. These arches are also known as

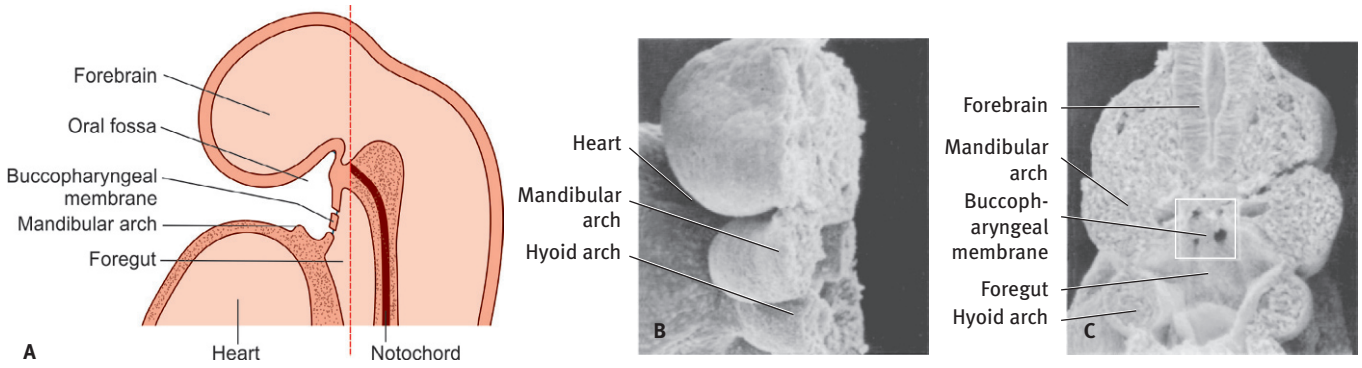

Figure 2.13 Oropharyngeal development. (**A**) Diagram of sagittal section through head of 3½- to 4-week-old human embryo. Oral fossa is separated from foregut by double layer of epithelium (buccopharyngeal membrane), which is in its early stages of breakdown. (**B**) and (**C**) Scanning electron micrographs (SEMs) of mouse head sectioned in plane indicated by broken line in **A**. **B** More lateral view of specimen while in **C** it is viewed from its posterior aspect. Rupturing buccopharyngeal membrane is outlined by rectangle in this figure.

pharyngeal or branchial arches. The gills (branchii) of the fish are modified to give rise to these arches. The proximal portion of the first (mandibular) arch becomes the maxillary prominence (Fig. 2.1). As the heart recedes caudally, the mandibular and hyoid arches develop further at their distal portions to become consolidated in the ventral midline (Figs. 2.7 and 2.13). As noted previously, the mesodermal core of each visceral arch (Fig. 2.7A) is concerned primarily with the formation of vascular endothelial cells. As noted below, these cells appear to be later replaced by cells that eventually form visceral arch myoblasts.

The first (mandibular) and second (hyoid) visceral arches undergo further developmental changes. As the heart recedes caudally, both arches send out bilateral processes that merge with their opposite members in the ventral midline (Fig. 2.7).

Nerve fibers from the fifth, seventh, ninth, and tenth cranial nerves extend into the mesoderm of the first four visceral arches. The mesoderm of the definitive mandibular and hyoid arches gives rise to the fifth and seventh nerve musculature, while mesoderm associated with the less well developed third and fourth arches forms the ninth and tenth nerve musculature. Recent studies show that myoblast cells in the visceral arches actually originate from mesoderm more closely associated with the neural tube (as do the cells that form the hypoglossal and extrinsic eye musculature; Fig. 2.6). They would then migrate into the visceral arches and replace the mesodermal cells that initiated blood vessel formation earlier. It therefore appears that myoblasts forming voluntary striated muscle fibers of the facial region would then originate from mesoderm *adjacent to the neural tube.*

Groups of visceral arch myoblasts that are destined to form individual muscles each take a branch of the appropriate visceral arch nerve. Myoblasts from the second visceral arch, for example, take branches of the seventh cranial nerve and migrate very extensively throughout the head and neck to form the contractile components of the 'muscles of facial expression.' Myoblasts from the first arch contribute mostly to the muscles of mastication, while those from the third and fourth arches contribute to the pharyngeal and soft palate musculature. As noted earlier, connective tissue components of each muscle in the facial region are provided by mesenchymal cells of crest origin.

The crest mesenchymal cells of the visceral arches give rise to skeletal components such as the temporary visceral arch cartilages (e.g. Meckel's cartilage; Fig. 2.11), middle ear cartilages, and mandibular bones. Also visceral arch crest cells form connective tissues such as dermis and the connective tissue components of the tongue.

The tongue forms in the ventral floor of the pharynx after arrival of the hypoglossal muscle cells. The significance of the lateral lingual tubercles (Fig. 2.14) and other swellings in the forming tongue has not been carefully documented. It is known that the anterior two thirds of the tongue is covered by ectoderm whereas endoderm covers the posterior one third. The thyroid gland forms by invagination of the most anterior endoderm (thyroglossal duct). A residual pit (the foramen cecum; Fig. 2.14C) left in the epithelium at the site of invagination marks the junction between the anterior two thirds and posterior one third of the tongue, which are, respectively, covered by epithelia of ectodermal and endodermal origin. It is also known that the connective tissue components of the anterior two thirds of the tongue are derived from first-arch mesenchyme, whereas those of the posterior one third appear to be primarily derived from the third-arch mesenchyme.

The epithelial components of a number of glands are derived from the endodermal lining of the pharynx. In addition to the thyroid, these include the parathyroid and thymus. The epithelial components of the salivary and anterior pituitary glands are derived from oral ectoderm.

Finally, a lateral extension from the inner groove between the first and second arch gives rise to the eustachian tube, which connects the pharynx with the ear. The external ear or pinna is formed at least partially from tissues of the first and second arches (Fig. 2.1, day 44) (Table 2.1).

FINAL DIFFERENTIATION OF FACIAL TISSUES

The extensive cell migrations referred to above bring cell populations into new relationships and lead to further inductive interactions, which, in turn, lead to progressively more differentiated cell types. For example, some of the crest cells coming into contact with pharyngeal

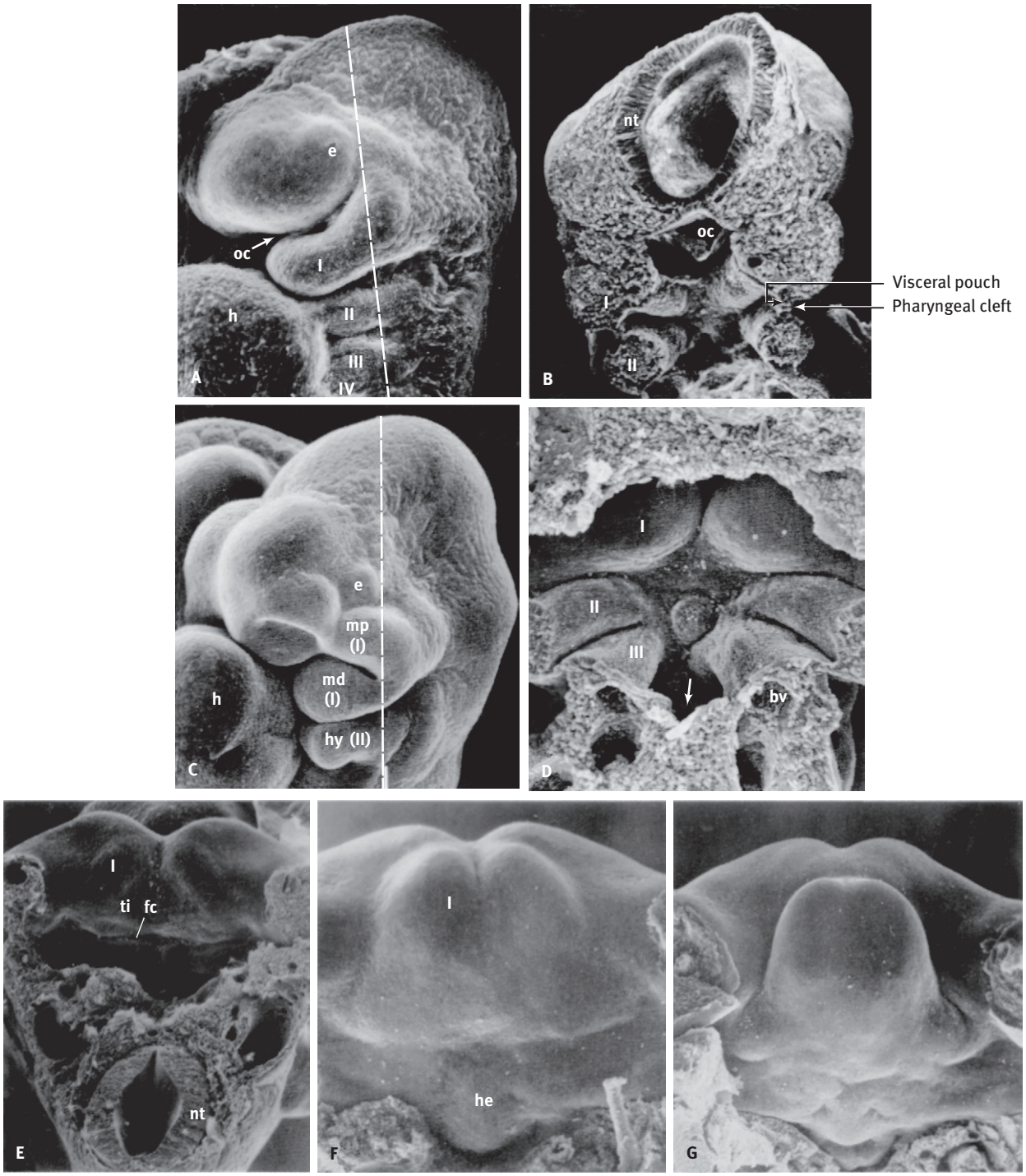

Figure 2.14 Scanning electron micrographs of developing visceral arches and tongue of mouse embryos. Planes of section illustrated in **A** and **C** (dorsal views of floor of pharynx) are shown in **B** and **D**. (**A**) and (**B**) Embryos whose developmental age is approximately equivalent to that of human 30-day-old embryos (see Fig. 2.1). Development of medial and lateral nasal prominences has yet to be initiated. Visceral arches are indicated by Roman numerals. First (mandibular) arch is almost separated from heart (h). Other structures indicated are eye (e), oral cavity (oc; compare to buccopharyngeal membrane in Fig. 2.15**C**), and neural tube (nt). (**C**)and (**D**) These are comparable to 35-day-old human embryos. The mandibular arch now has two distinct prominences, maxillary prominence (mp) and mandibular prominence (md). Second arch is called hyoid arch (hy). In **D** blood vessel exiting from third arch is labeled (bv). Arrow indicates entry into lower pharynx. (**E**)to (**G**) Older specimens, prepared in a manner similar to **B** and **D**, illustrate development of tongue. Lingual swellings (I) presumably represent accumulations of myoblasts derived from hypoglossal cord. Tuberculum impar (ti) also contributes to anterior two thirds of tongue. Foramen cecum (fc) is site of endodermal invagination that gives rise to epithelial components of thyroid gland. It lies at junction between anterior two thirds and posterior one third of tongue. Hypobranchial eminence (he) is primordium of epiglottis (From Johnston MC and Sulik KK: Embryology of the head and neck. In Serafin D, and Georgiade NG, editors: Pediatric plastic surgery, vol I, St Louis, 1984, The CV Mosby Co).

Table 2.1 Pharyngeal Arch Derivatives of First Three Arches

Arch	Muscles	Cartilage	Nerve Supply	Arterial Supply
First (mandibular arch)	Muscles of mastication Mylohyoid Anterior belly of digastric Tensor veli P & tensor tympani	Meckel's cartilage- Symphysis region of mandible Malleus, Incus , anterior ligament of malleus & Sphenomandibular ligament	Mandibular nerve (post-trematic nerve) supplies muscles of mastication Chorda tympani nerve (pre-trematic nerve)	Maxillary artery
Second (hyoid arch)	Muscles of facial expression Post belly of digastric Stylohyoid Stapedius	Reichert's cartilage: stapes, stylohyoid ligament, lesser cornu and upper half of body of hyoid bone Styloid process	Facial nerve	Stapedial artery
Third	Stylopharyngeus	Greater horn and lower part of body of hyoid bone	Glossopharyngeal	Common carotid and its terminal branches

Note:Total of six arches, fifth disappears.
From IV & VI arches laryngeal cartilages develop

endoderm are induced by the endoderm to form visceral arch cartilages (see Chapter 9). Recent studies indicate the early epithelial interactions are also involved in bone formation. The exact interactions involved in tooth formation are somewhat controversial. Mesenchymal cells of crest origin must be involved, and these cells form the dental papilla and the mesenchyme surrounding the epithelial enamel organ. Whether the epithelium or mesenchyme is initially responsible for determining which tooth (e.g. incisor or molar) forms from a tooth germ is controversial. Interestingly, epithelia from species that ceased forming teeth many centuries ago (e.g. the chick) can still form enamel under experimental conditions.

In many instances, such as those cited above, only crest mesenchymal cells and not mesodermal mesenchymal cells will respond to inducing tissues such as pharyngeal endoderm. In other cases, as in the differentiation of dermis and meninges, it appears that the origin of the mesenchyme is of no consequence. In any case it is clear that one function, the formation of skeletal and connective tissues, ordinarily performed by mesodermal cells in other regions, has been usurped by neural crest cells in the facial region. The crest cells therefore play a very dominant role in facial development, since they form all nonepithelial components except endothelial cells and the contractile elements of skeletal (voluntary) muscle.

The onset of bone formation or the establishment of all the organ systems (about the eighth week of development) is considered as the termination of the embryonic period. Bone formation and other aspects of the final differentiation of facial tissues will be considered in detail elsewhere in this text.

CLINICAL CONSIDERATIONS

Aberrations in embryonic facial development lead to a wide variety of defects. Although any step may be impaired, defects of primary and secondary palate development are most common. There is evidence that other developmental defects may be even more common but they are not compatible with completion of intrauterine life and are therefore not as well documented.

Facial Clefts

Most cases of clefts of the lip with or without associated cleft palate (Fig. 2.15) appear to form a group etiologically different from clefts involving only the secondary palate. For example, when more than one child in a family has facial clefts, the clefts are almost always found to belong only to one group.

Some evidence now indicates that there are two major etiologically and developmentally distinct types of cleft lips and palate. In the larger group, deficient medial nasal prominences appear to be the major developmental alteration, whereas in the smaller group the major developmental alteration appears to be underdevelopment of the maxillary prominence. Increases in clefting rates have been associated with children born to epileptic mothers undergoing phenytoin (Dilantin) therapy and to mothers who smoke cigarettes; in the latter case the embryonic effects are thought to result from hypoxia. When pregnant mice are exposed to hypoxia, the portion of the olfactory placode undergoing morphogenetic movements (Fig. 2.1) breaks down, and this is associated with underdevelopment of the lateral nasal prominence. Reduction in the size of the lateral nasal prominence that is more severe than that of other facial prominences also has been observed in an animal model of phenytoin-induced cleft lip and palate. Combination of developmental alterations (e.g. placodal breakdown associated with medial nasal prominence deficiency) may relate to the multifactorial etiology thought to be responsible for many human cleft cases.

About two thirds of patients with clefts of the primary palate also have clefts of the secondary palate. Studies of experimental animals suggest that excessive separation of jaw segments as a result of the primary palate cleft prevents the palatal shelves from contacting after elevation. The degree of clefting is highly variable. Clefts may

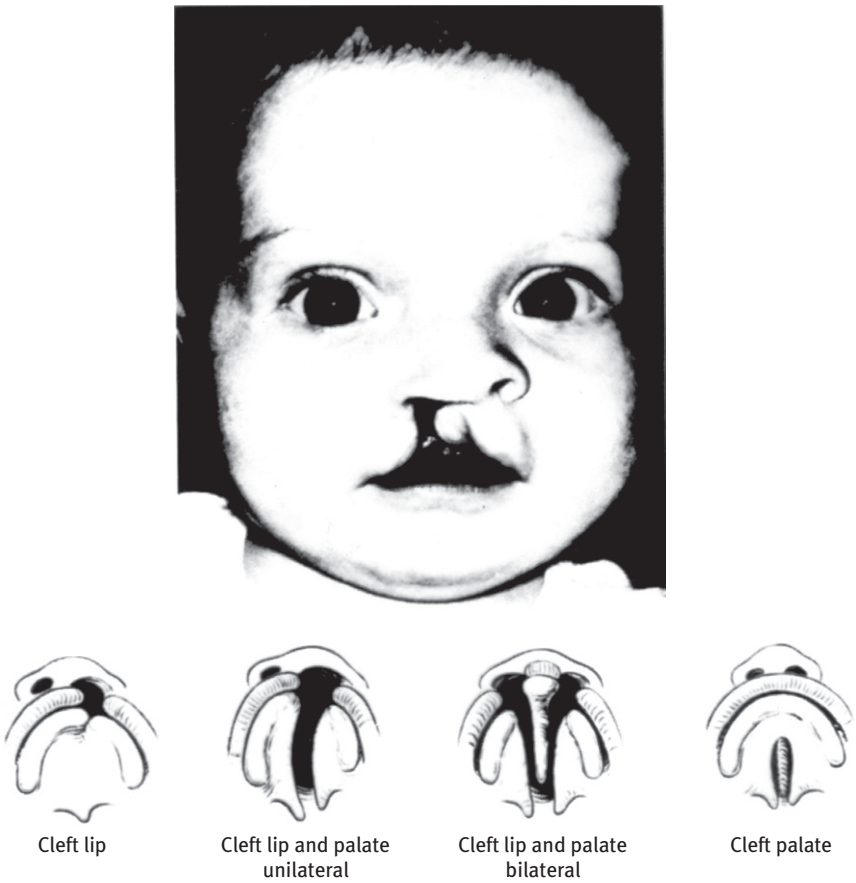

Figure 2.15 Clefts of lip and palate in infants. Infant in photograph has complete unilateral cleft of lip and palate (From Ross RB, and Johnston MC: Cleft lip and palate, Baltimore, 1972, The Williams & Wilkins Co).

be either bilateral or unilateral (Fig. 2.15) and complete or incomplete. Most of this variation results from differing degrees of fusion and may be explained by variable degrees of mesenchyme in the facial prominences. Some of the variations may represent different initiating events.

Clefts involving only the secondary palate (cleft palate, Fig. 2.15) constitute, after clefts involving the primary palate, the second most frequent facial malformation in humans. Cleft palate can also be produced in experimental animals with a wide variety of chemical agents or other manipulations affecting the embryo. Usually, such agents retard or prevent shelf elevation. In other cases, however, it is shelf growth that is retarded so that, although elevation occurs, the shelves are too small to make contact. There is also some evidence that indicates that failure of the epithelial seam or failure of it to be replaced by mesenchyme occurs after the application of some environmental agents. Cleft formation could then result from rupture of the persisting seam, which would not have sufficient strength to prevent such rupture indefinitely.

Less frequently, other types of facial clefting are observed. In most instances they can be explained by failure of fusion or merging between facial prominences of reduced size, and similar clefts can be produced experimentally. Examples include failure of merging and fusion between the maxillary prominence and the lateral nasal prominence, leading to oblique facial clefts, or failure of merging of the maxillary prominence and mandibular arch, leading to lateral facial clefts (macrostomia). Many of the variations in the position or degree of these rare facial clefts may depend on the timing or position of arrest of growth of the maxillary prominence that normally merges and fuses with adjacent structures (Fig. 2.8). Other rare facial malformations (including oblique facial clefts) may also result from abnormal pressures or fusions with folds in the fetal (e.g. amniotic) membranes.

Also new evidence regarding the apparent role of epithelial–mesenchymal interactions via the mesenchymal cell process meshwork (CPM) may help to explain the frequent association between facial abnormalities, especially clefts and limb defects. Genetic and/or environmental influences on this interaction might well affect both areas in the same individual.

Hemifacial Microsomia

The term ‘hemifacial microsomia’ is used to describe malformations involving underdevelopment and other abnormalities of the temporomandibular joint, the external and middle ear, and other structures in this region, such as the parotid gland and muscles of mastication. Substantial numbers of cases have associated malformations of the

vertebrae and clefts of the lip and/or palate. The combination with vertebral anomalies is often considered to denote a distinct etiologic syndrome (oculoauriculovertebral syndrome, etc). As a group these malformations constitute the third most common group of major craniofacial malformations, after the two major groups of facial clefts.

Somewhat similar malformations have resulted from inadvertent use of the acne drug retinoic acid (Accutane) in pregnant women. Animal models using this drug have produced very similar malformations, many of which appear to result from major effects on neural crest cells. This has resulted in re-evaluation of an earlier animal model that indicated that the malformation resulted from hemorrhage at the point where the external carotid artery fuses with the stapedial artery (Fig. 2.5). It now appears probable that at least some aspects of many hemifacial microsomia cases result from primary effects on crest cells. Malformations similar to hemifacial microsomia occurred in the fetuses of women who had taken the drug thalidomide.

Treacher Collins' Syndrome

Treacher Collins' syndrome (mandibulofacial dysostosis) is an inherited disorder that results from the action of a dominant gene and may be almost as common as hemifacial microsomia. The syndrome consists of underdevelopment of the tissues derived from the maxillary, mandibular, and hyoid prominences. The external, middle, and inner ear are often defective, and clefts of the secondary palate are found in about one third of the cases. Defects of a similar nature result from the action of an abnormal gene in mice and can also be produced experimentally with excessive doses of retinoic acid (Accutane) administered at a later stage in development. Here, the primary effect appears to be on ganglionic placodal cells (Fig. 2.4). Although not limited to placodal cells of the massive trigeminal ganglion, most of the characteristic alterations in development appear to result from secondary effects on crest cells in this area.

Labial Pits

Small pits may persist on either side of the midline of the lower lip. They are caused by the failure of the embryonic labial pits to disappear.

Lingual Anomalies

Median rhomboid glossitis, an innocuous, red, rhomboidal smooth zone of the tongue in the midline in front of the foramen cecum, is considered the result of persistence of the tuberculum impar. Lack of fusion between the two lateral lingual prominences may produce a bifid tongue. Thyroid tissue may be present in the base of the tongue.

Developmental Cysts

Epithelial rests in lines of union, of facial or oral prominences or from epithelial organs, (e.g. *vestigial nasopalatine ducts*) may give rise to cysts lined with epithelium.

Branchial cleft (cervical) cysts or fistulas may arise from the rests of epithelium in the visceral arch area. They usually are laterally disposed on the neck. Thyroglossal duct cysts may occur at any place along the course of the duct, usually at or near the midline.

Cysts may arise from epithelial rests after the fusion of medial, maxillary, and lateral nasal prominences. They are called globulomaxillary cysts and are lined with pseudostratified columnar epithelium and squamous epithelium. They may, however, develop as primordial cysts from a supernumerary tooth germ.

Anterior palatine cysts are situated in the midline of the maxillary alveolar prominence. Once believed to be from remnants of the fusion of two prominences, they may be primordial cysts of odontogenic origin; their true nature is a subject of discussion.

Nasolabial cysts, originating in the base of the wing of the nose and bulging into the nasal and oral vestibule and the root of the upper lip, sometimes causing a flat depression on the anterior surface of the alveolar prominence, are also explained as originating from epithelial remnants in the cleft-lip line. It is, however, more probable that they derive from excessive epithelial proliferations that normally, for some time in embryonic life, plug the nostrils. It is also possible that they are retention cysts of vestibular nasal glands or that they develop from the epithelium of the nasolacrimal duct.

The malformations in the development of head may indicate the defective formations in the heart as the spiral septum, which divides the conus cordis and truncus arteriosus, is derived from neural crest cells.

SUMMARY

Early Development of the Fetus

The cleavage or cell division is one of the effects of the fertilization of the ovum. Morula is formed following series of cell divisions. The outer cell mass (trophoblast cells) of the morula differentiates into the structures that nourish the embryo. Most of the inner cell mass (embryoblast) differentiates into the embryo. The initial, two layered (epiblast and hypoblast) embryonic disk is converted into three layered disk. This happens by the proliferation and migration of primitive streak cells into the region between ectoderm and endoderm, except over the region of prechordal plate that has only two layers. The primitive streak is the result of proliferation of the cells of epiblast (the later ectoderm). The cells from the cranial part of the primitive streak known as *primitive knot*, migrate in the midline between ectoderm and endoderm up to the prechordal plate giving rise to the notochord. The notochordal cells induce the overlying ectoderm to form neural plate that forms neural groove with neural crest cells at its edges. Interaction between the cells causes the mesodermal cells differentiate into paraxial, intermediate, and lateral plate of mesoderm. The paraxial mesodermal

cells give rise to somites into which dermatome (dermis), myotome (muscles), and sclerotome (bones) are differentiated.

Neural Crest Cells

The neural crest cells are multipotent cells. They give rise to variety of cells like odontoblasts, melanocytes, ganglia, suprarenal medulla, parafollicular cells of thyroid gland, connective tissue, and blood vessels of head and neck region, conotruncal septum that results in the formation of ascending aorta and pulmonary trunk, etc. The enamel organ develops from the ectoderm. The ectomesenchyme consists of neural crest cells and mesodermal cells. The migration of sufficient number of neural crest cells is essential for the normal growth of head region.

Development of Pharyngeal Arches

The foldings of embryo, craniocaudal and lateral foldings, alter the positions of developing head such that it lies cranial to the cardiac bulge with stomodeum (primitive mouth) between them. The gradual appearance of pharyngeal (branchial) arches contributes to the development of the face and neck. In each arch a skeletal element, artery, muscles supplied by the nerve of that arch is formed. Ectodermal clefts and endodermal pouches thus formed between the arches give rise to various structures.

Development of Face

The facial prominences, namely, frontonasal, maxillary, and mandibular, gives rise to the formation of the face. The olfactory placodes are formed in the frontonasal process as a result of ectodermal proliferation. Olfactory epithelium is derived from the placodes. Medial and lateral nasal prominences make the olfactory placodes to occupy the depth of the nasal pits which form nasal sacs. The fusion of the prominences bounding the stomodeum results in the formation of the face.

Derivatives of Pharyngeal Arches

Mesodermal proliferation adjoining the primitive pharynx gives rise to pharyngeal arches. Out of six arches found, the fifth one disappears soon. The first pharyngeal arch (mandibular arch) mesoderm gives rise to the muscles of mastication, mylohyoid, anterior belly of digastric, tensor veli palatini and tensor tympani muscles. All these are supplied by the post-trematic nerve of the arch, the mandibular nerve. The pre-trematric nerve of this arch is the chorda tympani nerve. The Meckel's cartilage of the arch gives rise to malleus, incus, anterior ligament of malleus, sphenomandibular ligament and small part of mandible near the chin. The artery of the arch forms a part of maxillary artery.

The second pharyngeal arch (hyoid arch) mesoderm gives rise to the muscles of facial expression, posterior belly of digastric, stylohyoid muscle and stapedius muscle. These muscles are supplied by the nerve of the second arch, the facial nerve. The cartilage of this arch, the Reichert's cartilage, gives rise to stapes, stylohyoid ligament, lesser cornu and upper half of body of hyoid bone. The artery of this arch forms the stapedial artery.

In the third pharyngeal arch mesoderm, stylopharyngeus muscle is formed and is supplied by the glossopharyngeal nerve. The lower part of body and greater horn of the hyoid bone are formed in the cartilage of this arch. The common carotid artery and parts of its terminal branches are formed from the artery of this arch.

From the mesoderm of the fourth and sixth pharyngeal arches cartilages of the larynx are formed. The superior laryngeal and recurrent laryngeal nerves are the nerves of these arches respectively.

Of the pharyngeal clefts (ectodermal) the first one gives rise to external acoustic meatus and the remaining get submerged deep to the caudally growing second arch. The cervical sinus found deep to the second arch may persist abnormally with its opening along the line of anterior border of the sternocleidomastoid muscle.

From the first pharyngeal pouch auditory tube and middle ear cavity are formed; the intra-tonsillar cleft is the remnant of the second pouch; the third pouch gives rise to the inferior parathyroid gland and the thymus; the fourth pouch gives rise to superior parathyroid gland. The parafollicular cells of the thyroid gland develop from the ultimobranchial body.

Development of the Tongue

The tongue is the result of fusion of tuberculum impar, the lingual swellings (first arch) and cranial part of the hypobranchial eminence (third and fourth arches). This fusion is seen as 'V' shaped sulcus terminalis on the tongue. The sensory nerve supply thus can be correlated with its development. The muscles of the tongue are formed in the occipital myotomes with their hypoglossal nerve.

Development of the Palate

The palate is formed by the union of primary and secondary palates, the former being formed by the frontonasal process and the latter by palatal process of maxillary prominences.

Clinical Considerations

Aberration in facial development leads to a wide variety of defects of which cleft lip and palate and anomalies of the tongue are more common than others.

Cleft lip and palate may be due to a combination of genetic and environmental factors. These have been observed in pregnant mothers who smoke cigarettes and in those who take drugs like phenytoin. In experimental animals deficient medial or lateral nasal process or underdevelopment of maxillary process causes clefting. Cleft lip may be unilateral or bilateral and may be associated with cleft palate. Rarely oblique facial cleft due to failure of fusion of maxillary process with lateral nasal process and lateral facial cleft due to failure of fusion of maxillary prominence and mandibular arch occurs.

Experimentally retinoic acid affects neural crest cells leading to malformation of external and middle ear as seen in hemifacial microsomia. These anomalies in association with cleft palate are seen in Treacher Collins' syndrome.

Persistence of tuberculum impar is said to cause median rhomboid glossitis. Failure of fusion of lateral lingual prominence leads to bifid tongue. Thyroid tissue may persist at the base of the tongue. Rarely labial pits may be seen.

Remnants of epithelial cells in the line of fusion of facial or oral prominence proliferate and give rise to cysts like branchial cleft cyst (in the neck), anterior palatine cyst and nasolabial cyst.

REVIEW QUESTIONS

1. What is the role of the notochord?
2. What are the derivatives of the neural crest cells?
3. What are the muscular, skeletal, nerve, and arterial elements formed in each pharyngeal arch?
4. What is the fate of pharyngeal clefts and pouches?
5. How can the correlation between the nerve supply and development of the tongue be made?

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3 Development and Growth of Teeth

CHAPTER CONTENTS

The primitive oral cavity, or stomodeum, is lined by stratified squamous epithelium called the oral ectoderm or primitive oral epithelium. The oral ectoderm contacts the endoderm of the foregut to form the buccopharyngeal membrane. At about the twenty-seventh day of gestation this membrane ruptures and the primitive oral cavity establishes a connection with the foregut. Most of the connective tissue cells underlying the oral ectoderm are of neural crest or ectomesenchyme in origin. These cells are thought to instruct or induce the overlying ectoderm to start tooth development, which begins in the anterior portion of what will be the future maxilla and mandible and proceeds posteriorly (see Chapter 2 for more details on embryonic induction).

DENTAL LAMINA

Two or three weeks after the rupture of the buccopharyngeal membrane, when the embryo is about 6 weeks old, certain areas of basal cells of the oral ectoderm proliferate more rapidly than do the cells of the adjacent areas. This leads to the formation of the *Primary epithelial band* which is a band of epithelium that has invaded the underlying ectomesenchyme along each of the horseshoe-shaped future dental arches (Figs 3.1A, and 3.3). At about 7th week the primary epithelial band divides into an inner (lingual) process called *Dental lamina* and an outer (buccal) process called *Vestibular lamina*. The dental laminae serve as the primordium for the ectodermal portion of the deciduous teeth. Later, during the development of the jaws, the permanent molars arise directly from a distal extension of the dental lamina.

The development of the first permanent molar is initiated at the fourth month in utero. The second molar is initiated at about the first year after birth, the third molar at the fourth or fifth years. The distal proliferation of the dental lamina is responsible for the location of the germs of the permanent molars in the ramus of the mandible and the tuberosity of the maxilla. The successors of the deciduous teeth develop from a lingual extension of the free end of the dental lamina opposite to the enamel organ of each deciduous tooth (Fig. 3.2C). The lingual extension of the dental lamina is named the successional lamina and develops from the fifth month in utero (permanent central incisor) to the tenth month of age (second premolar).

Fate of Dental Lamina

It is evident that the total activity of the dental lamina extends over a period of at least 5 years.

Any particular portion of the dental lamina functions for a much briefer period since only a relatively short time elapses after initiation of tooth development before the dental lamina begins to degenerate at that particular

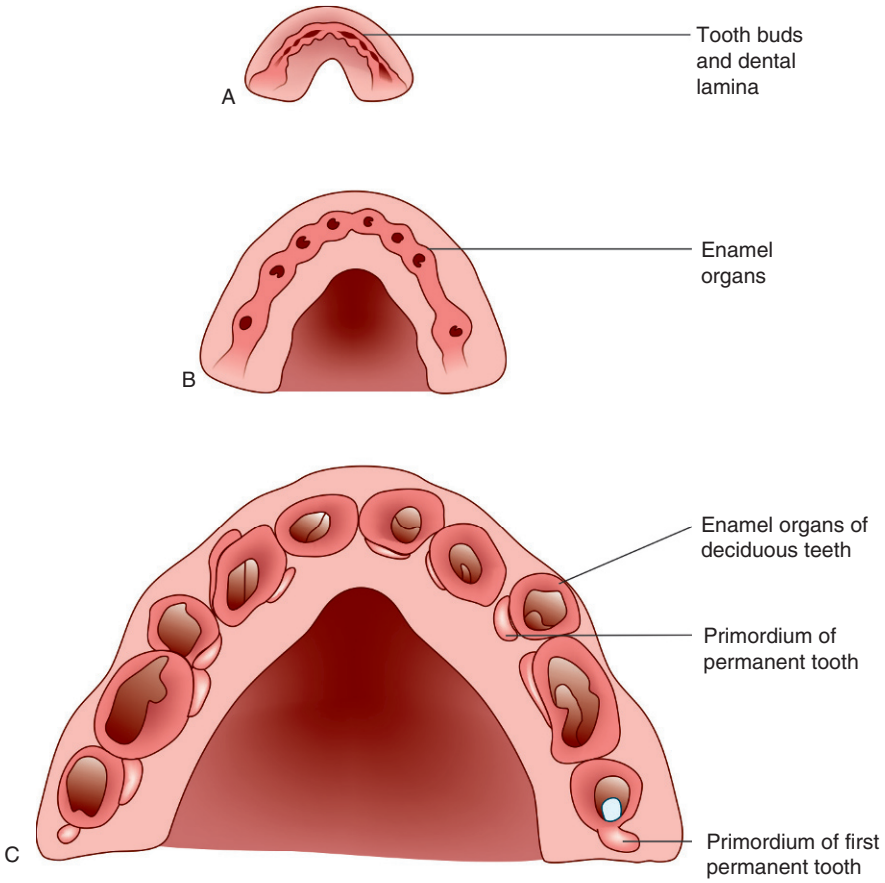


Figure 3.1 Diagrammatic reconstruction of dental lamina and enamel organs of mandible. (**A**) 22 mm embryo, bud stage (8th week). (**B**) 43 mm embryo, cap stage (10th week). (**C**) 163 mm embryo, bell stage (about 4 months). Primordia of permanent teeth are seen as thickenings of dental lamina on lingual side of each tooth germ. Distal extension of dental lamina with primordium of first molar.

location. However, the dental lamina may still be active in the third molar region after it has disappeared elsewhere, except for occasional epithelial remnants. As the teeth continue to develop, they lose their connection with the dental lamina. They later break up by mesenchymal invasion, which is at first incomplete and does not perforate the total thickness of the lamina. Remnants of the dental lamina persist as epithelial pearls or islands within the jaw as well as in the gingiva. These are referred to as cell *rest of Serres*.

Vestibular Lamina

Labial and buccal to the dental lamina in each dental arch, another epithelial thickening develops independently and somewhat later. It is the vestibular lamina, also termed the lip furrow band (Figs 3.6 and 3.7). It subsequently hollows and forms the oral vestibule between the alveolar portion of the jaws and the lips and cheeks (Figs 3.10 and 3.11; Flowchart 3.1).

TOOTH DEVELOPMENT

At certain points along the dental lamina, each representing the location of one of the 10 mandibular and 10 maxillary deciduous teeth, the ectodermal cells multiply still more rapidly and form little knobs that grow into the underlying mesenchyme (Figs 3.2 and 3.4). Each of these little downgrowths from the dental lamina represents the beginning of the enamel organ of the tooth bud of a deciduous tooth. Not all of these enamel organs start to develop at the same time, and the first to appear are those of the anterior mandibular region.

As cell proliferation continues, each enamel organ increases in size, sinks deeper into the ectomesenchyme and due to differential growth changes its shape. As it develops, it takes on a shape that resembles a cap, with an outer convex surface facing the oral cavity and an inner concavity (Figs 3.5 and 3.7).

On the inside of the cap (i.e. inside the depression of the enamel organ), the ectomesenchymal cells increase in number. The tissue appears more dense than the surrounding mesenchyme and represents the beginning of the *dental papilla*. Surrounding the combined enamel organ and dental papilla, the third part of the tooth bud forms. It is the *dental sac* or *dental follicle*, and it consists of ectomesenchymal cells and fibers that surround the dental papilla and the enamel organ (Fig. 3.8). Thus the tooth germ consists of the ectodermal component—the enamel organ and the ectomesenchymal components—the dental papilla and the dental follicle. The tooth and

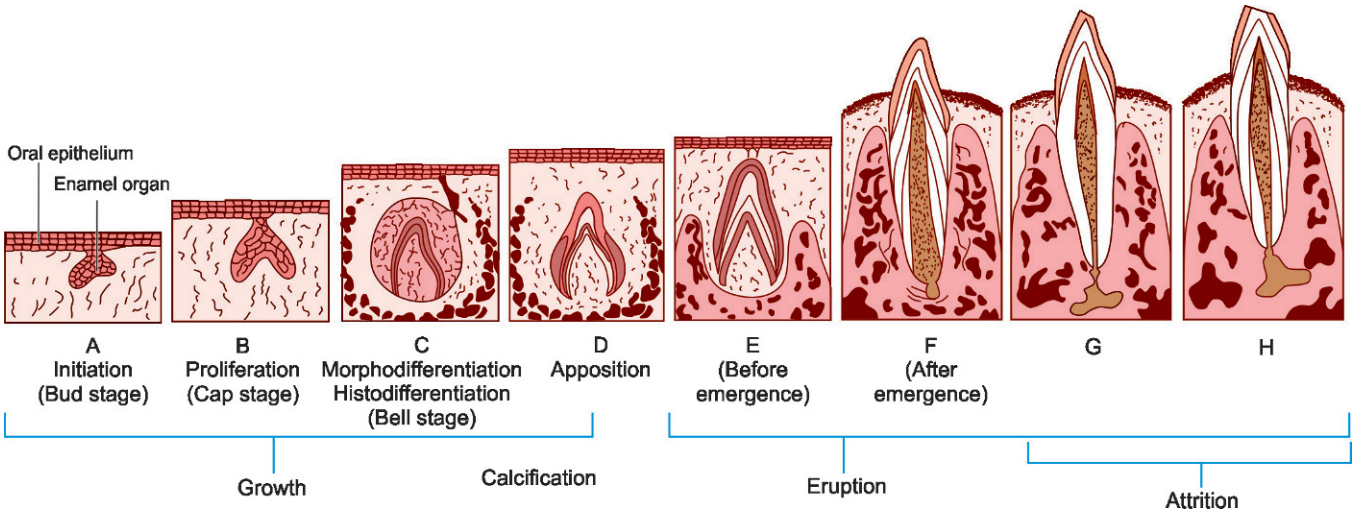
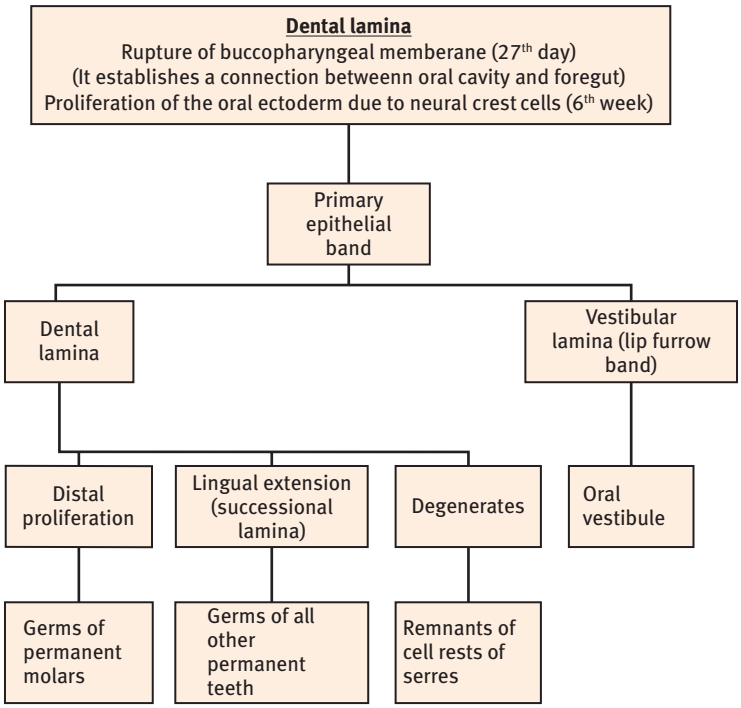


Figure 3.2 Diagram of life cycle of tooth (Modified from Schour I and Massler M: J Am Dent Assoc 27:1785, 1940).



Flowchart 3.1 Development of dental lamina.

its supporting structures are formed from the tooth germ. The enamel is formed from the enamel organ, the dentin and pulp from the dental papilla and the supporting tissues namely the cementum, periodontal ligament and the alveolar bone from the dental follicle.

During and after these developments, the shape of the enamel organ continues to change. The depression occupied by the dental papilla deepens until the enamel organ assumes a shape resembling a bell. As this development takes place, the dental lamina, which had thus far connected the enamel organ to the oral epithelium, becomes longer and thinner and finally breaks up and the tooth bud loses its connection with the epithelium of the primitive oral cavity.

Development of tooth results from interaction of the epithelium derived from the first arch and ectomesenchymal cells derived from the neural crest cells. Up to 12 days the first arch epithelium retains the ability to form tooth like structures when combined with neural crest cells of other regions. Afterwards this potential is lost but transferred to neural crest cells as revealed in various recombination experiments of first arch ectomesenchyme with various epithelia to produce tooth like structures. Like any other organ development in our

body numerous and complex gene expression occurs to control the development process through molecular signals. In odontogenesis, many of the genes involved or the molecular signals directed by them are common to other developing organs like kidney and lung or structures like the limb. Experimental studies to understand the genetic control and molecular signaling have been done on mice as it is amenable for genetic manipulations like to produce 'knock-out mice' 'or null mice.'

DEVELOPMENTAL STAGES

Although tooth development is a continuous process, the developmental history of a tooth is divided into several morphologic 'stages' for descriptive purposes. While the size and shape of individual teeth are different, they pass through similar stages of development. They are named after the shape of the enamel organ (epithelial part of the tooth germ), and are called the bud, cap, and bell stages (Fig. 3.2A to C).

Bud Stage

The epithelium of the dental laminae is separated from the underlying ectomesenchyme by a basement membrane (Fig. 3.3). Simultaneous with the differentiation of each dental lamina, round or ovoid swellings arise from the basement membrane at 10 different points, corresponding to the future positions of the deciduous teeth. These are the primordia of the enamel organs, the tooth buds (Fig. 3.4). Thus the development of tooth germs is initiated, and the cells continue to proliferate faster than adjacent cells. The dental lamina is shallow, and microscopic sections often show tooth buds close to the oral epithelium. Since the main function of certain epithelial cells of the tooth bud is to form the tooth enamel, these cells constitute the enamel organ, which is critical to normal tooth development. In the bud stage, the enamel organ consists of peripherally located low columnar cells and centrally located polygonal cells (Fig. 3.4). Many cells of the tooth bud and the surrounding mesenchyme undergo mitosis (Fig. 3.4). As a result of the increased mitotic activity and the migration of neural crest cells into the area the ectomesenchymal cells surrounding the tooth bud condense. The area of ectomesenchymal condensation immediately subjacent to the enamel organ is the dental papilla. The condensed ectomesenchyme that surrounds the tooth bud and the dental papilla is the dental sac (Figs 3.6 to 3.8). Both the dental papilla and the dental sac become more well defined as the enamel organ grows into the cap and bell shapes (Fig. 3.8).

Cap Stage

As the tooth bud continues to proliferate, it does not expand uniformly into a larger sphere. Instead, unequal growth in different parts of the tooth bud leads to the cap stage, which is characterized by a shallow invagination on the deep surface of the bud (Figs 3.2B and 3.5).

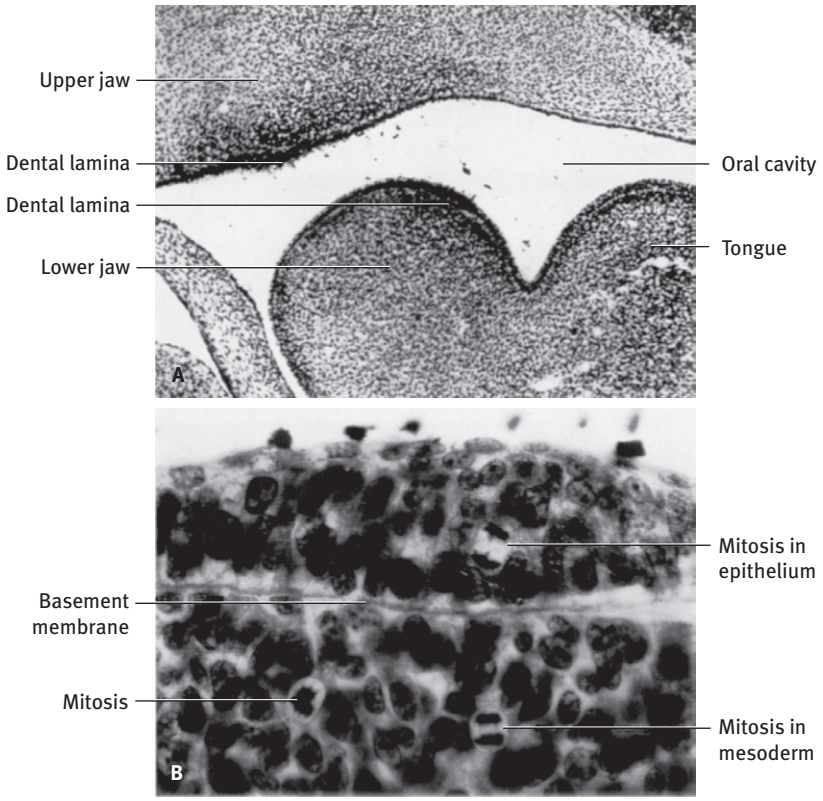


Figure 3.3 Initiation of tooth development.Human embryo 13.5 mm in length, 5th week. (**A**) Sagittal section through upper and lower jaws. (**B**) High magnification of thickened oral epithelium (From Orban B: Dental histology and embryology, Philadelphia, 1929, P Blakiston's Son & Co).

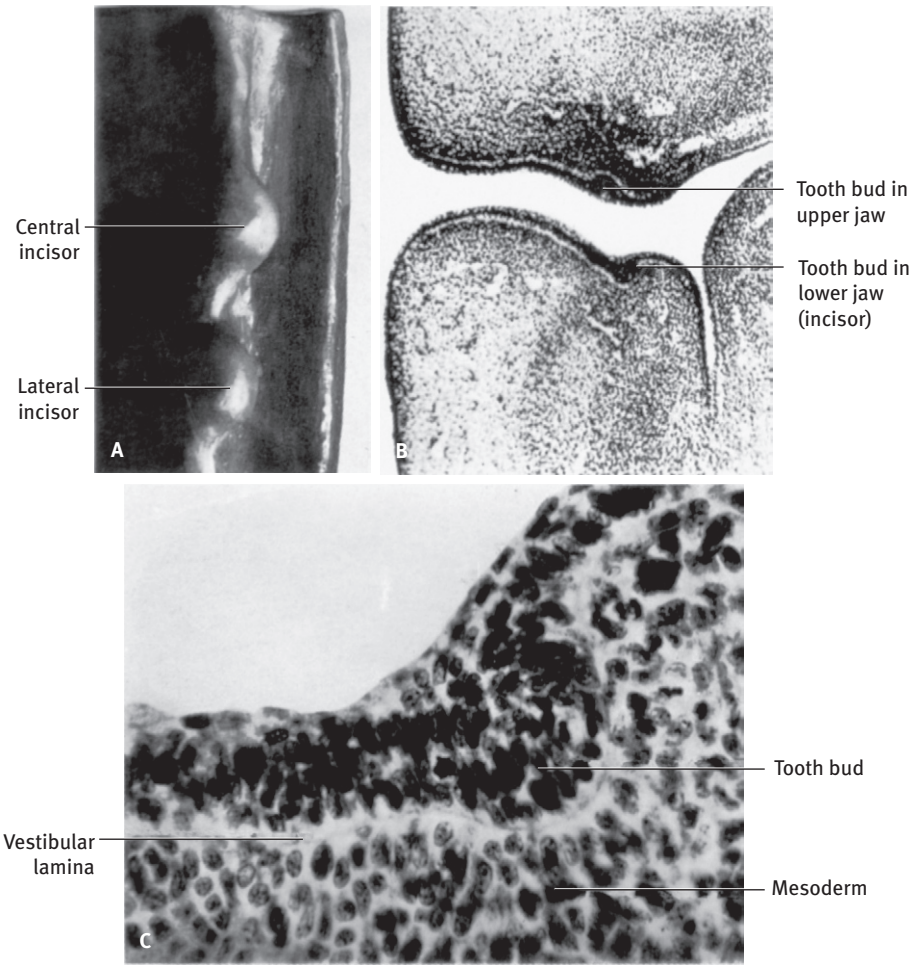


Figure 3.4 Bud stage of tooth development, proliferation stage. Human embryo 16 mm in length, 6th week. (**A**) Wax reconstruction of germs of lower central and lateral incisors. (**B**) Sagittal section through upper and lower jaws. (**C**) High magnification of tooth germ of lower incisor in bud stage (From Orban B: Dental histology and embryology, Philadelphia, 1929, P Blakiston's Son & Co).

Outer and inner enamel epithelium

The peripheral cells of the cap stage are cuboidal, cover the convexity of the 'cap,' and are called the outer enamel (dental) epithelium. The cells in the concavity of the 'cap' become tall, columnar cells and represent the inner enamel (dental) epithelium (Figs 3.6 and 3.7). The outer enamel epithelium is separated from the dental sac, and the inner enamel epithelium from the dental papilla, by a delicate basement membrane. Hemidesmosomes anchor the cells to the basal lamina. The enamel organ may be seen to have a double attachment of dental lamina to the overlying oral epithelium enclosing ectomesenchyme called enamel niche between them. This appearance is due to a funnel-shaped depression of the dental lamina.

Stellate reticulum

Polygonal cells located in the center of the epithelial enamel organ, between the outer and inner enamel epithelia, begin to separate due to water being drawn into the enamel organ from the surrounding dental papilla as a result of osmotic force exerted by glycosaminoglycans contained in the ground substance. As a result the polygonal cells become star shaped but maintain contact with each other by their cytoplasmic process. As these star-shaped cells form a cellular network, they are called the *stellate reticulum* (Figs 3.8, 3.9). This gives the stellate reticulum a cushion like consistency and acts as a shock absorber that may support and protect the delicate enamel-forming cells.

The cells in the center of the enamel organ are densely packed and form the *enamel knot* (Fig. 3.5). This knot projects in part toward the underlying dental papilla, so that the center of the epithelial invagination shows a slightly knob-like enlargement that is bordered by the labial and lingual enamel grooves (Fig. 3.5). At the same time a vertical extension of the enamel knot, called the *enamel cord* occurs (Fig. 3.8). When the enamel cord extends to meet the outer enamel epithelium it is termed as *enamel septum*, for it would divide the stellate reticulum into two parts. The outer enamel epithelium at the point of meeting shows a small depression and this is termed *enamel navel* as it resembles the umbilicus. These are temporary structures (transitory structures) that disappear before enamel formation begins. The function of the enamel knot and cord may act as a reservoir of dividing cells for the growing enamel organ. Recent studies have shown that enamel knot acts as a signaling center as

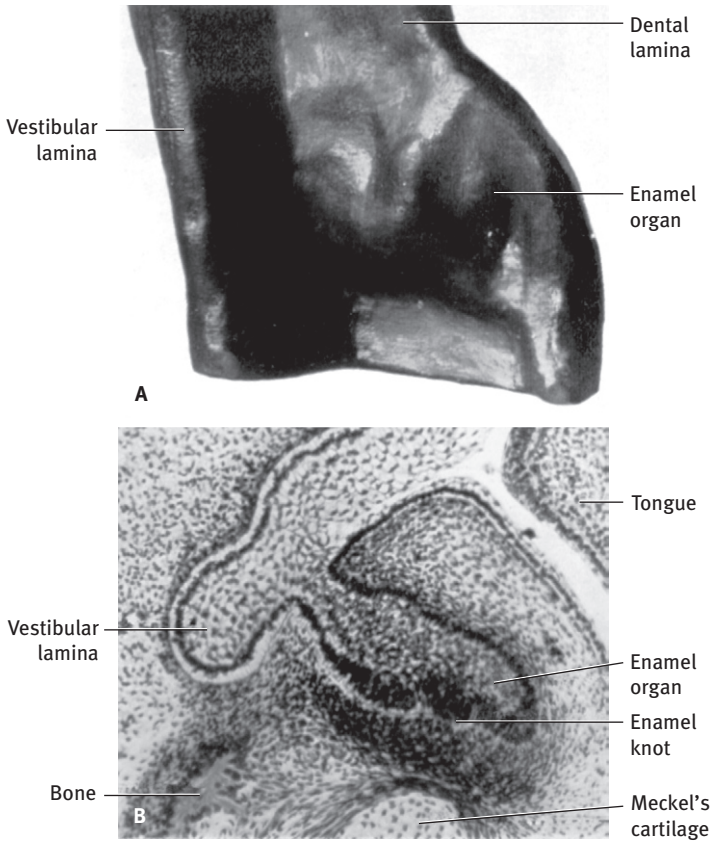


Figure 3.5 Cap stage of tooth development. Human embryo 31.5 mm in length, 9th week. (**A**) Wax reconstruction of enamel organ of lower lateral incisor. (**B**) Labiolingual section through same tooth (From Orban B: Dental histology and embryology, Philadelphia, 1929, P Blakiston's Son & Co).

many important growth factors are expressed by the cells of the enamel knot and thus they play an important part in determining the shape of the tooth. These are discussed in detail in the section on molecular insights in tooth morphogenesis.

Dental papilla

Under the organizing influence of the proliferating epithelium of the enamel organ, the ectomesenchyme (neural crest cells) that is partially enclosed by the invaginated portion of the inner enamel epithelium proliferates. It condenses to form the dental papilla, which is the formative organ of the dentin and the primordium of the pulp (Figs 3.5 and 3.6). The changes in the dental papilla occur concomitantly with the development of the epithelial enamel organ. Although the epithelium exerts a dominating influence over the adjacent connective tissue, the condensation of the latter is not a passive crowding by the proliferating epithelium. The dental papilla shows active budding of capillaries and mitotic figures, and its peripheral cells adjacent to the inner enamel epithelium enlarge and later differentiate into the odontoblasts.

Dental sac (dental follicle)

Concomitant with the development of the enamel organ and the dental papilla, there is a marginal condensation in the ectomesenchyme surrounding the enamel organ and dental papilla. Gradually, in this zone, a denser and more fibrous layer develops, which is the primitive dental sac.

Bell Stage

As the invagination of the epithelium deepens and its margins continue to grow, the enamel organ assumes a bell shape (Figs 3.2C, 3.8). In the bell stage, crown shape is determined. It was thought that the shape of the crown is due to the pressure exerted by the growing dental papilla cells on the inner enamel epithelium. This pressure however was shown to be opposed equally by the pressure exerted by the fluid present in the stellate reticulum. The folding of enamel organ to cause different crown shapes is shown to be due to differential rates of mitosis and differences in cell differentiation time. Cells begin to differentiate only when cells cease to divide. The inner enamel epithelial cells which lie in the future cusp tip or incisor region stop dividing earlier and begin to differentiate first. The pressure exerted by the continuous cell division on these differentiating cells from other areas of the enamel organ cause these cells to be pushed out into the enamel organ in the form of a cusp tip. The cells in another future cusp area begin to differentiate, and by a similar process results in a cusp tip form. The area between two cusp tips, i.e. the cuspal slopes extent and therefore of cusp height are due to cell

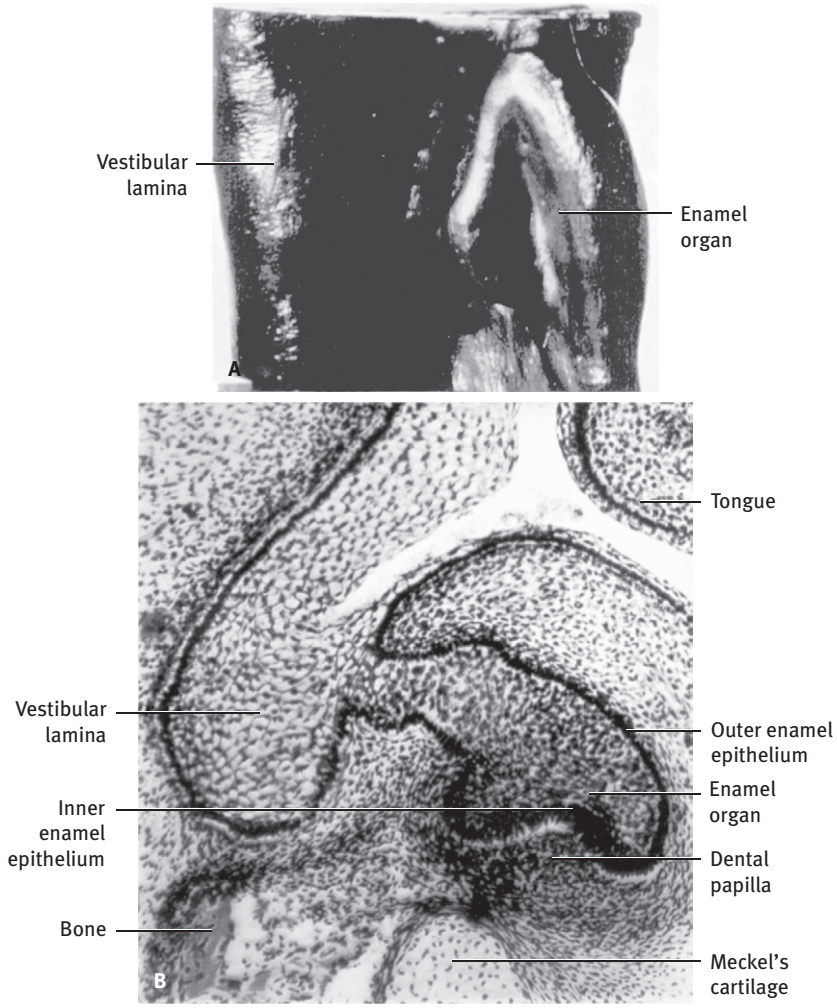


Figure 3.6 Cap stage of tooth development. Human embryo 41.5 mm in length, 10th week. (**A**) Wax reconstruction of enamel organ of lower central incisor. (**B**) Labiolingual section through same tooth (From Orban B: Dental histology and embryology, Philadelphia, 1929, P Blakiston's Son & Co).

proliferation and differentiation occurring gradually from cusp tips to the depth of the sulcus. Cell differentiation also proceeds gradually cervically, those at the cervix are last to differentiate. The determination of crown shape (tooth morphogenesis) is under the control of genes and their signaling molecules and growth factors. These have been dealt in detail in the section on molecular insights in tooth morphogenesis.

Four different types of epithelial cells can be distinguished on light microscopic examination of the bell stage of the enamel organ. The cells form the inner enamel epithelium, the stratum intermedium, the stellate reticulum, and the outer enamel epithelium. The junction between inner and outer enamel epithelium is called *cervical loop* and it is an area of intense mitotic activity.

Inner enamel epithelium

The inner enamel epithelium consists of a single layer of cells that differentiate prior to amelogenesis into tall columnar cells called ameloblasts (Figs 3.8 and 3.9). These cells are 4 to 5 micrometers (μm) in diameter and about 40 μm high. These elongated cells are attached to one another by junctional complexeslaterally and to cells in the stratum intermedium by desmosomes (Fig. 3.9). The fine structure of inner enamel epithelium and ameloblasts is described in Chapter 4.

The cells of the inner enamel epithelium exert an organizing influence on the underlying mesenchymal cells in the dental papilla, which later differentiate into odontoblasts.

Stratum intermedium

A few layers of squamous cells form the stratum intermedium between the inner enamel epithelium and the stellate reticulum (Fig. 3.9). These cells are closely attached by desmosomes and gap junctions. Desmosomal junctions are also observed between cells of stratum intermedium, stellate reticulum and inner enamel epithelium. The well-developed cytoplasmic organelles, acid mucopolysaccharides, and glycogen deposits indicate a high degree of metabolic activity. Also the cells of this layer are associated with high activity of alkaline phosphatase. The cells of stratum intermedium work synergistically with cells of inner enamel epithelium as a single functional unit and form enamel. It is absent in the part of the tooth germ that outlines the root portions of the tooth which does not form enamel.

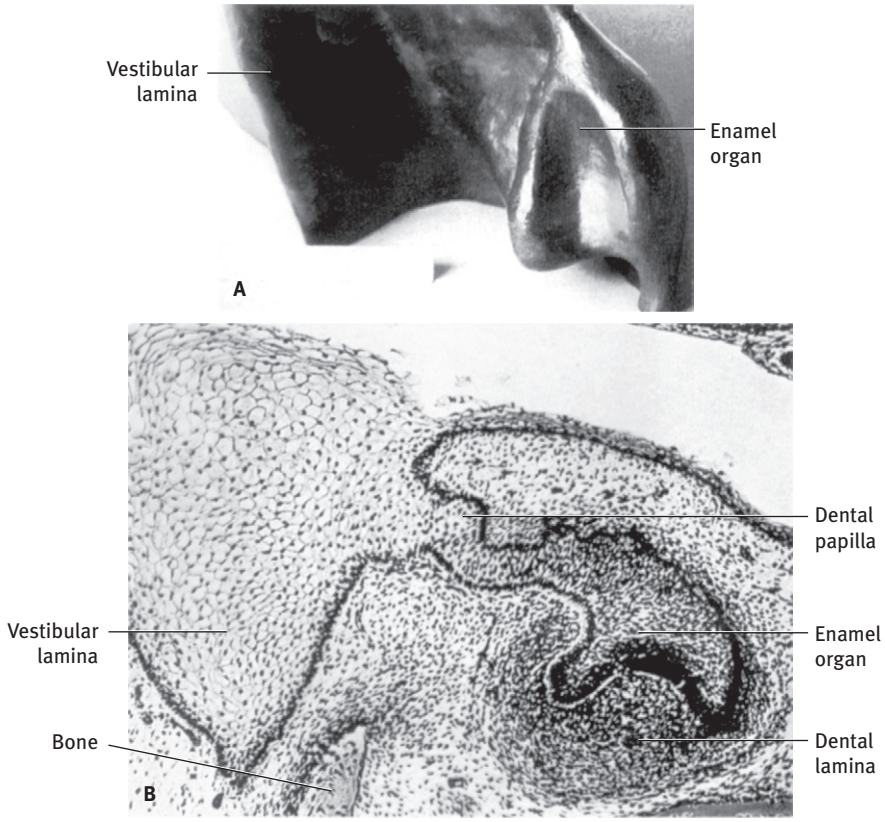


Figure 3.7 Cap stage of tooth development. Human embryo 60 mm in length, 11th week. (**A**) Wax reconstruction of enamel organ of lower lateral incisor. (**B**) Labiolingual section through same tooth (From Orban B: Dental histology and embryology, Philadelphia, 1929, P Blakiston's Son & Co).

Stellate reticulum

The stellate reticulum expands further, mainly by an increase in the amount of intercellular fluid. The cells are star shaped, with long processes that anastomose with those of adjacent cells (Fig. 3.9). Desmosomal junctions are observed between cells of stellate reticulum, stratum intermedium and outer enamel epithelium. Before enamel formation begins, the stellate reticulum collapses, reducing the distance between the centrally situated ameloblasts and the nutrient capillaries near the outer enamel epithelium. Its cells then are hardly distinguishable from those of the stratum intermedium. This change begins at the height of the cusp or the incisal edge and progresses cervically (see Fig. 4.37).

Outer enamel epithelium

The cells of the outer enamel epithelium flatten to a low cuboidal form. At the end of the bell stage, preparatory to and during the formation of enamel, the formerly smooth surface of the outer enamel epithelium is laid in folds. Between the folds the adjacent mesenchyme of the dental sac forms papillae that contain capillary loops and thus provide a rich nutritional supply for the intense metabolic activity of the avascular enamel organ. This would adequately compensate the loss of nutritional supply from dental papilla owing to the formation of mineralized dentin.

Dental lamina

The dental lamina is seen to extend lingually and is termed successional dental lamina as it gives rise to enamel organs of permanent successors of deciduous teeth (permanent incisors, canines and premolars—Figs 3.10, 3.11). The enamel organs of deciduous teeth in the bell stage show successional lamina and their permanent successor teeth in the bud stage.

Dental papilla

The dental papilla is enclosed in the invaginated portion of the enamel organ. Before the inner enamel epithelium begins to produce enamel, the peripheral cells of the mesenchymal dental papilla differentiate into odontoblasts under the organizing influence of the epithelium. First, they assume a cuboidal form; later they assume a columnar form and acquire the specific potential to produce dentin. The dental papilla ultimately gives rise to dental pulp, once the dentin formation begins at the cuspal tip of the bell stage tooth germ.

The basement membrane that separates the enamel organ and the dental papilla just prior to dentin formation is called the *membrana preformativa*.

Dental sac

Before formation of dental tissues begins, the dental sac shows a circular arrangement of its fibers and resembles a capsular structure. With the development of the root,

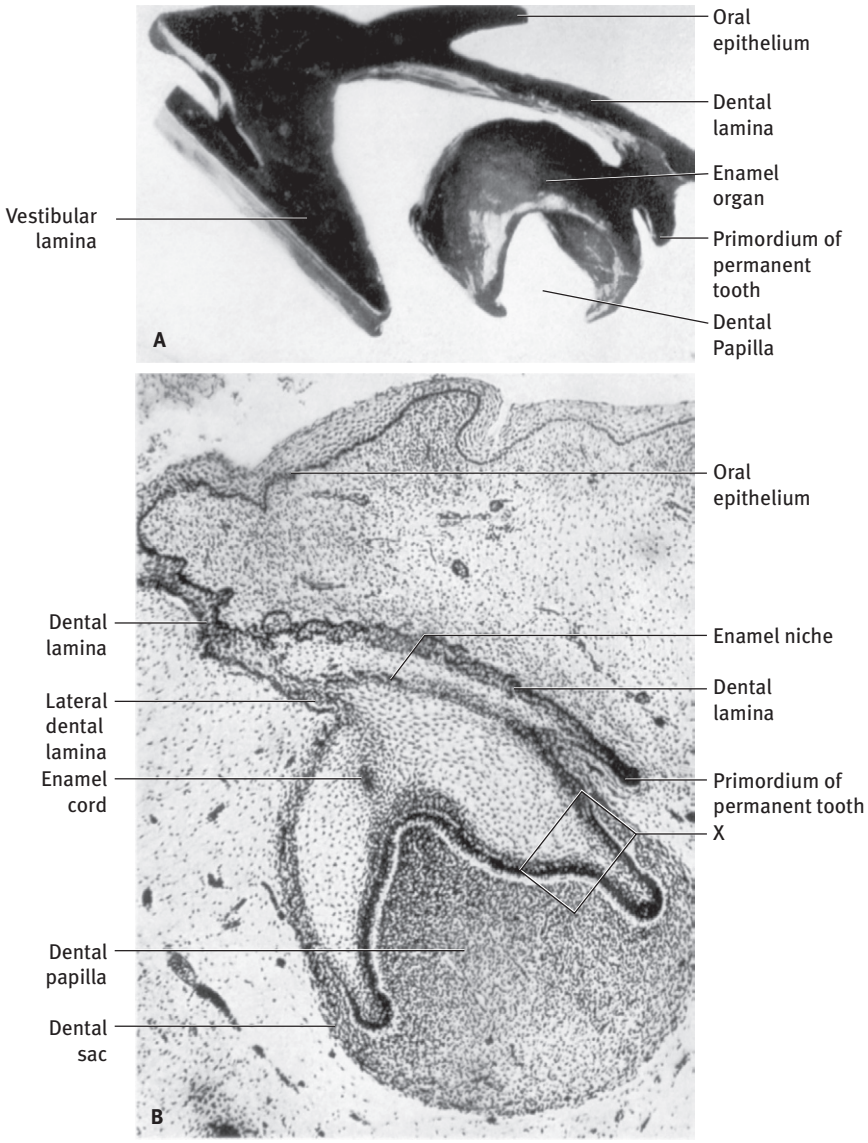


Figure 3.8 Bell stage of tooth development. Human embryo 105 mm in length, 14th week. (**A**) Wax reconstruction of lower central incisor. (**B**) Labiolingual section of the same tooth. X, See Fig. 3.9 (From Orban B: Dental histology and embryology, Philadelphia, 1929, P Blakiston's Son & Co).

the fibers of the dental sac differentiate into the periodontal fibers that become embedded in the developing cementum and alveolar bone.

Advanced bell stage

This stage is characterized by the commencement of mineralization and root formation. During the advanced bell stage, the boundary between inner enamel epithelium and odontoblasts outlines the future dentinoenamel junction (Figs 3.8, 3.10). The formation of dentin occurs first as a layer along the future dentinoenamel junction in the region of future cusps and proceeds pulpally and apically. After the first layer of dentin is formed, the ameloblast which has already differentiated from inner enamel epithelial cells lay down enamel over the dentin in the future incisal and cuspal areas. The enamel formation then proceeds coronally and cervically, in all regions from the dentinoenamel junction (DEJ) towards the surface. In addition, the cervical portion of the enamel organ gives rise to the epithelial root sheath of Hertwig. The Hertwig's epithelial root sheath (HERS) outlines the future root and is thus responsible for the shape, length, size, and number of roots (Table 3.1).

Hertwig's Epithelial Root Sheath and Root Formation

The development of the roots begins after enamel and dentin formation has reached the future cementoenamel junction.

The enamel organ plays an important part in root development by forming HERS, which molds the shape of the roots and initiates radicular dentin formation. Hertwig's root sheath consists of the outer and inner enamel epithelia only, and therefore it does not include the stratum

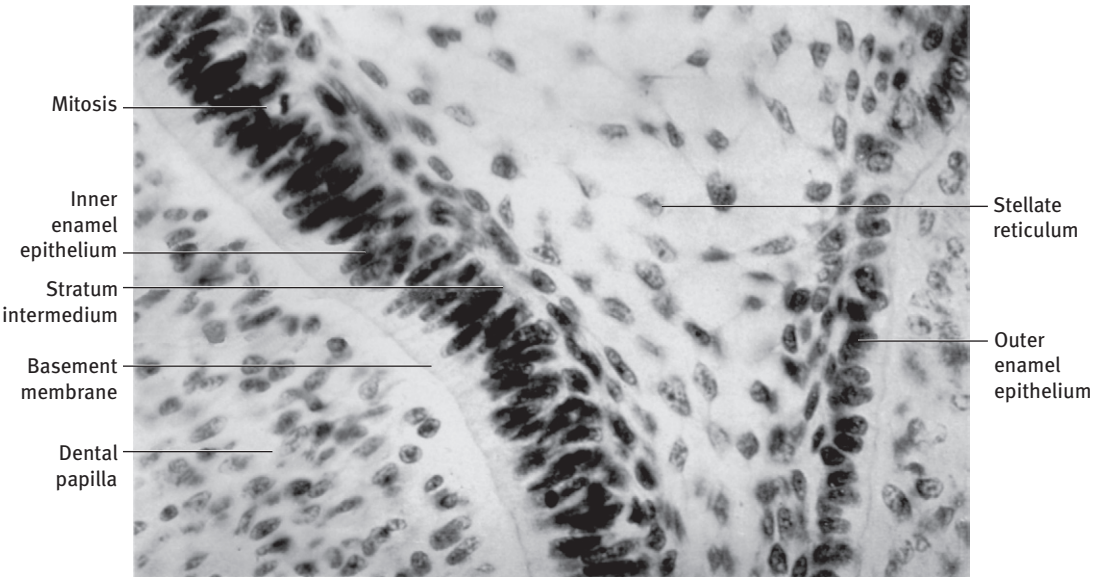


Figure 3.9 Layers of epithelial enamel organ at high magnification. Area X of Figure 3.8.

intermedium and stellate reticulum. The cells of the inner layer remain short and normally do not produce enamel. When these cells have induced the differentiation of radicular dental papilla cells into odontoblasts and the first layer of dentin has been laid down, the epithelial root sheath loses its structural continuity and its close relation to the surface of the root. Its remnants persist as an epithelial network of strands or clumps near the external surface of the root. These epithelial remnants are found in the periodontal ligament of erupted teeth and are called *rests of Malassez* (see Chapter 8).

There is a pronounced difference in the development of HERS in teeth with one root and in those with two or more roots. Prior to the beginning of root formation, the root sheath forms the epithelial diaphragm (Fig. 3.12). The outer and inner enamel epithelia bend at the future cementoenamel junction into a horizontal plane, narrowing the wide cervical opening of the tooth germ. The plane of the diaphragm remains relatively fixed during the development and growth of the root. The proliferation of the cells of the epithelial diaphragm is accompanied by proliferation of the cells of the connective tissue of the pulp, which occurs in the area adjacent to the diaphragm. The free end of the diaphragm does not grow into the connective tissue, but the epithelium proliferates coronal to the epithelial diaphragm (Fig. 3.12B). The differentiation of odontoblasts and the formation of dentin follow the lengthening of the root sheath. At the same time the connective tissue of the dental sac surrounding the root sheath proliferates and invades the continuous double epithelial layer (Fig. 3.12C) dividing it into a network of epithelial strands (Fig. 3.12D). The epithelium is moved away from the surface of the dentin so that connective tissue cells come into contact with the outer surface of the dentin and differentiate into cementoblasts that deposit a layer of cementum onto the surface of the dentin. The rapid sequence of proliferation and destruction of Hertwig's root sheath explains the fact that it cannot be seen as a continuous layer on the surface of the developing root (Figs 3.12D and 3.14). In the last stages of root development, the proliferation of the epithelium in the diaphragm lags behind that of the pulpal connective tissue. The wide apical foramen is reduced first to the width of the diaphragmatic opening itself and later is further narrowed by apposition of dentin and cementum to the apex of the root.

Differential growth of the epithelial diaphragm in multi-rooted teeth causes the division of the root trunk into two or three roots. During the general growth of the enamel organ the expansion of its cervical opening occurs in such a way that long tongue like extensions of the horizontal diaphragm develop (Fig. 3.13). Two such extensions are found in the germs of lower molars and three in the germs of upper molars. Before division of the root trunk occurs, the free ends of these horizontal epithelial flaps grow toward each other and fuse. The single cervical opening of the coronal enamel organ is then divided into two or three openings. On the pulpal surface of the dividing epithelial bridges, dentin formation starts (Fig. 3.14A), and on the periphery of each opening, root development follows in the same way as described for single-rooted teeth (Fig. 3.14B).

If cells of the epithelial root sheath remain adherent to the dentin surface, they may differentiate into fully functioning ameloblasts and produce enamel. Such droplets of enamel, called *enamel pearls*, are sometimes found in the area of furcation of the roots of permanent molars. If the continuity of HERS is broken or is not established prior to dentin formation, a defect in the dentinal wall of the pulp ensues. Such defects are found in the pulpal floor corresponding to the furcation or on any point of the root itself if the fusion of the horizontal extensions of the diaphragm remains incomplete. This accounts for the development of accessory root canals opening on the periodontal surface of the root (Flowchart 3.2) (see Chapter 6).

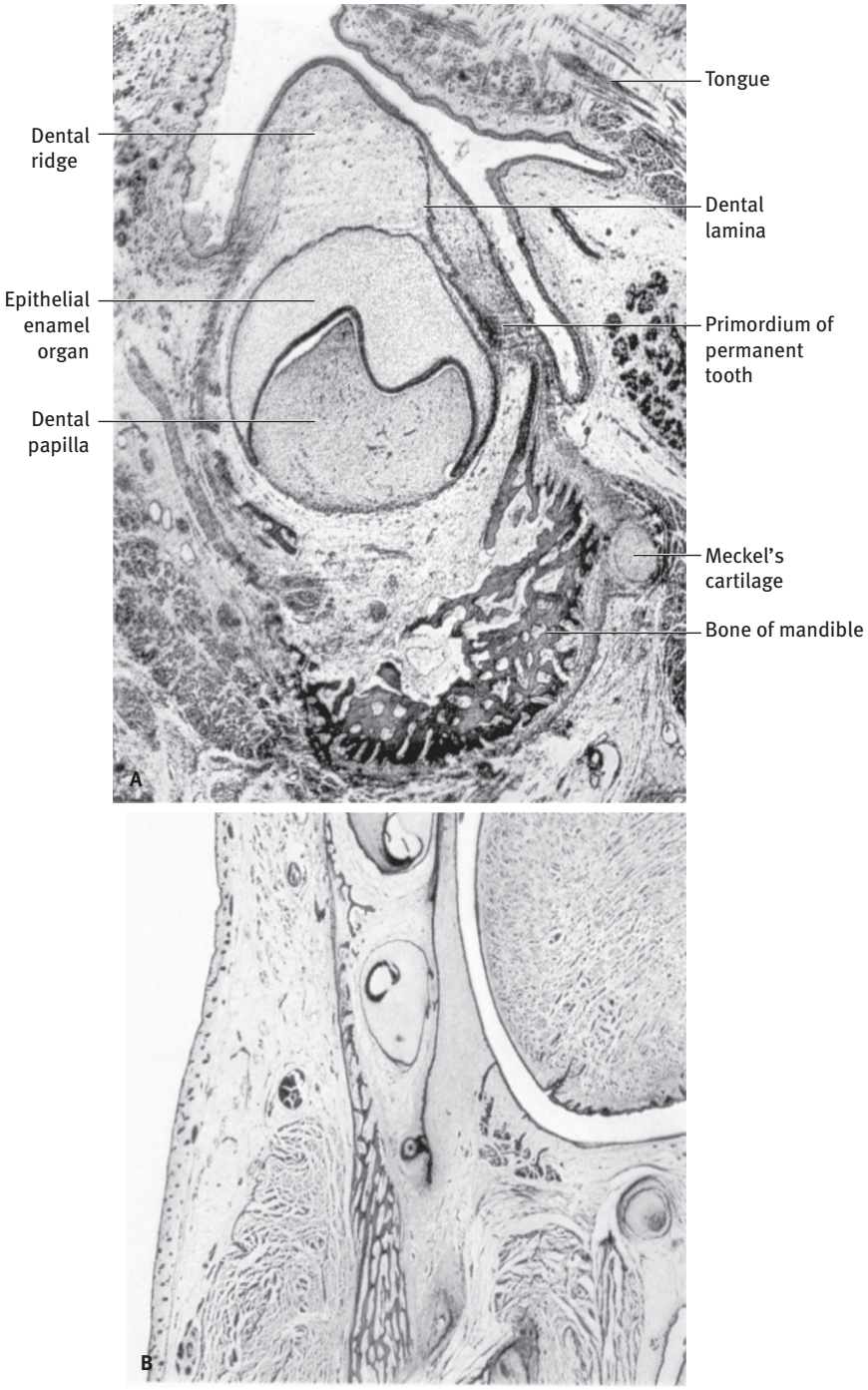


Figure 3.10 (**A**) Advanced bell stage of tooth development. Human embryo 200 mm in length, about 18 weeks. Labiolingual section through deciduous lower first molar. (**B**) Horizontal section through human embryo about 20 mm in length showing extension of dental lamina distal to second deciduous molar and formation of permanent first molar tooth germ (**B** from Bhaskar SN: Synopsis of oral histology, ed 5, St Louis, 1977, The CV Mosby Co).

HISTOPHYSIOLOGY

A number of physiologic growth processes participate in the progressive development of the teeth (Table 3.2). Except for their initiation, which is a momentary event, these processes overlap considerably, and many are continuous throughout the various morphologic stages of odontogenesis. Nevertheless, each physiologic process tends to predominate in one stage more than in another.

For example, the process of histodifferentiation characterizes the bell stage, in which the cells of the inner enamel epithelium differentiate into functional ameloblasts. However, proliferation still progresses at the deeper portion of the enamel organ.

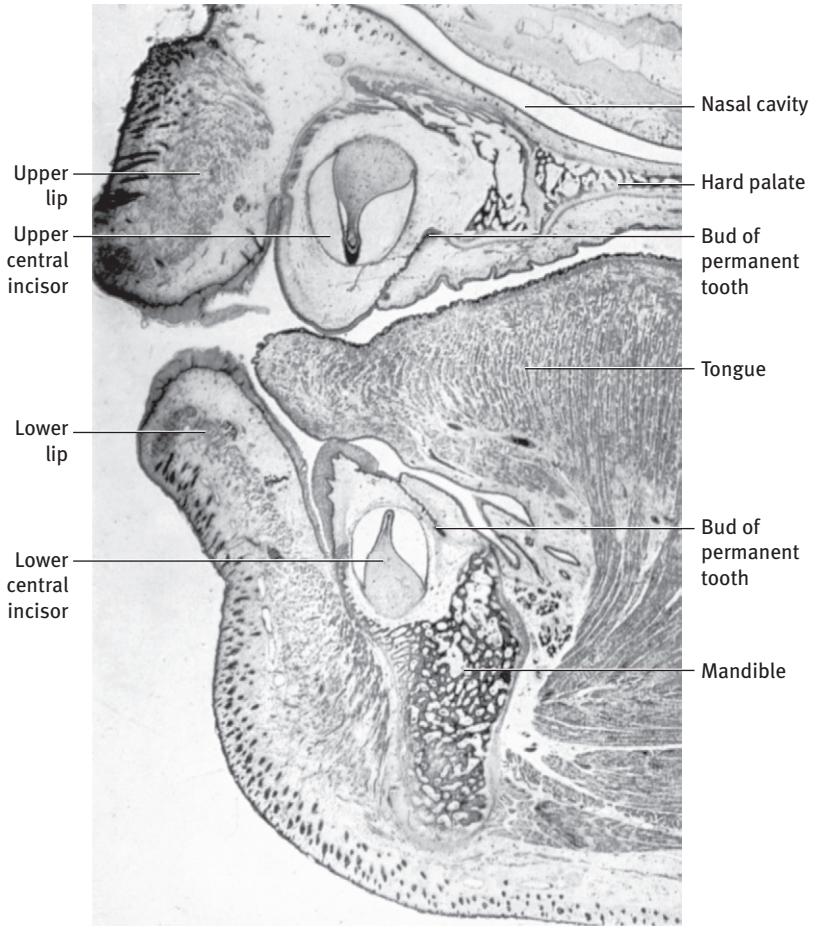


Figure 3.11 Sagittal section through head of human fetus 200 mm in length, about 18 weeks, in region of central incisors.

Table 3.1 Developmental Stages of Tooth Development

SL NO	STAGES	LAYERS	CELLS	EVENTS
1	BUD STAGE	-	• Peripheral: Low columnar cells • Central: Polygonal cells	• Formation of dental papilla and dental sac
2	CAP STAGE	• OEE • Stellate reticulum	• Low cuboidal cells • Star shaped cells which form cellular network	• Formation of enamel knot, enamel cord and enamel septa
3	EARLY BELL STAGE	• OEE • Stellate reticulum • Stratum intermedium	• Low cuboidal cells • Star shaped cells • Few layers of squamouns cells	• Stratum intermedium which is rich in glycogen helps in enamel formation • Collapse of stellate reticulum reduces the distance between ameloblasts and capillaries at OEE • OEE is laid down into folds in between of which the mesenchyme of dental sac condense to form papillae rich in capillaries which provide nutritional supply
4	ADVANCED BELL STAGE	• OEE • Stellate reticulum • Formation of future DEJ • Straum intermedium • IEE	• -AS ABOVE-	• Commencement of mineralization and root formation • Formation of future DEJ • Cervical portion of enamel organ gives rise to HERS

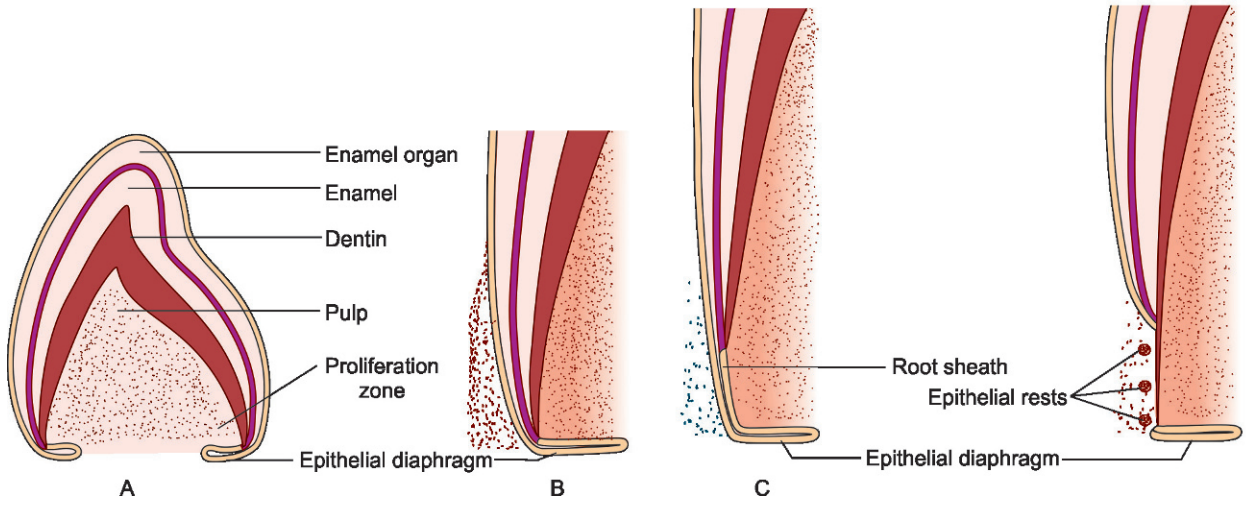


Figure 3.12 Diagrams showing three stages in root development. (**A**) Section through tooth germ. Note epithelial diaphragm and proliferation zone of pulp. (**B**) Higher magnification of cervical region of A. (**C**) 'Imaginary' stage showing elongation of Hertwig's epithelial sheath coronal to diaphragm. Differentiation of odontoblasts in elongated pulp. (**D**) In area of proliferation, dentin has been formed. Root sheath is broken up into epithelial rest and is separated from dentinal surface by connective tissue. Differentiation of cementoblasts.

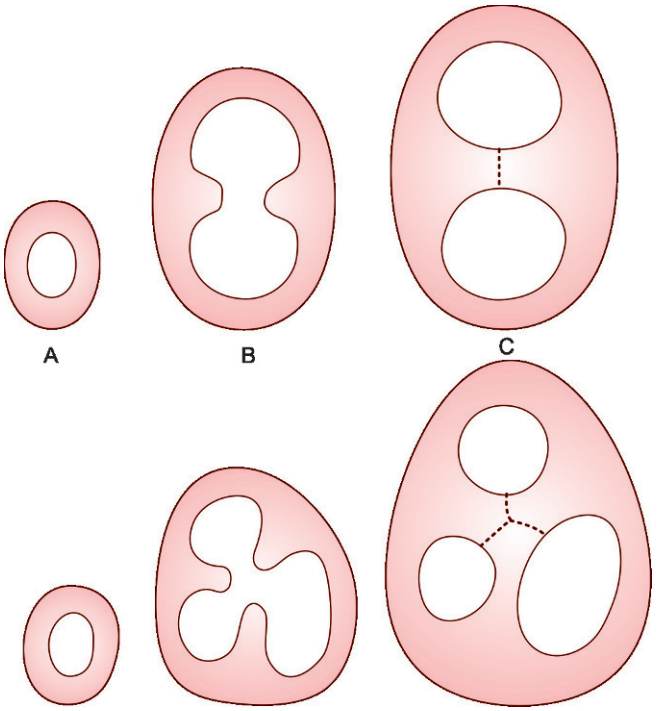


Figure 3.13 Three stages in development of tooth with two roots and one with three roots. Surface view of epithelial diaphragm. During growth of tooth germ, simple diaphragm, (**A**) expands eccentrically so that horizontal epithelial flaps are formed. (**B**) Later these flaps proliferate and unite (dotted lines in **C**) and divide single cervical opening into two or three openings.

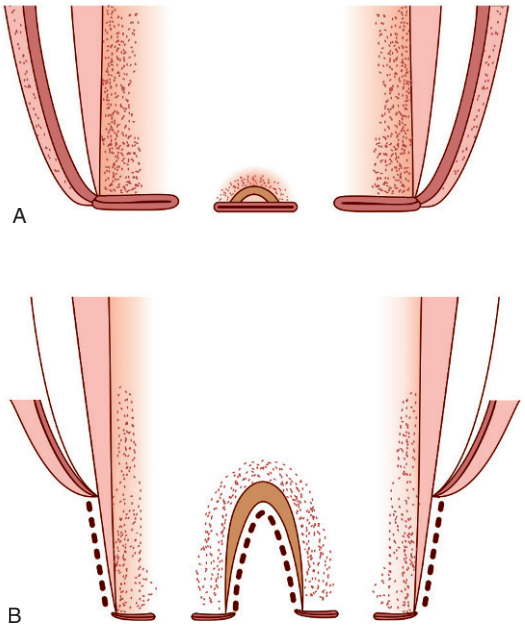
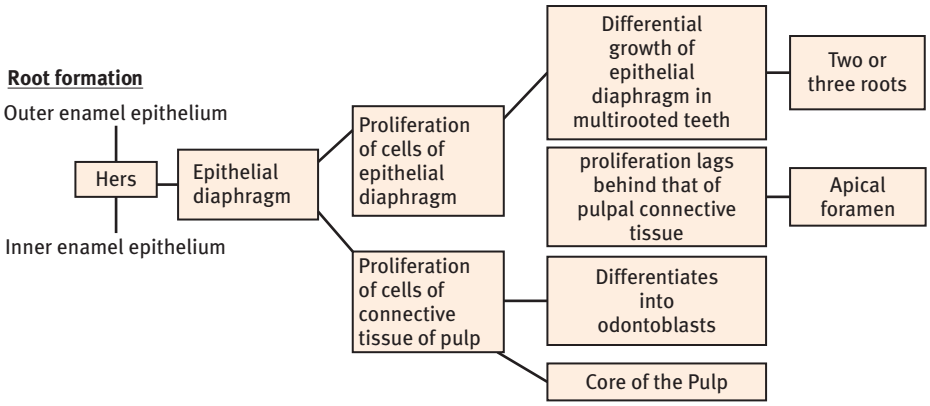


Figure 3.14 Two stages in development of two-rooted tooth. Diagrammatic mesiodistal sections of lower molar. (**A**) Beginning of dentin formation at bifurcation. (**B**) Formation of two roots in progress (Details as shown in Fig. 3.12).

Initiation

The dental laminae and associated tooth buds represent those parts of the oral epithelium that have the potential for tooth formation. Specific cells within the horseshoe-shaped dental laminae have the potential to form the enamel organ of certain teeth by responding to those factors that initiate or induce tooth development. Different teeth are initiated at definite times. Initiation induction requires ectomesenchymal–epithelial interaction. The mechanism of such interaction is not clearly understood. However, it has been demonstrated that dental papilla mesenchyme can induce or instruct tooth epithelium and even nontooth epithelium to form enamel.



Flowchart 3.2 Mechanism of root formation.

Table 3.2 Stages in Tooth Growth

Morphologic stages	Physiologic processes
Dental lamina	Initiation
Bud stage, Cup stage, Bell stage (early), Bell stage (advanced)	Proliferation, Histodifferentiation, Morphodifferentiation
Formation of enamel and dentin matrix	Apposition

Proliferation

Enhanced proliferative activity ensues at the points of initiation and results successively in the bud, cap, and bell stages of the odontogenic organ. Proliferative growth causes regular changes in the size and proportions of the growing tooth germ (Figs 3.3 and 3.7).

Even during the stage of proliferation, the tooth germ already has the potential to become more highly developed. This is illustrated by the fact that explants of these early stages continue to develop in tissue culture through the subsequent stages of histodifferentiation and appositional growth. A disturbance or experimental interference has entirely different effects, according to the time of occurrence and the stage of development that it affects.

Histodifferentiation

Histodifferentiation succeeds the proliferative stage. The formative cells of the tooth germs developing during the proliferative stage undergo definite morphologic as well as functional changes and acquire their functional assignment (the appositional growth potential). The cells become restricted in their functions. They differentiate and give up their capacity to multiply as they assume their new function; this law governs all differentiating cells. This phase reaches its highest development in the bell stage of the enamel organ, just preceding the beginning of formation and apposition of dentin and enamel (Fig. 3.8).

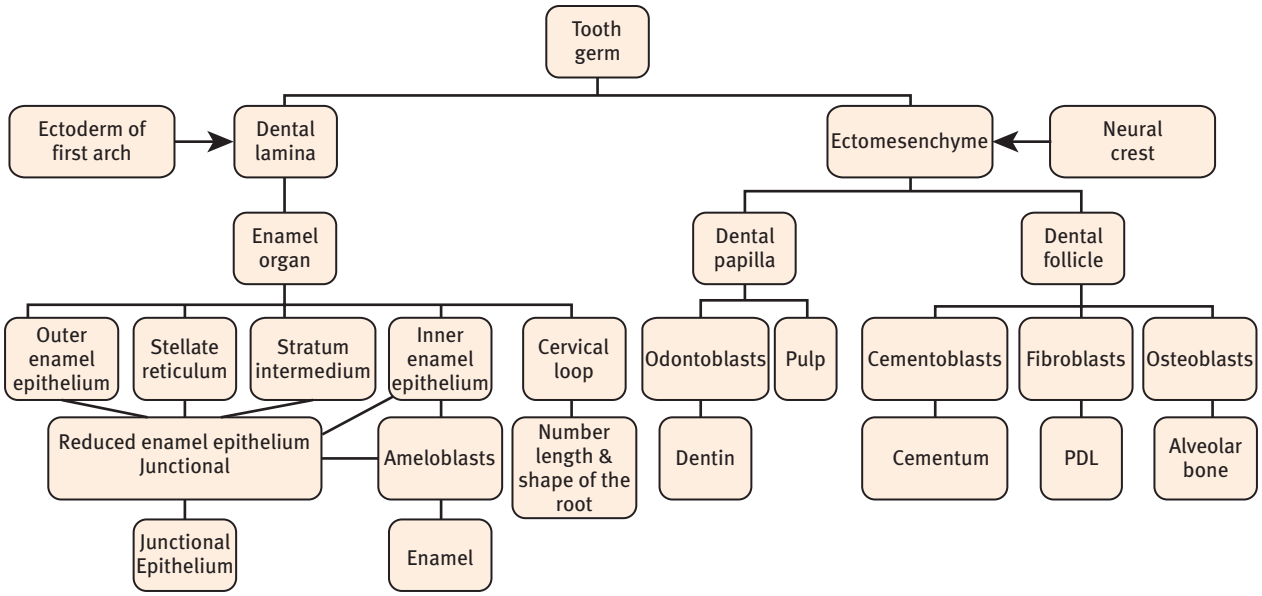
The organizing influence of the inner enamel epithelium on the mesenchyme is evident in the bell stage and causes the differentiation of the adjacent cells of the dental papilla into odontoblasts. With the formation of dentin, the cells of the inner enamel epithelium differentiate into ameloblasts and enamel matrix is formed opposite the dentin. Enamel does not form in the absence of dentin, as demonstrated by the failure of transplanted ameloblasts to form enamel when dentin is not present. Dentin formation therefore precedes and is essential to enamel formation. The differentiation of the epithelial cells precedes and is essential to the differentiation of the odontoblasts and the initiation of dentin formation.

In vitro studies on tooth development have provided vital information concerning the interaction of dermal–epidermal components of tooth tissues on differentiation of odontoblasts and ameloblasts. The importance of the basement membrane of this interface has been recognized. However, the criteria for the development of this complex organ system will have to await the delineation of the precise roles of the stellate reticulum, the stratum intermedium, and the outer enamel epithelial components. One of the models that has been suggested for the interactions that may occur between tissues during the development of a tooth is presented in Flowchart 3.3.

Morphodifferentiation

The morphologic pattern, or basic form and relative size of the future tooth, is established by morphodifferentiation, that is, by differential growth. Morphodifferentiation therefore is impossible without proliferation. The advanced bell stage marks not only active histodifferentiation but also an important stage of morphodifferentiation in the crown, outlining the future dentinoenamel junction (Figs 3.8 and 3.10).

The dentinoenamel and dentinocemental junctions, which are different and characteristic for each type of tooth, act as a blueprint pattern. In conformity with this pattern the ameloblasts, odontoblasts, and cementoblasts deposit enamel, dentin, and cementum, respectively, and



Flowchart 3.3 An outline of tooth development.

thus give the completed tooth its characteristic form and size. For example, the size and form of the cuspal portion of the crown of the first permanent molar are established at birth long before the formation of hard tissues begin.

Apposition

Apposition is the deposition of the matrix of the hard dental structures. It will be described in separate chapters on enamel, dentin, and cementum. This chapter deals with certain aspects of apposition in order to complete the discussion of the physiologic processes concerned in the growth of teeth.

Appositional growth of enamel and dentin is a layer like deposition of an extracellular matrix. This type of growth is therefore additive. It is the fulfillment of the plans outlined at the stages of histodifferentiation and morphodifferentiation. Appositional growth is characterized by regular and rhythmic deposition of the extracellular matrix, which is of itself incapable of further growth. Periods of activity and rest alternate at definite intervals during tooth formation.

MOLECULAR INSIGHTS IN TOOTH MORPHOGENESIS

The details of genetic control of tooth development are dealt in detail in the Appendix section on Molecular Aspects in Oral Histology (see Online Resource). However a brief outline of the important events is as given.

Like any other organogenesis, the tooth development requires systematic cascading interactions between branchial arch ectoderm (epithelial component) and ectomesenchyme (mesenchymal component). The ectoderm shows independent competency during initial stages of tooth development (intiation) during which ectomesenchyme remains responsive to external signals from the dental epithelium.

Numerous genes interact, either act in conjunction or antagonize each other in odontogenesis. The expression of *Fgf*-8 in the first branchial arch epithelium, *Lhx*-6/7 in the adjacent ectomesenchyme of the first branchial arch along with the restricted expression of *Gsc* in the ectomesenchyme finely regulate the oral–aboral axis.

The position of tooth germ in the established oral–aboral axis is determined by the expression of *Fgf*-8, *Pitx*-2 and *Bmp*-4 in oral epithelium and *Pax*-9 in tooth mesenchyme. While *Pitx*-2 positively regulates *Fgf*-8 expression, both *Bmp*-4 and *Bmp*-2 antagonize *Pax*-9 expression in tooth mesenchyme. Similarly, *Shh* expression is seen specifically in dental ectoderm while *Wnt*-7b is excluded from this zone.

After establishing the tooth position, the tooth type determination is regulated through a subfamily of Homeobox genes that include *Msx* gene and *Dlx* gene. They are seen expressed in a spatially and temporally patterned manner within the first branch tooth ectomesenchyme. The *Dlx*-1, *Dlx*-2 and *Barx*-1 are seen in the posterior regions while *Msx*-1, *Msx*-2 and *Dlx*-2 are seen in the anterior region. Ectopic expression of *Lef*-1 in oral epithelium results in ectopic tooth formation.

Enamel knot, a transient structure seen in the enamel organ plays the prime role in controlling the growth as well as the designing of tooth cusps.

CLINICAL CONSIDERATIONS

A lack of initiation results in the absence of either a single tooth or multiple teeth (partial anodontia), most

frequently the permanent upper lateral incisors, third molars, and lower second premolars. There also may be a complete lack of teeth (anodontia). On the other hand, abnormal initiation may result in the development of single or multiple supernumerary teeth.

Teeth may develop in abnormal locations, for example, in the ovary (dermoid tumors or cysts) or in the hypophysis. In such instances the tooth undergoes stages of development similar to those in the jaws.

In vitamin A deficiency the ameloblasts fail to differentiate properly. Consequently, their organizing influence on the adjacent mesenchymal cells is disturbed, and atypical dentin, known as osteodentin, is formed.

Endocrine disturbances affect the size or form of the crown of teeth if such effects occur during morphodifferentiation, that is, in utero or in the first year of life. Size and shape of the root, however, may be altered by disturbances in later periods. Clinical examinations show that the retarded eruption that occurs in persons with hypopituitarism and hypothyroidism results in a small clinical crown that is often mistaken for a small anatomic crown. Abnormal curvatures in the root, termed dilacerations may be due to trauma sustained during development of the root.

Disturbances in morphodifferentiation may affect the form and size of the tooth without impairing the function of the ameloblasts or odontoblasts. New parts may be differentiated like formation of supernumerary cusps or roots. Twinning, i.e. two similar teeth may be produced as a result of splitting of one tooth germ. Fusion of teeth produced from two tooth germ joined together before mineralization may occur. A suppression of parts may occur like loss of cusps or roots. An abnormality in shape may result in a peg or malformed tooth with enamel and dentin that may be normal in structure. Peg shaped teeth (screw-driver shaped) with the permanent upper central incisor showing a notched incisal edge may be seen in individuals born with congenital syphilis. This condition is known as Hutchinson's incisor.

Genetic and environmental factors may disturb the normal synthesis and secretion of the organic matrix of enamel leading to a condition called *enamel hypoplasia.*

If the organic matrix is normal but its mineralization is defective, then the enamel or dentin is said to be hypocalcified or hypomineralized. Both hypoplasia and hypocalcification can occur as a result of an insult to the cells responsible for the apposition stage of tooth development.

SUMMARY

Formation of Dental Lamina

Odontogenesis occurs in the 6th week of intrauterine life with the formation of a primary epithelial band. At about the 7th week the primary epithelial band divides into a lingual process called dental lamina and a buccal process called vestibular lamina.

Enamel Organs

Initiated odontogenic cells continue to proliferate forming ovoid swellings called enamel organs in areas where teeth are going to form. All deciduous teeth arise from this dental lamina, later the permanent successors arise from its lingual extension and the permanent molars from its distal extension.

Parts of the Tooth Germ

The tooth germ consists of an ectodermal component—the enamel organ and the ectomesenchymal components—the dental papilla and the dental follicle. The enamel is formed from the enamel organ, the dentin and pulp from the dental papilla and the supporting tissues, the cementum, periodontal ligament and the alveolar bone from the dental follicle. Reciprocal epithelial and ectomesenchymal interactions are responsible for formation of dental tissues although the mechanisms of these interactions are not clearly understood.

Stages of Tooth Development

Unequal proliferation results in different shapes of the enamel organ with enclosure of increasing amounts of dental papilla. Depending on the shape of the enamel organ, the stages of tooth formation may be classified as the bud, cap and the bell stage.

Cap Stage

The peripheral cuboidal cells that line the convexity of the enamel organ are called the outer enamel epithelium while those lining the inner concavity are called the inner enamel epithelium. The star-shaped cells in the center forming a network are called stellate reticulum. The stellate reticulum acts as a shock absorber.

Bell Stage

Invagination and deepening of the inner enamel epithelium results in the bell stage of tooth development in which four different types of cells can be identified at a light microscopic level namely the inner enamel epithelium, stratum intermedium, stellate reticulum and the outer enamel epithelium. The stratum intermedium are 2 to 4 rows of squamous cells which help in enamel formation. Transient structures namely the enamel knot, enamel cord and enamel septum are seen in the late cap stage. Except for enamel knot which acts as a signaling center, the functions of other structures are not known. The junction of inner and outer enamel epithelium is called cervical loop which marks the future CEJ and it is an area of intense mitotic activity. Anticipating blood supply cut off from dental papilla side due to dentin formation, the outer enamel epithelium becomes thrown into folds in which numerous blood vessels are seen. These changes are called reversal of nutritional source. Inner enamel epithelial cells become tall columnar and acquire organelles for protein synthesis under the influence of dental papilla cells. These are then known as ameloblasts. They then cause differentiation of dental papilla cells into odontoblasts. All differentiation and formation of enamel and dentin in crown of the tooth takes place in the future incisal/cuspal regions.

Advanced Bell Stage

Advanced bell stage is characterized by the commencement of mineralization and root formation. The formation of dentin

occurs first as a layer along the future dentinoenamel junction (DEJ) in the region of future cusps and proceeds pulpally and apically. After the first layer of dentin is formed, the ameloblasts lay down enamel over the dentin. The enamel formation then proceeds coronally and cervically, from the DEJ towards the surface. In addition, the cervical portion of the enamel organ gives rise to the bilayered epithelial root sheath of Hertwig.

Root Development

The Hertwig's epithelial root sheath (HERS) outlines the future root and is thus responsible for the shape, length, size and number of roots.

The development of the roots begins after enamel and dentin formation has reached the future cementoenamel junction (CEJ). The radicular dental papilla cells differentiate under the influence of HERS cells into odontoblasts and lay down the first layer of dentin. The epithelium is moved away from the surface of the dentin allowing connective tissue cells of the dental sac to come into contact with the outer surface of the dentin which differentiate into cementoblasts, and deposit a layer of cementum. The epithelial root sheath loses its structural continuity and disintegrates. Differential growth of the epithelial diaphragm in multirooted teeth causes the division of the root trunk into two or three roots.

The outermost dental follicle cells differentiate into osteoblast form the alveolar bone and the middle portion of dental sac cells become fibroblast and forms the periodontal ligament fibers which gain attachment to cementum and alveolar bone. The tooth develops within the bony crypt.

Genetic Control of Tooth Development

Numerous genes interact, either act in conjunction or antagonize each other in odontogenesis. The expression of *Fgf*-8 in the first branchial arch epithelium, *Lhx-6/7* in the adjacent ectomesenchyme of the first branchial arch along with the restricted expression of *Gsc* in the ectomesenchyme finely regulate the oral–aboral axis.

The position of tooth germ in the established oral–aboral axis is determined by the expression of *Fgf*-8, *Pitx*-2 and *Bmp*-4 in oral epithelium and *Pax*-9 in tooth mesenchyme. After establishing the tooth position, the tooth type determination is regulated through a subfamily of Homeobox genes that include *Msx* gene and *Dlx* gene. The *Dlx*-1, *Dlx*-2 and *Barx*-1 are seen in the posterior regions while *Msx*-1, *Msx*-2 and *Dlx*-2 are seen in the anterior region. Ectopic expression of *Lef*-1 in oral epithelium results in ectopic tooth formation.

Enamel knot, a transient structure seen in the enamel organ plays the prime role in controlling the growth as well as the designing of tooth cusps.

REVIEW QUESTIONS

1. Describe the early bell stage of tooth development.
2. Describe the advanced bell stage of tooth development.
3. Describe the epithelial–mesenchymal interactions that take place during tooth morphogenesis.
4. Describe the development of roots of teeth.
5. Write notes on: Dental lamina, HERS, enamel knot, stellate reticulum and dental papilla.
6. How is the oral–aboral axis established?
7. How is the tooth type determined?
8. Describe the role of enamel knot in control of tooth morphogenesis.

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4 Enamel

CHAPTER CONTENTS

HISTOLOGY

Physical Characteristics

Enamel forms a protective covering of variable thickness over the entire surface of the crown. On the cusps of human molars and premolars the enamel attains a maximum thickness of about 2 to 2.5 mm, thinning down to almost a knife edge at the neck of the tooth (Fig. 4.1). The enamel was found to be thicker in the lingual surfaces of maxillary molars and in the buccal surfaces of mandibular molars. As these are supporting cusps, it is suggested that the increased thickness in these areas may be viewed as an adaptation to functional demands.

The shape and contour of the cusps receive their final modeling in the enamel.

Because of its high content of mineral salts and their crystalline arrangement, enamel is the hardest calcified tissue in the human body. The function of the enamel is to form a resistant covering of the teeth, rendering them suitable for mastication. The structure and hardness of the enamel render it brittle, which is particularly apparent when the enamel loses its foundation of sound dentin. The complex microstructure of enamel leads to large variations in mechanical behavior. These are particularly pronounced when comparing the mechanical properties of the occlusal surface, to those close to the dentinoenamel junction. At the surface the modulus of elasticity is higher and the hardness is more than on dentinoenamel junction. The specific gravity of enamel is 2.8. The density decreases from the surface to the deeper regions and from cuspal to incisal region. Enamel has always been observed as a non-electrical conductive material: it is in fact an insulator at room temperature. Temperature resistance of enamel measured by AC impedance spectroscopy, is in the frequency range from 5 to 13 Hz. Electrical resistance ranges from 10^{15} to 10^{5} ohms.

Another physical property of enamel is its permeability. It has been found with radioactive tracers that the enamel can act in a sense like a semipermeable membrane, permitting complete or partial passage of certain molecules: ^{14}C-labeled urea, I, etc. The same phenomenon has also been demonstrated by means of dyes.

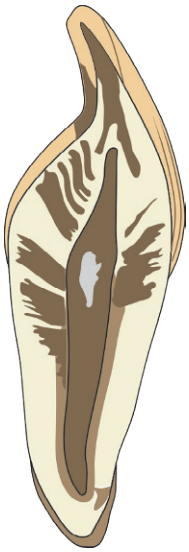

Figure 4.1 Ground section of incisor tooth. Note the variation in the thickness of enamel from incisal to cervical regions.

The color of the enamel-covered crown ranges from yellowish white to grayish white. It has been suggested that the color is determined by differences in the translucency of enamel, yellowish teeth having a thin, translucent enamel through which the yellow color of the dentin is visible and grayish teeth having a more opaque enamel. The translucency may be attributable to variations in the degree of calcification and homogeneity of the enamel. Grayish teeth frequently show a slightly yellowish color at the cervical areas, presumably because the thinness of the enamel permits the light to strike the underlying yellow dentin and be reflected. Incisal areas may have a bluish tinge where the thin edge consists only of a double layer of enamel. The translucency of enamel increased with increasing wavelengths. The transmission coefficient at 525 nm was 0.481 mm^{-1}. Dehydration decreased the translucency but it was reversed on rehydration.

Chemical Properties

The enamel consists mainly of inorganic material (96%) and only a small amount of organic substance and water (4%).

The organic material consists of some unique proteins, found exclusively in the enamel and lipids. The proteins found in the enamel are of two main groups—the *amelogenins* and the *nonamelogenins*. Amelogenins, are a heterogeneous group of low molecular weight proteins, accounting for about 90% of the enamel proteins. They are hydrophobic and are rich in proline, histidine, glutamine and leucine. Nonamelogenins constitute about 10% of enamel matrix proteins. *Enamelin, ameloblastin* and *tuftelin* are the important proteins of this group. Nonamelogenins are high molecular weight proteins and are rich in glycine, aspartic acid and serine.

The inorganic material of the enamel is hydroxyapatite. Its chemical formula is $Ca_{10}(PO_4)_6(OH_4)_2$. The crystals of hydroxyapatite are hexagonal in cross-section. The shape of a single crystal was observed by high resolution SEM to be a rod with an equilateral hexagon base. The crystals are arranged to form enamel rods or enamel prisms. The hydroxyapatite crystal has a central core or C axis of hydroxyl ion around which calcium and phosphorus ions are arranged in the form of triangles (Fig. 4.2A). During the formation, magnesium can replace calcium and carbonate can replace hydroxyl ion. Both these substitutions destabilize the lattice due to poorer fit of these ions in the lattice structure. The concentrations of these ions increase from the surface of enamel towards dentin whereas the concentration of fluoride decreases from surface towards dentin. Thus, the core of the crystals are richer in Mg and carbonate and this accounts for their greater solubility in acids than the peripheral portions. The average concentrations in dry weight % of human tooth enamel of oxygen, calcium and phosphorus, which are the three major constituents are 43.4, 36.6, and 17.7, respectively. The minor constituents together account for 2.3%, of which Na (0.67) and carbon (0.64) and magnesium (0.35) are the principal constituents. The concentration of carbonate is 3.2% which is important as carbonate rich crystals are preferentially attacked by acids in caries.

Water is present as a part of the crystal (hydroxyapatite), between crystals and between rods and surrounding the rods. Pores are present between the crystals, especially at the boundaries of the rods and these are filled with water. Enamel proteins do not contribute to structuring of enamel. This is in contrast to collagen, which is the principal protein of dentin or bone, having a structuring function.

The bar graph in Figure 4.2B indicates the composition by volume of mineralized tissues in which odontoblast processes have been replaced with peritubular dentin (sclerotic dentin) and the equivalent situation in bone in which osteocyte lacunae are filled with mineral.

The origins shown at the left of Figure 4.2B reflect the facts that enamel matrix mineralization begins immediately after it is secreted and that the lag in mineralization after matrix formation is greater in dentin than in bone. Enamel primary mineralization and secondary mineralization (maturation) increase mineral content in a relatively smooth curve. In both bone and dentin, well over one half of the mineral accumulates rapidly (primary mineralization). The curves then flatten as secondary mineralization occurs. The curves continue to rise slowly as cell-occupied space is filled with mineralized matrix (secondary matrix formation) in bone and dentin.

Structure

Rods

The enamel is composed of enamel rods or prisms, rod sheaths, and in some regions a cementing interprismatic substance. The enamel prisms are cylindrical, in longitudinal section, therefore the term rods is more apt. The number of enamel rods has been estimated as ranging from 5 million in the lower lateral incisors to 12 million in the upper first molars. From the dentinoenamel junction the rods run somewhat tortuous courses outward to the surface of the tooth. The length of most rods is greater than the thickness of the enamel because of the oblique direction and the wavy course of the rods. The rods located in the cusps, the thickest part of the enamel, are longer than those at the cervical areas of the teeth. The increase in the area of enamel at the surface compared to their area at the DE junction is not due to increase in the number of prisms but it is explained on the basis of increased prism diameter near the tooth surface and

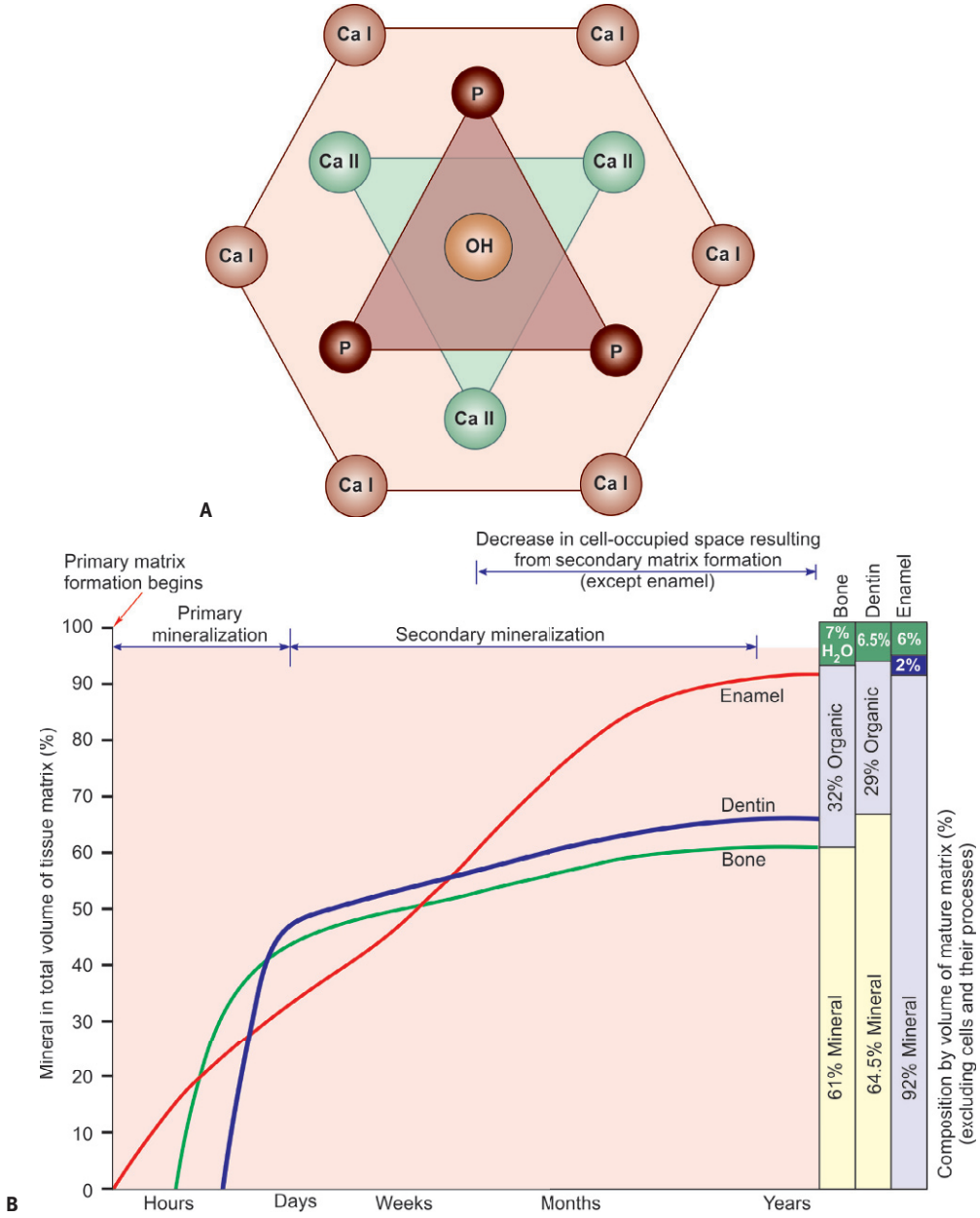

Figure 4.2 (**A**) Arrangement of calcium and phosphorus ions around the central hydroxyl ions in the planar hexagonal structure of hydroxyapatite crystal. (**B**) Formation, mineralization, and maturation of some mineralized tissues (**B,** Figures for bone from Robinson RA: In Rodahl K, Nicholson JT, and Brown EM, editors: Bone as a tissue. New York, 1960, The Blakiston Division, McGraw-Hill Book Co, pp. 186–250. Figures for dentin and enamel from Brudevold F: In Sognnaes RF, editor: Chemistry and prevention of dental caries, Springfield, Ill, 1962, Charles C Thomas, Publisher, pp. 32–88).

the oblique orientation of prisms towards the surface. It is stated generally that, as observed with the light microscope, the diameter of the rods averages 4 µm, but this measurement necessarily varies, since the outer surface of the enamel is greater than the dentinal surface where the rods originate. It is claimed that the diameter of the rods increases from the dentinoenamel junction toward the surface of the enamel at a ratio of about 1:2.

The enamel rods normally have a clear crystalline appearance, permitting light to pass through them. In cross-section under the light microscope they occasionally appear hexagonal. Sometimes they appear round or oval. In cross-sections of human enamel, many rods resemble fish scales (Fig. 4.3). Recently, using 3D images obtained from confocal laser scanning microscope were reconstructed and the path of a single and groups of rods were studied. It was found that the rods did not maintain their same outline throughout, arcade outlines were seen near DE junction and keyhole-shaped outlines were seen at the enamel surface.

Ultrastructure

Since many features of enamel rods are below the limit of resolution of the light microscope, many questions concerning their morphology can only be answered by electron microscopy. Although many areas of human enamel seem to contain rods surrounded by rod sheaths and separated by interrod substance (Fig. 4.4), a more

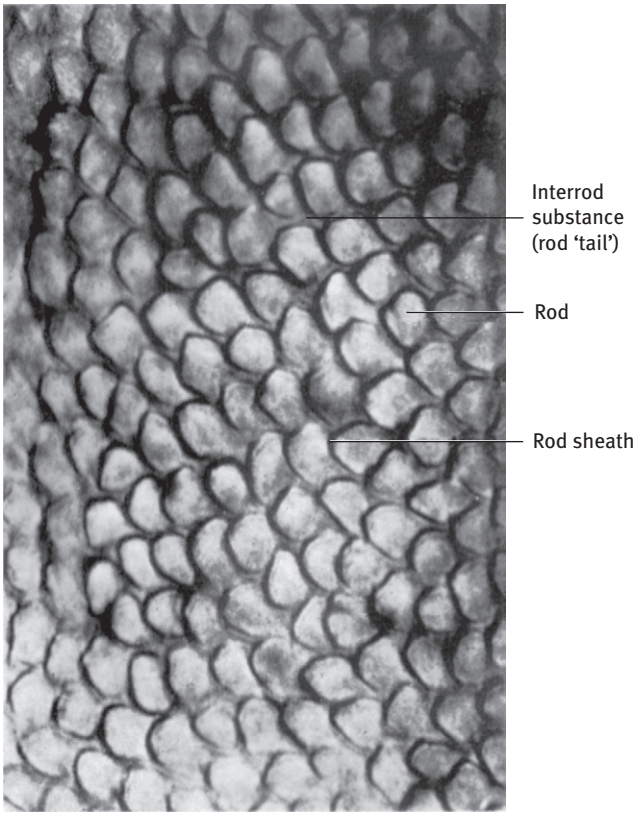

Figure 4.3 Decalcified section of enamel of human tooth germ. Rods cut transversely have appearance of fish scales.

common pattern is a keyhole or paddle-shaped prism in human enamel (Fig. 4.5). When cut longitudinally (Fig. 4.6), sections pass through the 'heads' or 'bodies' of one row of rods and the 'tails' of an adjacent row. This produces an appearance of rods separated by interrod substance. These rods measure about 5 µm in breadth and 9 µm in length. Rods of this shape can be packed tightly together (Fig. 4.7), and enamel with this structure explains many bizarre patterns seen with the electron microscope. The 'bodies' of the rods are nearer occlusal and incisal surfaces, whereas the 'tails' point cervically.

Studies with polarized light and X-ray diffraction have indicated that the apatite crystals are arranged approximately parallel to the long axis of the prisms, although deviations of up to 40 degrees have been reported. Careful electron microscopic studies have made it possible to describe more precisely the orientation of these crystals. They are approximately parallel to the long axes of the rods in their 'bodies' or 'heads' and deviate about 65 degrees from this axis as they fan out into the 'tails' of the prisms (Fig. 4.8). Since it is extremely difficult to prepare a section that is exactly parallel to the long axes of the crystals, there is some question about their length, but they are estimated to vary between 0.05 and 1 µm. Fusion of the lateral branches of the crystals were observed so that the crystals assumed pyramidal shape with their bases towards DE junction. When cut in cross-section, the crystals of human enamel are somewhat irregular in shape (Fig. 4.9) and have an average thickness of about 30 nm and an average width of about 90 nm.

Early investigators using electron microscope described a network of fine organic fibrils running throughout the rods and interred substance. Recent improvements in preparative methods have disclosed that the organic matrix probably forms an envelope surrounding each apatite crystal (Fig. 4.10). In electron micrographs the surfaces of rods are visible because of abrupt changes in crystal orientation from one rod to another. For this reason, the crystals are not as tightly packed and there may be more space for organic matrix at these surfaces. This accounts for the rod sheath visible in the light microscope (Fig. 4.3).

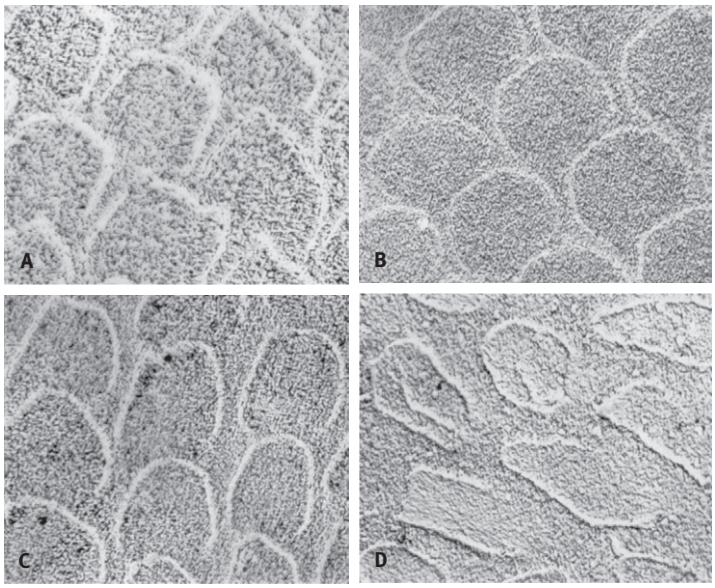

Figure 4.4 Electron micrographs of replicas of polished and etched human subsurface enamel. Rods are cut in cross-section. Various patterns are apparent. (**A**) 'Keyholes.' (**B**) 'Staggered arches.' (**C**) 'Stacked arches.' (**D**) Irregular rods near dentinoenamel junction (Approximately × 3000) (From Swancar VR, Scott DB, and Njemirovskij Z: J Dent Res 49:1025, 1970. Copyright by the American Dental Association. Reprinted by permission).

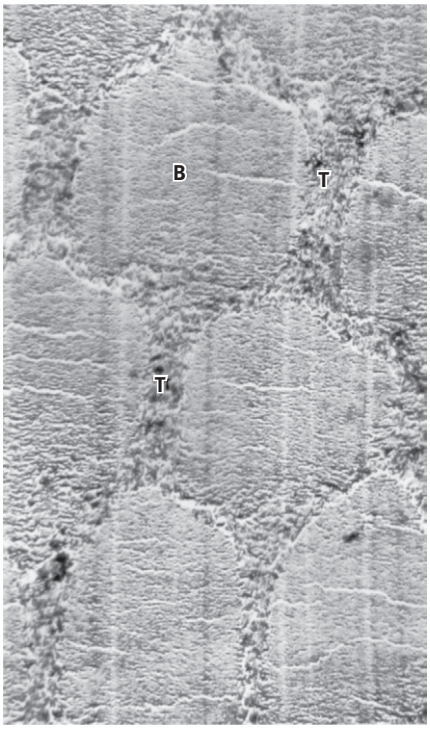

Figure 4.5 Electron micrograph of cross-sections of rods in mature human enamel. Rods are keyhole shaped, and crystal orientation is different in 'bodies,' B, than in 'tails,' T (Approximately ×5000) (From Meckel AH, Griebstein WJ, and Neal RJ: Arch Oral Biol 10:775, 1965).

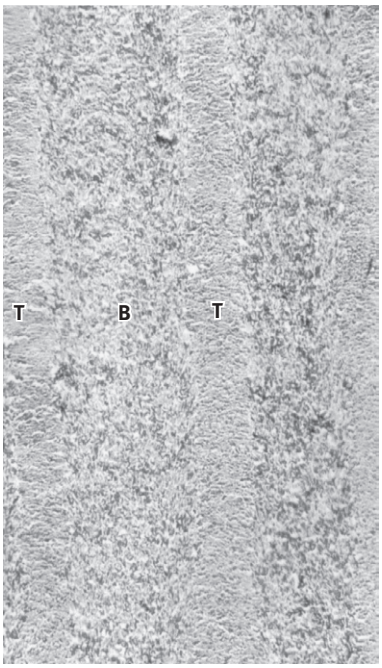

Figure 4.6 Electron micrograph of longitudinal section through mature human enamel. Alternating 'tails,' T, and 'bodies,' B, of rods are defined by abrupt changes in crystal direction where they meet. (Approximately ×5000) (From Meckel AH, Griebstein WJ, and Neal RJ: Arch Oral Biol 10:775, 1965).

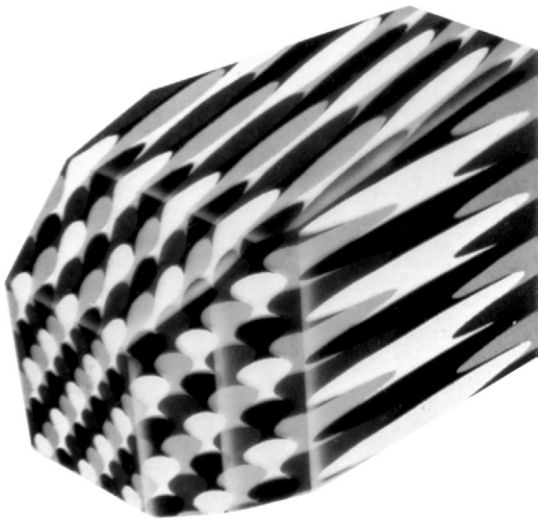

Figure 4.7 Model indicating packing of keyhole-shaped rods in human enamel. Various patterns can be produced by changing plane of sectioning (From Meckel AH, Griebstein WJ, and Neal RJ: Arch Oral Biol 10:775, 1965).

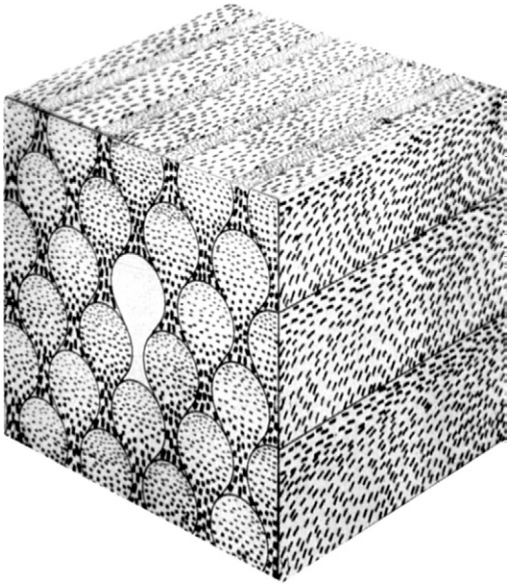

Figure 4.8 Drawing of keyhole pattern of human enamel indicating orientation of apatite crystals within individual rods. Crystals are oriented parallel to long axes of 'bodies' of rods and fan out at an angle of approximately 65 degrees in 'tails' of rods (From Griebstein WJ: In Stack MV and Fearnhead RW, editors: Tooth enamel, Bristol, 1965, John Wright & Sons, Ltd, p 190).

Striations

Each enamel rod is built up of segments separated by dark lines that give it a striated appearance (Fig. 4.11). These *cross striations* demarcate rod segments and become more visible by the action of mild acids. The striations are more pronounced in enamel that is insufficiently calcified. The rods are segmented because the enamel matrix is formed in a rhythmic manner. In humans these segments seem to be a uniform length of about 4 μm. The cross- striations seen in light microscope is suggested to be due to a diurnal rhythm in the enamel formation and

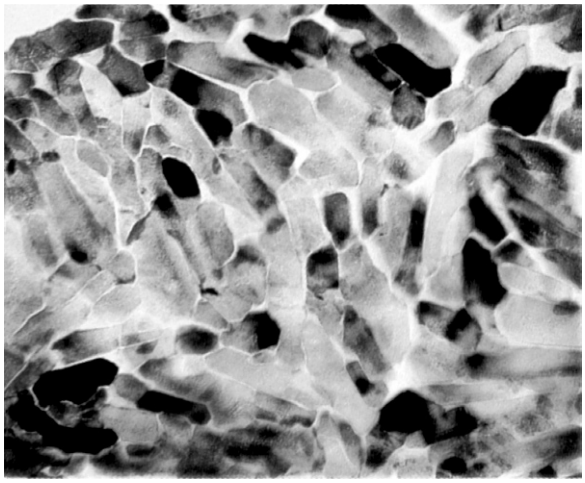

Figure 4.9 Cross-section of apatite crystals within enamel rod in human enamel. Crystals are tightly packed and irregular in shape (Approximately ×1,68,000) (From Frazier PD: J Ultrastruct Res 22:1, 1968).

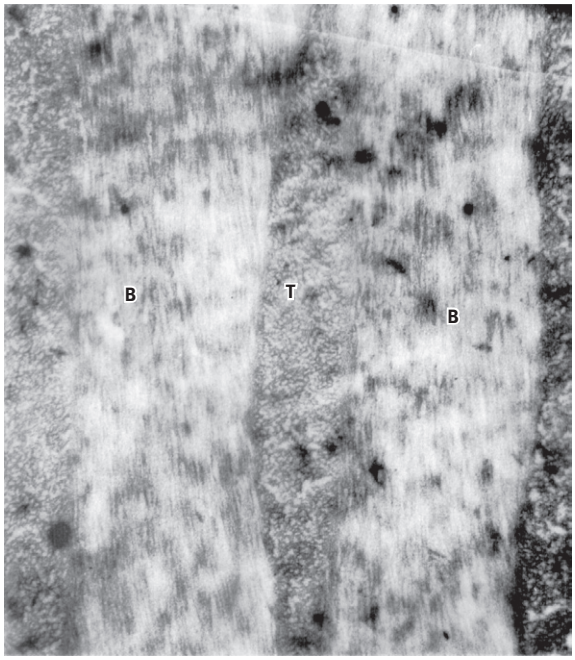

Figure 4.10 Electron micrograph of decalcified section of immature bovine enamel. Although shape of rods in bovine enamel is not clearly established, this electron micrograph reproduces pattern one would expect in longitudinal sections through human enamel. Organic sheaths around individual apatite crystals are oriented parallel to long axes of rods in their 'bodies,' B, and more nearly perpendicular to long axes in their 'tails,' T (Approximately × 38,000) (From Travis DF and Glimcher MJ: J Cell Biol 23:447, 1964).

that in these areas rods show varicosities and variation in composition.

Direction of rods

Generally the rods are oriented at right angles to the dentin surface. In the cervical and central parts of the crown of a deciduous tooth they are approximately horizontal

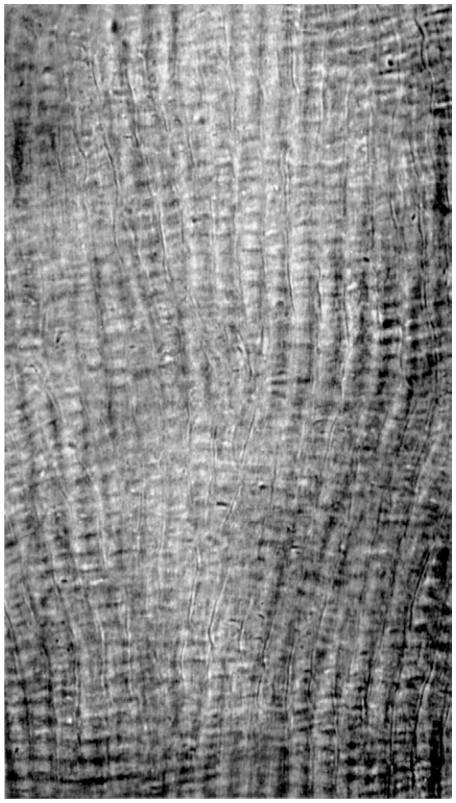

Figure 4.11 Ground section through enamel. Rods cut longitudinally. Cross-striation of rods.

(Fig. 4.12A). Near the incisal edge or tip of the cusps, they change gradually to an increasingly oblique direction until they are almost vertical in the region of the edge or tip of the cusps. The arrangement of the rods in permanent teeth is similar in the occlusal two thirds of the crown. In the cervical region, however, the rods deviate from the horizontal in an apical direction (Fig. 4.12B).

The rods are rarely, if ever, straight throughout. They follow a wavy course from the dentin to the enamel surface. The most significant deviations from a straight radial course can be described as follows. If the middle part of the crown is divided into thin horizontal disks, the rods in the adjacent disks bend in opposite directions. For instance, in one disk the rods start from the dentin in an oblique direction and bend more or less sharply to the left side (Fig. 4.13A), whereas in the adjacent disk the rods bend toward the right (Fig. 4.13B). This alternating clockwise and counter-clockwise deviation of the rods from the radial direction can be observed at all levels of the crown if the disks are cut in the planes of the general rod direction.

If the disks are cut in an oblique plane, especially near the dentin in the region of the cusps or incisal edges, the rod arrangement appears to be further complicated—the bundles of rods seem to intertwine more irregularly. This optical appearance of enamel is called *gnarled enamel.*

The enamel rods forming the developmental fissures and pits, as on the occlusal surface of molar and premolars, converge in their outward course.

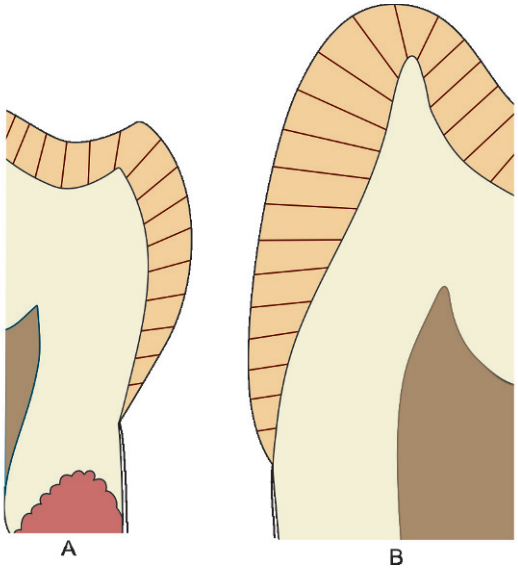

Figure 4.12 Diagrams indicating general direction of enamel rods. (**A**) Deciduous tooth. (**B**) Permanent tooth.

Hunter–Schreger bands

The more or less regular change in the direction of rods may be regarded as a functional adaptation, minimizing the risk of cleavage in the axial direction under the influence of occlusal masticatory forces. The change in the direction of rods is responsible for the appearance of the Hunter–Schreger bands. These are alternating dark and light strips of varying widths (Fig. 4.14A) that can best be seen in a longitudinal ground section under oblique reflected light. They originate at the dentinoenamel border and pass outward, ending at some distance from the outer enamel surface. The prisms which are cut longitudinally to produce the dark bands are called *diazones*, while the prisms which are cut transversely to produce light bands are called *parazones*. The angle between the parazones and the diazones is about 40 degrees. The enamel crystals aggregate in each zone of the Hunter–Schreger bands deviated not only in the opposite directions but also tilted to about 50 with respect to the central axis. Some investigators claim that there are variations in calcification of the enamel that coincide with the distribution of the bands of Hunter–Schreger. Careful decalcification and staining of the enamel have provided further evidence that these structures may not be the result solely of an optical phenomenon but that they are composed of alternate zones having a slightly different permeability and a different content of organic material (Fig. 4.14B).

However, the opinion that Hunter–Schreger bands are a result of change in direction of rods is largely prevalent and widely accepted.

Incremental Lines of Retzius

The incremental lines of Retzius appear as brownish bands in ground sections of the enamel. They illustrate the incremental pattern of the enamel, that is, the successive apposition of layers of enamel during formation of the crown. In longitudinal sections they surround the tip of the dentin (Fig. 4.15A). In the cervical parts of the crown they run obliquely. From the dentinoenamel junction to the surface they deviate occlusally (Fig. 4.15B). In transverse sections of a tooth the incremental lines of Retzius appear as concentric circles (Fig. 4.16). They may be compared to the growth rings in the cross-section of a tree. The term 'incremental lines' designates these structures appropriately, for they do, in fact, reflect variations in structure and mineralization, either hypomineralization or hypermineralization, that occur during growth of the enamel. The exact nature of these developmental changes is not known. The incremental lines have been attributed to periodic bending of the enamel rods, to variations in the basic organic structure (Fig. 4.17), or to a physiologic calcification rhythm. The mean daily rate of enamel formation of about 3.5 microns increases from inner to outer enamel and decreases from incisal/cuspal region to cervical enamel. The evenly spaced striae of Retzius was shown to represent a 6–11 day rhythm in enamel formation while other Retzius lines are suggested to be due to stress. It is estimated that about 25–30 striae do not reach the surface.

The incremental lines of Retzius, if present in moderate intensity, are considered normal. However, the rhythmic alteration of periods of enamel matrix formation

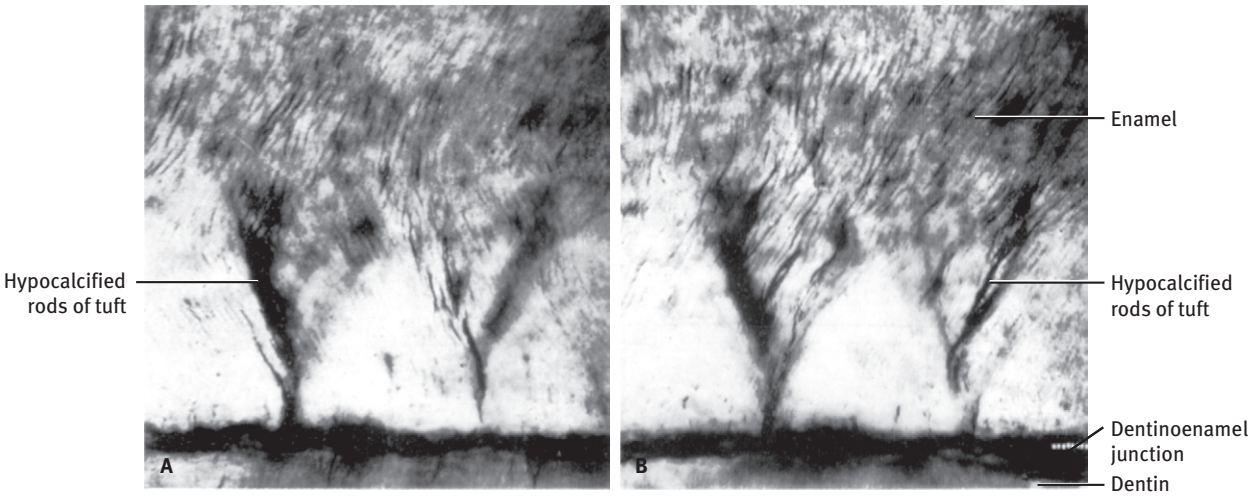

Figure 4.13 Horizontal ground section through enamel near dentinoenamel junction. (**A**) and (**B**) show change in direction of rods in two adjacent layers of enamel, which is made visible by change in focus of microscope.

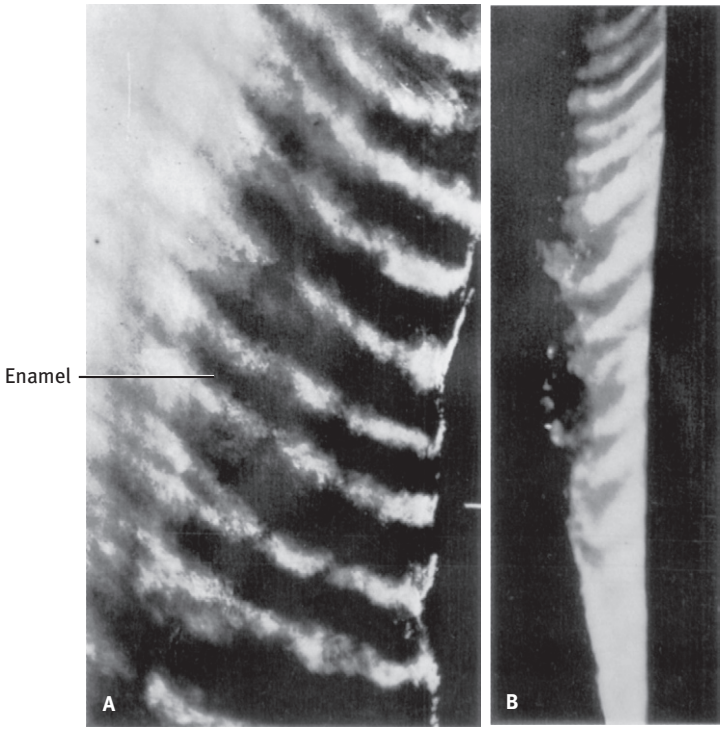

Figure 4.14 (**A**) Longitudinal ground section through enamel photographed by reflected light. Hunter–Schreger bands. (**B**) Decalcified enamel, photographed by reflected light, showing Hunter–Schreger bands.

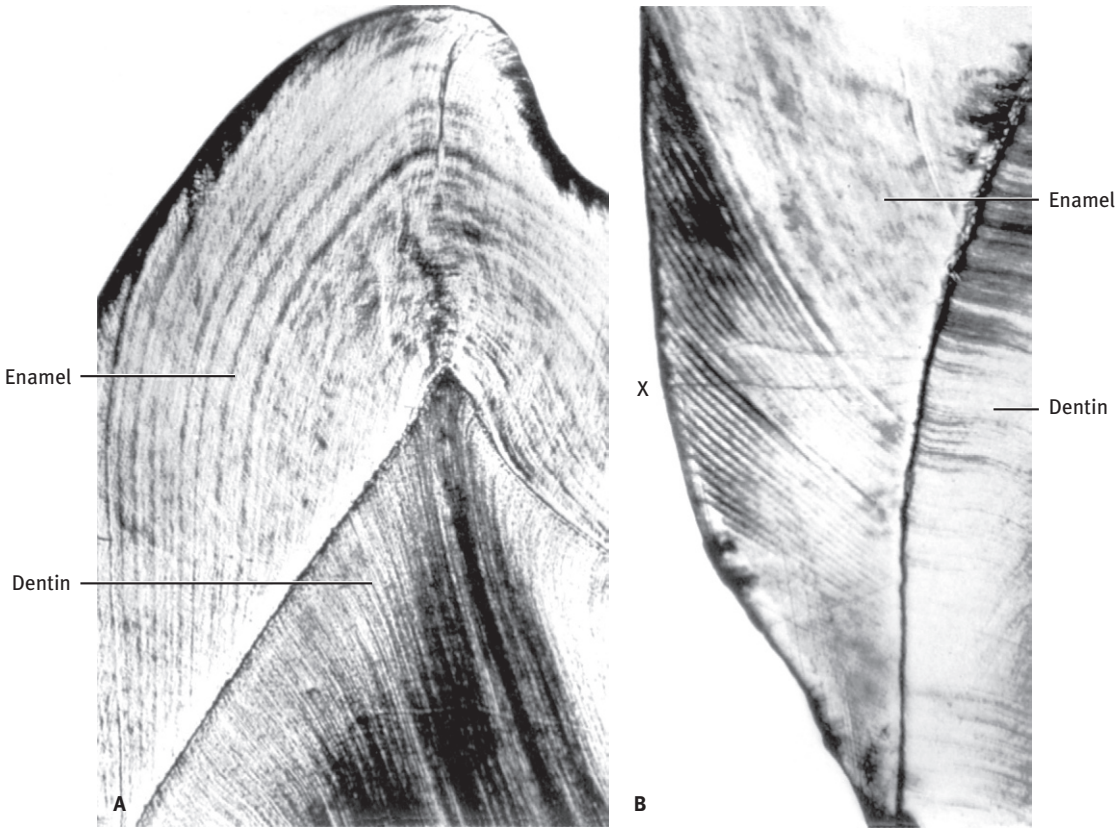

Figure 4.15 Incremental lines of Retzius in longitudinal ground sections. (**A**) Cuspal region. (**B**) Cervical region, X.

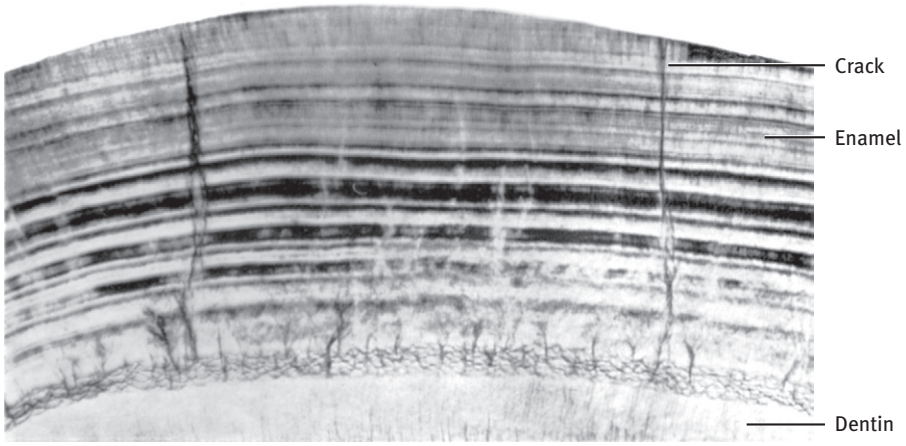

Figure 4.16 Incremental lines of Retzius in transverse ground section, arranged concentrically.

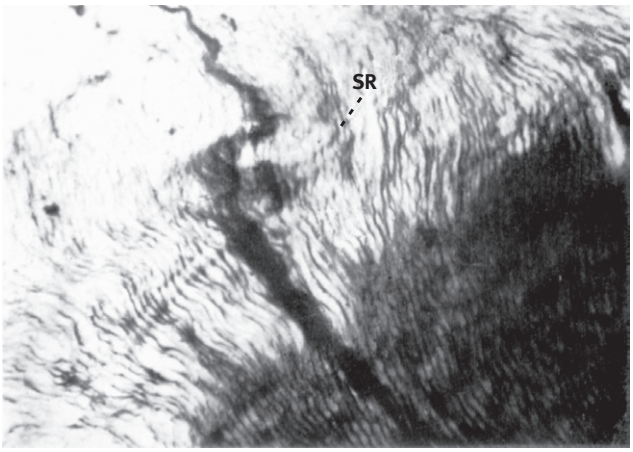

Figure 4.17 Carefully decalcified section through enamel. Thickening of sheath substance, SR, in Retzius lines (From Bodecker CF: Dent Rev 20:317, 1906).

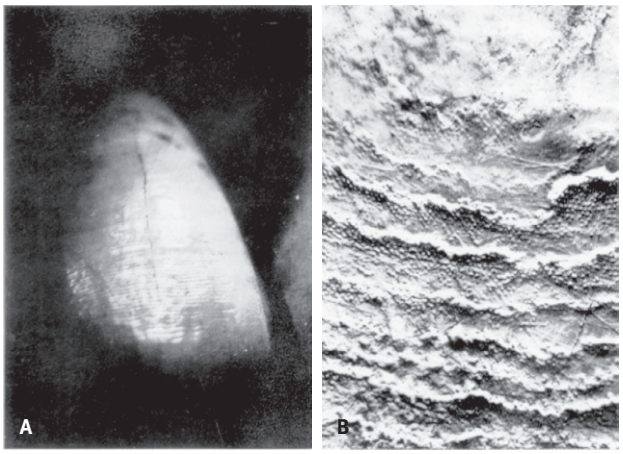

Figure 4.18 (**A**) Perikymata on lateral incisor. (**B**) Shadowed replica of surface of intact enamel (buccal surface of upper left second molar showing perikymata) (× 1500) (**B** from Scott DB and Wyckoff RWG: Public Health Rep 61:1397, 1946).

and of rest can be upset by metabolic disturbances, causing the rest periods to be unduly prolonged and lines closer together. Such an abnormal condition is responsible for the broadening of the incremental lines of Retzius, rendering them more prominent.

Surface structures

A relatively structureless layer of enamel, approximately 30 µm thick, called *prismless enamel*, has been described in 70% of permanent teeth and all deciduous teeth. This structureless enamel is found least often over the cusp tips and most commonly toward the cervical areas of the enamel surface. In this surface layer no prism outlines are visible, and all of the apatite crystals are parallel to one another and perpendicular to the striae of Retzius. It is also somewhat more heavily mineralized than the bulk of enamel beneath it. Other microscopic details that have been observed on outer enamel surfaces of newly erupted teeth are perikymata, rod ends, and cracks (lamellae).

Perikymata are transverse, wave-like grooves, believed to be the external manifestations of the striae of Retzius. They are continuous around a tooth and usually lie parallel to each other and to the cementoenamel junction (Figs 4.18 and 4.19). Ordinarily there are about 30 perikymata per millimeter in the region of the cementoenamel junction, and their concentration gradually decreases to about 10 per millimeter near the occlusal or incisal edge of a surface. Their course usually is fairly regular, but in the cervical region it may be quite irregular. The terms perikymata and imbrication lines are used without distinction to the surface structures of ridges or grooves. To avoid confusion in the usage of these terms it has been suggested that the terms perikymata or imbrication lines be suffixed by the words ridge or crest to denote elevations and the terms groove or furrow to denote depressions, as for example, perikymata ridge.

The enamel rod ends are concave and vary in depth and shape. They are shallowest in the cervical regions of surfaces and deepest near the incisal or occlusal edges (Fig. 4.18B).

Ultrastructurally, the surface of the enamel appears very uneven. *Pits* of about 1–1.5 µm in diameter and small elevations of about 10–15 µm called *enamel caps* are seen. The surface pits are said to represent the ends of ameloblast and the caps are due to enamel deposition on

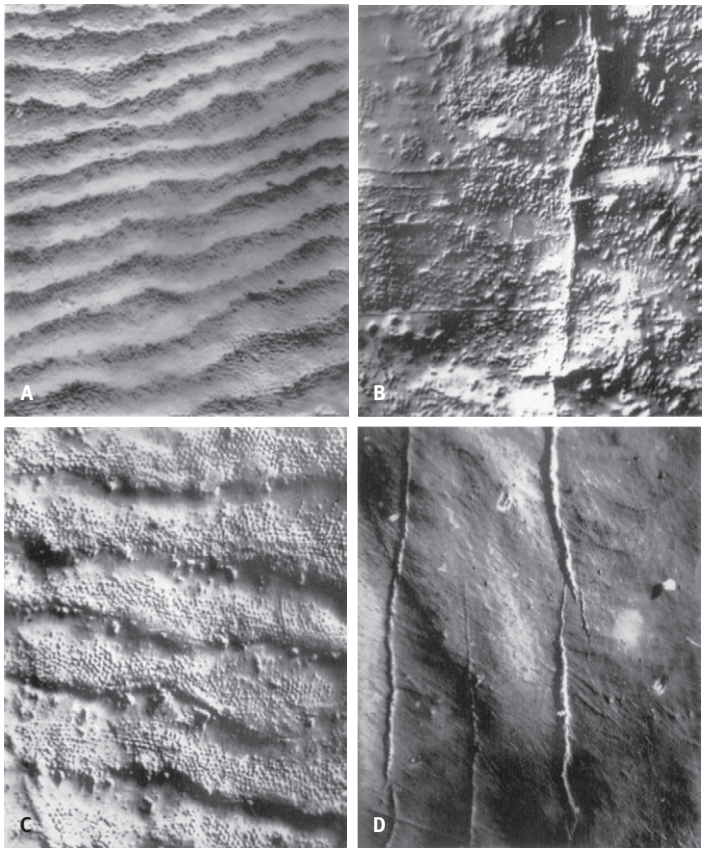

Figure 4.19 Progressive loss of surface structure with advancing age. (**A**) Surface of recently erupted tooth showing pronounced enamel prism ends and perikymata. Patient is 12 years of age. (**B**) Early stage of structural loss that occurs during first few years (wear is more rapid on anterior teeth than on posterior teeth and more rapid on facial or lingual surfaces than on proximal surfaces). Note small regions where prism ends are worn away. Patient is 25 years of age. (**C**) Later stage. Here elevated parts between perikymata are worn smooth, while structural detail in depths of grooves is still more or less intact. Eventually wearing proceeds to point where all prism ends and perikymata disappear. Patient is 52 years of age (Since these are negative replicas, surface details appear inverted. Raised structures represent depressions in actual surface). (**D**) Surface worn completely smooth and showing only 'cracks,' which actually represent outer edges of lamellae. Patient is 50 years of age (All magnifications × 105) (From Scott DB and Wyckoff RWG: J Am Dent Assoc 39:275, 1949).

nonmineralizable debris. Larger enamel elevations are termed *enamel brochs.*

The term 'cracks' originally was used to describe the narrow, fissure-like structures that are seen on almost all surfaces (Fig. 4.19D). It has since been demonstrated that they are actually the outer edges of lamellae (see discussion of enamel lamellae). They extend for varying distances along the surface, at right angles to the dentinoenamel junction, from which they originate. Most of them are less than a millimeter in length, but some are longer, and a few reach the occlusal or incisal edge of a surface. They are fairly evenly spaced, but long lamellae appear thicker than short ones.

The enamel of the deciduous teeth develops partly before and partly after birth. The boundary between the two portions of enamel in the deciduous teeth is marked by an accentuated incremental line of Retzius, the *neonatal line* or *neonatal ring* (Fig. 4.20). It appears to be the result of the abrupt change in the environment and nutrition of the newborn infant. The prenatal enamel usually is better developed than the postnatal enamel. This is explained by the fact that the fetus develops in a well-protected environment with an adequate supply of all the essential materials, even at the expense of the mother. Because of the undisturbed and even development of the enamel prior to birth, perikymata are absent in the occlusal parts of the deciduous teeth, whereas they are present in the postnatal cervical parts.

Neonatal lines were found to be more frequently absent in permanent first molars of boys than girls indicating that boys were less dentally mature than girls at the time of their birth. The location of neonatal line also varied in preterm and in postterm births.

Enamel cuticle

A delicate membrane called *Nasmyth's membrane,* after its first investigator, or the *primary enamel cuticle* covers the entire crown of the newly erupted tooth but is probably soon removed by mastication. Electron microscopic studies have indicated that this membrane is a typical basal lamina found beneath most epithelia (Fig. 4.21). It is probably visible with the light microscope because of its wavy course. This basal lamina is apparently secreted by the ameloblasts when enamel formation is completed. The

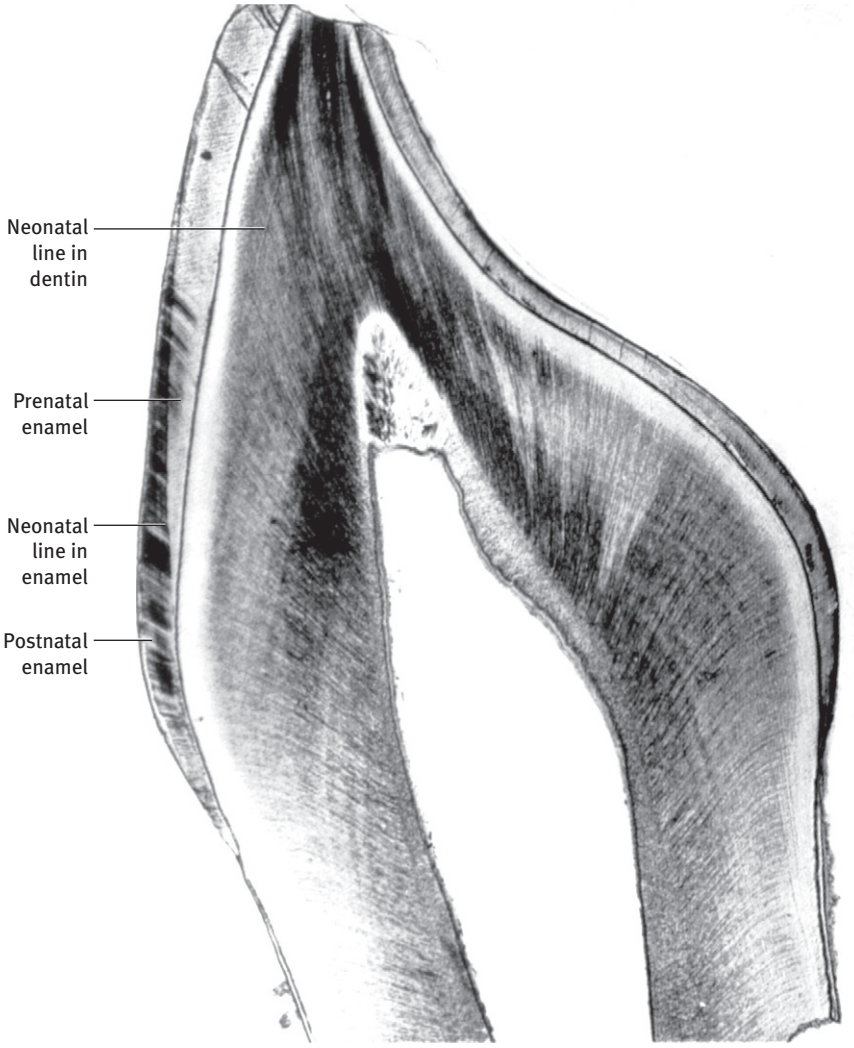

Figure 4.20 Neonatal line in enamel. Longitudinal ground section of deciduous canine (From Schour I: J Am Dent Assoc 23:1946, 1936).

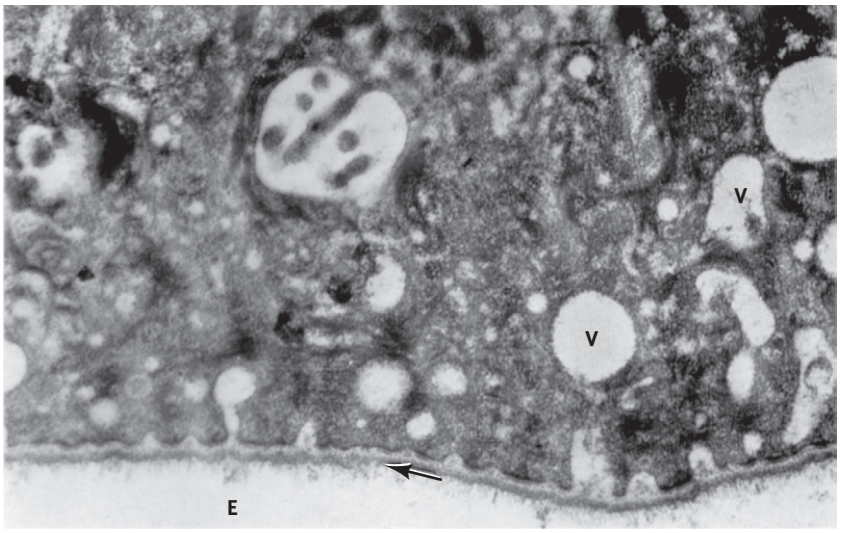

Figure 4.21 Electron micrograph of reduced enamel epithelium covering surface of unerupted human tooth. Enamel has been removed by demineralization, E. Typical basal lamina separates enamel space from epithelium (arrow). Epithelial cells contain a number of intracytoplasmic vacuoles, V (Approximately × 24,000) (From Listgarten MA: Arch Oral Biol 11:999, 1966).

function of enamel cuticle is to protect the surface of enamel from the resorptive activity of the adjacent vascular tissue prior to the eruption of the teeth. It has also been reported that the cervical area of the enamel is covered by *afibrillar cementum*, continuous with the cementum and probably of mesodermal origin (Fig. 4.22). This cuticle is apparently secreted after the epithelial enamel organ retracts from the cervical region during tooth development.

Finally, erupted enamel is normally covered by a *pellicle*, which is apparently a precipitate of salivary proteins (Fig. 4.23). This pellicle reforms within hours after an enamel surface is mechanically cleaned. Within a day or two after the pellicle has formed, it becomes colonized by microorganisms to form a bacterial plaque (Fig. 4.24).

Enamel lamellae

Enamel lamellae are thin, leaf-like structures that extend from the enamel surface toward the dentinoenamel junction (Fig. 4.25). They may extend to, and sometimes penetrate into, the dentin. They consist of organic material, with but little mineral content. In ground sections, these structures may be confused with cracks caused by grinding of the specimen (Fig. 4.16). Careful decalcification of ground sections of enamel makes possible the distinction between cracks and enamel lamellae. The former disappear, whereas the latter persist (Figs. 4.25A).

Lamellae may develop in planes of tension. Where rods cross such a plane, a short segment of the rod may not fully calcify. If the disturbance is more severe, a crack may

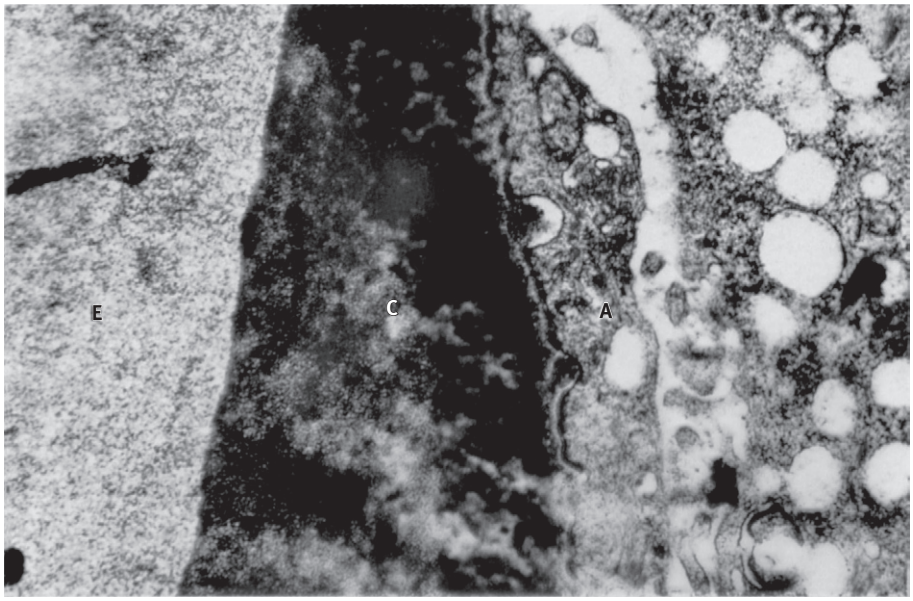

Figure 4.22 Electron micrograph of gingival area of erupted human tooth. Remnants of enamel matrix appear at left, E. Cuticle, C, separates enamel matrix from epithelial cells of attached epithelial cuff, A. Inner layers of cuticle (afibrillar cementum) are deposited before eruption; origin of outer layers is not known (Approximately × 37,000) (From Listgarten MA: Am J Anat 119:147, 1966).

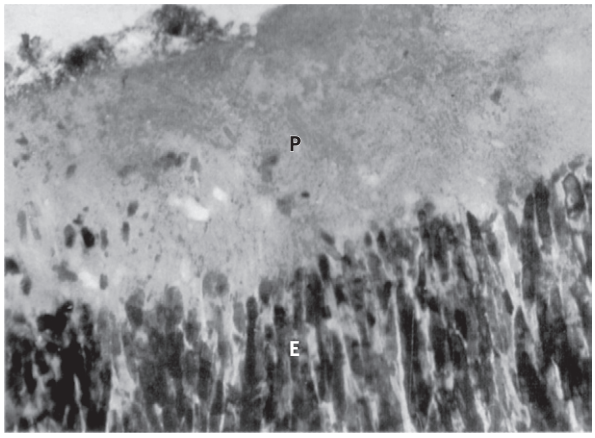

Figure 4.23 Electron micrograph of surface of undemineralized human enamel. Enamel surface, E, is covered by pellicle, P. Individual crystals can be seen in enamel (Approximately ×58,000) (From Houver G and Frank RM: Arch Oral Biol 12:1209, 1967).

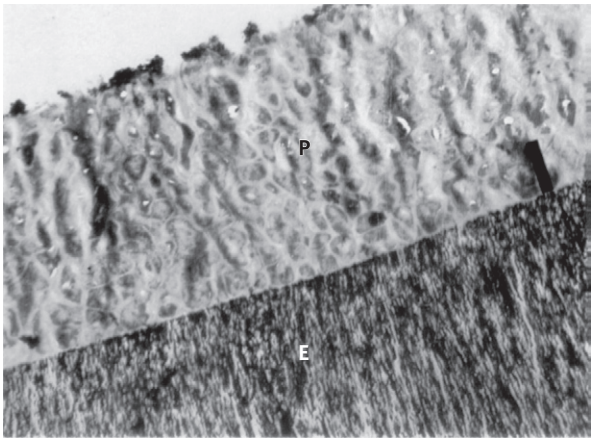

Figure 4.24 Electron micrograph of undemineralized human enamel surface. Enamel, E, is covered by a bacterial plaque, P. Black bar at right, Thickness of pellicle seen in Fig. 4.23 (Approximately × 12,000) (From Frank RM and Brendel A: Arch Oral Biol 11:883, 1966).

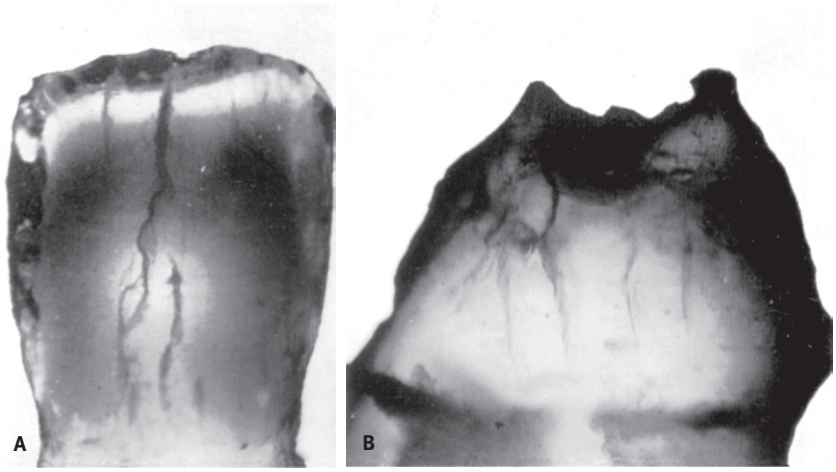

Figure 4.25 (**A**) Decalcified incisor with moderately severe mottled enamel. Numerous lamellae can be observed (× 8). (**B**) Maxillary first permanent molar of caries-free 2-year-old rhesus monkey. Numerous bands of organic matter, lamellae, can be seen after decalcification (× 8) (**B** from Sognnaes RF: J Dent Res 29:260, 1950).

develop that is filled either by surrounding cells, if the crack occurred in the unerupted tooth, or by organic substances from the oral cavity, if the crack developed after eruption. Three types of lamellae can thus be differentiated: type A, lamellae composed of poorly calcified rod segments; type B, lamellae consisting of degenerated cells; and type C, lamellae arising in erupted teeth where the cracks are filled with organic matter, presumably originating from saliva. The last type may be more common than formerly believed. Although lamellae of type A are restricted to the enamel, those of types B and C may reach into the dentin (Fig. 4.27). If cells from the enamel organ fill a crack in the enamel, those in the depth degenerate, whereas those close to the surface may remain vital for a time and produce a hornified cuticle in the cleft. In such cases the inner parts of the lamella consist of an organic cell detritus, the outer parts of a double layer of the cuticle. If connective tissue invades a crack in the enamel, cementum may be formed. In such cases lamellae consist entirely or partly of cementum.

Lamellae extend in the longitudinal and radial direction of the tooth, from the tip of the crown toward the cervical region (Fig. 4.25). This arrangement explains why they can be observed better in horizontal sections. It has been suggested that enamel lamellae may be a site of weakness in a tooth and may form a road of entry for bacteria that initiate caries. This suggestion was contested. Later it was shown that enamel cracks or lamellae showed uptake of dyes suggesting that these structures may act as pathways for caries producing bacteria.

Enamel tufts

Enamel tufts (Fig. 4.27) arise at the dentinoenamel junction and reach into the enamel to about one fifth to one third of its thickness. They were so termed because they resemble tufts of grass when viewed in ground sections. This picture is erroneous. An enamel tuft does not spring from a single small area but is a narrow, ribbon-like structure, the inner end of which arises at the dentin. The impression of a tuft of grass is created by examining such structures in thick sections under low magnification. Under these circumstances the imperfections, lying in different planes and curving in different directions (Fig. 4.13), are projected into one plane (Fig. 4.27).

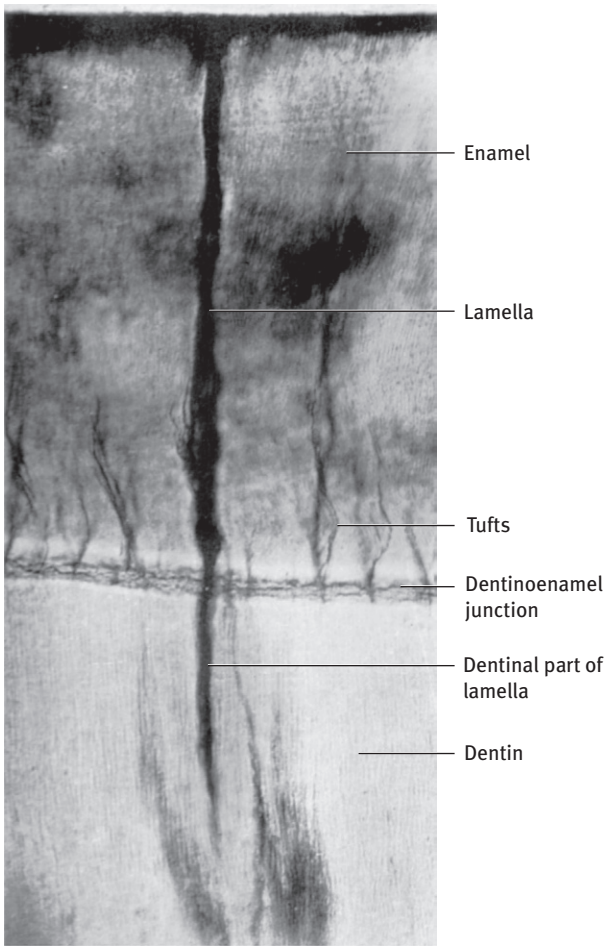

Figure 4.26 Transverse ground section through lamella reaching from surface into dentin.

Figure 4.27 Transverse ground section through tooth under low magnification. Numerous tufts extend from dentinoenamel junction into enamel.

Tufts consist of hypocalcified enamel rods and interprismatic substance. Like the lamellae, they extend in the direction of the long axis of the crown. Therefore they are seen abundantly in horizontal, and rarely in longitudinal, sections. Their presence and their development are a consequence of, or an adaptation to, the spatial conditions in the enamel.

SEM studies revealed enamel tufts to be tubular structures with cross-striations. TEM studies showed plate-like structures in the center of the tufts. These plates originated from the superficial layer of the dentin and entered the tufts after crossing the DE junction. In another study needle-like crystals and granular or amorphous material was found partially occluding the channels or the voids. These studies concluded that the tufts were hypomineralized structures. The major organic component of the tuft was a 13.17 kd protein, rather than amelogenin.

Dentinoenamel junction

The surface of the dentin at the dentinoenamel junctions is pitted. Into the shallow depressions of the dentin fit rounded projections of the enamel. This relation assures the firm hold of the enamel cap on the dentin. In sections, therefore, the dentinoenamel junction appears not as a straight but as a scalloped line (Figs. 4.27 and 4.28). The convexities of the scallops are directed toward the dentin. The pitted dentinoenamel junction is preformed even before the development of hard tissues and is evident in the arrangement of the ameloblasts and the basement membrane of the dental papilla (Fig. 4.38). In the dentinoenamel junction (DEJ) the crystals of dentin and enamel mix with each other. The DEJ, which is a series of ridges is more pronounced in the occlusal area, where masticatory stresses are greater.

In microradiographs of ground sections a hypermineralized zone about 30 µm thick can sometimes be demonstrated at the dentinoenamel junction. It is most prominent before mineralization is complete.

Odontoblast processes and enamel spindles

Occasionally odontoblast processes pass across the dentinoenamel junction into the enamel. Since many are thickened at their end (Fig. 4.29), they have been termed

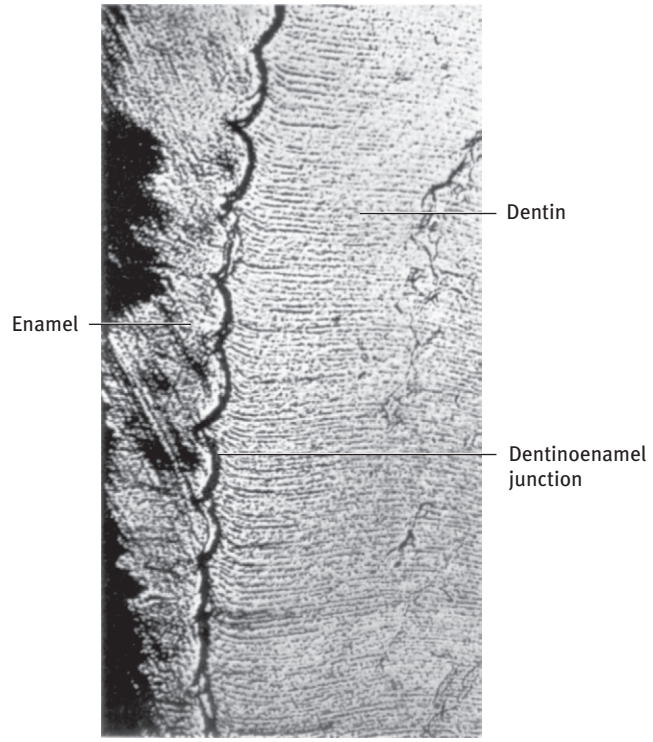

Figure 4.28 Longitudinal ground section. Scalloped dentinoenamel junction.

enamel spindles. They seem to originate from processes of odontoblasts that extended into the enamel epithelium before hard substances were formed. The direction of the odontoblast processes and spindles in the enamel corresponds to the original direction of the ameloblasts—at right angles to the surface of the dentin. Since the enamel rods are formed at an angle to the axis of the ameloblasts, the direction of spindles and rods is divergent. In ground sections of dried teeth the organic content of the spindles disintegrates and is replaced by air, and the spaces appear dark in transmitted light.

TEM studies showed spindles to be channels of about 2 microns in diameter containing small needle-like crystals

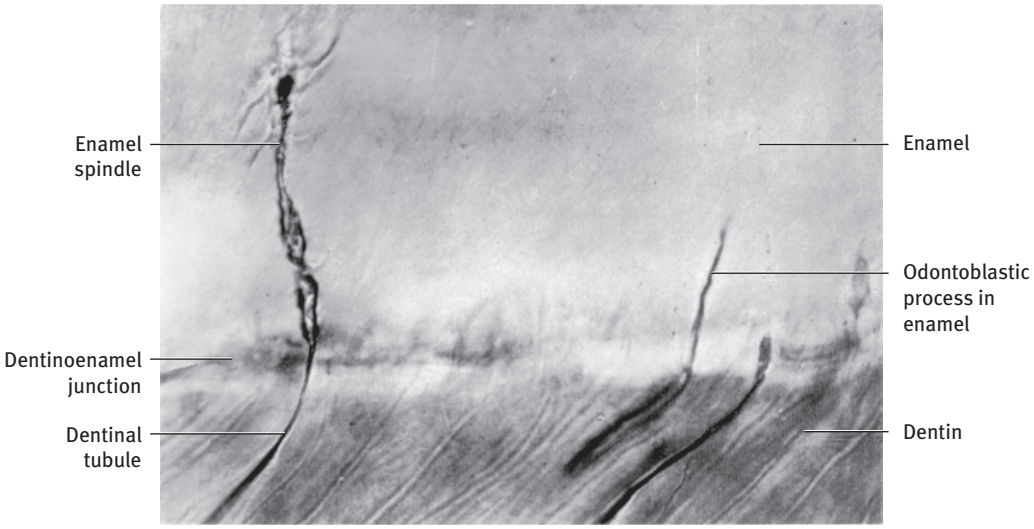

Figure 4.29 Ground section. Odontoblast processes extend into enamel as enamel spindles.

of about 70 nm in length and about 5 nm in width, or granular and/or amorphous material. The structure of the spindles were similar to enamel tufts and that both of them were hypomineralized or partially mineralized structures. They are found mainly in the cup tip regions. Energy dispersive X-ray microscopy showed that enamel tufts, lamellae and spindles contained less calcium and phosphorus than enamel prisms. Both SEM and TEM studies did not reveal peritubular dentin, membranous structures or lamina limitans surrounding the enamel spindles.

Age Changes

Age changes in enamel are dealt in detail in Chapter 17 on Age Changes in Oral Tissues (Ch 17). However, the age changes are briefly outlined below.

Enamel undergoes wear due to mastication termed attrition. Perikymata is lost after eruption. Fluoride uptake by superficial layers of enamel is noted and permeability of enamel is decreased.

CLINICAL CONSIDERATIONS

The course of the enamel rods is of importance in cavity preparations. The choice of instruments depends on the location of the cavity in the tooth. Generally the rods run at a right angle to the underlying dentin or tooth surface. Close to the cementoenamel junction the rods run in a more horizontal direction (Fig. 4.12B). In preparing cavities, it is important that unsupported enamel rods are not left at the cavity margins because they would soon break and produce leakage. Bacteria would lodge in these spaces, inducing secondary dental caries. Enamel is brittle and does not withstand forces in thin layers or in areas where it is not supported by the underlying dentin (Fig. 4.30A).

Deep enamel fissures predispose teeth to caries. Although these deep clefts between adjoining cusps cannot be regarded as pathologic, they afford areas for retention

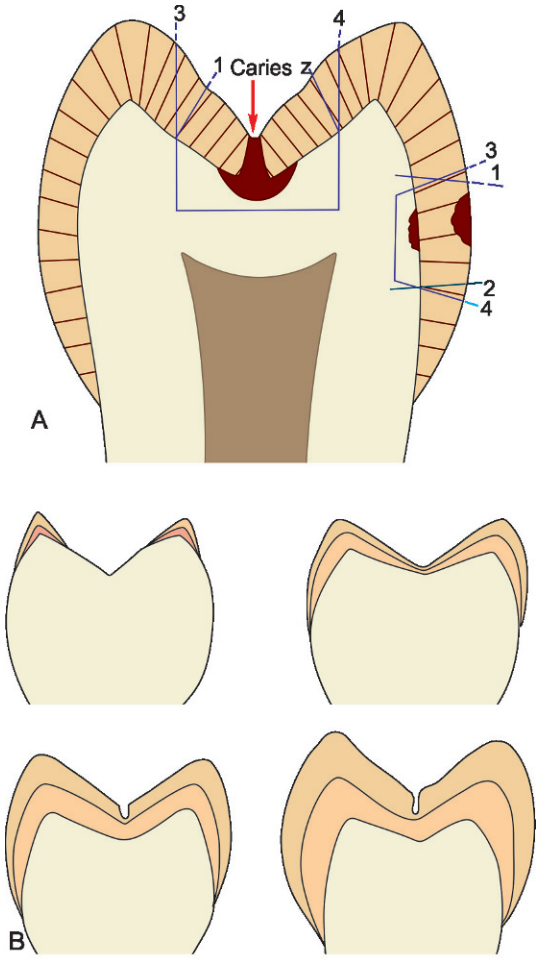

Figure 4.30 (**A**) Diagram of course of enamel rods in molar in relation to cavity preparation. 1 and 2 indicate wrong preparation of cavity margins. 3 and 4 indicate correct preparation. (**B**) Diagram of development of deep enamel fissure. Note thin enamel layer forming floor of fissure (**B** from Kronfeld R: J Am Dent Assoc 22:1131, 1935).

of caries-producing agents. Caries penetrate the floor of fissures rapidly because the enamel in these areas is very thin (Fig. 4.30B). As the destructive process reaches the dentin, it spreads along the dentinoenamel junction, undermining the enamel. An extensive area of dentin becomes carious without giving any warning to the patient because the entrance to the cavity is minute. Careful examination is necessary to discover such cavities because most enamel fissures are more minute than a single toothbrush bristle and cannot be detected with the dental probe.

Dental lamellae may also be predisposing locations for caries because they contain much organic material. Primarily from the standpoint of protection against caries, the structure and reactions of the outer enamel surface are subject to much current research. In vitro tests have shown that the acid solubility of enamel can be greatly reduced by treatment with fluoride compounds. Clinical trials based on these studies have demonstrated reductions of 40% or more in the incidence of caries in children after topical applications of sodium or stannous fluoride. Incorporation of fluorides in dentifrices is now a well-accepted means of caries prevention. Fluoride-containing mixtures such as stannous fluoride pastes, sodium fluoride rinses, and acidulated phosphate fluoride are also used by the dentist to alter the outer surface of the enamel in such a manner that it becomes more resistant to decay.

The most effective means for mass control of dental caries to date has been adjustment of the fluoride level in communal water supplies to 1 part per million. Epidemiologic studies in areas in which the drinking water contained natural fluoride revealed that the caries prevalence in both children and adults was about 65% lower than in nonfluoride areas, and long-term studies have demonstrated that the same order of protection is afforded through water fluoridation programs. The mechanisms of action are believed to be primarily a combination of changes in enamel resistance, brought about by incorporation of fluoride during calcification, and alterations in the environment of the teeth, particularly with respect to the oral bacterial flora.

The surface of the enamel in the cervical region should be kept smooth and well polished by proper home care and by regular cleansing by the dentist. If the surface of the cervical enamel becomes decalcified or otherwise roughened, food debris, bacterial plaques, and so on accumulate on this surface. The gingiva in contact with this roughened, debris covered enamel surface undergoes inflammatory changes. The ensuing gingivitis, unless promptly treated, may lead to more serious periodontal disease.

One of the more recently developed techniques in operative dentistry consists of the use of composite resins. These materials can be mechanically 'bonded' directly to the enamel surface. In this procedure the enamel surface is first etched with an acid (phosphoric acid 37%) to remove the smear layer on the enamel that was created during cavity preparation. Smear layers are about 1 µm thick and are made up of burnished cutting debris. Because the particles that constitute the smear layer are very small, the layer is very acid labile. Acid etching of enamel removes this smear layer. This produces an uneven dissolution of the enamel rods and their 'sheaths' or enamel 'heads' and their 'tails' so that a relatively smooth enamel surface becomes pitted and irregular. When a composite resin is put on this irregular surface, it can achieve mechanical bonding with the enamel. The same principle is used in coating the susceptible areas of the enamel with the so-called pit fissure sealants.

Depending on the crystal orientation to the surface three types of etching patterns are produced. Crystals dissolve more readily at their ends than on their sides. Hence, those crystals which are perpendicular to the surface, are removed preferentially. This results in crystal removal from the rods and is called type I pattern. In the type II pattern the interrod crystals are preferentially removed. The type III pattern is irregular. Prismless enamel found on the surface does not provide enough mechanical retention so etching should go beyond the prismless enamel to the prismatic enamel below it.

DEVELOPMENT

Epithelial Enamel Organ

The early development of the enamel organ and its differentiation have been discussed in Chapter 3. At the stage preceding the formation of hard structures (dentin and enamel) the enamel organ, originating from the stratified epithelium of the primitive oral cavity, consists of four distinct layers: outer enamel epithelium, stellate reticulum, stratum intermedium, and inner enamel epithelium (ameloblastic layer) (Fig. 4.31). The borderline between the inner enamel epithelium and the connective tissue of the dental papilla is the subsequent dentinoenamel junction. Thus its outline determines the pattern of the occlusal or incisal part of the crown. At the border of the wide basal opening of the enamel organ, the inner enamel epithelium reflects onto the outer enamel epithelium. This is the *cervical loop*. The inner and outer enamel epithelia are elsewhere separated from each other by a large mass of cells differentiated into two distinct layers. The layer that is close to the inner enamel epithelium consists of two or three rows of flat polyhedral cells—the stratum intermedium. The other layer, which is more loosely arranged, constitutes the stellate reticulum.

Outer enamel epithelium

In the early stages of development of the enamel organ the outer enamel epithelium consists of a single layer of cuboid cells, separated from the surrounding connective tissue of the dental sac by a delicate basement membrane (Fig. 4.32). Prior to the formation of hard structures, this regular arrangement of the outer enamel epithelium is maintained only in the cervical parts of the enamel organ. At the highest convexity of the organ (Fig. 4.31) the cells of the outer enamel epithelium become irregular in shape and cannot be distinguished easily from the outer portion of the stellate reticulum. The capillaries in the connective tissue surrounding the epithelial enamel organ proliferate and protrude toward it (Fig. 4.32). Immediately before enamel formation commences, capillaries

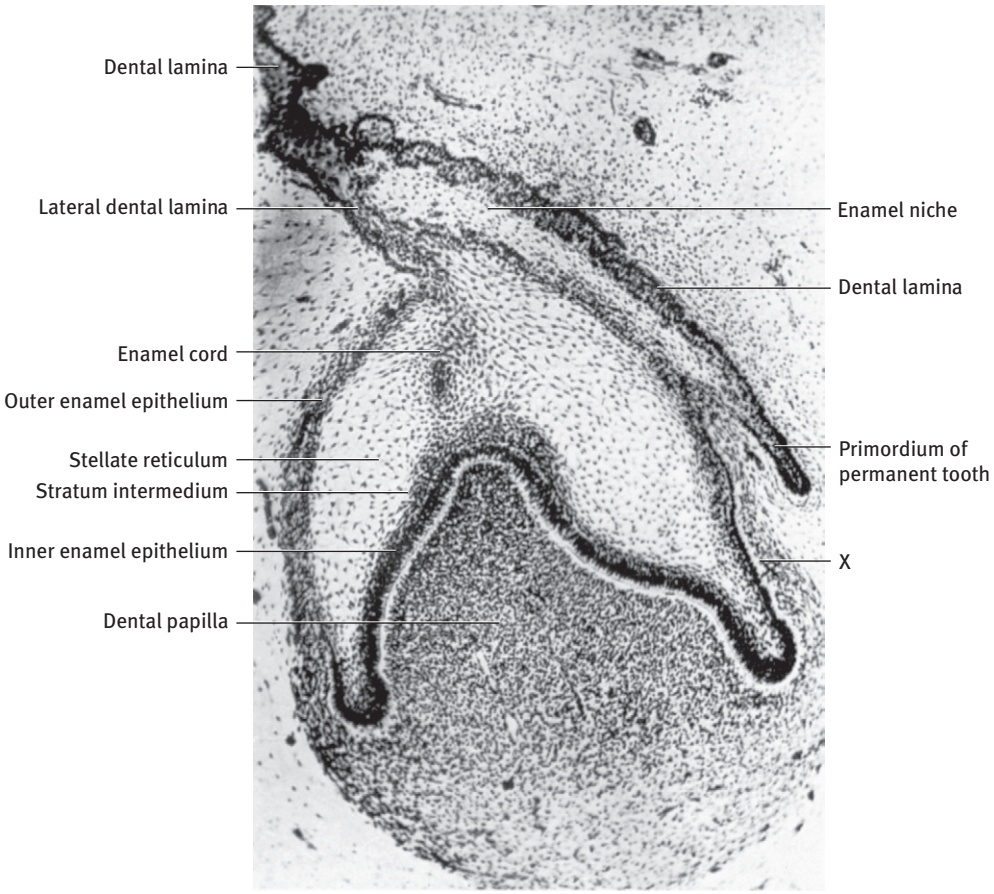

Figure 4.31 Tooth germ (deciduous lower incisor) of human embryo 105 mm, fourth month. Four layers of enamel organ. Area at X is shown at a higher magnification in Figure 4.33.

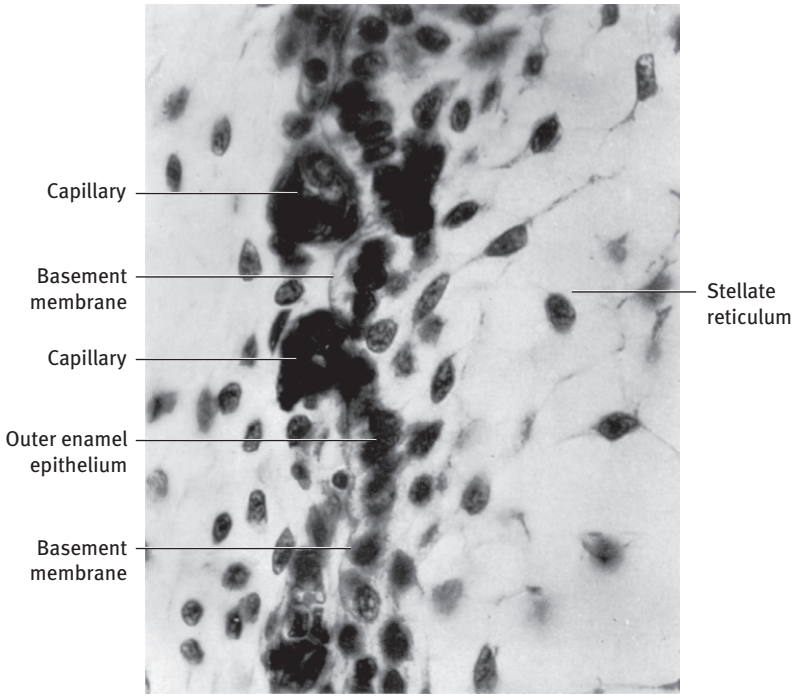

Figure 4.32 Capillaries in contact with outer enamel epithelium. Basement membrane separates outer enamel epithelium from connective tissue.

may even indent the stellate reticulum. This increased vascularity ensures a rich metabolism when a plentiful supply of substances from the bloodstream to the inner enamel epithelium is required.

During enamel formation, cells of the outer enamel epithelium develop villi and cytoplasmic vesicles and large numbers of mitochondria, all indicating cell specialization for the active transport of materials. The capillaries in contact with the outer enamel epithelium show areas with very thin walls, a structural modification also commonly found in areas of active transport.

Stellate reticulum

In the stellate reticulum, which forms the middle part of the enamel organ, the neighboring cells are separated by wide intercellular spaces filled by a large amount of intercellular substance. The cells are star shaped, with long processes reaching in all directions from a central body (Figs. 4.32 and 4.33). They are connected with each other and with the cells of the outer enamel epithelium and the stratum intermedium by desmosomes.

The structure of the stellate reticulum renders it resistant and elastic. Therefore it seems probable that it acts as a buffer against physical forces that might distort the conformation of the developing dentinoenamel junction, giving rise to gross morphologic changes. It seems to permit only a limited flow of nutritional elements from the outlying blood vessels to the formative cells. Indicative of this is the fact that the stellate reticulum is noticeably reduced in thickness when the first layers of dentin are laid down, and the inner enamel epithelium is thereby cut off from the dental papilla, its original source of supply (Fig. 4.34).

Stratum intermedium

The cells of the stratum intermedium are situated between the stellate reticulum and the inner enamel epithelium. They are flat to cuboid in shape and are arranged in one to three layers. They are connected with each other and with the neighboring cells of the stellate reticulum and the inner enamel epithelium by desmosomes. Tonofibrils, with an orientation parallel to the surface of the developing enamel, are found in the cytoplasm. The function of the stratum intermedium is not understood, but it is believed to play a role in production of the enamel itself, either through control of fluid diffusion into and out of the ameloblasts or by the actual contribution of necessary formative elements or enzymes. The cells of the stratum intermedium show mitotic division even after the cells of the inner enamel epithelium cease to divide.

Inner enamel epithelium

The cells of the inner enamel epithelium are derived from the basal cell layer of the oral epithelium. Before enamel formation begins, these cells assume a columnar form and differentiate into ameloblasts that produce the enamel matrix. The changes in shape and structure that the cells of the inner enamel epithelium undergo will be

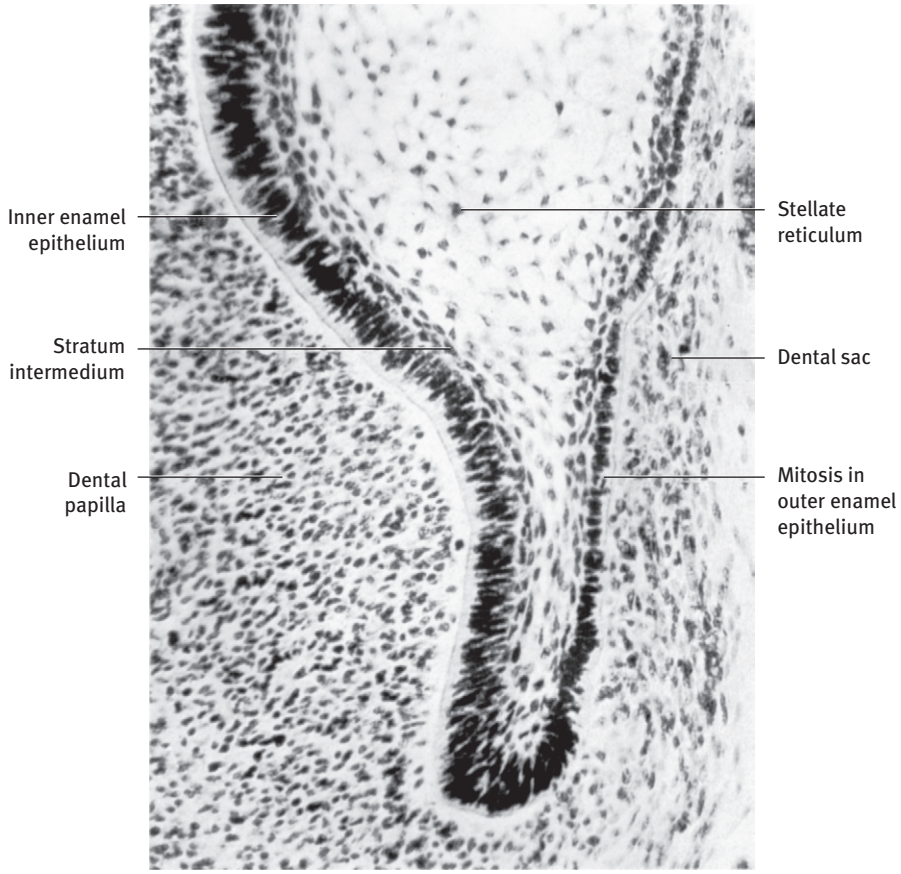

Figure 4.33 Region of cervical loop (higher magnification of area X in Fig. 4.31). Transition of outer into inner enamel epithelium.

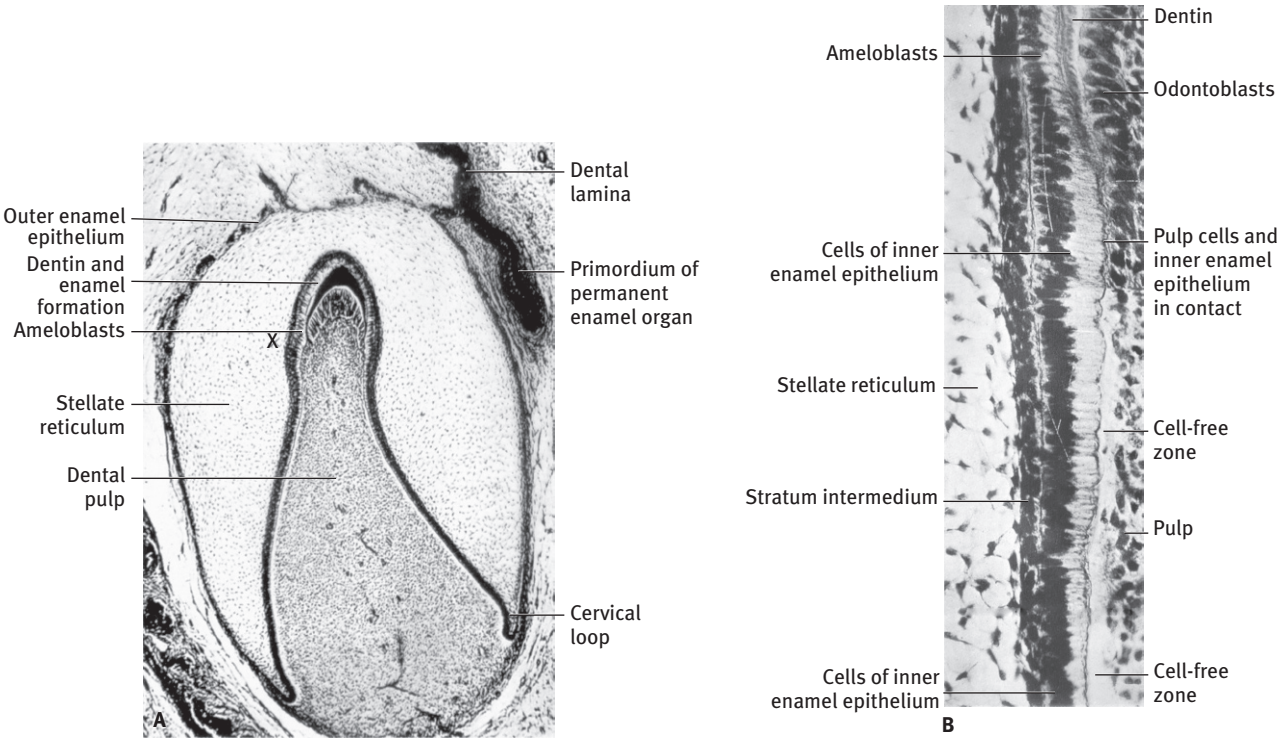

Figure 4.34 (**A**) Tooth germ (lower incisor) of human fetus (fifth month). Beginning of dentin and enamel formation. Stellate reticulum at tip of crown is reduced in thickness. (**B**) High magnification of inner enamel epithelium from area X in (**A**). In cervical region, cells are short, and outermost layer of pulp is cell free. Occlusally cells are long, and cell-free zone of pulp has disappeared. Ameloblasts are again shorter where dentin formation has begun and enamel formation is imminent (**B**) (from Diamond M and Weinmann JP: J Dent Res 21:403, 1942).

described in detail in the discussion of the life cycle of the ameloblasts. It should be mentioned, however, that cell differentiation occurs earlier in the region of the incisal edge or cusps than in the area of the cervical loop.

Cervical loop

At the free border of the enamel organ the outer and inner enamel epithelial layers are continuous and reflected into one another as the cervical loop (Fig. 4.31). In this zone of transition between the outer enamel epithelium and the inner enamel epithelium the cuboid cells gradually gain in length. When the crown has been formed, the cells of this portion give rise to Hertwig's epithelial root sheath (see Chapter 3).

Life Cycle of the Ameloblasts

According to their function, the life span of the cells of the inner enamel epithelium can be divided into six stages: (1) morphogenic, (2) organizing, (3) formative, (4) maturative, (5) protective, and (6) desmolytic. Since the differentiation of ameloblasts is most advanced in the region of the incisal edge or tips of the cusps and least advanced in the region of the cervical loop, all or some stages of the developing ameloblasts can be observed in one tooth germ. Amelogenesis which is the formation of enamel occurs during formative and maturative stages of the ameloblasts and is discussed in detail under the section on amelogenesis.

Morphogenic stage

Before the ameloblasts are fully differentiated and produce enamel, they interact with the adjacent mesenchymal cells, determining the shape of the dentinoenamel junction and the crown (Fig. 4.34A). During this morphogenic stage the cells are short and columnar, with large oval nuclei that almost fill the cell body.

The Golgi apparatus and the centrioles are located in the proximal end of the cell,[1] whereas the mitochondria are evenly dispersed throughout the cytoplasm. During ameloblast differentiation, terminal bars appear concomitantly with the migration of the mitochondria to the basal region of the cell. The terminal bars represent points of close contact between cells. They were previously believed to consist of dense intercellular substance, but under the electron microscope it has been found that they comprise thickening of the opposing cell membranes, associated with condensations of the underlying cytoplasm.

The inner enamel epithelium is separated from the connective tissue of the dental papilla by a delicate basal lamina. The adjacent pulpal layer is a cell-free, narrow, light zone containing fine argyrophil fibers and the cytoplasmic processes of the superficial cells of the pulp (Fig. 4.34B).

[1]In modern usage, to conform with the terminology applied to other secretory cells, the dentinal end of the ameloblast, at which enamel is formed, is called *distal*, and the end facing the stratum intermedium is called *basal* or *proximal*.

Organizing stage

In the organizing stage of development the inner enamel epithelium interacts with the adjacent connective tissue cells, which differentiate into odontoblasts. This stage is characterized by a change in the appearance of the cells of the inner enamel epithelium. They become longer, and the nucleus-free zones at the distal ends of the cells become almost as long as the proximal parts containing the nuclei (Fig. 4.34B). In preparation for this development a reversal of the functional polarity of these cells takes place by the migration of the centrioles and Golgi regions from the proximal ends of the cells into their distal ends.

Special staining methods reveal the presence of fine acidophil granules in the proximal part of the cell. Electron microscopic studies have shown that these granules are actually the mitochondria, which have become concentrated in this part of the cell. At the same time the clear cell-free zone between the inner enamel epithelium and the dental papilla disappears (Fig. 4.34B), probably because of elongation of the epithelial cells toward the papilla. Thus the epithelial cells come into close contact with the connective tissue cells of the pulp, which differentiate into odontoblasts. Recently it has been shown that the preameloblasts secrete proteins similar to those of enamel matrix. These proteins appear to be phagocytosed by developing odontoblast. It is suggested that these proteins may play a role in epithelial mesenchymal interaction.

During the terminal phase of the organizing stage the formation of the dentin by the odontoblasts begins (Fig. 4.34B).

The first appearance of dentin seems to be a critical phase in the life cycle of the inner enamel epithelium. As long as it is in contact with the connective tissue of the dental papilla, it receives nutrient material from the blood vessels of this tissue. When dentin forms, however, it cuts off the ameloblasts from their original source of nourishment, and from then on they are supplied by the capillaries that surround and may even penetrate the outer enamel epithelium. This reversal of nutritional source is characterized by proliferation of capillaries of the dental sac and by reduction and gradual disappearance of the stellate reticulum (Fig. 4.34A). Thus the distance between the capillaries and the stratum intermedium and the ameloblast layer is shortened. Experiments with vital stains demonstrate this reversal of the nutritional stream.

Formative stage

The ameloblasts enter their formative stage after the first layer of dentin has been formed. The presence of dentin seems to be necessary for the beginning of enamel matrix formation just as it was necessary for the epithelial cells to come into close contact with the connective tissue of the pulp during differentiation of the odontoblasts and the beginning of dentin formation. This mutual interaction between one group of cells and another is one of the fundamental laws of organogenesis and histodifferentiation.

During formation of the enamel matrix the ameloblasts retain approximately the same length and arrangement. Changes in the organization and number of cytoplasmic organelles and inclusions are related to the initiation of secretion of enamel matrix.

The earliest apparent change is the development of blunt cell processes on the ameloblast surfaces, which penetrate the basal lamina and enter the predentin (Fig. 4.35).

Maturative stage

Enamel maturation (full mineralization) occurs after most of the thickness of the enamel matrix has been formed in the occlusal or incisal area. In the cervical parts of the crown, enamel matrix formation is still progressing at this time. During enamel maturation the ameloblasts are slightly reduced in length and are closely attached to enamel matrix. The cells of the stratum intermedium lose their cuboidal shape and regular arrangement and assume a spindle shape. It is certain that the ameloblasts also play a part in the maturation of the enamel. During maturation, ameloblasts display microvilli at their distal extremities, and cytoplasmic vacuoles containing material resembling enamel matrix are present (Figs. 4.44 and 4.46). These structures indicate an absorptive function of these cells.

Protective stage

When the enamel has completely developed and has fully calcified, the ameloblasts cease to be arranged in a well-defined layer and can no longer be differentiated from the cells of the stratum intermedium and outer enamel epithelium (Fig. 4.44). These cell layers then form a stratified epithelial covering of the enamel, the so-called reduced enamel epithelium. The function of the reduced enamel epithelium is that of protecting the mature enamel by separating it from the connective tissue until the tooth erupts. If connective tissue comes in contact with the enamel, anomalies may develop. Under such conditions the enamel may be either resorbed or covered by a layer of cementum.

During this phase of the life cycle of ameloblasts the epithelial enamel organ may retract from the cervical edge of the enamel. The adjacent mesenchymal cells may then deposit afibrillar cementum on the enamel surface (Fig. 4.36).

Desmolytic stage

The reduced enamel epithelium proliferates and seems to induce atrophy of the connective tissue separating it from the oral epithelium, so that fusion of the two epithelia can occur (see Chapter 10). It is probable that the epithelial cells elaborate enzymes that are able to destroy connective tissue fibers by desmolysis. Premature degeneration of the reduced enamel epithelium may prevent the eruption of a tooth.

Amelogenesis

On the basis of ultrastructure and composition, two processes are involved in the development of enamel: organic matrix formation and mineralization. Although the inception of mineralization does not await the completion of matrix formation, the two processes will be treated separately.

Formation of the enamel matrix

The ameloblasts begin their secretory activity when a small amount of dentin has been laid down. The ameloblasts lose the projections that had penetrated the basal

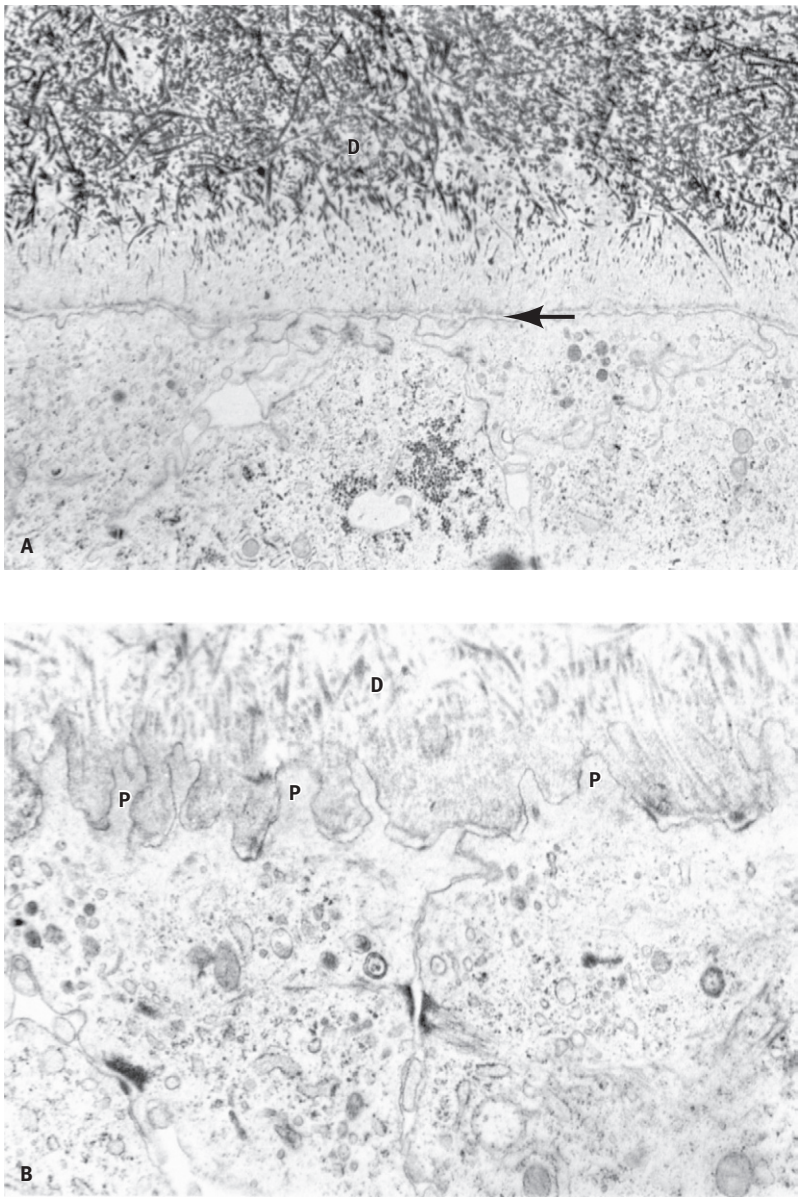

Figure 4.35 Electron micrographs of distal (secretory) ends of ameloblasts in stage of differentiation shortly before enamel formation begins. (**A**) Relatively smooth ameloblast surfaces are separated from predentin (D) by basal lamina (arrow). (Approximately × 11,000.) (**B**) At slightly later stage, ameloblast cell processes (P) have penetrated basal lamina and protrude into predentin (D) (Approximately × 16,000) (From Kallenbach E: Am J Anat 145:283, 1976).

lamina separating them from the predentin (compare Figs 4.35B and 4.37A), and islands of enamel matrix are deposited along the predentin (Fig. 4.37B). As enamel deposition proceeds, a thin, continuous layer of enamel is formed along the dentin (Figs 4.34B and 4.38).

Amelogenin is the major component of enamel matrix proteins. It undergoes extracellular degradation by proteolytic enzymes like matix metalloproteinases into smaller low molecular weight fragments, like tyrosine rich amelogenin protein, and leucine rich amelogenin polypeptide which are suggested to have specific functions as in regulating crystal growth. Many isoforms of amelogenins are produced with similar terminal amino acid sequences but with differing sequences in the central portions of the protein. The genes coding for amelogenin is present in X and Y chromosomes. Hence variations in amino acid sequences also exist between the sexes in humans. Most of the secreted amelogenin is removed during maturation. It is suggested that amelogenins form thixotropic gels in that they can be easily squeezed out by the pressure from the growing crystals. Amelogenins have been shown to form minute nanospheres between which enamel crystals form. Thus, it is suggested to have a functional role in maintaining spaces between the crystals. In the fully formed enamel amelogenin remains in between the crystals and also surrounding them. Experimentally, absence of amelogenin, resulted in the formation of hypoplastic teeth.

Ameloblastin and enamelin are the other important proteins of the enamel matrix. They also undergo extracellular

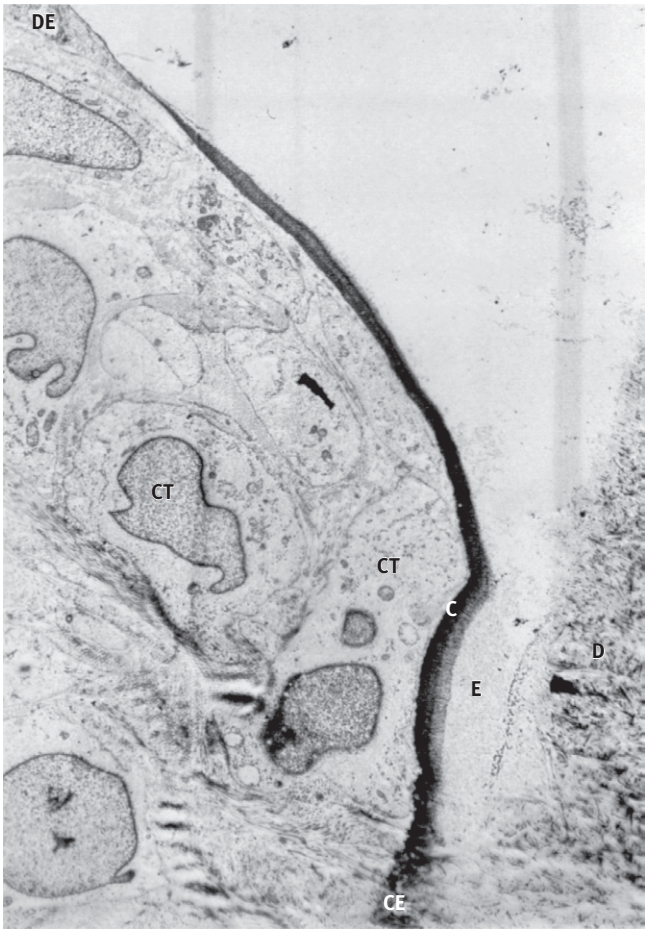

Figure 4.36 Electron micrograph of cervical region of unerupted human tooth. Dentin matrix, D, and remnants of demineralized enamel matrix, E, are at right. Afibrillar cementum, apparently of mesodermal origin, runs through center of figure, C, and is continuous with cementum, CE. Cells of adjacent connective tissue, CT, and retracted end of enamel organ, DE, are at left (Approximately × 6500) (From Listgarten MA: Arch Oral Biol 11:999, 1966).

processing like amelogenin, but at a rapid rate. Therefore time for interaction between nonamelogenin and amelogenin is limited. Ameloblastin and enamelin are reported to help in nucleation and growth of crystals. Tuftelin, on the hand, is localized to DE junction and is suggested to be involved in cell signaling. Experimentally, in the absence of ameloblastin, no structural layer of enamel formation is observed.

Recently, amelotin, a new protein was reported to be secreted by maturative ameloblast. This protein is suggested to help in enamel formation.

During early amelogenesis, ameloblasts transiently express dentin sialoprotein and dentin phosphoproteins. These proteins are usually expressed by odontoblast. These proteins localized to DEJ, suggest that the DEJ may be regarded as a transition zone between dentin and enamel.

Amelogenins were also found in the acellular cementum. It was reported to be involved in the formation of acellular cementum and help in the regeneration of cementum in teeth affected by periodontal diseases.

Development of Tomes' processes

The surfaces of the ameloblasts facing the developing enamel are not smooth. There is an interdigitation of the cells and the enamel rods that they produce (Fig. 4.39). This interdigitation is partly a result of the fact that the long axes of the ameloblasts are not parallel to the long axes of the rods (Figs 4.40 and 4.41). The projections of the ameloblasts into the enamel matrix have been named Tomes' processes. It was once believed that these processes were transformed into enamel matrix, but more recent electron microscopic studies have demonstrated that matrix synthesis and secretion by ameloblasts are very similar to the same processes occurring in other protein-secreting cells. Although Tomes' processes are partly delineated by incomplete septa (Fig. 4.40), they also contain typical secretion granules as well as rough endoplasmic reticulum and mitochondria (Figs. 4.42B and 4.47B).

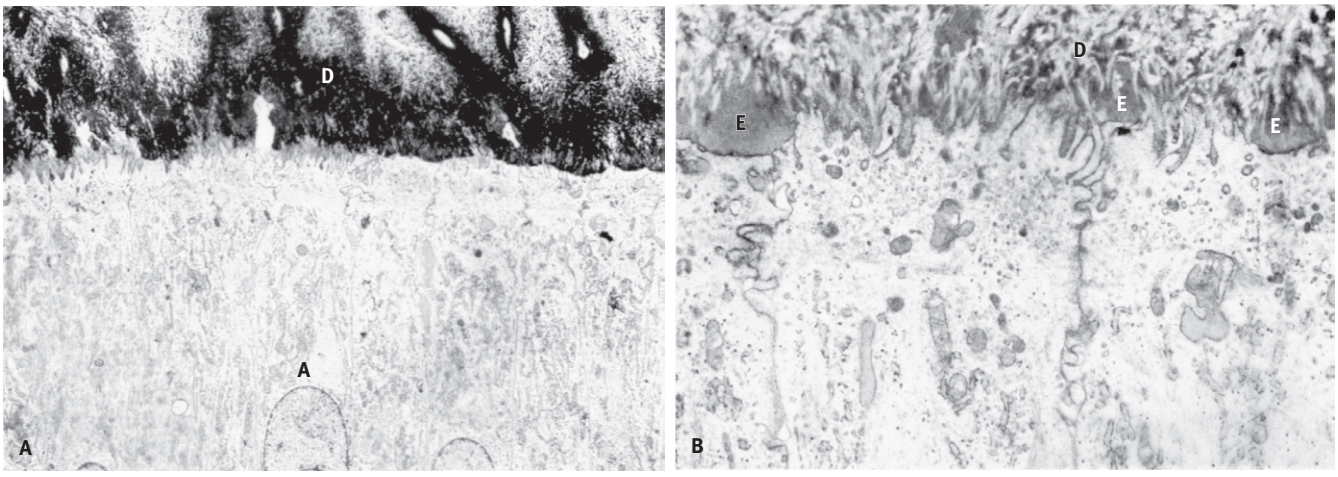

Figure 4.37 Electron micrographs of ameloblasts in later stage of differentiation than those in Fig. 4.35. (**A**) Ameloblasts (A) at left still retain their processes, while those at right, at a slightly later stage of differentiation, have smooth surfaces facing dentin (D). (Approximately × 2700) (**B**) Higher magnification of region similar to that in (**A**). In this decalcified section, dentin (D) is pale and islands of enamel matrix (E) are more darkly stained (Approximately × 12,000) (From Kallenbach E: Am J Anat 145:283, 1976).

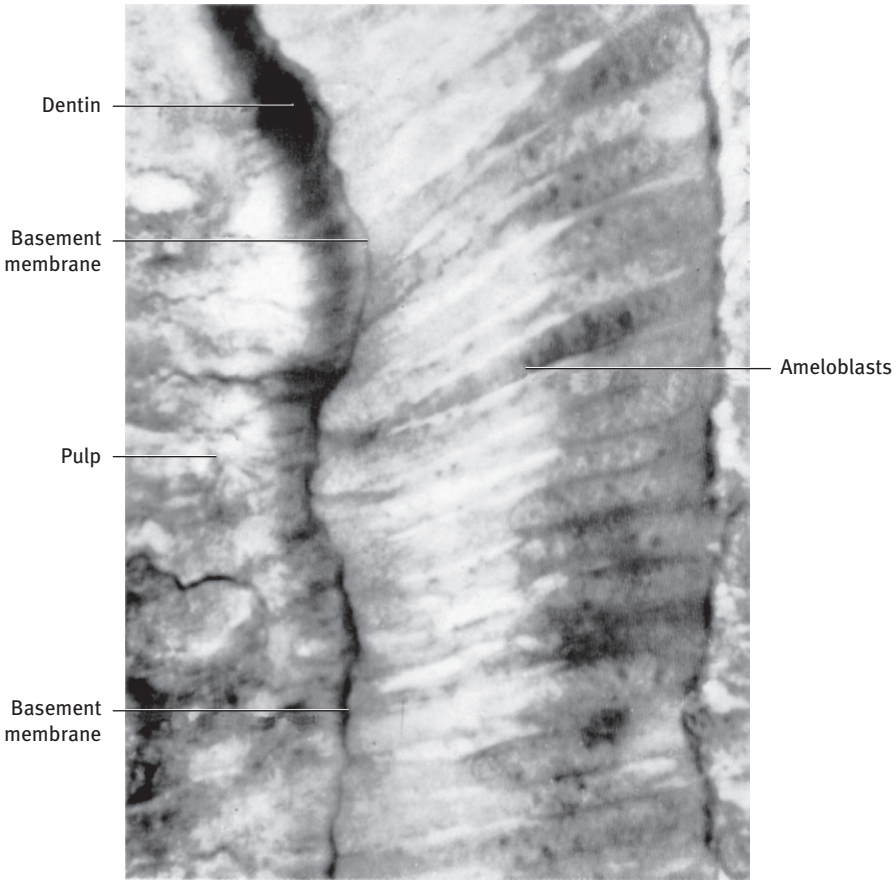

Figure 4.38 Basement membrane of dental papilla can be followed on outer surface of dentin, forming dentinoenamel membrane (From Orban B. Sicher H, and Weinmann JP: J Am Coll Dent 10:13, 1943).

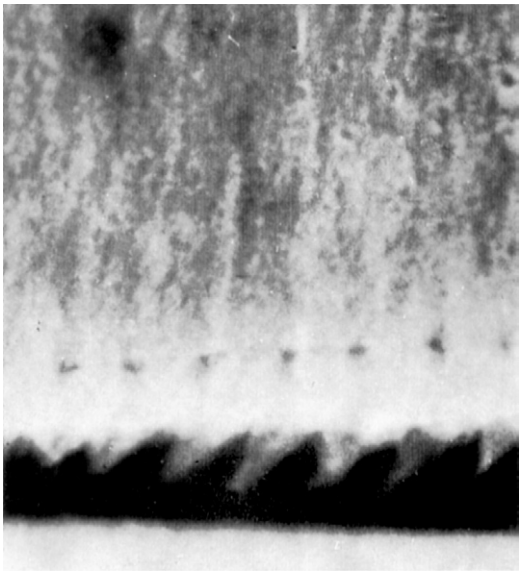

Figure 4.39 'Picket fence' arrangement of Tomes' processes. Rods are at angle to ameloblasts and Tomes' processes. (From Orban B, Sicher H, and Weinmann JP: J Am Coll Dent 10:13, 1943).

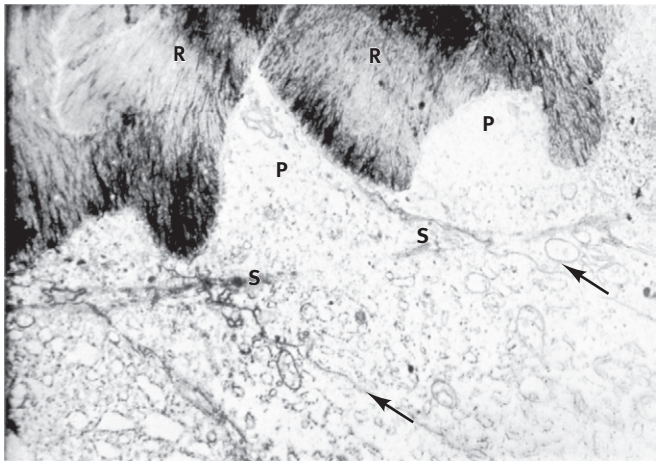

Figure 4.40 Electron micrograph of ends of ameloblasts and adjacent enamel in developing human deciduous tooth. Positions of ameloblast cell membranes (arrows) indicate that cells are nearly perpendicular to long axes of rods, R. An incomplete septum, S, can be seen, indicating approximate position of Tomes' processes, P (Approximately ×16,000) (From Rönnholm E: J Ultrastruct Res 6:249, 1962).

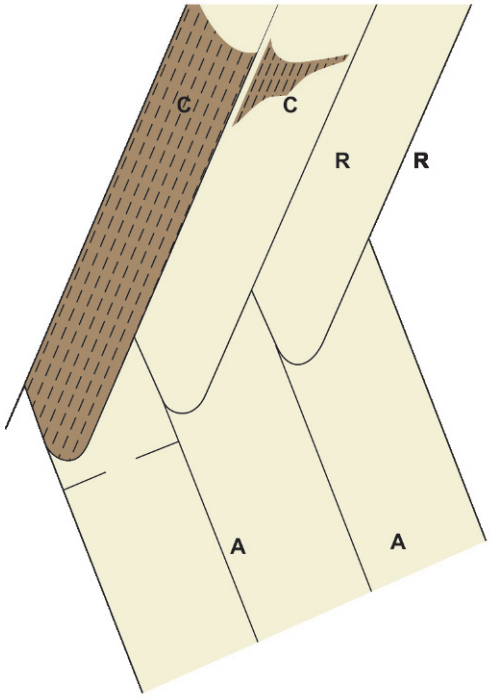

Figure 4.41 Drawing derived from Fig. 4.40. Dark lines indicate rod boundaries, R, and ameloblast cell surfaces, A, as well as incomplete septum near distal end of ameloblast at left. Gray lines indicate approximate orientation of apatite crystals, C.

The junctional complexes which encircle the ameloblast at their distal and proximal ends have fine radiating actin filaments extending into the cytoplasm, forming webs. These serve to control the substances that pass between ameloblast and enamel. The juctional complexes which form at the distal end are called *distal terminal bars*. These terminal bars separate the Tomes' processes from the cell proper (Fig. 4.42A).

Secretions from areas close to junctional complexes and from adjacent ameloblasts form the interrod enamel. They occur earlier and serve to outline the pit into which secretions from Tomes' process occur later to form the enamel rod. The distal portion of the Tomes' process lengthens and becomes narrower due to growth of crystals pressing against the wall of the pit, narrowing it, resulting in narrower rods.

The shape of the Tomes' process in the outer enamel gets altered, they appear to have ovoid profiles and its orientation to the cell body changes in that they become straighter.

Figure 4.41 is a drawing derived from the electron micrograph in Figure 4.40. It is clear from this sketch that at least two ameloblasts are involved in the synthesis of each enamel rod. If the surface of developing enamel is examined in the scanning electron microscope, which permits a three-dimensional visualization of the surface, the depressions resulting from the presence of Tomes' processes are quite obvious. One interpretation of the relationships between the key hole-shaped enamel rods and the roughly hexagonal ameloblasts is indicated in Figure 4.42. The

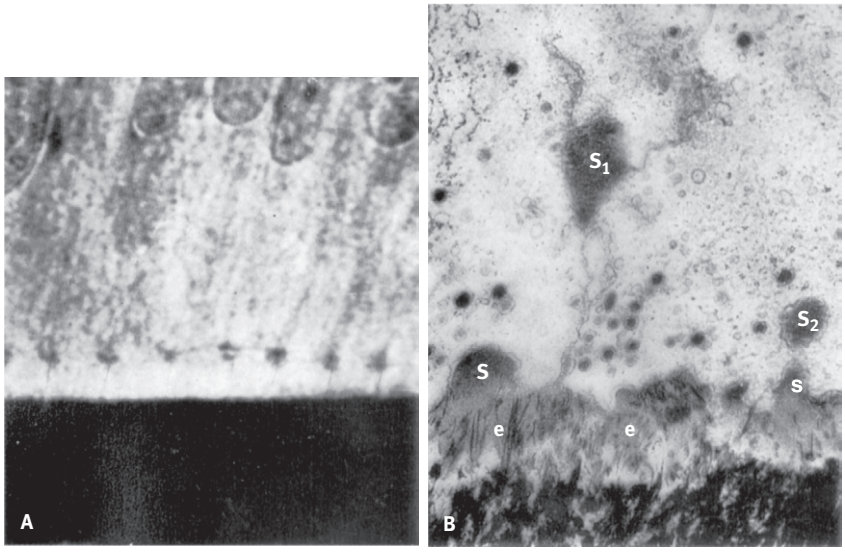

Figure 4.42 (**A**) Formation of Tomes' processes and terminal bars as first step in enamel rod formation. Rat incisor. (**B**) Electron photomicrograph showing an early stage in formation of enamel in lower incisor of rat. At this stage, dentin (at bottom of photomicrograph) is well developed. Enamel, e, appears as a less dense layer on surface of dentin and consists of thin, ribbon-shaped elements running more or less perpendicular to dentinoenamel junction and masses of a less dense stippled material, S. Separating enamel from cytoplasm of ameloblasts, which occupies most of upper part of photomicrograph, is ameloblast plasma membrane. Parts of three ameloblasts are shown. In middle of photomicrograph in region bounded by membranes of three ameloblasts lies another mass of stippled material, S1, while a second mass, S2, lies at right, surrounded by membrane, but within bounds of ameloblast. Numerous small, membrane-bound granules lie within cytoplasm. Contents of these have same general consistency as stippled material, but rather higher density. It is possible that these represent unsecreted granules of stippled material, which in turn is a precursor of enamel matrix (× 24,000) (**A** from Orban B, Sicher H, and Weinmann JP: J Am Coll Dent 10:13, 1943; B from Watson ML: J Biophys Biochem Cytol 7:489, 1960).

bulk of the 'head' of each rod is formed by one ameloblast, whereas three others contribute components to the 'tail' of each rod. According to this interpretation, each rod is formed by four ameloblasts, and each ameloblast contributes to four different rods.

Ameloblasts covering maturing enamel

At the light microscope level one can see that the ameloblasts over maturing enamel are considerably shorter than the ameloblasts over incompletely formed enamel (Fig. 4.44). In the electron microscope, several substages can be identified in the transition of ameloblasts from the formative stage through the maturative stage (Fig. 4.47). The changes occurring in the ameloblasts after secretory stage and prior to the onset of maturation process are called transition stage. During this stage ameloblasts reduce in height, enamel secretion stops completely and the process of amelogenin removal starts. About 50% of ameloblasts undergo apoptosis. The organelles involved in protein synthesis undergo autophagocytosis. Ameloblasts deposit a basal lamina, which adheres to the enamel surface and the ameloblast attaches to the basal lamina by hemidesmosomes.

Ameloblasts alternate cyclically in developing smooth and ruffled borders in the apical cytoplasm during the maturative stage in a cervicoincisal direction. These changes are referred to as *modulation*. Modulation between the two forms occur many times in a day (every 5 to 7 hours). The period of maturation is much longer (about twice/thrice) than the secretory phase. The ruffle ended ameloblasts have their villous surface packed with mitochondria (Figs 4.45 and 4.46). They have proximal junctions leaky and distal junctions tight while the arrangement is just the opposite in the smooth ended ameloblast, they possess leaky distal and tight proximal junctions. The ruffle ended ameloblasts show numerous lysosomes and possess endocytic activity. They also promote calcium entry into the forming enamel. The smooth ended ameloblasts have little endocytic activity but leak small proteins and water into the forming enamel. They do not show calcium pumping activity. Calcium ions pass actively through the ruffle ended ameloblasts and passively through the sides of the smooth ended ameloblast to the mineralizing front. Ruffle ended ameloblasts secrete bicarbonate ion to keep the mineralizing front alkaline, prevent acidification and thereby helps to keep the mineralization process to continue.

Ameloblasts secrete proteases of different types of which metalloproteinases and serine proteases are important. Metalloproteinases alter the basic structure of amelogenenins by hydrolysis into many low molecular weight fragments. Serine proteases function as bulk digestive enzymes to clear matrix proteins from intercrystal spaces. It degrades the enamel proteins into small polypeptides to be absorbed by the ruffle ended ameloblast and through leaky distal junctions as well as through the basolateral surfaces of the smooth ended ameloblasts.

Membrane bound proteins present in the ameloblasts like CD63, annexin A2 and lysosomal associated glycoprotein 1 interact with secreted proteins to initiate removal of the organic matrix. The understanding of these signaling system are of great importance in understanding experimental tooth regeneration.

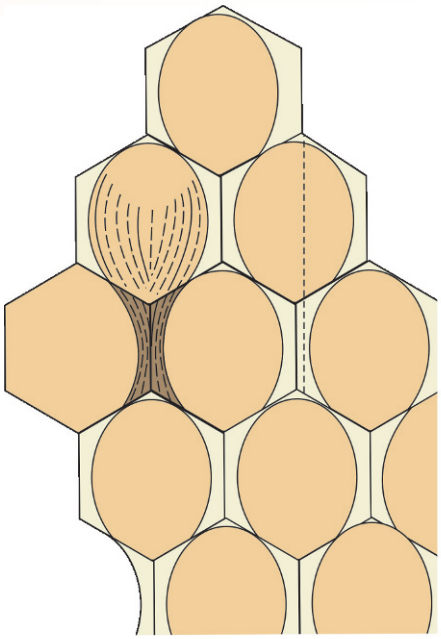

Figure 4.43 Drawing illustrating one interpretation of relationships between enamel rods and ameloblasts. Cross-sections of ameloblasts are indicated by thin lines arranged in regular hexagonal array. Enamel rods are indicated by thicker curved black lines, outlining keyhole-or paddle-shaped rods. Gray lines indicate approximate orientation of enamel crystals, which are parallel to long axes of rods in their 'bodies' and approach a position perpendicular to long axes in 'tails.' One can see that each rod is formed by four ameloblasts and that each ameloblast contributes to four different rods (Modified from Boyde A: In Stack MV and Fearnhead RW, editors: Tooth enamel, Bristol, 1965, John Wright & Sons Ltd).

The fact that organic components as well as water are lost in mineralization is a striking difference between enamel and other mineralized tissues. Over 90% of the initially secreted protein is lost during enamel maturation, and that which remains forms envelopes around individual crystals (Fig. 4.10), although there may be a higher content of organic matter in the area of the prism sheath where the abrupt change in crystal orientation occurs.

Mineralization and maturation of the enamel matrix

Mineralization of the enamel matrix takes place in two stages, although the time interval between the two appears to be very small. In the first stage an immediate partial mineralization occurs in the matrix segments and the interprismatic substance as they are laid down. No matrix vesicles are observed in enamel formation and no unmineralized matrix like that of predentin or osteoid is seen during enamel formation. Therefore, apatite crystals are not preformed when they are released by the secretory granules. Nucleation is initiated by the apatite crystallites of dentin on which enamel is laid.

Chemical analyses indicate that the initial influx may amount to 25–30% of the eventual total mineral content. It has been shown recently by electron microscopy and diffraction that this first mineral actually is in the form of crystalline apatite (Fig. 4.50A). However, other studies have shown that the initial mineral is octacalcium phosphate. The octacalcium may act as a template for hydroxyapatite.

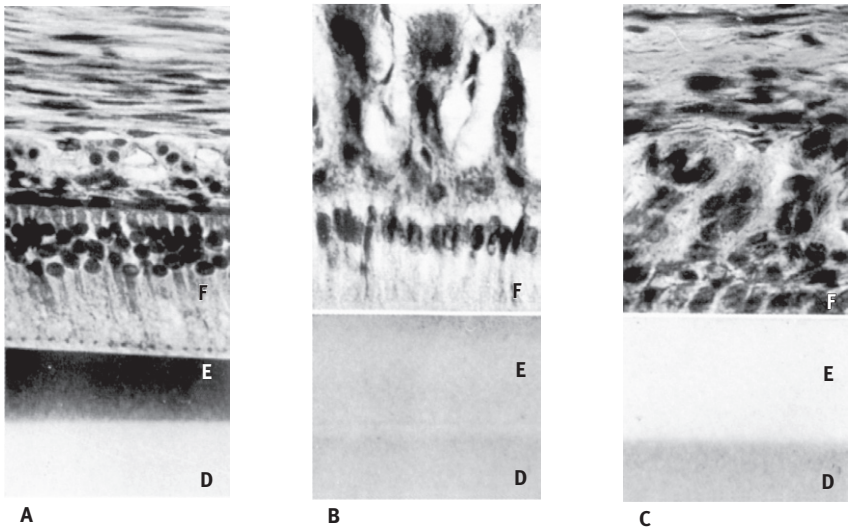

Figure 4.44 Light micrographs of various stages in life cycle of ameloblasts, F, in rat incisor matched with microradiographs of corresponding adjacent enamel, E, and dentin, D. (**A**) Ameloblasts are secreting enamel, which is incompletely formed. Enamel is less radiopaque than dentin, indicating that it is less mineralized. (**B**) In area of enamel maturation, ameloblasts are shorter, and enamel matrix is about as heavily mineralized as dentin. (**C**) In area in which ameloblasts are in protective stage, enamel is fully mineralized and is much more radiopaque than underlying dentin (All approximately × 260).

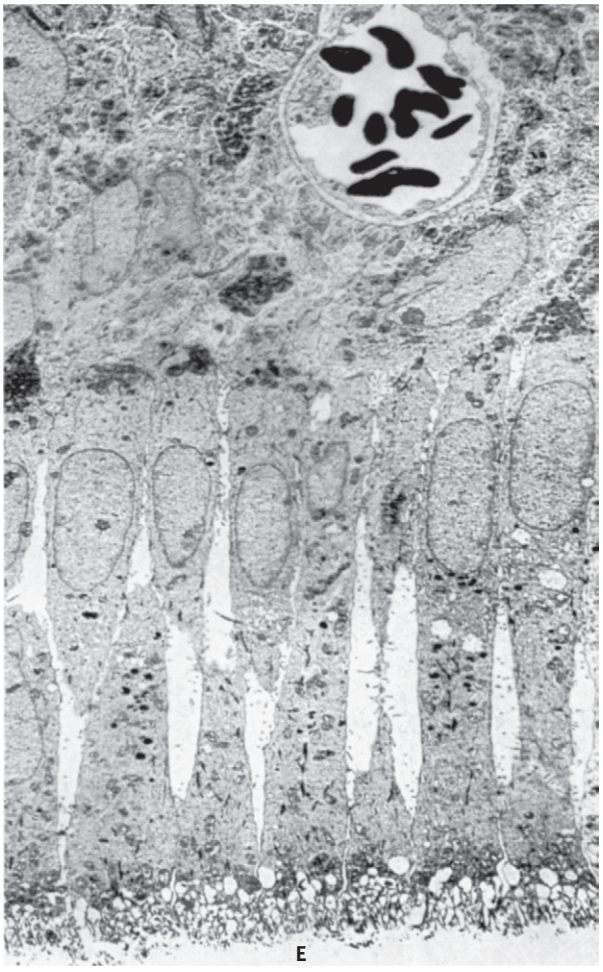

Figure 4.45 Electron micrograph of ameloblasts during stage of enamel maturation. Enamel has been lost during demineralization. Cells are covered at their surfaces adjoining enamel, E, and on their lateral surfaces by numerous microvilli (Approximately × 3400) (From Reith EJ: J Biophys Biochem Cytol 9:825, 1961).

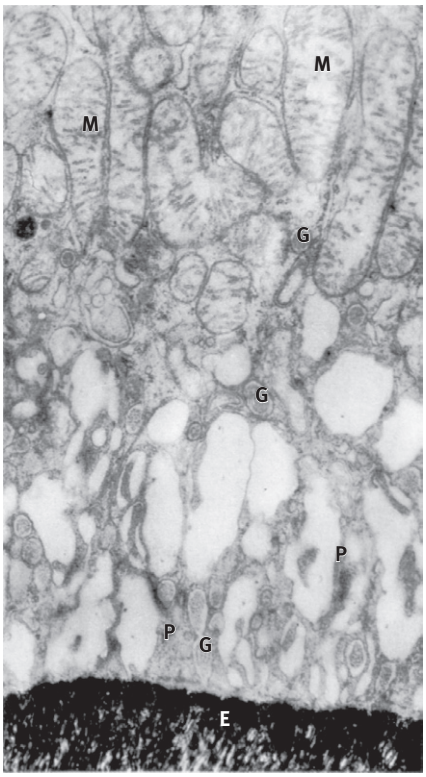

Figure 4.46 Higher magnification of electron micrograph of ends of ameloblasts during stage of enamel maturation. Adjacent to enamel, E, are elaborate cell processes of ameloblasts, P, as well as numerous mitochondria within ameloblast cytoplasm, M. Granular material, possibly being resorbed, is seen both between cell processes and within ameloblast cytoplasm, G. This structure is typical of resorptive cells (Approximately × 36,000) (From Reith EJ: J Cell Biol 18:691, 1963).

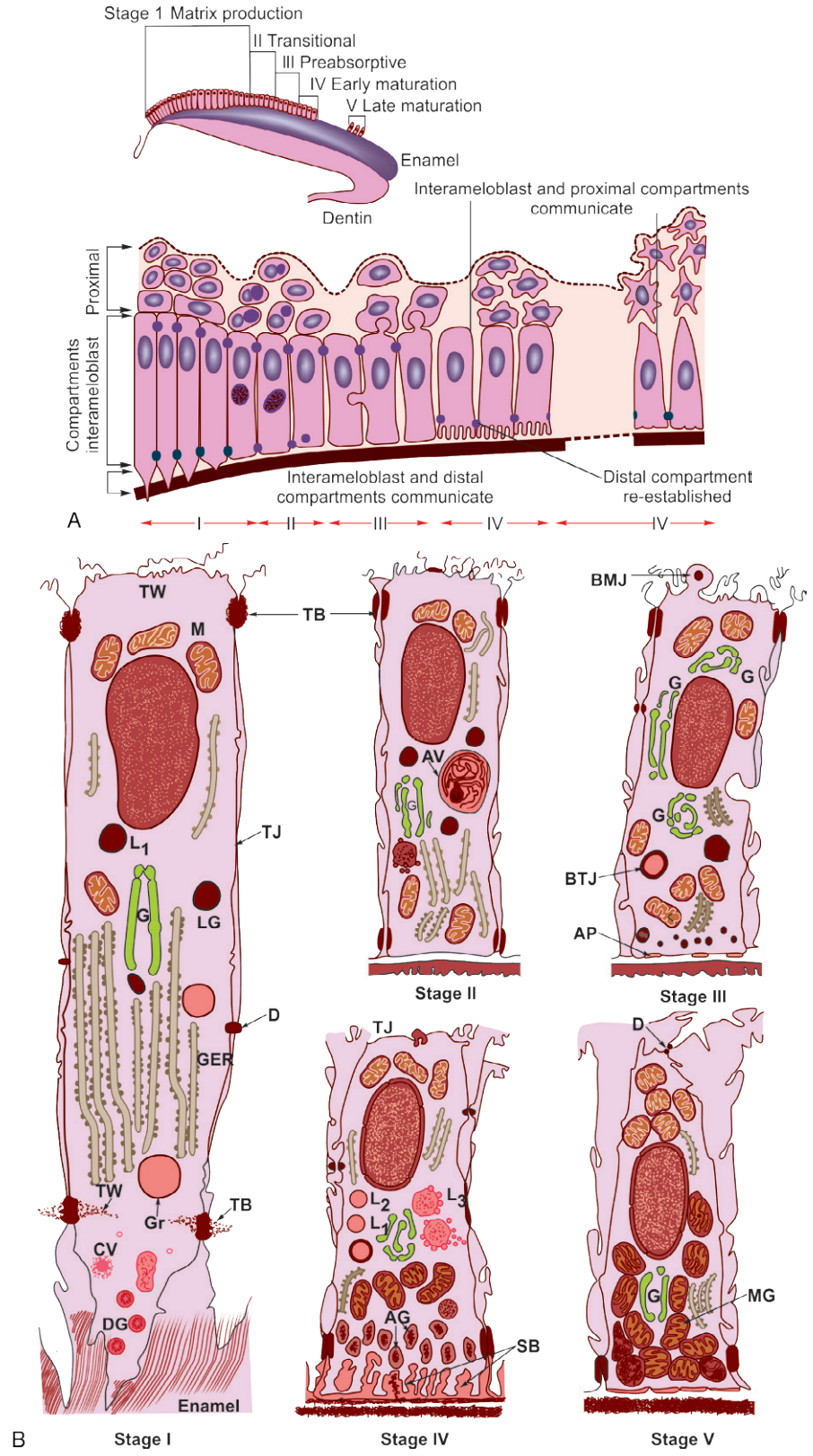

Figure 4.47 Drawings of electron micrographs of enamel organ of rat incisor. Five substages have been identified from formative to maturative. (**A**) Overview of enamel organ. (**B**) Individual ameloblasts from five substages. Organelles: AG, absorption granules; AP, apical contact specialization (hemidesmosomes); AV, autophagic vacuoles (lysosomes); BTJ, bulb type of contacts; CV, coated (absorptive?) vesicles; D, desmosomes; DG, dense (secretory) granules; G, Golgi apparatus; GER, granular (rough) endoplasmic reticulum; Gr, pale (secretory?) granules; L_1, L_2, L_3, lysosomes; LG, lipid granules; M, mitochondria; MG, mitochondrial granules; SB, striated border; TB, terminal bars; TJ, tight junctions; TW, terminal web (From Reith EJ: J Ultrastruct Res 30:111, 1970).

It is however unstable and convert into hydroxyapatite; one unit of it forming two units of hydroxyapatite.

The second stage, or *maturation*, is characterized by the gradual completion of mineralization (Fig. 4.44). The process of maturation starts from the height of the crown and progresses cervically (Fig. 4.48). However, at each level, maturation seems to begin at the dentinal end of the rods. Thus there is an integration of two processes: each rod matures from the depth to the surface, and the sequence of maturing rods is from cusps or incisal edge toward the cervical line.

Maturation begins before the matrix has reached its full thickness. Thus it is going on in the inner, first-formed matrix at the same time as initial mineralization is taking place in the outer, recently formed matrix. The advancing front is at first parallel to the dentinoenamel junction and later to the outer enamel surface. Following this basic pattern, the incisal and occlusal regions reach maturity ahead of the cervical regions (Fig. 4.49).

At the ultrastructural level, maturation is characterized by growth of the crystals seen in the primary phase (Fig. 4.50A). The original ribbon-shaped crystals increase in thickness more rapidly than in width (Fig. 4.51). The crystals increase in size from 1.5 to 25 µm during the maturative phase. Tuftelin an acidic enamel protein localized to the DEJ has been reported to participate in the nucleation of enamel crystals. Other enamel proteins regulate enamel mineralization by binding to specific surfaces of the crystal and inhibiting further deposition.

The rate of formation of enamel is 4 µm/day, therefore to form a layer of enamel of 1 mm thickness it would take about 240 days. The rate of enamel formation is more in permanent teeth than in deciduous teeth.

The crystal sizes increase further after tooth eruption due to ionic exchange with saliva.

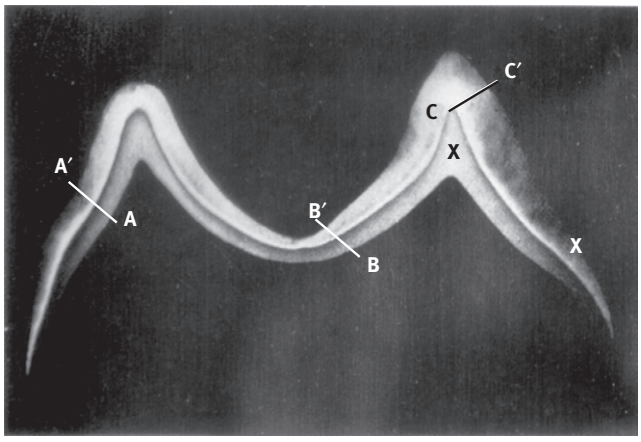

Figure 4.48 Microradiograph of ground section through developing deciduous molar. From gradation in radiopacity, maturation can be seen to progress from dentinoenamel junction toward enamel surface. Mineralization is more advanced occlusally than in cervical region. Lines A, B, and C indicate planes in which actual microdensitometric tracings were made. Black X, Cusp area. White X, Cervical area (×15) (From Hammarlund-Essler E: Trans R Schools Dent, Stockholm and Umea 4:15, 1958).

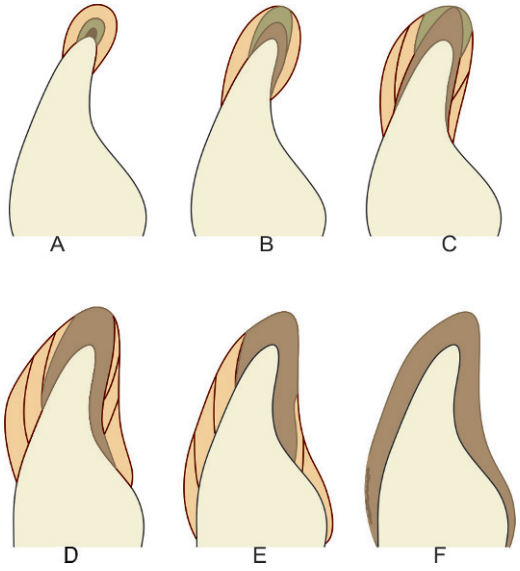

Figure 4.49 Diagram showing pattern of mineralization of incisor tooth. Stippled zones, Consecutive layers of partly mineralized enamel matrix. Black areas, Advance of final mineralization during maturation (From Crabb HSM: Proc R Soc Med 52:118, 1959; and Crabb HSM and Darling AI: Arch Oral Biol 2:308, 1960).

Concomitantly the organic matrix gradually becomes thinned and more widely spaced to make room for the growing crystals. Chemical analysis shows that the loss in volume of the organic matrix is caused by withdrawal of a substantial amount of protein as well as water.

Amelogenesis is unique in many ways. The secretory cell is an epithelial cell whereas all other secretory cells of hard tissues are ectomesenchymal. Noncollagenous proteins are involved in mineralization of enamel whereas in all other hard tissues collagen plays an important role. The matrix of enamel does not contain collagen; in other hard tissues collagen is the major protein. The matrix of enamel is partially mineralized; in other hard tissues the matrix is nonmineralized. Enamel therefore lacks a distinct organic phase like osteoid, predentin or cementoid. There is no absorption of secreted matrix in other hard tissues but in enamel formation 90% of secreted matrix is absorbed and this activity is done by ameloblasts itself. After formation of enamel, ameloblasts undergo apoptosis; hence enamel formation does not occur later on. In other hard tissues formation occurs throughout life.

CLINICAL CONSIDERATIONS

Clinical interest in amelogenesis is centered primarily on the perfection of enamel formation. Although there is relatively little the dentist can do directly to alter the course of events in amelogenesis, it may be possible to minimize certain factors believed to be associated with the etiology of defective enamel structure. The principal expressions of pathologic amelogenesis are hypoplasia, which is manifested by pitting, furrowing, or even total absence of the enamel, and hypocalcification, in the

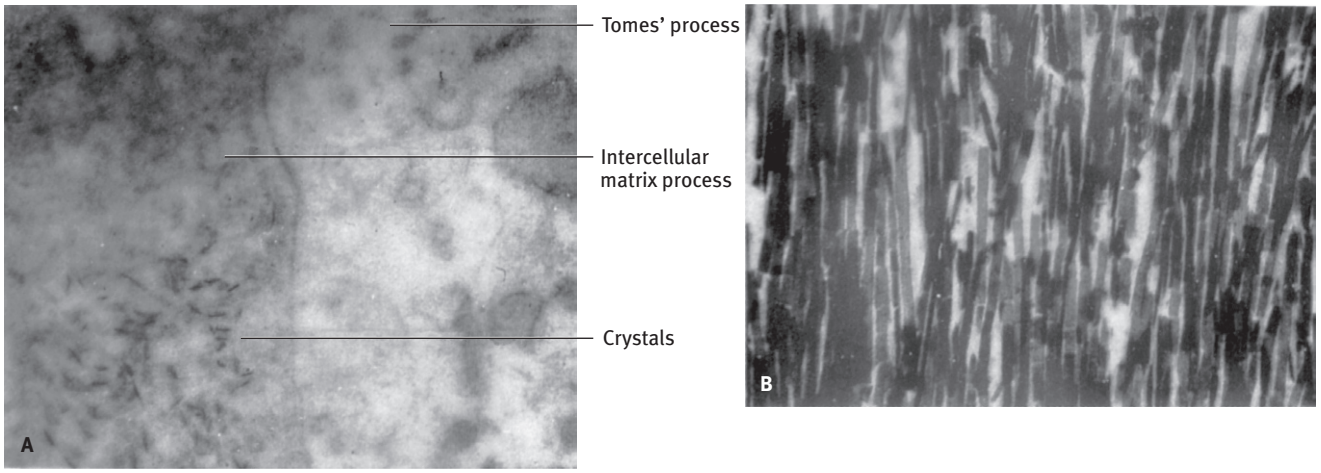

Figure 4.50 Electron photomicrographs illustrating difference between short, needle-like crystals laid down in newly deposited enamel matrix (**A**) and long, ribbon-like crystals seen in mature enamel (**B**) (× 70,000).

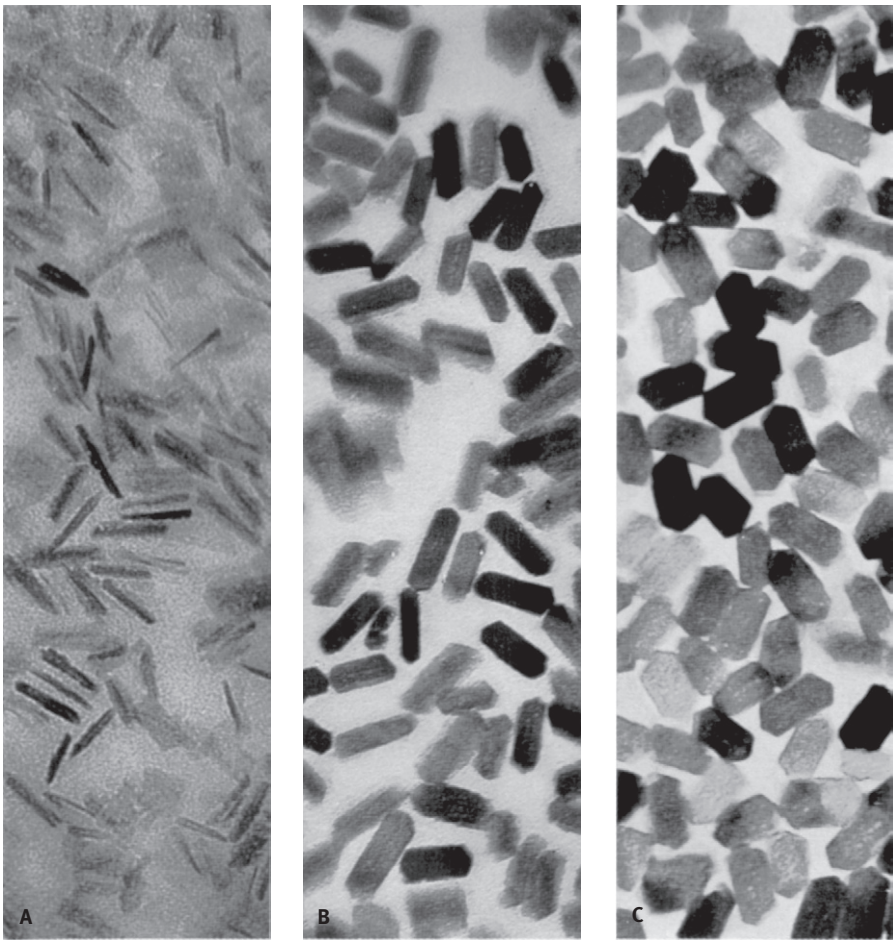

Figure 4.51 Electron photomicrographs of transverse sections through enamel rods in rat incisor showing three stages in growth of apatite crystals during enamel maturation. From (**A**) (recently formed enamel) through (**C**) (more mature enamel) crystals increase in thickness more rapidly than in width. Spaces between crystals will become even smaller as maturation is completed (×240,000) (Modified from Nylen MU, Eanes ED, and Omnell K-Å: J Cell Biol 18:109, 1963).

form of opaque or chalky areas on normally contoured enamel surfaces. The causes of such defective enamel formation can be generally classified as systemic, local, or genetic. The most common systemic influences are nutritional deficiencies, endocrinopathies, febrile diseases, and certain chemical intoxications. It thus stands to reason that the dentist should exert his or her influence to ensure sound nutritional practices and recommended immunization procedures during periods of gestation and postnatal amelogenesis. Chemical intoxication of the ameloblasts is not prevalent and is limited essentially to the ingestion of excessive amounts of water borne fluoride. Where the drinking water contains fluoride in excess of 1.5 parts per million, chronic endemic fluorosis may occur as a result of continuous use throughout the period of amelogenesis. In such areas it is important to urge substitution of water with levels of fluoride (about 1 part per million) well below the threshold for fluorosis, yet optimal with regard to protection against dental caries (see discussion of clinical considerations in section on histology).

Since it has been realized that enamel development occurs in two phases, that is, matrix formation and maturation, developmental disturbances of the enamel can be understood more fully. If matrix formation is affected, enamel hypoplasia will ensue. If maturation is lacking or incomplete, hypocalcification of the enamel results. In the case of hypoplasia a defect of the enamel is found. In the case of hypocalcification a deficiency in the mineral content of the enamel is found. In the latter the enamel persists as enamel matrix and is therefore soft and acid insoluble in routine preparation after formalin fixation.

Hypoplasia as well as hypocalcification may be caused by systemic, local, or hereditary factors. Hypoplasia of systemic origin is termed 'chronologic hypoplasia' because the lesion is found in the areas of those teeth where the enamel was formed during the systemic (metabolic) disturbance. Since the formation of enamel extends over a longer period and the systemic disturbance is, in most cases, of short duration, the defect is limited to a circumscribed area of the affected teeth. A single narrow zone of hypoplasia (smooth or pitted) may be indicative of a disturbance of enamel formation during a short period in which only those ameloblasts that at that time had just started enamel formation were affected. Multiple hypoplasia develops if enamel formation is interrupted on more than one occasion.

No specific cause of chronologic hypoplasia has been established as yet. Recent investigations have demonstrated that exanthematous diseases are not so frequently a cause of enamel hypoplasia as was commonly believed. The more frequent causes are said to be rickets and hypoparathyroidism, but hypoplasia cannot be predicted with any reliability even in the most severe forms of those diseases.

The systemic influences causing enamel hypoplasia are, in the majority of cases, active during the first year of life. Therefore the teeth most frequently affected are the incisors, canines, and first molars. The upper lateral incisor is sometimes found to be unaffected because its development starts later than that of the other teeth mentioned.

Local factors affect single teeth, in most cases only one tooth. If more than one tooth is affected by local hypoplasia, the location of the defects shows no relation to chronology of development. The cause of local hypoplasia may be an infection of the pulp with subsequent infection of the periapical tissues of a deciduous tooth if the irritation occurred during the period of enamel formation of its permanent successor.

The hereditary type of enamel hypoplasia is probably a generalized disturbance of the ameloblasts. Therefore the entire enamel of all the teeth, deciduous as well as permanent, is affected rather than merely a belt-like zone of the enamel of a group of teeth, as in systemic cases. The anomaly is transmitted as a mendelian dominant character. The enamel of such teeth is so thin that it cannot be noticed clinically or in radiographs. The crowns of the teeth of affected family members are yellow-brown, smooth, glossy, and hard, and their shape resembles teeth prepared for jacket crowns.

An example of systemic hypocalcification of the enamel is the so-called mottled enamel. A high fluoride content in the water is the cause of the deficiency in calcification. Fluoride hypocalcification is endemic; that is, it is limited in its distribution to definite areas in which the drinking water contains more than 1 part of fluoride per 1 million parts of water. It has been demonstrated that a small amount of fluoride (about 1 to 1.2 parts per million) reduces susceptibility to dental caries without causing mottling. For this reason many communities are adding small quantities of fluoride to the community water supplies.

The same local causes that might affect the formation of the enamel can disturb maturation. If the injury occurs in the formative stage of enamel development, hypoplasia of the enamel will result. An injury during the maturation stage will cause a deficiency in calcification.

The hereditary type of hypocalcification is characterized by the formation of a normal amount of enamel matrix that, however, does not fully mature. Such teeth, if investigated before or shortly after eruption, show a normal shape. Their surfaces do not have the luster of normal enamel but appear dull. The enamel is opaque. The hypocalcified soft enamel matrix is soon discolored, abraded by mastication, or peeled off in layers. When parts of the soft enamel are lost, the teeth show an irregular, rough surface. When the enamel is altogether lost, the teeth are small and brown, and the exposed dentin is extremely sensitive.

The discoloration of teeth from administration of tetracyclines during childhood is a very common clinical problem. Whereas usually this discoloration is because of deposition of tetracycline in the dentin, a small amount of the drug may be deposited in the enamel. In mild cases, the use of some of the newly developed surface-binding restorative materials can produce good esthetic results.

SUMMARY

Inorganic and Organic Constituents

The inorganic part of enamel containing hydroxyapatite makes up to 96% by weight. The organic part consists mainly of protein and water. The organic part is present between and around the crystals. The proteins of enamel are of two main types—amelogenins and the nonamelogenins. Amelogenins constitute for 90% of enamel proteins. They are hydrophobic and are of low molecular weight whereas nonamelogenins are high molecular weight proteins. The crystals of hydroxyapatite are hexagonal in cross-section.

Structure of Enamel Rods

The crystals are grouped into enamel prisms or rods. The number of enamel prisms in a tooth like permanent maxillary first molar is estimated to be around 12 million and the average thickness of each rod is 4 μm. The enamel prisms appear as segmental rods in longitudinal section and in cross-section they appear as oval, fish scale or keyhole shaped. The prisms are covered by prism sheath or rod sheath and interprismatic substance is said to be present between prisms. These areas are rich in organic matter. In LS cross-striations, demarcate the prisms and they represent secretion of enamel in a time interval.

Incremental Lines of Retzius

The incremental lines called striae of Retzius, which denote rest periods between periods of activity, appear concentric in cross-section and run obliquely from dentinoenamel junction to the surface (incomplete striae) or course around the tooth (complete striae). The meeting of incomplete striae produces many surface elevations and between it are depressions. These are known as perikymata. Apart from these there are other surface structures like enamel caps, brochs, and pits. A delicate membrane called Nasmyth's membrane or primary enamel cuticle covers the entire newly formed crown. Neonatal line is also a prominent incremental line denoting the prolonged period of rest during birth. Hence it is seen in deciduous teeth and in first permanent molars only.

Course of Prisms and Hunter – Schreger Bands

The prisms follow a wavy course from dentinoenamel junction alternatively bending to left and right. The prisms on the adjacent rows, bends in opposite directions giving rise to alternating dark and light bands, when viewed in reflected or polarized light. The bands are called Hunter–Schreger bands. In the outer third of enamel, the prisms do not bend, hence these bands are absent. The prisms are arranged in a direction perpendicular to the dentin surface and in such a way that the heads are directed towards occlusal and tail towards cervical.

Prismless Enamel

The outermost layer and in the first layer of enamel the hydroxyapatite crystals are not arranged in the form of prisms, hence they are referred to as prismless enamel. In certain areas, the prisms appear to be twisted and these are known as gnarled enamel.

Structures Related to Dentino enamel Junction-Tufts, Lamella and Spindles

From the dentinoenamel junction certain structures, rich in organic matter hence collectively called hypocalcified structures, are seen. These are enamel tufts, enamel lamella and enamel spindles. Enamel tufts are ribbon shaped and are due to adaptations to spatial conditions of enamel and are well appreciated in cross-sections. The enamel spindles are extension of odontoblastic process into the enamel. Enamel lamellae are leaf-like structures but appears as cracks and develop in planes of tension. These are of three types A, B and C and extend from surface up to or beyond dentinoenamel junction into dentin. Type A and B are produced during enamel formation and type C is produced after eruption. Enamel lamellae may predispose to caries.

Age Changes

The age changes in the enamel include decrease in permeability and loss of enamel due to mechanical wear (abrasion), physiological wear (attrition) and chemical wear (erosion).

Amelogenesis

Amelogenesis is the formation of enamel, which includes enamel matrix production and mineralization. It occurs during the advanced bell stage of tooth development and the entire process is under genetic control. Reciprocal epithelial–mesenchymal interactions lead to differentiation of odontoblasts and ameloblasts. Message is sent by preameloblasts to newly differentiated odontoblasts, which lay down predentin. This, in turn, causes the differentiation of secretory ameloblasts, which secrete enamel matrix over the newly formed dentin. The apatite crystals of the matrix are not organized into prisms, hence they are referred to as aprismatic enamel.

Tomes' Process

After the initial enamel matrix is laid down, ameloblasts develop cone-like projections called Tomes' process. All enamel matrix secretions from then on occurs through the Tomes' process. The enamel matrix is a partially mineralized matrix (25 to 30% calcified). The important proteins secreted by the ameloblasts are amelogenins, ameloblastin and enamelin. The secreted proteins undergo degradation by enzymes like metalloproteinases and serine proteases into smaller low molecular weight fragments.

Formation of Prisms

From each Tomes' process parts of four prisms are formed: head of one prism and tails of the other three. For the formation of one prism four Tomes' process are involved. The ameloblasts retreat towards the surface at an angle. Since the matrix is partially calcified this retreating movement causes the formation of prisms. The interprismatic substance is from areas close to junctional complexes and from adjacent ameloblasts.

Maturation of Enamel

After the full thickness of enamel matrix is laid down, it undergoes complete mineralization or maturation. During this process the ameloblasts develop villous projections on their secretory surface. They are now known as ruffle ended ameloblasts. Most of the proteins secreted (over 90%) especially amelogenins and water are absorbed. The pressure from the growing crystals squeezes out the matrix which is in the form of thixotropic gel. The crystals increase in size from 1.5 to 25 μm. During maturation the ameloblasts alternate cyclically from ruffle ended to smooth ended ameloblasts. This process is called modulation. Enamel proteins

like tuftelin help in mineralization while other proteins regulate the growth of crystals. The minerals find their way first between and then through the ameloblasts to the mineralizing front. The last layer of enamel formed is not from Tomes' process, hence it is aprismatic enamel.

Unique Features of Amelogenesis

Amelogenesis is unique in that the secretory cell is an epithelial cell while all other secretory cells producing hard tissues are ectomesenchymal. In enamel mineralization only noncollagenous proteins are involved; in all other hard tissues collagen plays an important part. After formation of enamel the ameloblasts undergo apoptosis, therefore there is no formation later in life unlike in other hard tissues.

Life Cycle of Ameloblasts

The ameloblast's life cycle is divided into six stages: morphogenetic; which is prior to differentiation, organizing stage in which the cell differentiates into ameloblasts; a formative stage in which enamel formation occurs; a maturative stage in which complete mineralization occurs, protective stage; in which ameloblasts become part of condensed enamel organ (reduced enamel epithelium) to protect the newly formed crown and the desmolytic stage in which the reduced enamel epithelium creates a pathway for the eruption of the teeth. In the differentiation into ameloblasts the cell undergoes morphological change (becomes tall columnar), cytological changes (acquires organelles for protein synthesis) and biochemical changes (enzymes involved in mineralization identified).

Defective Amelogenesis

Infections, trauma, chemicals like fluoride and metabolic changes involving calcium or phosphorus affect ameloblasts easily. They produce decreased thickness of enamel called enamel hypoplasia or decreased mineralization called enamel hypomineralization.

REVIEW QUESTIONS

1. Describe the microscopic ultrastructure and course of enamel rods.
2. Write notes on:
   Physical properties of enamel
   Striae of Retzius
   Neonatal line
   Hunter–Schreger bands
   Enamel lamellae
   Enamel tufts
   Perikymata
   Enamel spindles
   Nasmyth's membrane
   Age changes in the enamel
   Dentinoenamel junction.
3. Describe the hypocalcified structures in the enamel.
4. Describe the life cycle of the ameloblasts.
5. Describe amelogenesis.
6. Write notes on:
   Tomes' process
   Prismless enamel.

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5 Dentin

CHAPTER CONTENTS

The dentin provides the bulk and general form of the tooth and is characterized as a hard tissue with tubules throughout its thickness. Since it begins to form slightly before the enamel, it determines the shape of the crown, including the cusps and ridges, and the number and size of the roots. As a living tissue it contains within its tubules the processes of the specialized cells, the odontoblasts. Although the cell bodies of the odontoblast are arranged along the pulpal surface of the dentin, the cells are morphologically cells of the dentin, because the odontoblasts produce the dentin as well as the odontoblast processes casting within it. Physically and chemically the dentin closely resembles bone. The main morphologic difference between bone and dentin is that some of the osteoblasts exist on the surface of bone, and when one of these cells becomes enclosed within its matrix, it is called an osteocyte. The odontoblasts' cell bodies remain external to dentin, but their processes exist within tubules in dentin. Both are considered vital tissues because they contain living protoplasm.

PHYSICAL AND CHEMICAL PROPERTIES

In the teeth of young individuals the dentin usually is light yellowish in color, becoming darker with age. Unlike enamel, which is very hard and brittle, dentin is viscoelastic and subject to slight deformation. It is somewhat harder than bone but considerably softer than enamel. Dentin hardness varies slightly between tooth types and between crown and root dentin. Dentin is somewhat harder in its central part than near the pulp or on its periphery. The dentin of primary teeth is slightly less hard than that of permanent teeth. The lower content of mineral salts in dentin renders it more radiolucent than enamel. Dentin consists of 35% organic matter and water and 65% inorganic material.

The organic substance consists of collagenous fibrils embedded in the ground substance of mucopolysaccharides (proteoglycans and glycosaminoglycans). Type I collagen is the principal type of collagen found in the dentin. The important constituents of the ground substance are the proteoglycans: chondroitin sulfates, decorin and biglycan; glycoproteins: dentin sialoprotein (DSP), osteonectin, osteopontin; phosphoproteins: dentin phosphoprotein (DPP), gamma carboxyglutamate containing proteins (Gla-proteins) and phospholipids. The protein of dentin matrix and bone are similar, but dentin sialoprotein and dentin phosphoprotein are present only in dentin. In addition, the matrix contains growth factors like transforming growth factor (TGF), fibroblast growth factor (FGF), insulin-like growth factors (IGFs), bone morphogenic proteins (BMPs), epidermal growth factor (EGF), platelet derived growth factor (PDGF), placenta growth factor (PLGF), vascular endothelial growth factor (VEGF), and angiogenic growth factor (AGF). The matrix components have important roles to play in mineralization of dentin.

The inorganic component has been shown by X-ray diffraction to consist of hydroxyapatite, as in bone, cementum, and enamel. Each hydroxyapatite crystal is composed of several thousand unit cells. The unit cells have a formula of $3Ca_3\ (PO_4)_2 \cdot Ca\ (OH)_2$. The crystals are plate shaped and much smaller than the hydroxyapatite crystals

in enamel. Dentin also contains small amounts of phosphates, carbonates, and sulfates. The crystals are poor in calcium but rich in carbon when compared to enamel.

Organic and inorganic substances can be separated by either decalcification or incineration. In the process of decalcification the organic constituents can be retained and maintain the shape of the dentin. This is why decalcified teeth and bone can be sectioned and provide clear histologic visualization. The enamel, being over 90% mineral in composition, is lost after decalcification.

STRUCTURE

The dentinal matrix of collagen fibers is arranged in a network. As dentin calcifies, the hydroxyapatite crystals mask the individual collagen fibers. Collagen fibers are only visible at the electron microscopic level.

The bodies of the odontoblasts are arranged in a layer on the pulpal surface of the dentin, and only their cytoplasmic processes are included in the tubules in the mineralized matrix. Each cell gives rise to one process, which traverses the predentin and calcified dentin within one tubule and terminates in a branching network at the junction with enamel or cementum. Tubules are found throughout normal dentin and are therefore characteristic of it.

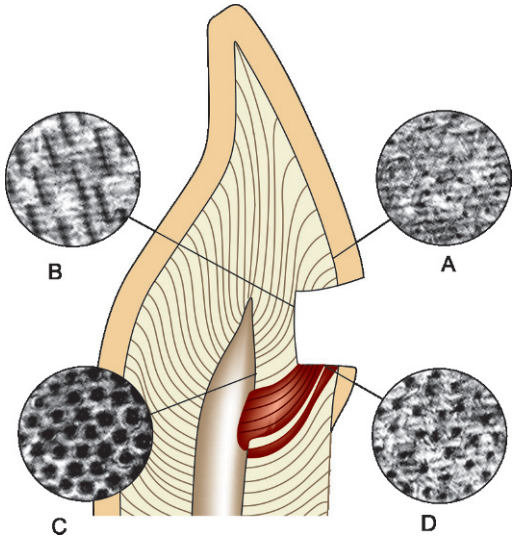

Figure 5.1 Diagram illustrating the curvature, size, and distance between dentinal tubules in human outer (**A**), mid (**B**), and inner dentin (**C**). The tubules are approximately 1 µm in diameter at the dentinoenamel junction (DEJ), 1.5 to 2 µm midway through dentin, and 1.5 to 3 µm at the pulp. Bacterial penetration (**D**) follows line of least resistance to reach the pulp. Note that cut tubules in the floor and walls ofa cavity may be different; 1 mm^2 of cavity exposes 30,000 tubules.

Dentinal tubules

The course of the dentinal tubules follows a gentle curve in the crown, less so in the root, where it resembles a gentle S (sigmoid course) in shape (Fig. 5.1). These curvatures are called *primary curvatures*. Starting at right angles from the pulpal surface, the first convexity of this doubly curved course is directed toward the apex of the tooth. These tubules end perpendicular to the dentinoenamel and dentinocementum junctions. Branches of the dentinal tubules near the terminals are referred to as *terminal branches*. The terminal branching is more profuse in the root dentin than in the coronal dentin. Near the root tip and along the incisal edges and cusps the tubules are almost straight. Over their entire lengths the tubules exhibit minute, relatively regular *secondary curvatures* that are sinusoidal in shape. The tubules are longer than the dentin, and are thick because they curve through dentin. The dentin ranges in thickness from 3 to 10 mm or more. Dentin showed variation in thickness between the sexes, it was thicker in boys than girls of similar age group and this increased during puberty. Dentin thickness varied not only from tooth to tooth but also in different surfaces of the same tooth. The buccal surfaces showed maximum thickness, followed by lingual, and there was no difference in thickness between mesial and distal surfaces.

The ratio between the outer and inner surfaces of dentin is about 5:1. Accordingly, the tubules are farther apart in the peripheral layers and are more closely packed near the pulp (Fig. 5.1). In addition, they are larger in diameter near the pulpal cavity (3 to 4 µm) and smaller at their outer ends (1µm). The ratio between the numbers of tubules per unit area on the pulpal and outer surfaces of the dentin is about 4:1. Near the pulpal surface of the dentin the number per square millimeter varies between 50,000 and 90,000. There are more tubules per unit area in the crown than in the root. The dentinal tubules have *lateral branches* throughout dentin, which are termed canaliculi or microtubules. These canaliculi are 1 µm or less in diameter and originate more or less at right angles to the main tubule every 1 to 2 µm along its length (Fig. 5.2A and B). Some of them enter adjacent or distant tubules while others end in the intertubular dentin. A few odontoblastic processes extend through the dentinoenamel junction into the enamel for several millimeters. These are termed *enamel spindles*.

Peritubular dentin

The dentin that immediately surrounds the dentinal tubules is termed *peritubular dentin*. This dentin forms the walls of the tubules in all but the dentin near the pulp. It is more highly mineralized (about 9%) than the dentin present between the tubules (the *intertubular dentin*). Peritubular dentin differs from intertubular dentin by its matrix composition. The crystal arrangement appears to be similar.

It is twice as thick in outer dentin (approximately 0.75 µm) than in inner dentin (0.4 µm). By its growth, it constricts the dentinal tubules to a diameter of 1 µm near the dentinoenamel junction. Studies with soft X-rays and with electron microscope show the increased mineral density in the peritubular dentin (Fig. 5.3). Since the deposition of the minerals occurs in the inner wall of the tubule rather on the outer wall, the term 'intratubular dentin' is considered to be more appropriate than the term peritubular dentin.

A very delicate organic matrix has been demonstrated in this dentin that along with the mineral is lost after

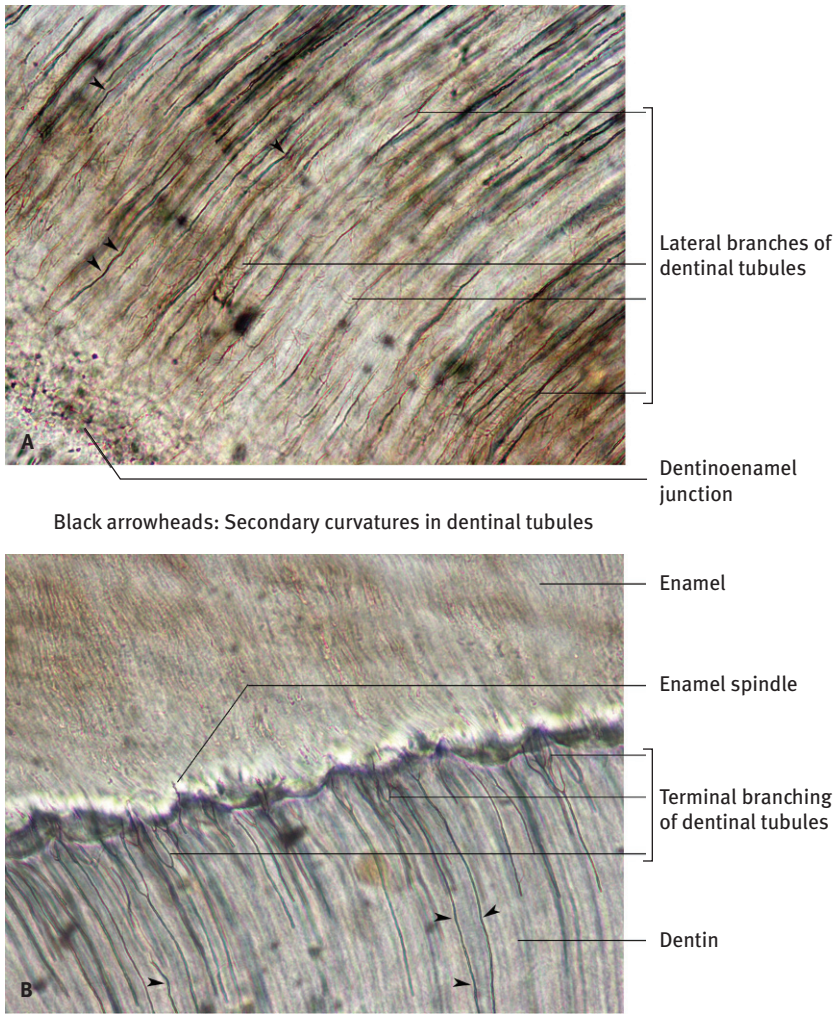

Figure 5.2 (**A**) Photomicrograph of the ground sections showing (**A**) secondary curvatures and lateral branches of dentinal tubules (x400). (**B**) Terminal Y-shaped branching of dentinal tubules and secondary curvatures (x400).

decalcification (Fig. 5.3B). After decalcification the odontoblast process appears to be surrounded by an empty space. In demineralized dentin visualized with a light microscope, the tubule diameter will therefore appear similar in inner and outer dentin because of the loss of the peritubular dentin. This is important clinically, as etching of a cavity floor will open up the tubules. When peritubular dentin is visualized ultrastructurally in a calcified section of a tooth, the densely mineralized peritubular dentin appears structurally different than the intertubular dentin. The collagen fibers in the tubule wall are masked in peripheral dentin. A comparison of the tubule wall in inner and outer dentin is shown in Figure 5.4. Several investigators believe the calcified tubule wall has an inner organic lining termed the *lamina limitans*. This is described as a thin organic membrane, high in glycosaminoglycan (GAG) and similar to the lining of lacunae in cartilage and bone. Other investigators believe this lining in the tubules is absent or limited and that instead only the plasma membrane of the odontoblast is present there.

Between the odontoblastic process and the peritubular dentin, a space known as *periodontoblastic space* is reported to be present. This space contains the *dentinal fluid*. The normal flow of the fluid is outwards from the pulp. The dentinal fluid has a higher K^+ and a lower Na^+ content. Dentin sensitivity is explained on the basis of this fluid movement.

Intertubular dentin

The main body of dentin is composed of intertubular dentin. It is located between the dentinal tubules or, more specifically, between the zones of peritubular dentin. Although it is highly mineralized, this matrix, like bone and cementum, is retained after decalcification, whereas peritubular dentin is not. About one half of its volume is organic matrix, specifically collagen fibers, which are randomly oriented around the dentinal tubules (Fig. 5.4). The fibrils range from 0.5 to 0.2 μm in diameter and exhibit cross banding at 64 μm (640 Å) intervals, which is typical for collagen (Fig. 5.4A). Hydroxyapatite crystals, which average 0.1 μm in length, are formed

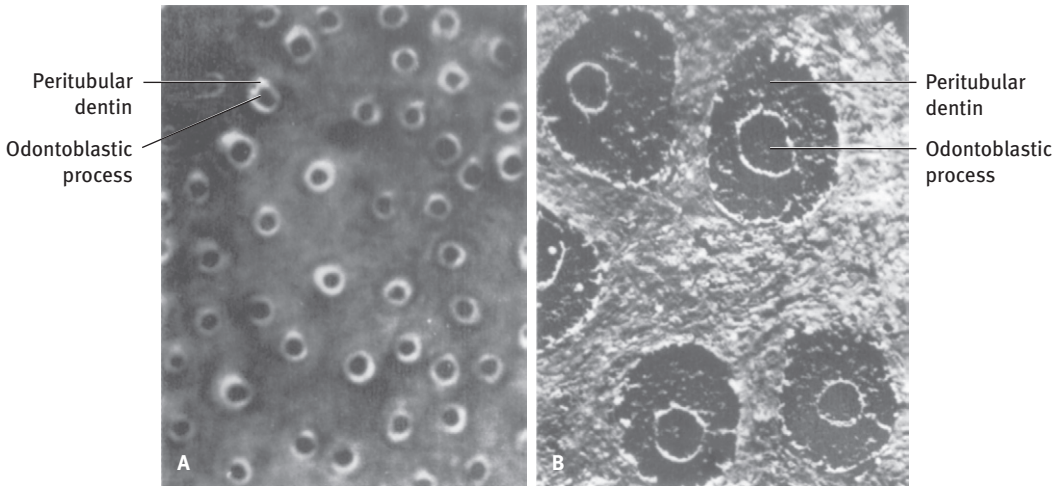

Figure 5.3 Microscopic appearance of peritubular dentin. (**A**) Undermineralized ground section of soft X-ray showing increased mineral density in peritubular zone (×1000). (**B**) Electron micrograph of demineralized section of dentin showing loss of mineral in peritubular zone. Organic matrix in peritubular zone is sparse (approximately ×4000).

along the fibers with their long axes oriented parallel to the collagen fibers.

Predentin

The predentin is located always adjacent to the pulp tissue and is 2 to 6 μm wide, depending on the extent of activity of the odontoblast. It is not mineralized (Fig. 5.5).

The predentin appears to be pale staining than the mineralized dentin owing to differences in composition of the matrix.

As the collagen fibers undergo mineralization at the predentin-dentin junction, the predentin becomes dentin and a new layer of predentin forms circumpulpally.

Odontoblast process

The odontoblast processes are the cytoplasmic extensions of the odontoblasts. The odontoblast cells reside in the peripheral pulp at the pulp-predentin border and their processes extend into the dentinal tubules (Fig. 5.5). The processes are largest in diameter near the pulp (3 to 4 μm) and taper to approximately 1 μm further into the dentin. The odontoblast cell bodies are approximately 7 μm in diameter and 40 μm in length. The junctions between odontoblasts may be of gap junctions, tight junctions, and desmosomal junctions. The life span of the odontoblasts is equal to the age of the tooth as once differentiated, they cannot undergo further division. The odontoblastic processes narrow to about half the size of the cell as they enter the tubules (Fig. 5.5). There is disagreement among investigators even of now, whether the odontoblast processes extend through the thickness of mature human dentin. Good evidence is shown by transmission electron microscope that dentinal tubules 200 to 300 μm from the pulp contain processes (Fig. 5.6A). Other investigators, using scanning electron microscope, have shown what appear to be processes at

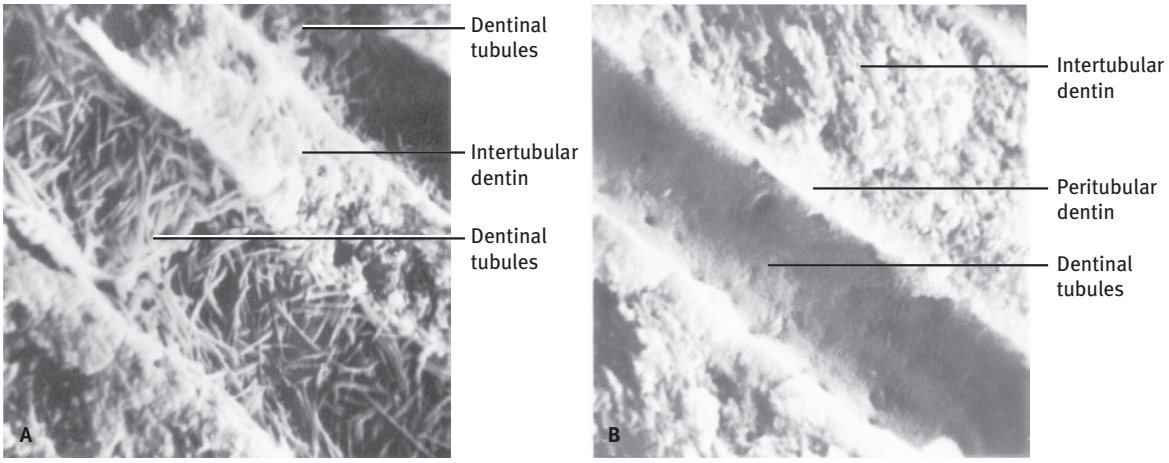

Figure 5.4 (**A**) Dentinal tubule representative of inner dentin near formative front as seen by scanning electron microscope. Collagen fibers are evident, composing the walls of the dentinal tubules (×18,000). (**B**) Same dentinal tubule as in (**A**), further peripheral in calcified dentin viewed by scanning electron microscope. Peritubular dentin masks collagen fibers in the tubule wall. Observe numerous side branches (canaliculi) of dentinal tubule (×15,000) (From Boyde A: Beitr Electronmikroskop Direktabb Oberfl 1[S]:213, 1968).

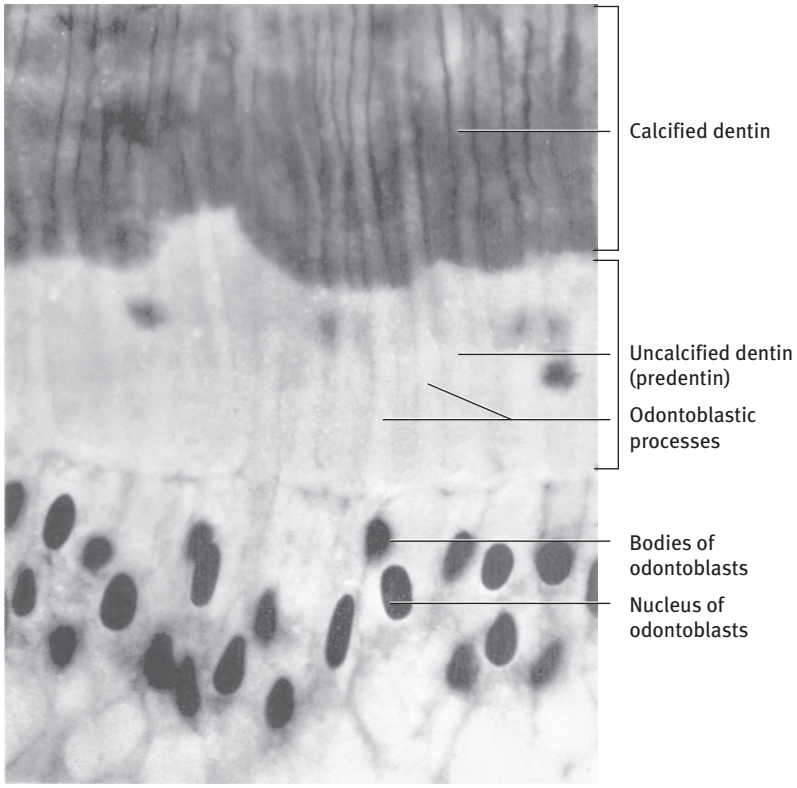

Figure 5.5 Odontoblast processes (Tomes' fibers) within dentinal tubules. They extend from the cell body below at the pulp-predentin junction into the dentin above.

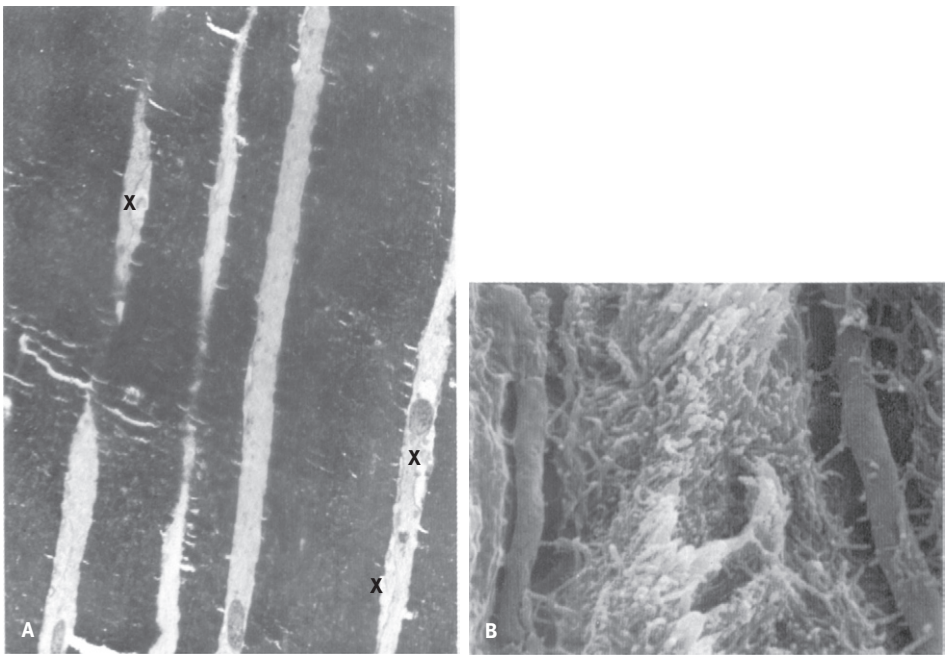

Figure 5.6 (**A**) Transmission electron micrograph of odontoblast processes in dentin tubules approximately 200 to 300 μm from the pulp. These processes contain microfilaments, a few vesicles, and an occasional mitochondrion enclosed in the plasma membrane of the process (x = artifact, × 6000). (**B**) Scanning electron micrograph of odontoblast process in dentinal tubules. Side branches of the process are seen entering the peritubular dentin.

the dentinoenamel junctions (Fig. 5.6B). Cryofractured human teeth revealed the odontoblast process to extend to the dentinoenamel junction. Recent studies with scanning electron microscope showed that odontoblastic process did not extend beyond the inner dentin. The initial groups of investigators believe the findings in Figure 5.6 represent the organic lining membrane of the tubule (lamina limitans) and not the living process of the odontoblast. Further investigations using immunofluorescent techniques revealed tubulin (an intracellular

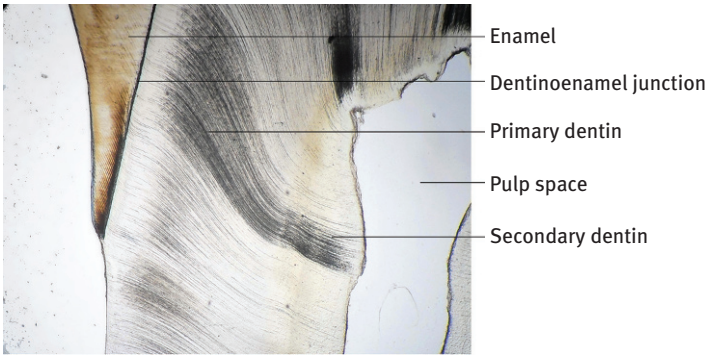

Figure 5.7 Photomicrograph of ground section of tooth shows sharp bend of dentinal tubules as they pass from primary into secondary dentin (x40).

TABLE 5.1 Differences between Mantle Dentin and Predentin

Mantle Dentin	Predentin
First formed dentin in the crown underlying the DEJ	Located adjacent to pulp tissue
20 µm wide	2– 6 µm wide
Fibrils are perpendicular to DEJ	Fibrils are of mixed orientation
Organic matrix composed of larger collagen fibrils, called KORFF'S fibers	Collagen fibrils are smaller than mantle dentin
Mantle dentin undergoes globular mineralization	Unmineralized dentin

protein of microtubules) throughout the thickness of dentin. It is appropriate to consider that some odontoblast processes traverse the thickness of dentin. In other areas a shortened process may be characteristic in tubules that are narrow or obliterated by mineral deposit.

The odontoblast process is composed of microtubules of 20 µm (200 to 250 Å) in diameter and small filaments 5 to 7.5 µm (50 to 75 Å) in diameter. The microtubules and intermediate filaments run longitudinally throughout the tubules. Occasionally mitochondria, dense bodies resembling lysosomes, microvesicles, and coated vesicles that may open to the extracellular space are also seen (Fig. 5.6A). The odontoblast processes divide near the dentinoenamel junction and may indeed extend into enamel in the *enamel spindles*. Periodically along the course of the processes side branches (lateral branches) appear that extend laterally into adjacent tubules.

PRIMARY DENTIN

Dentin which is formed before root completion is known as *primary dentin*. The primary dentin are of two types—mantle dentin and the circumpulpal dentin.

Mantle dentin is the name of the first-formed dentin in the crown underlying the dentinoenamel junction. This zone below the DE junction is soft and thus provides cushioning effect to the tooth. It is thus the outer or most peripheral part of the primary dentin and is about 20 µm thick. The fibrils formed in this zone are perpendicular to the dentinoenamel junction, and the organic matrix is composed of larger collagen fibrils than are present in the rest of the primary dentin (circumpulpal dentin). The larger diameter collagen fibers (0.1–0.2 µm in diameter) are argyrophilic (silver stained) and are known as von Korff's fibers. They contain mainly type III collagen. Compared to circumpulpal dentin, mantle dentin is less mineralized. Mantle dentin also has fewer defects than circumpulpal dentin. Unlike, rest of the dentin, matrix vesicles are involved in the mineralization of mantle dentin. Mantle dentin undergoes globular mineralization whereas the circumpulpal dentin mineralizes either by globular or linear pattern. When the rate of formation progresses slowly, the mineralization front appears more uniform and the process is said to be linear.

Circumpulpal dentin

Forms the remaining primary dentin or bulk of the tooth. The collagen fibrils in circumpulpal dentin are much smaller in diameter (0.05 µm) and are more closely packed together compared to the mantle dentin. The circumpulpal dentin may contain slightly more mineral than mantle dentin (Table 5.1).

SECONDARY DENTIN

Secondary dentin is a narrow band of dentin bordering the pulp and representing that dentin formed after root completion. This dentin contains fewer tubules than primary dentin. There is usually a bend in the tubules where primary and secondary dentin interface (Fig. 5.7). Many believe that secondary dentin is formed more slowly than primary dentin and that it looks similar to primary dentin but contains fewer tubules. Secondary dentin is not formed uniformly and appears in greater amounts on the roof and floor of the coronal pulp chamber, where it protects the pulp from exposure in older teeth. The secondary dentin formed is not in response to any external stimuli, and it appears very much like primary dentin. Due to the regular arrangement of dentinal tubules, it is known as *regular secondary dentin*.

The apical dentin shows irregularity in the dentinal tubules of both primary and secondary dentin.

TERTIARY DENTIN

Tertiary dentin is reparative, response, or reactive dentin. This is localized formation of dentin on the pulp-dentin border, formed in reaction to trauma such as caries or restorative procedures. This type of dentin is described in greater detail under reparative dentin.

INCREMENTAL LINES

The incremental lines of von Ebner, appear as fine lines or striations in dentin. They run at right angles to the dentinal tubules and correspond to the incremental lines in enamel or bone (Fig. 5.8). These lines reflect the daily rhythmic, recurrent deposition of dentin matrix as well

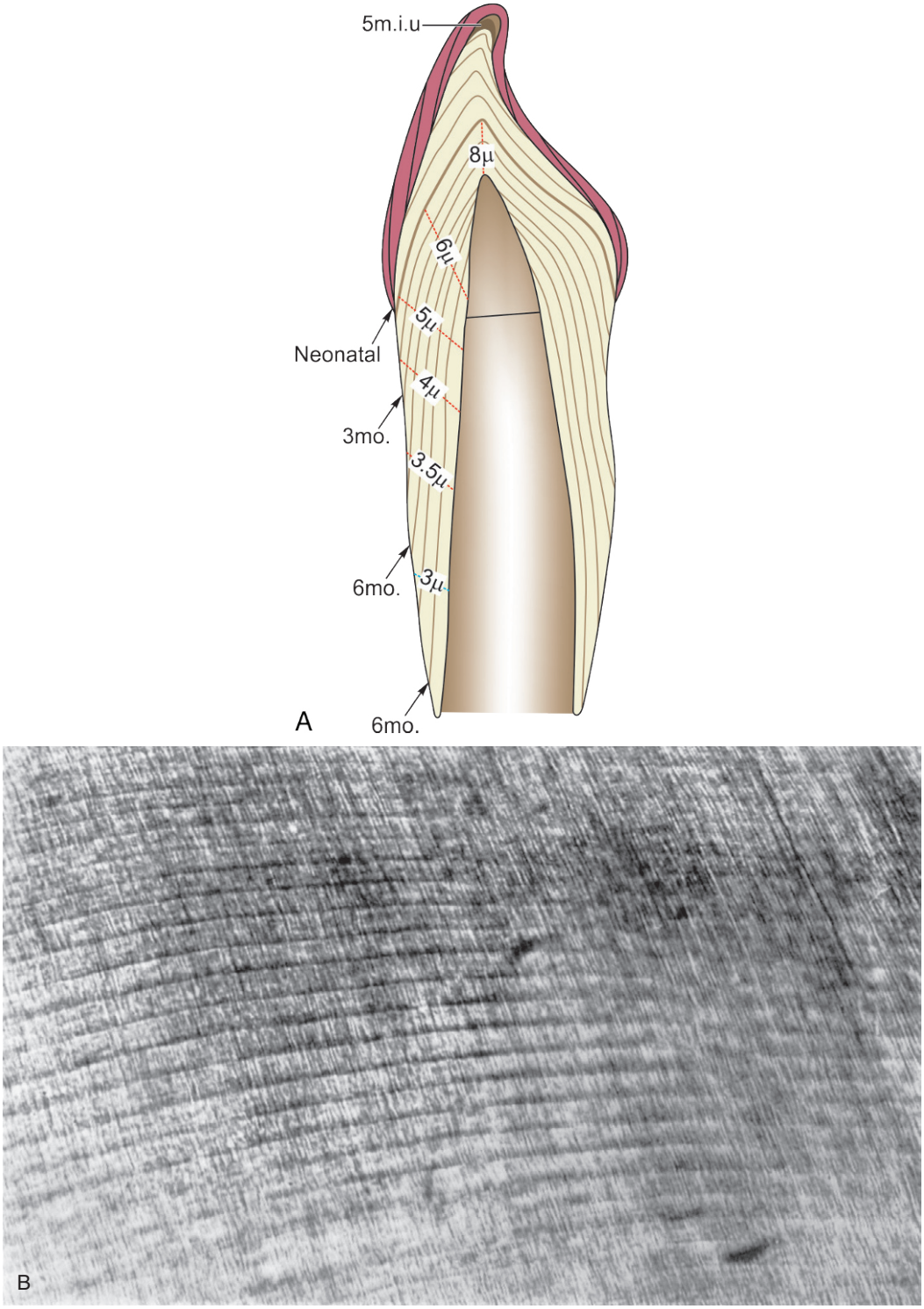

Figure 5.8 (**A**) Diagram of incremental appositional pattern in dentin in a human deciduous central incisor in a 5-month fetus. In the crown as much as 8 μm/day was deposited and in the root 3 to 4 μm. (**B**) Incremental lines in dentin. Also known as imbrication lines or incremental lines of von Ebner. Ground section human tooth (**A** from Schour I and Massler M: J Am Dent Assoc 23:1946, 1936).

as a hesitation in the daily formative process. The distance between lines varies from 4 to 8 mm in the crown to much less in the root. The daily increment decreases after a tooth reaches functional occlusion. The course of the lines indicates the growth pattern of the dentin. Opinions vary regarding the causes of the incremental lines. Some investigators are of the opinion that the lines are 20 μ apart and, they represent a 5 day interval in dentin formation. 2 μm organic matrix of dentin is deposited in a 12-hour cycle, so the daily deposition is

approximately 4 μm. Dentin is similarly mineralized in a 12-hour cycle.

Occasionally some of the incremental lines are accentuated because of disturbances in the matrix and mineralization process. Such lines are readily demonstrated in ground sections and are known as *contour lines of Owen,* (Fig. 5.9). Analysis withsoft X-ray has shown these lines to represent hypocalcified bands. Some investigators have shown that the contour lines of Owen are due to the coincidence of secondary curvatures.

In the deciduous teeth and in the first permanent molars, where dentin is formed partly before and partly after birth, the prenatal and postnatal dentins are separated by an accentuated contour line. This is termed the *neonatal line* and is seen in enamel as well as dentin (Fig. 5.10). This line reflects the abrupt change in environment that occurs at birth. The dentin matrix formed prior to birth is usually of better quality than that formed after birth, and the neonatal line may be a zone of hypocalcification.

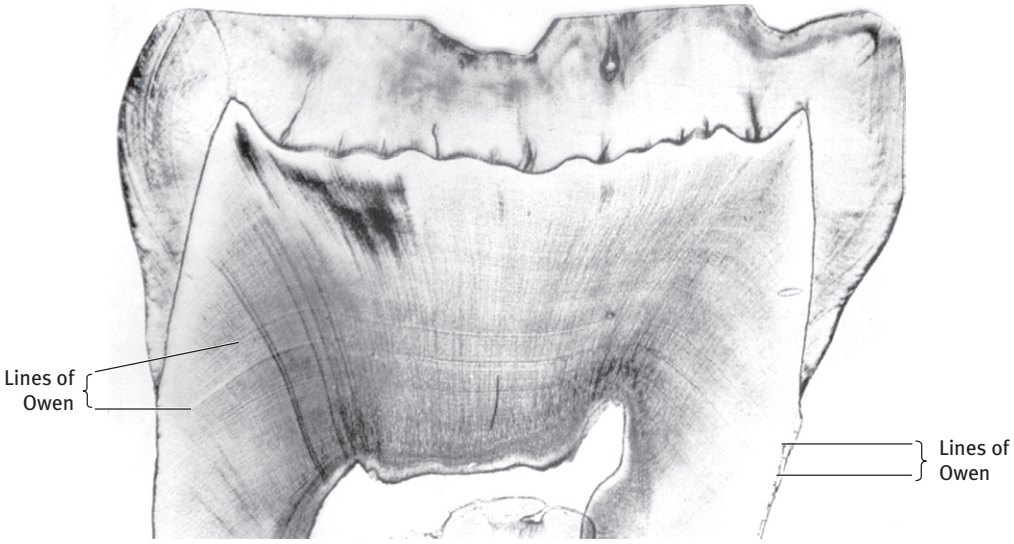

Figure 5.9 Accentuated incremental lines are termed contour lines (of Owen). Ground section human tooth.

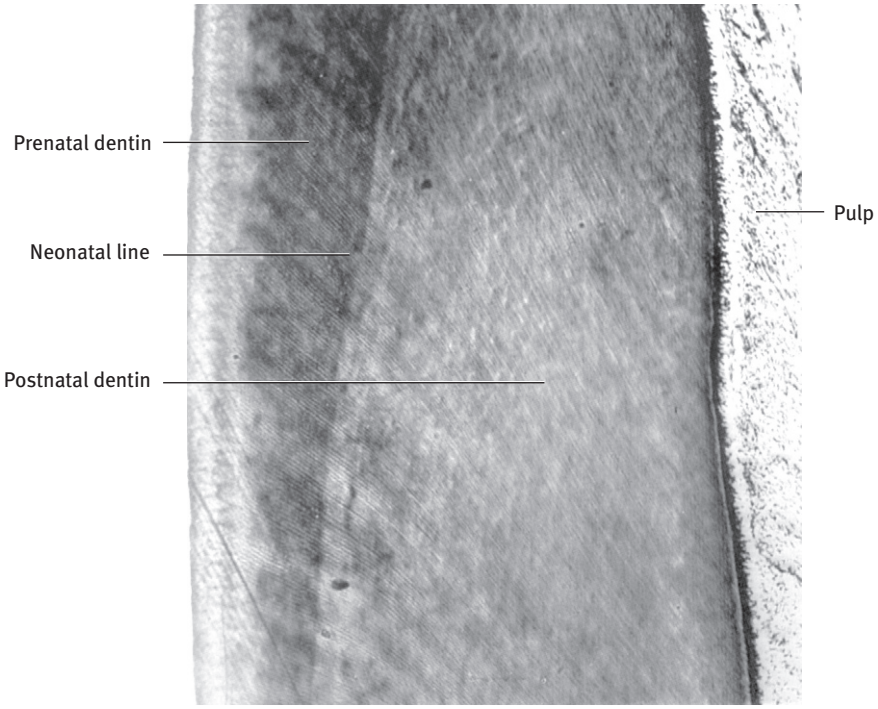

Figure 5.10 Postnatal formed dentin is separated from prenatal formed dentin by an accentuated incremental line termed the neonatal line (From Schour I and Poncher HG: Am J Dis Child 54:757, 1937).

INTERGLOBULAR DENTIN

Sometimes mineralization of dentin begins in small globular areas that fail to coalesce into a homogeneous mass. This results in zones of hypomineralization between the globules. These zones are known as *globular dentin* or *interglobular spaces*. This dentin forms in the crowns of teeth in the circumpulpal dentin just below the mantle dentin, and it follows the incremental pattern (Fig. 5.11). The dentinal tubules pass uninterruptedly through interglobular dentin, thus demonstrating defect of mineralization and not of matrix formation (Fig. 5.12). In dry ground sections, some of the globular dentin may be lost, and a space results that appears black in transmitted light (Fig. 5.13). Recent studies reveal that interglobular dentin occurs most frequently in the cervical and middle thirds followed by intercuspal and coronal third in the crown. In roots, the highest occurrence is seen in the cervical third followed by the middle third (Box 5.1).

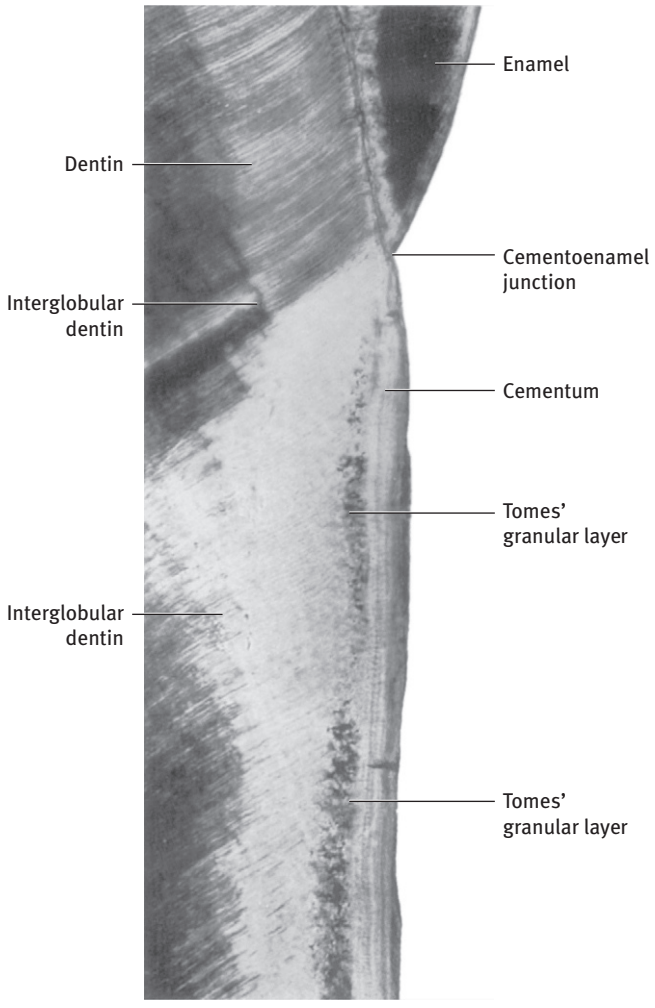

Figure 5.11 Granular layer (Tomes') appears in root dentin a short distance from the cementoenamel junction. The spaces are air filled and appear black in transmitted light in a ground section.

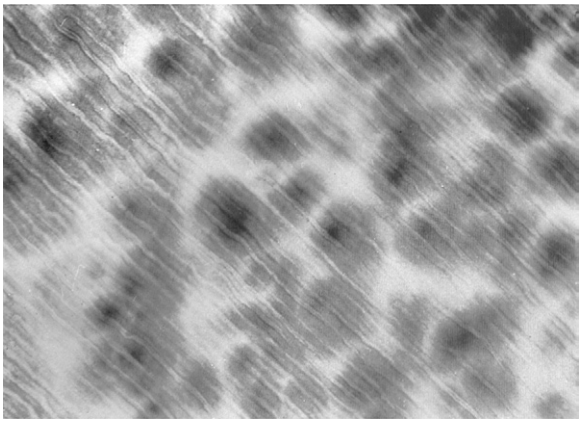

Figure 5.12 Globular dentin with interglobular spaces as seen in decalcified section of dentin. Dentin tubules pass uninterrupted through uncalcified and hypocalcified areas.

GRANULAR LAYER

When dry ground sections of the root dentin are visualized in transmitted light, a zone adjacent to the cementum appears granular (Fig. 5.11). This is known as (Tomes') granular layer. This zone increases slightly in amount from the cementoenamel junction to the root apex and is believed to be caused by a coalescing and looping of the terminal portions of the dentinal tubules. Such a process is considered possible as a result of the odontoblasts turning on themselves during early dentin formation. These areas remain unmineralized, like interglobular dentin. The cause of development of this zone is probably similar to the branching and beveling of the tubules at the dentinoenamel junction. Though Tomes' granular layer and interglobular dentin have similarities in their formation, they differ in their mineral content. Among the hypomineralized areas, Tomes' granular layer showed highest concentrations of calcium and phosphorus, while interglobular dentin showed a higher content of sulfur. Recent studies relate Tomes' granular layer as a special arrangement of collagen and noncollagenous matrix proteins at the interface between dentin and cementum.

In any case the differentiating odontoblast initially interacts with ameloblasts or the root sheath cells through the basal lamina. In the crown extensive branching of the odontoblast process occurs, and in the root there is branching and coalescing of adjacent processes.

INNERVATION OF DENTIN

Intratubular nerves

Nerve fibers were shown to accompany 30 to 70 % of the odontoblastic process and these are referred to as intratubular nerves. Dentinal tubules contain numerous nerve endings in the predentin and inner dentin no farther than 100 to 150 μm from the pulp. Most of these small vesiculated endings are located in tubules in the coronal zone, specifically in the pulp horns. The nerves and their terminals are found in close association with the odontoblast process within the tubule. There may be single terminals (Fig. 5.14) or several dilated and

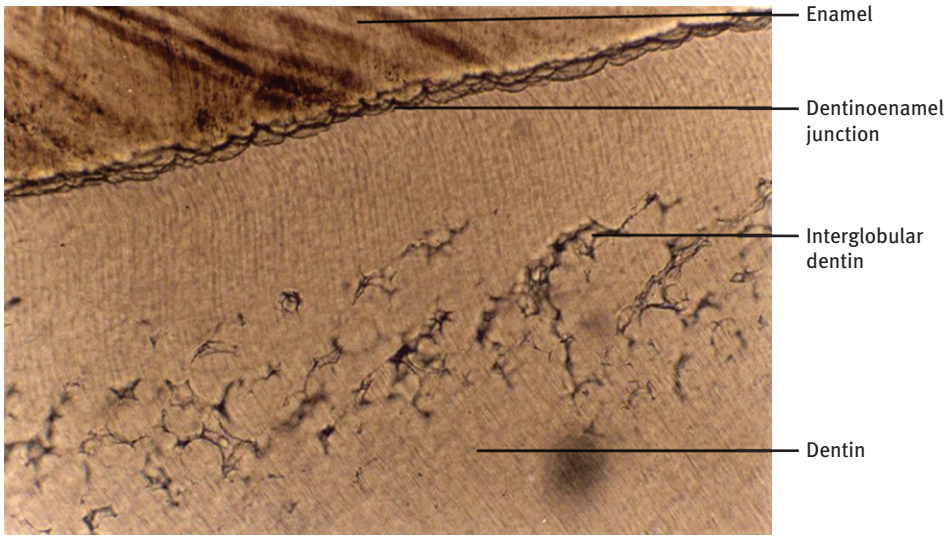

Figure 5.13 Photomicrogrph of ground section of tooth shows areas of interglobular dentin in the crown close to DE junction (x100).

Box 5.1

Interglobular dentin: "This is the unmineralized or hypomineralized dentin where in small globular areas of dentin fail to coalesce into a homogeneous mass. It appears as dark rounded masses usually with concave edges in transmitted light. The dentinal tubules pass through it without showing any change.

constricted portions (Fig. 5.15). In either case, the nerve endings are packed with small vesicles, either electron dense or lucent, which probably depends on whether there has been discharge of their neurotransmitter substance. In any case, they interdigitate with the odontoblast process, indicating an intimate relationship to this cell. Synapse like relation between the process and nerve fibers were demonstrated. It is believed that most of these are terminal processes of the myelinated nerve fibers of the dental pulp.

The primary afferent somatosensory nerves of the dentin and pulp project to the descending trigeminal nuclear complex (subnucleus caudalis).

Theories of pain transmission through dentin

There are three basic theories of pain conduction through dentin. The first is that of *direct neural stimulation*, by which the nerves in the dentin get stimulated. The nerves in the dentinal tubules are not commonly seen and even if they are present, they do not extend beyond the inner dentin. Topical application of local anesthetics do not abolish sensitivity. Hence this theory is not accepted. The second and most popular theory is the fluid or *hydrodynamic theory*. Various stimuli such

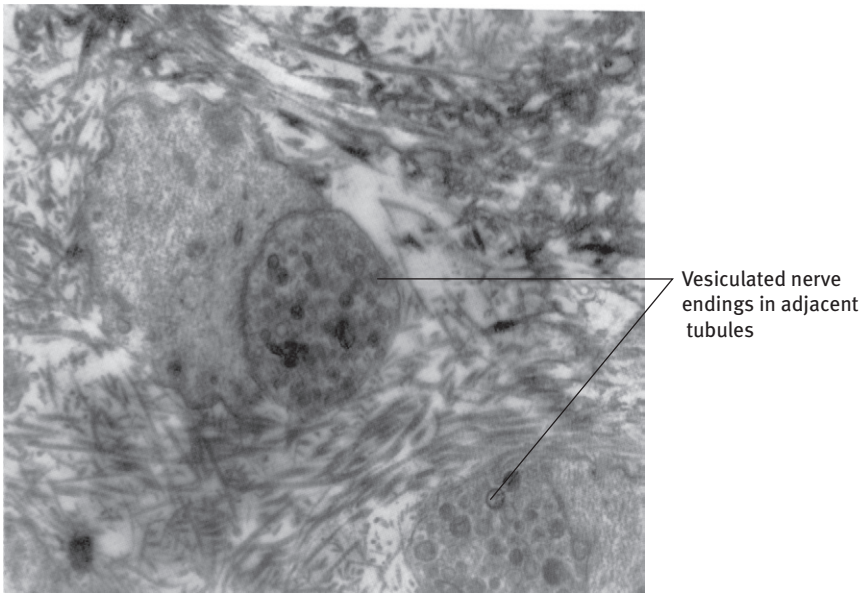

Figure 5.14 Nerve endings in dentinal tubules in region of predentin. The vesiculated endings are seen in adjacent tubules lying in contact with the odontoblast processes.

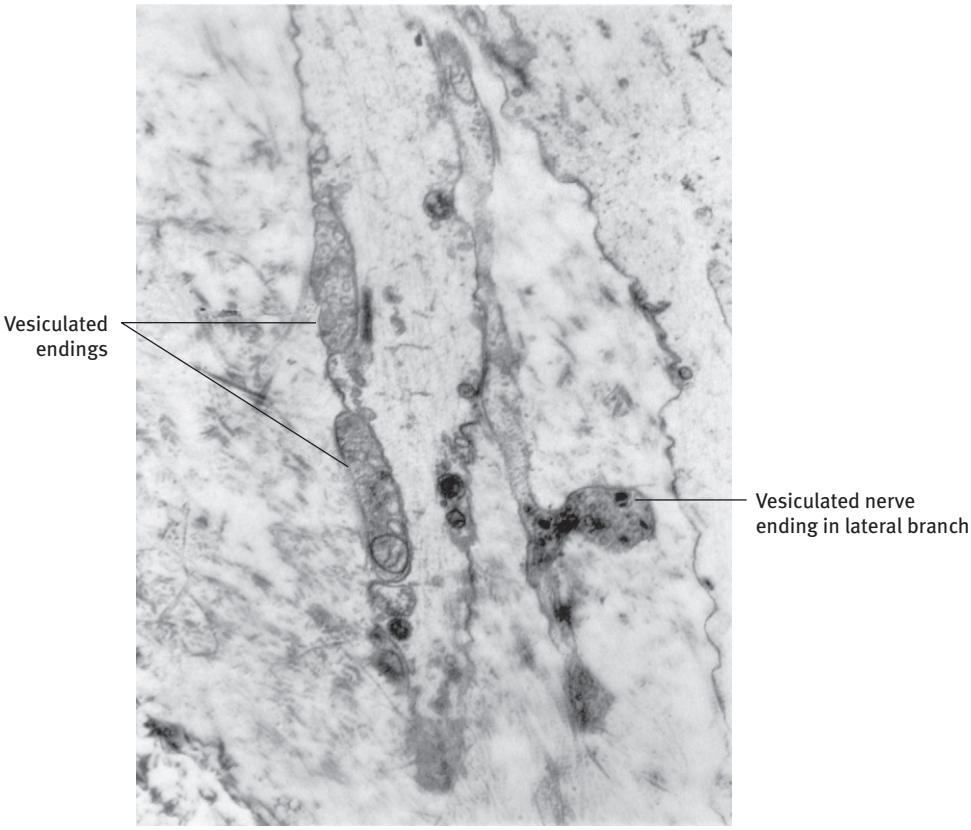

Figure 5.15 On the left two nerve endings in a dentinal tubule along with an odontoblast process. On the right a nerve ending extends into the side branch of a dentinal tubule in region of predentin (transmission electron micrograph).

as heat, cold, air blast desiccation, or mechanical or osmotic pressure affect fluid movement in the dentinal tubules. This fluid movement, either inward (due to cold stimuli) or outward (due to drying of exposed dentinal surface), stimulates the pain mechanism in the tubules by mechanical disturbance of the nerves closely associated with the odontoblast and its process. Thus these endings may act as mechanoreceptors as they are affected by mechanical displacement of the tubular fluid. The third theory is the *transduction theory*, which presumes that the odontoblast process is the primary structure excited by the stimulus and that the impulse is transmitted to the nerve endings in the inner dentin. This is not a popular theory since there are no neurotransmitter vesicles in the odontoblast process to facilitate the synapse or synaptic specialization. However, odontoblasts, by modifying the local ionic environment, alter the threshold of intradentinal nerves. The three theories are further explained in Figure 5.16.

PERMEABILITY OF DENTIN

Dentinal tubules become occluded by growth of peritubular dentin or by reprecipitation of minerals from demineralized areas of dental caries. Exposed dentinal surface becomes hypermineralized. Dentin permeability depends upon the patency of dentinal tubules. Therefore tubular occlusion, smear layer formation and lack of tubular communication between primary and irregular secondary dentin will result in reduced permeability.

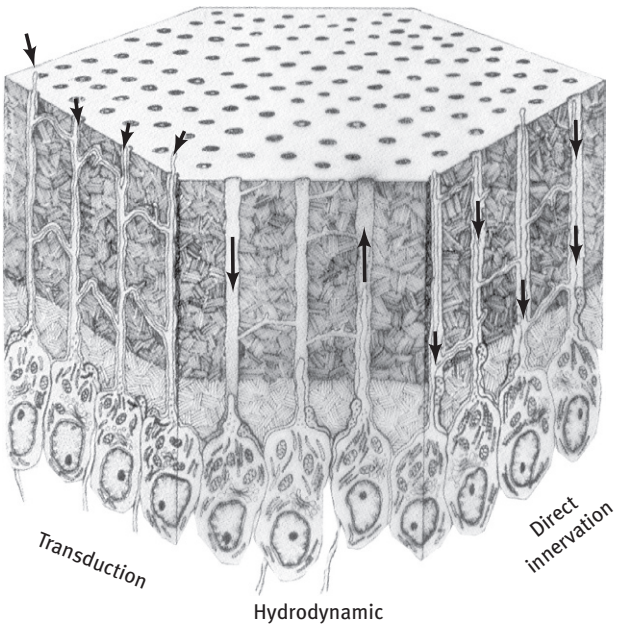

Figure 5.16 A diagram of the three main explanations of pain transmission through dentin. On the left is shown the transduction theory in which the membrane of the odontoblast process conducts an impulse to the nerve endings in the predentin, odontoblast zone, and pulp. In the center is the hydrodynamic theory. Stimuli cause an inward or outward movement of fluid in the tubule, which in turn produces movement of the odontoblast and its process. This in turn stimulates the nerve endings. On the right is the direct conduction theory in which stimuli directly affect the nerve endings in the tubules.

Reduction in dentin permeability would lessen the sensitivity of dentin.

Dentin permeability increases rapidly as the pulp chamber is approached because the number and diameter of the tubules are more per unit area towards pulp than towards periphery. The outward flow of dentinal fluid and the odontoblasts act as barriers for entry of bacteria or their toxins.

AGE AND FUNCTIONAL CHANGES

The changes in dentin due to age are dealt in detail in Chapter 17 on Age Changes in Oral Tissues. However, an outline of the age changes is described in the following paragraph.

It is rather difficult to separate age and functional changes in dentin. Dentin is laid down throughout life. This dentin is termed secondary dentin. If dentin forms as a result of pathological process, like caries, it is termed tertiary dentin. Tertiary dentin shows irregularity in size, shape, number and arrangement of dentinal tubules. Dentinal tubules degenerate due to injury resulting in the formation of dead tracts. Mineralization of dentinal tubules results in the formation of sclerotic dentin. This makes the tooth to appear transparent in these areas.

DEVELOPMENT

Dentinogenesis

Dentinogenesis begins at the cusp tips after the odontoblasts have differentiated and begin collagen production. In odontoblast differentiation, fibronectin, decorin, laminin and chondroitin sulfate may be involved. Recent studies showed that laminin α2, a subunit of laminin is essential for odontoblastic differentiation and to regulate the expression of dentin matrix proteins.

Dentinogenesis factors like TGF, IGF and BMP which are present in the inner enamel epithelium, are released and these are taken up by the preodontoblast. These factors help in the organization of odontoblast cytoskeleton assembly, which is important for relocation of organelles that occurs prior to morphological changes. As the odontoblasts differentiate they change from an ovoid to a columnar shape, and their nuclei become basally oriented at this early stage of development. One or several processes arise from the apical end of the cell in contact with the basal lamina. The length of the odontoblast then increases to approximately 40 µm, although its width remains constant (7 µm). Proline appears in the rough surface endoplasmic reticulum and Golgi apparatus. The proline then migrates into the cell process in dense granules, and is emptied into the extracellular collagenous matrix of the predentin.

Factors controlling odontoblast secretion and mineralization are not known. One of the key proteins involved in mineralization and secreted by the odontoblast is the dentin phosphoprotein(DPP). It is highly anionic and binds to calcium, transports it to the mineralization front and controls the growth of apatite crystals. Osteonectin secreted by the odontoblasts inhibits the growth of apatite crystals but promotes its binding to collagen. Osteopontin, a phosphoprotein, also promotes mineralization. Gla protein (gamma carboxyglutamic acid) containing protein and phospholipids act as seeds or nucleators to attract and concentrate calcium. Chondroitin sulfate, has opposite actions in mineralization. In predentin, they prevent the transport of apatite crystals, but in mineralized dentin they get adsorbed into the collagen and promote apatite binding to the collagen. Odontoblast takes up the calcium and maintains its concentration higher than in tissue fluid.

Matrix vesicles are involved in the mineralization of mantle dentin. The matrix vesicles, prior to its release by the odontoblast promotes the formation of apatite. It contains enzymes like alkaline phosphatase, which locally increases the concentration of phosphates, and these combine with calcium taken up from the tissue fluid to form apatite within it.

The role of effector nerves in dentin secretion is suggested due to the presence of synapse proteins; synapsin and synaptotagmin in the dentinal tubules.

As the cell recedes it leaves behind a single extension, and the several initial processes join into one, which becomes enclosed in a tubule. As the matrix formation continues, the odontoblast process lengthens, as does the dentinal tubule. Initially daily increments of approximately 4 µm of dentin are formed. This continues until the crown is formed and the teeth erupt and move into occlusion. After this time dentin production slows to about 1 µm/day. After root development is complete, dentin formation may decrease further, although reparative dentin may form at a rate of 4 µm/day for several months after a tooth is restored. Dentinogenesis is a two-phase sequence in that collagen matrix is first formed and then calcified. As each increment of predentin is formed along the pulp border, it remains a day before it is calcified and the next increment of predentin forms (Fig. 5.17). *Korff's fibers* have been described as the initial dentin deposition along the cusp tips. Because of the argyrophilic reaction (stain black with silver) it was long believed that bundles of collagen formed among the odontoblasts (Fig. 5.18). Recently, ultrastructural studies revealed that the staining is of the ground substance among the cells and not collagen. Consequently, all predentin is formed in the apical end of the cell and along the forming tubule wall (Fig. 5.17). The finding of formation of collagen fibers in the immediate vicinity of the apical ends of the cells is in agreement with the general concept of collagen synthesis in connective tissue and bone. The odontoblasts secrete both collagen and other components of the extracellular matrix.

The radicular dentin formation compared to coronal dentin, is slower, less mineralized with collagen fibers laid down parallel to the cementodentinal junction. These collagen fibers unlike in coronal dentin are laid adjacent to the noncollagenous matrix of (Hertwig's epithelial root sheath).

Mineralization

The mineralization sequence in dentin appears to be as follows. The earliest crystal deposition is in the form of very fine plates of hydroxyapatite on the surfaces of the collagen fibrils and in the ground substance (Fig. 5.17A). Subsequently, crystals are laid down within the fibrils themselves. The crystals associated with the collagen

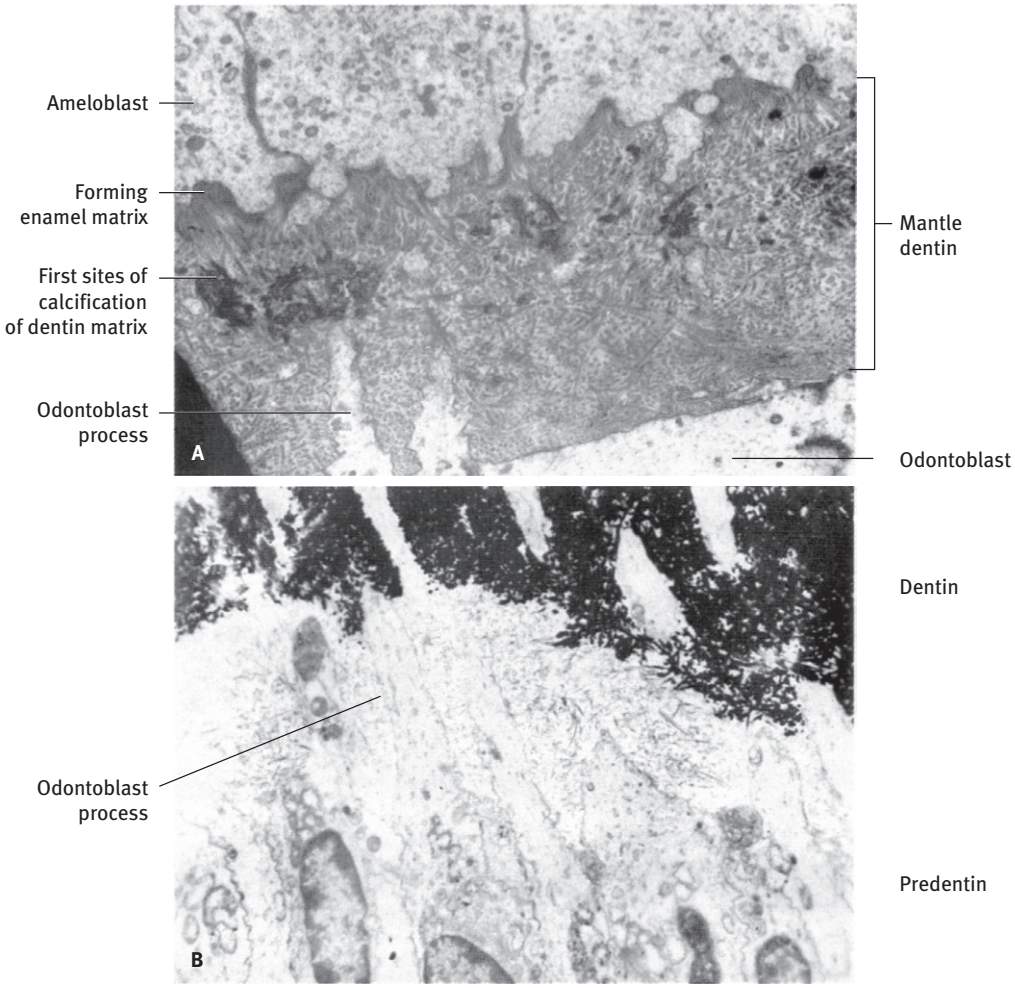

Figure 5.17 (**A**) First-formed dentin, showing cytoplasm of apical zone of ameloblast, above, and first-formed enamel matrix at the dentinoenamel junction. Below the junction collagen fibers of dentin matrix are seen with calcification sites appearing near the first-formed enamel. Predentin zone is seen below these sites with the odontoblast process extending from the odontoblasts at bottom of field. (**B**) Predentin and dentin as visualized in a later developing tooth. Observe calcified (black) dentin above, predentin composed of collagen fibers below, odontoblast processes, and the cell body (transmission electron micrographs).

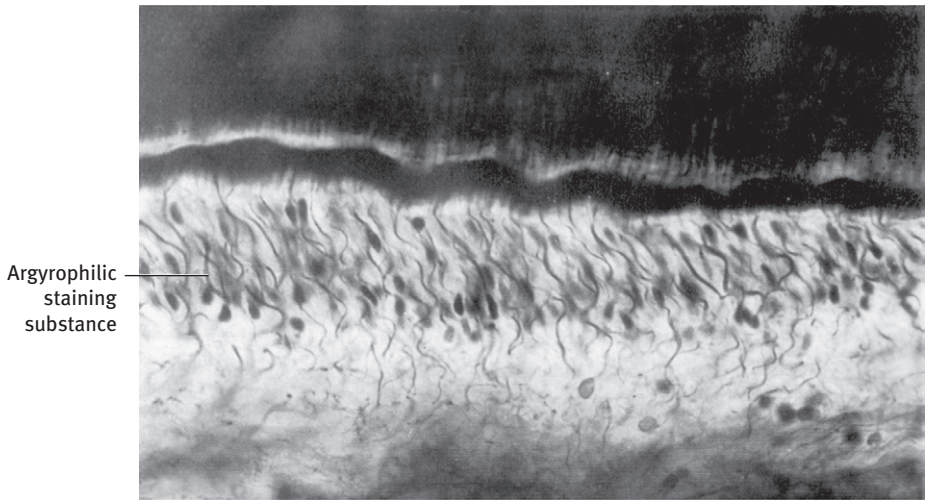

Figure 5.18 Light micrograph of a silver-stained section of early forming dentin. The argyrophilic nature of the ground substances among the odontoblasts appears like bundles of collagen fibers. Figure 5.17B illustrates that the collagen formed by the odontoblast is apical to the cell body in the area of the forming predentin.

fibrils are arranged in an orderly fashion, with their long axes paralleling the fibril long axes, and in rows conforming to the 64 nm (640 Å) striation pattern. Within the globular islands of mineralization, crystal deposition appears to take place radially from common centers, in a so-called spherulite form. These are seen as the first sites of calcification of dentin (Fig. 5.17A).

The general calcification process is gradual, but the peritubular region becomes highly mineralized at a very early stage. Although there is obviously some crystal growth as dentin matures, the ultimate crystal size remains very small, about 3 nm (30 Å) in thickness and 100 nm (1000 Å) in length. The apatite crystals of dentin resemble those found in bone and cementum. They are 300 times smaller than those formed in enamel (Fig. 5.19). It is interesting that two cells so closely allied at the dentinoenamel junction produce crystals of such a size difference but at the same time produce chemically the same hydroxyapatite crystals. Calcospherite mineralization is seen occasionally along the pulp-predentin-forming front (Fig. 5.20). Dentin sialoprotein present in mineralizing dentin affects the rate of mineral deposition while other proteoglycans present more in the predentin, inhibit calcification to prevent premature calcification of the predentin.

Many genes are implicated in dentinogenesis, the newer ones being MAP1B for odontoblast differentiation, and PHEX for dentin mineralization.

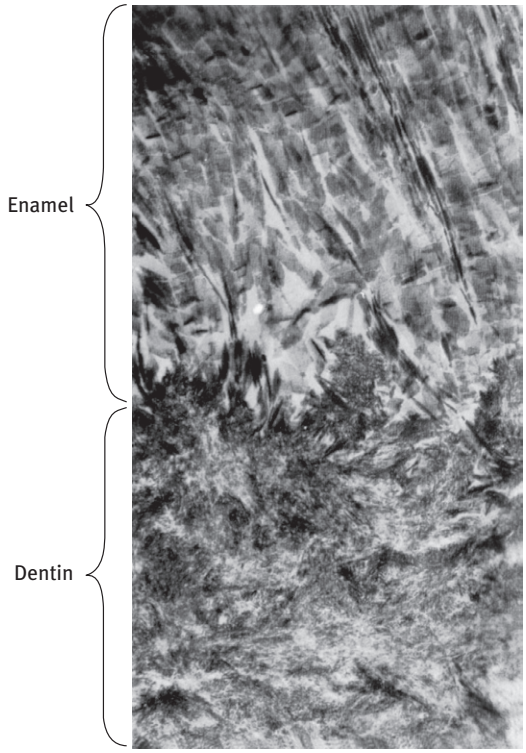

Figure 5.19 Dentinoenamel junction. Enamel is above and dentin below. Note difference in size and orientation between crystallites of enamel and dentin. Whereas crystals of human enamel may be 90 nm (900 Å) in width and 0.5 to 1 μm in length, those of dentin are only 3 nm (30 Å) in width and 100 nm (1000 Å) in length. Crystals of dentin are similar in size to bone (Electron microphotograph ×35,000).

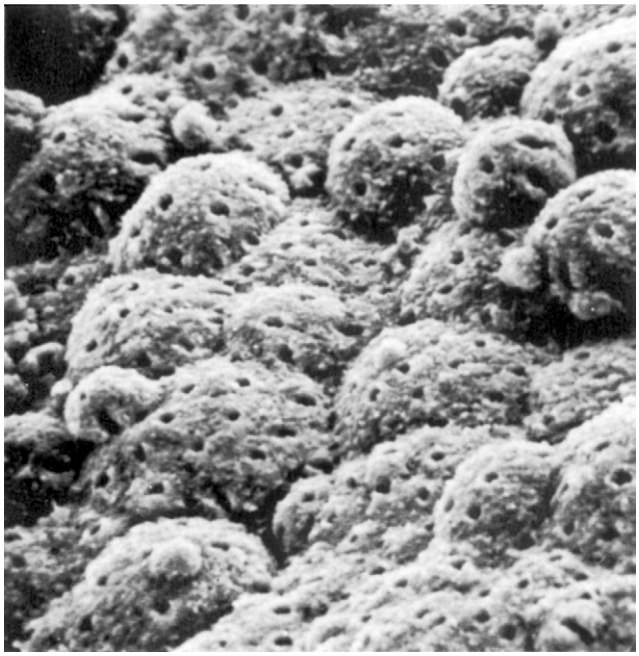

Figure 5.20 Scanning electron micrograph of globular dentin.

CLINICAL CONSIDERATIONS

The cells of the exposed dentin should not be insulted by bacterial toxins, strong drugs, undue operative trauma, unnecessary thermal changes, or irritating restorative materials. One should bear in mind that when 1 mm^2 of dentin is exposed, about 30,000 living cells are damaged. It is advisable to seal the exposed dentin surface with a nonirritating, insulating substance.

The rapid penetration and spread of caries in the dentin is the result of the tubule system in the dentin (Fig. 5.1). The enamel may be undermined at the dentinoenamel junction, even when caries in the enamel is confined to a small surface area. This is due in part to the spaces created at the dentino-enamel junction by enamel tufts, spindles, and open and branched dentinal tubules. The dentinal tubules provide a passage for invading bacteria and their products through either a thin or thick dentinal layer.

Electron micrographs of carious dentin show regions of massive bacterial invasion of dentinal tubules (Fig. 5.21). The tubules are enlarged by the destructive action of the micro-organisms. Dentin sensitivity of pain, unfortunately, may not be a symptom of caries until the pulp is infected and responds by the process of inflammation, leading to toothache. Thus patients are surprised at the extent of damage to their teeth with little or no warning from pain. Undue trauma from operative instruments also may damage the pulp. Air-driven cutting instruments cause dislodgement of the odontoblasts from the periphery of the pulp and their "aspiration" within the dentinal tubule. This could be an important factor in survival of the pulp if the pulp is already inflamed. Repair requires the mobilization of the macrophage system as healing takes place; as this progresses there is the

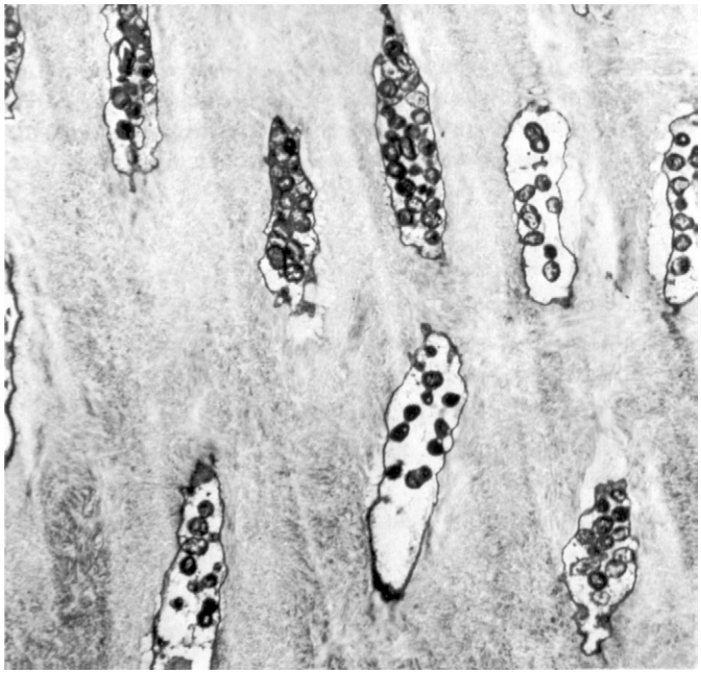

Figure 5.21 Electron micrograph of dentin underlying carious lesion.Coccoid bacteria are present in the tubules. The peritubular dentin has been destroyed causing the enlargement of the tubules (×10,000).

contribution of deeper pulpal cells, through cytodifferentiation into odontoblasts, which will be active in formation of reparative dentin.

The sensitivity of the dentin has been explained by the hydrodynamic theory, that alteration of the fluid and cellular contents of the dentinal tubules causes stimulation of the nerve endings in contact with these cells (Fig. 5.16). Most pain inducing stimuli increase centrifugal fluid flow within the dentinal tubules, giving rise to a pressure change throughout the entire dentin. This, in turn, activates the Aσ intradentinal nerves at the pulp–dentinal interface, or within the dentinal tubules thereby generating pain. Dentin sensitivity is seen more in patients with periodontal problems. The teeth most commonly affected are maxillary premolars followed by the maxillary first molars with the incisors being the least sensitive teeth. This theory explains pain throughout dentin since fluid movement will occur at the dentinoenamel junction as well as near the pulp. Erosion of peritubular dentin and smear plug removal accounts for dentin hypersensitivity caused by agents like acidic soft drinks. Brushing after acidic drink consumption induces smear layer formation, thus reducing sensitivity.

The basic principles of treatment of hypersensitivity are to block the patent tubules or to modify or block pulpal nerve response. The most inexpensive and first line of treatment is to block the patent tubules with dentifrice containing potassium nitrate and/or stannous fluoride. Lasers have been used in the treatment of hypersensitivity with varying success, ranging from 5.2 to 100%.

The permeability of radicular dentin near the pulp is only about 20% that of coronal dentin, and the permeability of outer radicular dentin is about 2% of coronal dentin. This suggests that the outer dentin of the root acts as a barrier to fluid movement across dentin in normal circumstances and recalls the correlation between root planing and hypersensitivity.

Smear layer consists of cut dentin surface along with the embedded bacteria and the debris. Though the smear layer occludes the tubules and reduces the permeability, it also prevents the adhesion of restorative materials to dentin. Therefore this layer has to be removed by etching and a rough porous surface should be created for bonding agent to penetrate.

Recent studies on tertiary dentin show that the patients treated with transforming growth factor beta 1 (TGF-β1) and to a lesser extent with osteogenic protein-1 (OP-1) showed significantly greater tertiary dentin formation and intratubular mineralization, over an 8-week period when compared with control group.

Dentin formation, unlike bone, is not affected by vitamin D deficiency states. Fluoride incorporated during active dentinogenesis, as occurring in dental fluorosis, increases the hardness of dentin.

A summary diagram illustrating the relationship of the odontoblast and its process to the dentin matrix is shown in Figure 5.22.

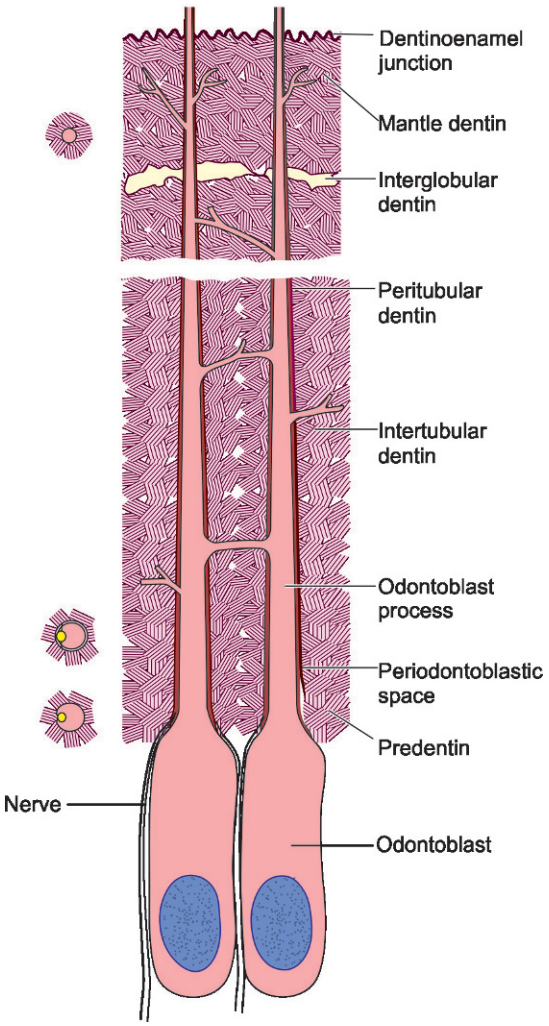

Figure 5.22 Diagram of the odontoblast and its process in the dental tubule. Note the relationship of the process to the periodontoblastic space and the peritubular dentin.

SUMMARY

Dentin forms bulk of the tooth tissues and occupies both the crown and the root. It is surrounded by the enamel in the crown and cementum in the root. Physically and chemically it resembles bone. Dentin consists of 65% inorganic and 35% organic material. The organic substance consists mainly of type I collagen fibers embedded in ground substance of mucopolysaccharides. The inorganic component consists of plate shaped hydroxyapatite crystals.

Structure of Dentin

Dentin is made up of dentinal tubules. The dentinal tubules contain the protoplasmic process of the odontoblasts called *odontoblastic process*. The inner wall of the tubule is highly calcified and is termed as *peritubular* or *intratubular dentin*. Between the tubules is the *intertubular dentin* and it is calcified to a lesser degree than peritubular dentin. Between the odontoblastic processes and the peritubular dentin is the periodontoblastic space which contains the dentinal fluid. The tubules are broader at their point of origin, i.e. at their pulpal end (3–4 μm) and become gradually narrower at their point of termination (1 μm), i.e. at dentinoenamel or cementoenamel junction. Also the tubules are closer together at the pulpal end (50–90,000 tubules/mm^2) and further apart at their terminal end. Therefore the number of tubules per unit area is more at the pulpal end by 4 times than at their terminal end. Hence the intertubular dentin is well appreciated at the terminal end. The tubules generally have a doubly convex course, the *primary curvature* (often called 'S'-shaped tubules), the first convexity of which is directed towards apex. This is well appreciated in the middle portion of the tooth because the convexity lessens and the tubules gradually become straighter near the incisal/cuspal regions and the apex. Apart from this primary curvature the tubules show smaller undulations called *secondary curvatures* all along their course. Also, all along the course the tubules show lateral branches and terminal branches at their terminal end. The odontoblastic process which contains microtubules, microfilaments, and mitochondria extends into these branches. Few of the odontoblastic process cross the DEJ and they are known as enamel spindles

Primary Dentin

The dentin which forms before root completion is called primary dentin. Primary dentin is of two types—the mantle dentin and the circumpulpal dentin. The *mantle dentin* found along the DEJ is about 20 μm thick, and it contains larger-diameter argyrophilic (silver stained) collagen fibers arranged perpendicularly to the DEJ. The *circumpulpal dentin* contains smaller diameter closely packed collagen fibers.

Secondary Dentin

The dentin which forms after root completion is called secondary dentin. This forms at a slower rate (1 μm/day) than primary dentin (4 μm/day) and regularly but not uniformly—more formation is seen on the roof and floor of the pulp chamber. Hence this dentin is also known as regular secondary dentin.

Tertiary Dentin (Reparative Dentin)

If the dentin forms as a reaction to an irritant (trauma or dental caries) to protect the pulp, it is called reparative/reactive/tertiary/irregular secondary dentin. The tertiary dentin shows irregularity in the number, size, and arrangement of tubules—the irregularity increases with the rapidity of formation.

Formation of Dentin

Whenever dentin forms, it forms in two phases—a distinct organic matrix known as *predentin* which calcifies subsequently but only after another layer of predentin is laid down. Hence a layer of predentin always exist adjacent to the odontoblast. Many factors are involved in differentiation of odontoblast, secretion of organic matrix, and in subsequent mineralization. Like other mineralized tissues dentin formation is not a continuous process, the periods of rest are denoted by incremental lines—the *incremental lines of Von Ebner* which are 4–8 mm apart run at right angles to the dentinal tubules. The accentuated incremental lines are known as *contour lines of Owen,* while those formed during the period of birth are referred to as *neonatal lines*. The mineralization of dentin occurs in relation to collagen fibers as linear deposits (linear mineralization) or by fusion of globules (globular mineralization). Incomplete fusion of globules leads to the formation of *interglobular dentin.* The interglobular dentin is found near DEJ, and are visible in ground sections as dark spaces under transmitted light. Similarly, the *Tomes' granular layer* is visible near cementodentinal junction as minute dark spaces, and it is related to looping and coalescing of terminal portions of dentinal tubules in that region.

Hypersensitivity of Dentin

The nerves which enter dentin do not extend beyond the inner two thirds of dentin. The stimulation of dentin by any agent causes a pain like sensation called hypersensitivity. The theories that explain sensitivity are based on the direct stimulation of the nerves (direct neural stimulation theory) or due to inward or outward movement of dentinal fluid exciting the nerve endings (hydrodynamic theory) or due to stimulation of odontoblastic process (transduction theory).

Age Changes in Dentin

The age changes in dentin include the formation of sclerotic dentin and dead tracts. *Sclerotic dentin occurs* when hydroxyapatite crystals are laid down in the intertubular dentin and within dentinal tubules to block the tubules against the entry of bacteria, etc. The dentin then becomes transparent in transmitted light hence often called transparent dentin. Permeability of dentin becomes reduced while hardness increases in these regions. Breakage of apical thirds of roots during extraction of teeth of elderly is due to the brittle nature of sclerotic dentin. *Dead tracts* are areas containing degenerated dentinal tubules which appear dark in transmitted light. These are often seen in attrited or abraded teeth or below the carious lesions.

REVIEW QUESTIONS

1. Describe the physical and chemical properties of dentin.
2. Describe the course, structure, and content of dentinal tubules.
3. Explain the terms mantle dentin, predentin, and circumpulpal dentin.
4. What are the lines seen in dentin? What is their significance?
5. What is Tomes' granular layer?
6. Write about interglobular dentin.
7. What factors are involved in odontoblasts differentiation, secretion and mineralization of dentin?
8. Explain the theories of dentin sensitivity. What are the principles underlying their treatment?

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6 Pulp

CHAPTER CONTENTS

The term 'pulpa', derived from Latin primarily indicates animal or plant tissues which are moist and soft, occurring in the form of a cohering mass. Dental pulp can be defined as a richly vascularized and innervated connective tissue of mesodermal origin enclosed by dentin with communications to the periodontal ligament.

ANATOMY

General features

The dental pulp occupies the center of each tooth and consists of soft connective tissue. The pulp is housed in the pulp chamber of the crown and in the root canal of the root. The pulp present in the crown is called coronal pulp and the pulp present in the root is called radicular pulp. The shape of the pulp therefore resembles the shape of the tooth in which it is housed. The total volume of all the permanent teeth pulp is 0.38 cc, and the mean volume of a single adult human pulp is 0.02 cc. Molar pulps are three to four times larger than incisor pulps. Table 6.1 gives the variation in the size of pulp in different permanent teeth.

Coronal pulp

The coronal pulp in young individuals resembles the shape of the outer surface of the crown dentin. The coronal pulp has six surfaces: the roof or occlusal, the mesial, the distal, the buccal, the lingual, and the floor. It has pulp horns, which are protrusions that extend into the cusps of each crown. The number of these horns thus depends on the cuspal number. The cervical region of the pulp organs constricts as does the contour of the crown, and at this zone the coronal pulp joins the radicular pulp. Because of continuous deposition of dentin, the pulp becomes smaller with age. This is not uniform through the coronal pulp but progresses faster on the floor than on the roof or side walls.

Radicular pulp

The radicular or root pulp is that pulp extending from the cervical region of the crown to the root apex. In the anterior teeth the radicular pulps are single and in posterior ones multiple. They are not always straight and vary in size, shape, and number. The radicular portions of the pulp are continuous with the periapical connective tissues through the apical foramen or foramina. The dentinal walls taper and the shape of the radicular pulp is tubular. During root formation the apical root end is a wide opening limited by an epithelial diaphragm (Fig. 6.1A). As growth proceeds, more dentin is formed, so that when the root of the tooth has matured the radicular pulp is narrower. The apical pulp canal becomes smaller also because of apical cementum deposition (Fig.6.1B).

Table 6.1 Pulp Volumes for the Permanent Human Teeth from a Preliminary Investigation of 160 Teeth*

	Maxillary (Cubic Centimeters)	Mandibular (Cubic Centimeters)
Central incisor	0.012	0.006
Lateral incisor	0.011	0.007
Canine	0.015	0.014
First premolar	0.018	0.015
Second premolar	0.017	0.015
First molar	0.068	0.053
Second molar	0.044	0.032
Third molar	0.023	0.031

*Figures for volumes from Fanibunda KB: Personal communication, University of Newcastle upon Tyne, Department of Oral Surgery, Newcastle upon Tyne, England.

Apical foramen

The average size of the apical foramen of the maxillary teeth in the adult is 0.4 mm. In the mandibular teeth it is slightly smaller, being 0.3 mm in diameter.

The location and shape of the apical foramen may undergo changes as a result of functional influences on the teeth. A tooth may be tipped from horizontal pressure, or it may migrate mesially, causing the apex to tilt in the opposite direction. Under these conditions the tissues entering the pulp through the apical foramen may exert pressure on one wall of the foramen, causing resorption. At the same time, cementum is laid down on the opposite side of the apical root canal, resulting in a relocation of the original foramen (Fig. 6.2A).

Sometimes the apical opening is found on the lateral side of the apex (Fig. 6.2B), although the root itself is not curved. Frequently, there are two or more foramina separated by a portion of dentin and cementum or by cementum only.

Accessory canals

Accessory canals leading from the radicular pulp laterally through the root dentin to the periodontal tissue may be seen anywhere along the root but are most numerous in the apical third of the root (Fig. 6.3A). They are clinically significant in spread of infection, either from the pulp to the periodontal ligament or vice versa. The mechanism by which they are formed is not known, but it is likely that they occur in areas where there is premature loss of root sheath cells because these cells induce the formation of the odontoblasts which form the dentin. Accessory canals may also occur where the developing root encounters a blood vessel. If the vessel is located in the area where the dentin is forming, the hard tissue may develop around it, making a lateral canal from the radicular pulp.

STRUCTURAL FEATURES

The central region of both the coronal and the radicular pulp contains large nerve trunks and blood vessels. Peripherally, the pulp is circumscribed by the specialized odontogenic region composed of: (1) the odontoblasts (the dentin-forming cells), (2) the cell-free zone (Weil's zone), and (3) the cell-rich zone (Fig. 6.4). The cell-free zone is a space in which the odontoblast may move pulpward during tooth development and later to a limited extent in functioning teeth. This may be why the zone is

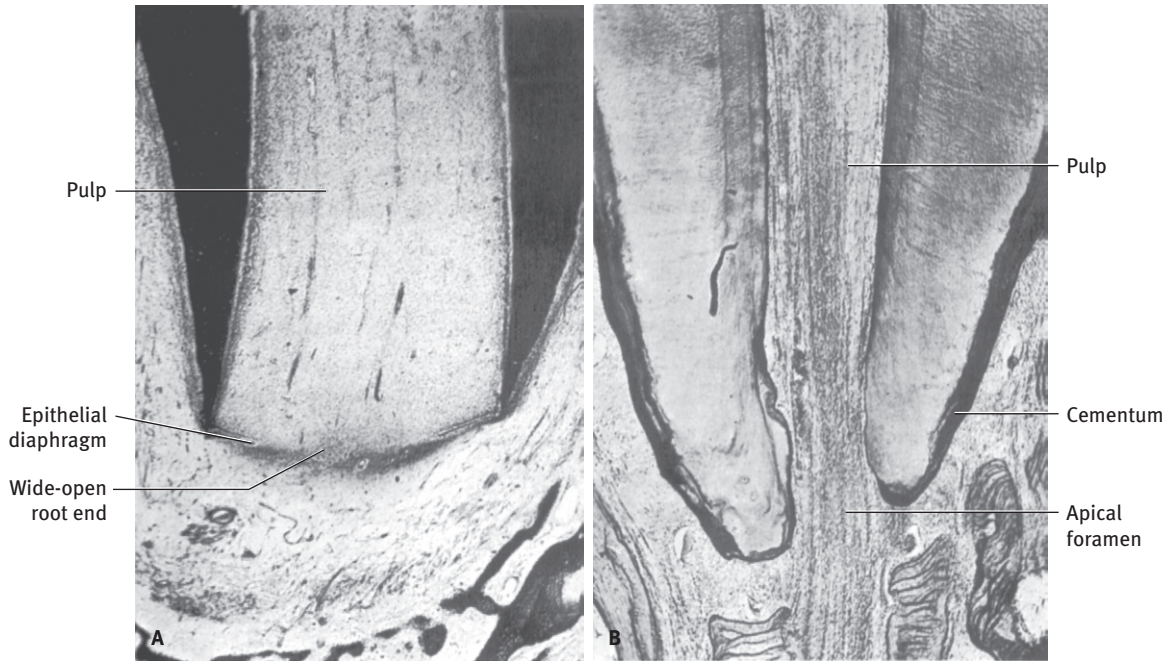

Figure 6.1 Development of apical foramen. (**A**) Undeveloped root end. Wide opening at end of root, partly limited by epithelial diaphragm. (**B**) Apical foramen fully formed. Root canal straight. Apical foramen surrounded by cementum (From Coolidge ED: J Am Dent Assoc 16:1456, 1929).

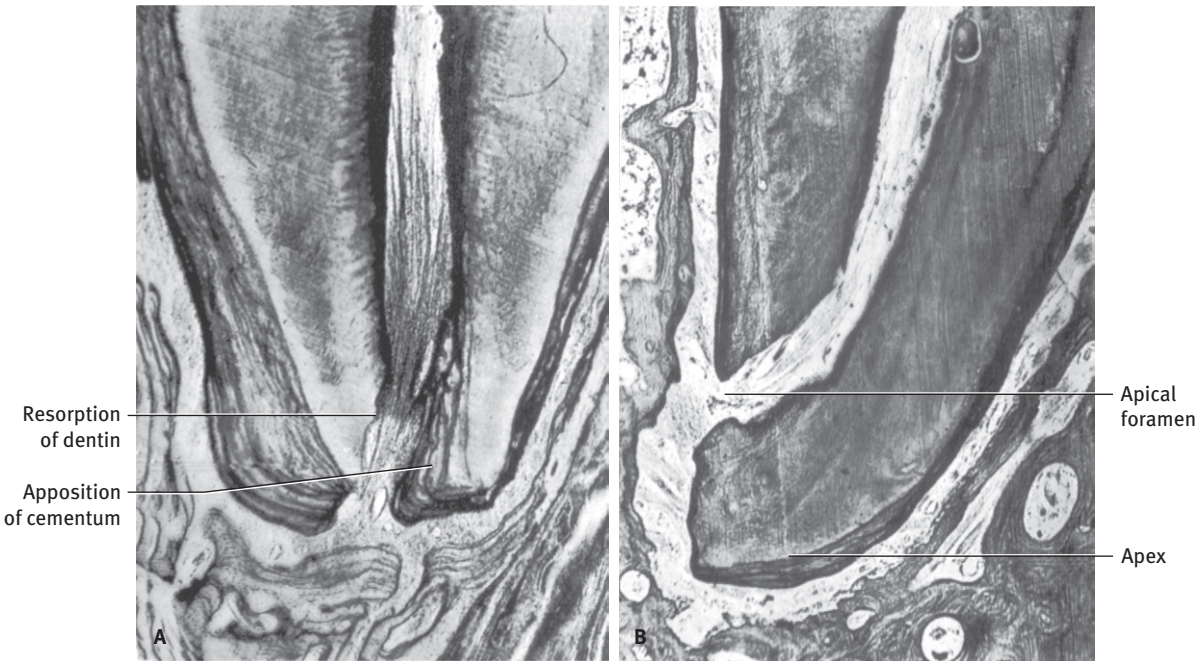

Figure 6.2 Variations of apical foramen. (**A**) Shift of apical foramen by resorption of dentin and cementum on one surface and apposition of cementum on the other. (**B**) Apical foramen on side of apex (From Coolidge ED: J Am Dent Assoc 16:1456, 1929).

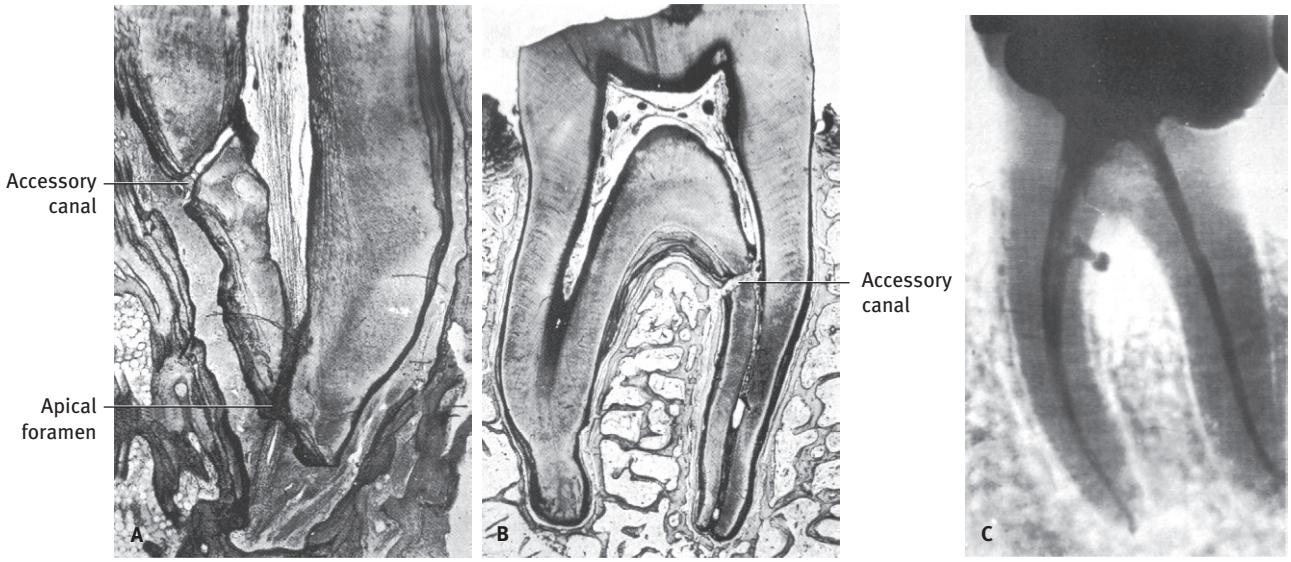

Figure 6.3 (**A** and **B**) Sections through teeth with accessory canals. (**A**) Close to apex. (**B**) Close to bifurcation. (**C**) Radiograph of lower molar with accessory canal filled. (**C** from Johnston HB and Orban B: J Endodont 3:21, 1948).

inconspicuous during early stages of rapid dentinogenesis since odontoblast migration would be greatest at that time. The cell-rich layer composed principally of fibroblasts and undifferentiated mesenchymal cells is restricted to the coronal regions, as it is formed during the pre-eruptive phase of the tooth. During early dentinogenesis there are also many young collagen fibers in this zone (Table 6.2).

Intercellular substance

The intercellular substance is dense and gel like in nature, varies in appearance from finely granular to fibrillar, and appears more dense in some areas, with clear spaces left between various aggregates. It is composed of both acid mucopolysaccharides and protein polysaccharide compounds (glycosaminoglycans and proteoglycans). During early development, the presence of chondroitin A, chondroitin B, and hyaluronic acid has been demonstrated in abundance. Glycoproteins are also present in the ground substance. The aging pulp contains less of all of these substances. The ground substance lends support to the cells of the pulp while it also serves as a means for transport of nutrients from the blood vessels to the cells, as well as for transport of metabolites from cells to blood vessels.

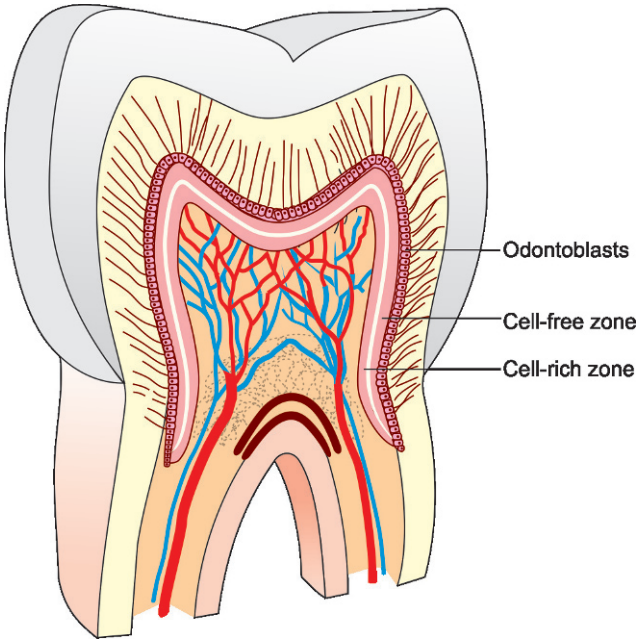

Figure 6.4 Diagram of pulp organ, illustrating architecture of large central nerve trunks (dark) and vessels (light) and peripheral cell-rich, cell-free, and odontoblast rows. Observe small nerves on blood vessels.

Table 6.2 Zones of Pulp

Zone	Major Component
Odontoblastic zone	Odontoblast cells
Cell-free zone (Weil's zone)	Relatively acellular, accommodate odontoblast during deveolpment & function of tooth
Cell-rich zone (primarily coronal)	Fibroblasts, undifferentiated mesenchymal cells
Pulp core	Predominantly fibrous tissue , major vessels & nerves, fibroblasts

Glycosaminoglycans being hydrophilic, forms a gel and contributes to high tissue fluid pressure of the pulp. *Hyaluronan*, in addition to mechanical function helps in cell migration. *Versican* forms the bulk of the proteoglycans. *Syndecan*, another important proteoglycan, attaches to the cell and acts as an adhesion molecule between fibroblast and collagen. It also binds signaling molecules like fibroblastic growth factor. *Tenascin* and *Fibronectin*, which promote cell adhesion and cell migration are absent in areas of inflammation. *Laminin*, which is present in the basement membrane of blood vessels, also coats the odontoblast cell membrane. *Integrins*, the glycoproteins, which interact to form cell surface adhesion receptors were found in pulp to get attached to biologically active molecules like laminin and fibronectin.

Fibroblasts

The pulp organ is said to be consist of specialized connective tissue because it lacks elastic fibers. Fibroblasts are the most numerous cell type in the pulp. As their name implies, they function in collagen fiber formation throughout the pulp during the life of the tooth. They have the typical stellate shape and extensive processes that contact and are joined by intercellular junctions to the processes of other fibroblasts (Fig. 6.5A). Under the light microscope the fibroblast nuclei stain deeply with basic dyes, and their cytoplasm is lighter stained and appears homogeneous. Electron micrographs reveal abundant rough-surfaced endoplasmic reticulum, mitochondria, and other organelles in the fibroblast cytoplasm (Fig. 6.5B). This indicates these cells are active in pulpal collagen production. There is some difference in appearance of these cells depending on the age of the pulp organ. In the young pulp the cells divide and are active in protein synthesis, but in the older pulp they appear rounded or spindle shaped with short processes and exhibit fewer intracellular organelles. They are then termed *fibrocytes*. In the course of development the relative number of cellular elements in the dental pulp decreases, whereas the fiber population increases (Fig. 6.6). In the embryonic and immature pulp the cellular elements predominate, while in the mature pulp the fibrous components predominate. The fibroblasts of the pulp, in addition to forming the pulp matrix, also have the capability of ingesting and degrading this same matrix. These cells thus have a dual function with pathways for both synthesis and degradation in the same cell.

Fibroblasts play an important role in inflammation and healing. Fibroblasts secrete angiogenic factors like FGF-2 and VEGF, especially after injury, which help in

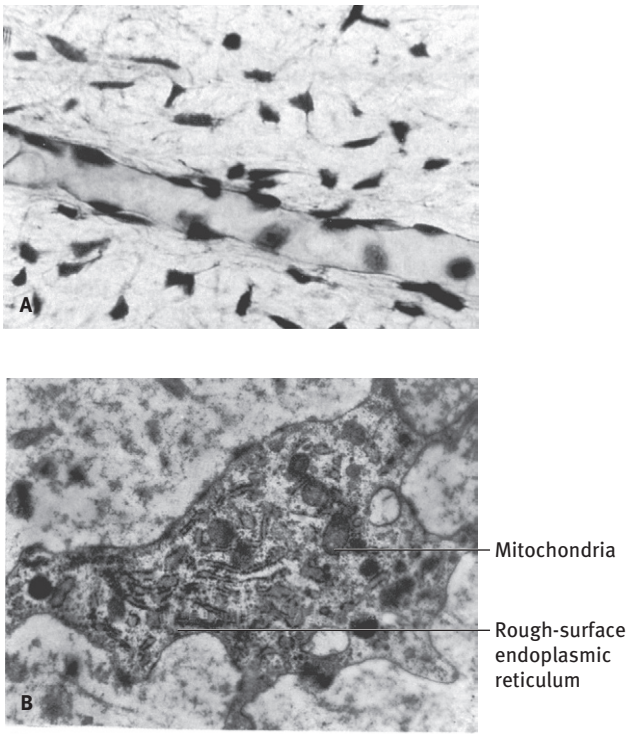

Figure 6.5 (**A**) Typical fibroblasts of pulp are stellate in shape with long processes. (**B**) Electron micrograph of pulp fibroblast.

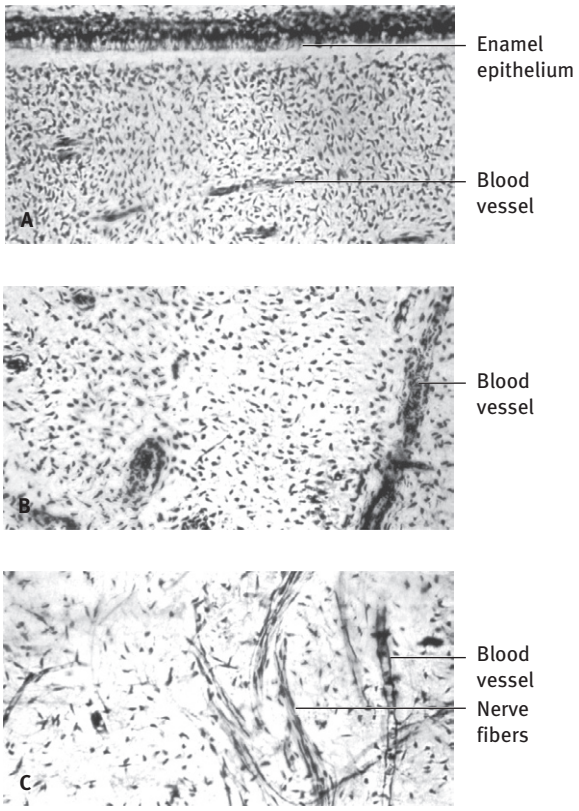

Figure 6.6 Age changes of dental pulp. Cellular elements decrease and fibrous intercellular substance increases with advancing age. (**A**) Newborn infant. (**B**) Infant 9 months of age. (**C**) Adult.

healing. They are also shown to secrete colony stimulating factors, which help in the migration of class II major histocompatibility expressing cells into the pulp tissue. They release inflammatory mediators cytokines and growth factors. In cell cultures they form mineralized tissue like bone on stimulation.

Fibers

The collagen fibers in the pulp exhibit typical cross striations at 64 nm (640 Å) and range in length from 10 to 100 nm or more (Fig. 6.7). The main type of collagen fiber in the pulp is type I. Type III collagen is also present. Bundles of these fibers appear throughout the pulp. In very young pulp fine fibers ranging in diameter from 10 to 12 nm (100 to 120 Å) have been observed. These fine fibers are called fibrillin. Downregulation and degradation of fibrillin helps in release of TGF β, which in turn promotes the formation of a mineralized tissue barrier in exposed pulps.

Pulp collagen fibers do not contribute to dentin matrix production, which is the function of the odontoblast. After root completion the pulp matures and bundles of collagen fibers increase in number. They may appear scattered throughout the coronal or radicular pulp, or they may appear in bundles. These are termed diffuse or bundle collagen depending on their appearance, and their presence may relate to environmental trauma. Fiber bundles are most prevalent in the root canals, especially near the apical region.

Undifferentiated mesenchymal cells

Undifferentiated mesenchymal cells are the primary cells in the very young pulp, but a few are seen in the pulps after root completion. They appear larger than fibroblasts and are polyhedral in shape with peripheral processes and large oval staining nuclei. The latter are distinctive because they lack a ribosome-studded endoplasmic reticulum and have mitochondria with readily discernible cisternae. They are found along pulp vessels, in the cell-rich zone and scattered throughout the central pulp. Viewed from the side, they appear spindle shaped (Fig. 6.8). They are believed to be a totipotent cell and when need arises they may become odontoblasts, fibroblasts, or macrophages. They decrease in number in old age.

Odontoblasts

Odontoblasts, the second most prominent cell in the pulp, reside adjacent to the predentin with cell bodies in the

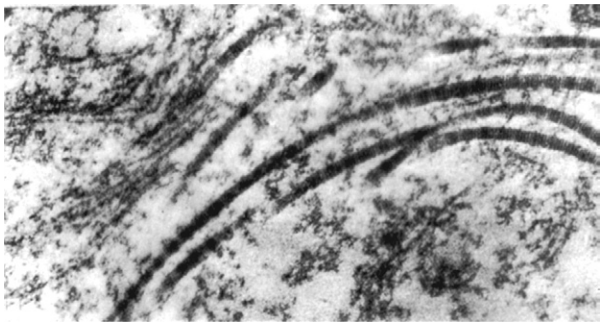

Figure 6.7 Typical collagen fibers of the pulp with 640 Å banding.

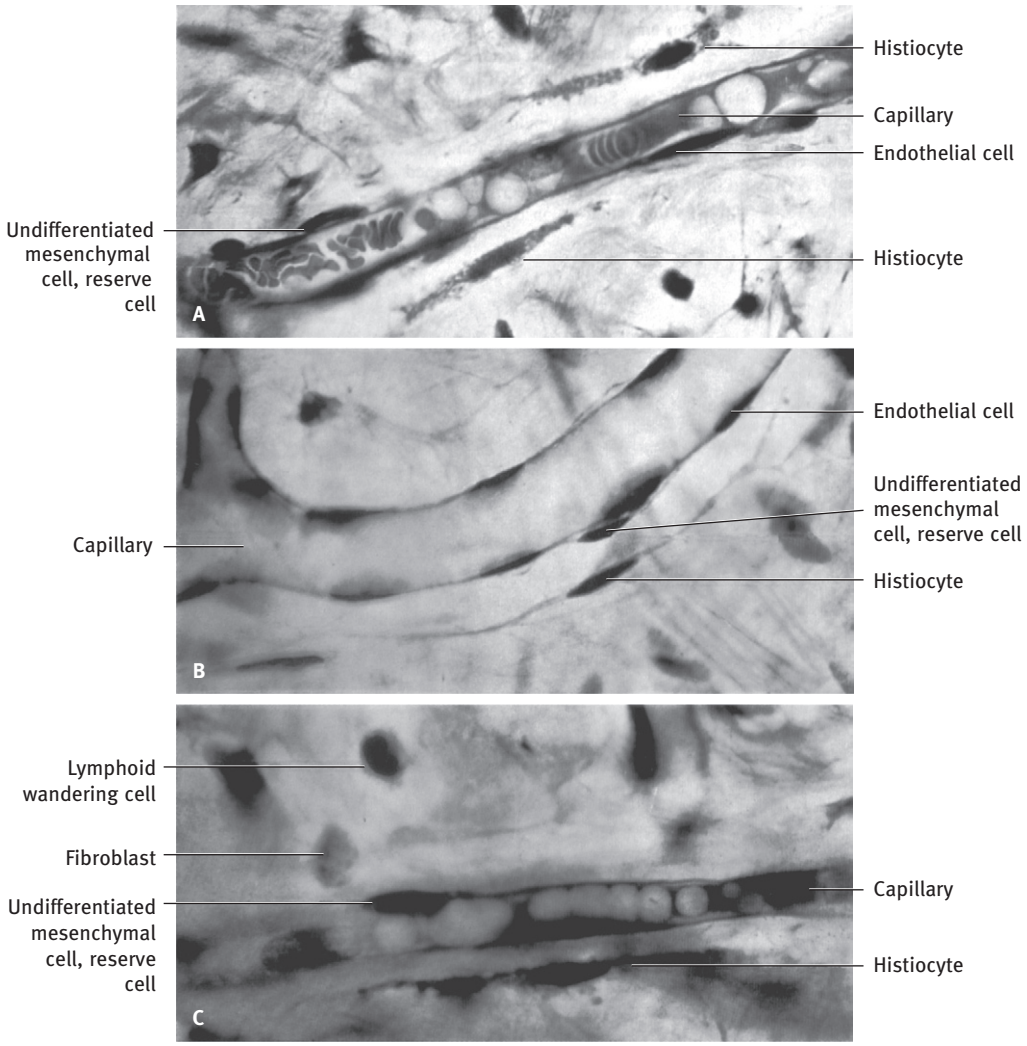

Figure 6.8 Defense cells in pulp.

pulp and cell processes in the dentinal tubules. The number of odontoblasts corresponds to the number of dentinal tubules. They are approximately 5 to 7 µm in diameter and 25 to 40 µm in length.They have a constant location adjacent to the predentin, in what is termed the 'odontogenic zone of the pulp' (Fig. 6.9). The cell bodies of the odontoblasts are columnar in appearance with large oval nuclei, which fill the basal part of the cell (Fig. 6.9). Immediately adjacent to the nucleus basally is rough-surfaced endoplasmic reticulum and the Golgi apparatus. The cells in the odontoblastic row lie very close to each other. Between odontoblasts gap, tight and desmosomal junctions exist (Fig. 6.10). Further toward the apex of the cell appears an abundance of rough-surfaced endoplasmic reticulum. Near the pupal-predentin junction the cell cytoplasm is devoid of organelles. Focal junctional complexes are present where the odontoblast cell body gives rise to the process. Actin filaments are inserted into this region. The clear terminal part of the cell body and the adjacent intercellular junction is described by some as the terminal bar apparatus of the odontoblast. At this zone the cell constricts to a diameter of 3 to 4 µm, where the cell process enters the predentinal tubule (Fig. 6.9).

The process of the cell contains no endoplasmic reticulum, but during the early period of active dentinogenesis it does contain occasional mitochondria and vesicles. During the later stages of dentinogenesis these are less frequently seen.

The odontoblast morphology and its organelles vary with the functional activity of the cell. An active cell is elongated whereas a resting cell is stubby. The active cell has a basally placed nucleus and a basophilic cytoplasm. The resting cell has little cytoplasm but a more hematoxyphilic nucleus.

There is also a striking difference in the cytoplasm of the young cell body, active in dentinogenesis, and the older cell. During this early active phase the Golgi apparatus is more prominent, the rough-surfaced endoplasmic reticulum is more abundant, and numerous mitochondria appear throughout the odontoblast. A great number of vesicles are seen along the periphery of the process where there is evidence of protein synthesis along the tubule wall. The cell actually increases in size as its process lengthens during dentin formation. When the cell process becomes 2 mm long, it is then many times greater in volume than the cell body. While the active cell

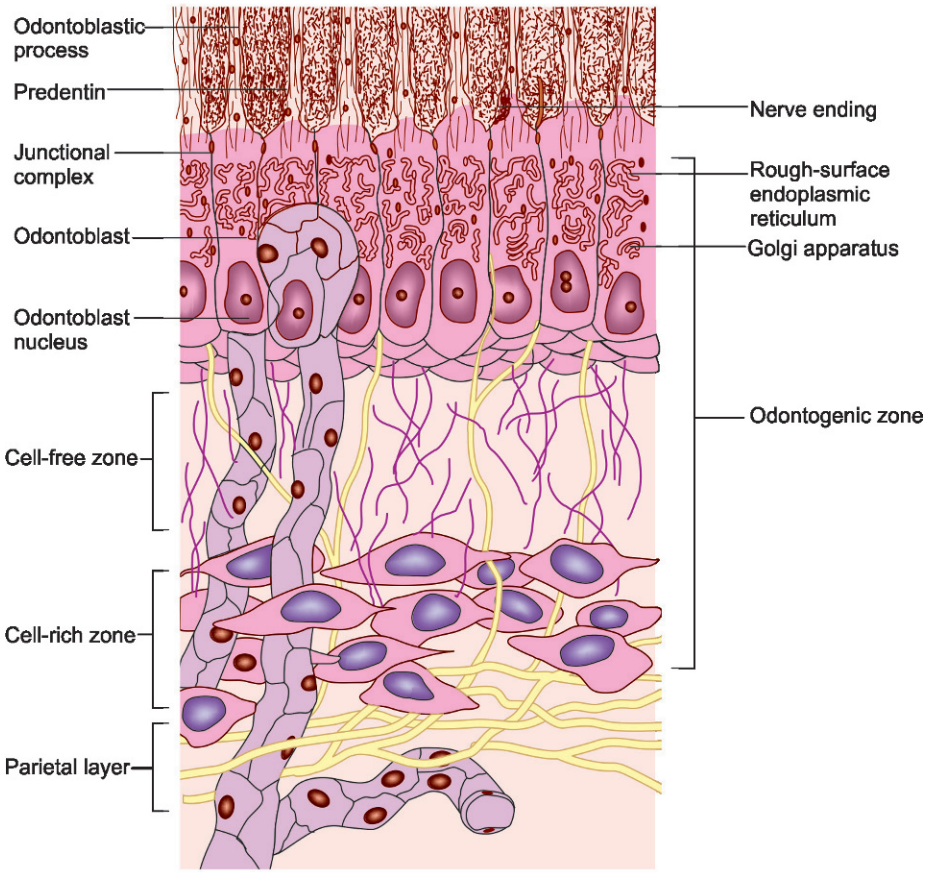

Figure 6.9 Diagram of odontogenic zone illustrating odontoblast, cell-free, and cell-rich zones, with blood vessels and nonmyelinated nerves among odontoblasts.

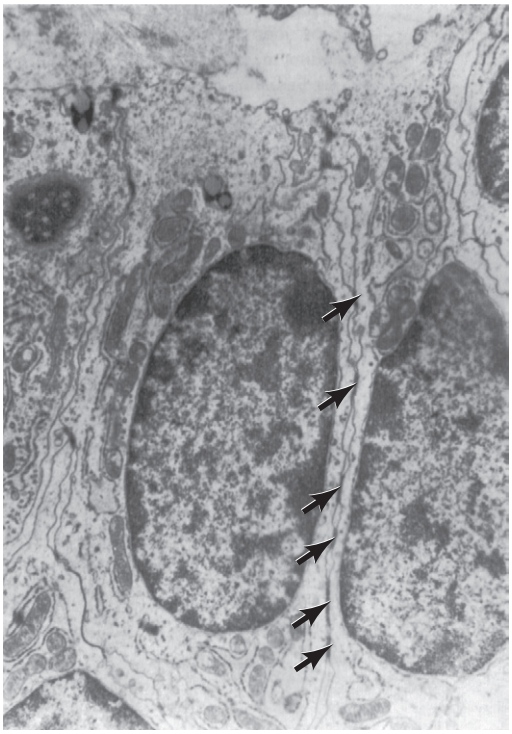

Figure 6.10 Close relation of adjacent odontoblasts. Note junctional complexes between cells (arrows).

is rich in organelles, the resting cell is devoid of organelles especially in the supranuclear region, where mainly lipid filled vacuoles are present. Ultrastructurally, an intermediate stage between active and resting called transitional stage is recognized. In this stage, the cells are narrower with fewer organelles and with the presence of autophagic vacuoles. Recently, primary cilia have been identified in odontoblast. These cilia may play a role in response of odontoblasts to external stimuli.

The form and arrangement of the bodies of the odontoblasts are not uniform throughout the pulp. They are more cylindrical and longer (tall columnar) in the crown (Fig. 6.11A) and more cuboid in the middle of the root (Fig. 6.11B). Close to the apex of an adult tooth the odontoblasts are ovoid and spindle shaped, appearing more like osteoblasts than odontoblasts, but they are recognized by their processes extending into the dentin. The pseudostratified arrangement seen in the coronal pulp is due to the crowding of cells in this region (Fig. 6.11C). Ultrastructurally, ring-layered structures have been observed between aging odontoblasts that might be characteristic of aging teeth.

Collagen is assembled in the odontoblast similar to that occurring in fibroblast. The noncollagenous proteins which are secreted by the odontoblast may be present in the same secretory granule along with the collagen.

Odontoblasts are end cells. They have lost the ability to divide. When they die they have to be replaced by cells,

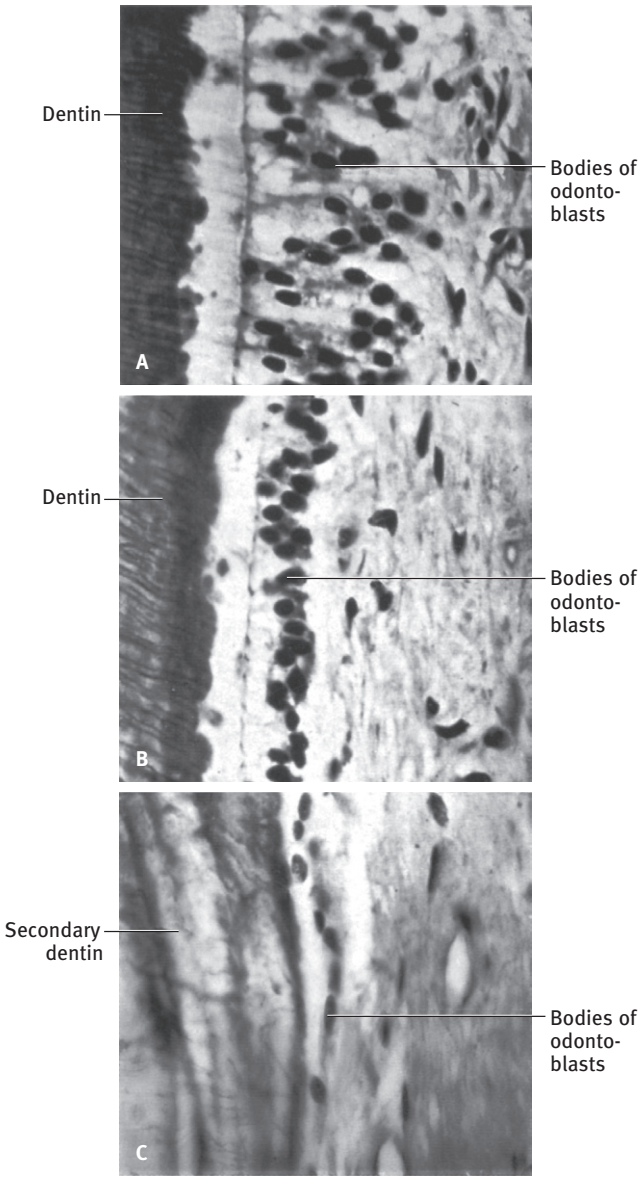

Figure 6.11 Variation of odontoblasts in different regions of one tooth.(**A**) High columnar odontoblasts in pulp chamber. (**B**) Low columnar odontoblasts in root canal. (**C**) Flat odontoblasts in apical region.

which differentiate from the cell-rich zone. Odontoblast and subodontoblastic cells have been shown to undergo apoptotic cell death by apoptotic cell markers like bcl-2.

Odontoblasts release inflammatory chemokine interleukin-8 which is chemotactic for neutrophils. Nerve growth factor and its receptor found in the odontoblasts are chemo attractants for neutrophils. Nitric oxide synthetase are important enzymes for vasodilatation and blood pressure regulation. These have been identified in odontoblasts and endothelial cells of the pulp. This finding suggests that they may have a role in mediating cell proliferation and vasodilatation.

Defense cells

In addition to fibroblasts, odontoblasts, and the cells that are a part of the neural and vascular systems of the pulp, there are cells important to the defense of the pulp. These are histiocytes or macrophages, dendritic cells, mast cells, and plasma cells. In addition, there are the blood vascular elements such as the neutrophils (PMNs), eosinophils, basophils, lymphocytes, and monocytes. These latter cells emigrate from the pulpal blood vessels and develop characteristics in response to inflammation.

The *histiocyte* or *macrophage*, is an irregularly shaped cell with short blunt processes (Figs 6.8 and 6.12). In the light microscope the nucleus is somewhat smaller, more rounded, and darker staining than that of fibroblasts, and it exhibits granular cytoplasm. When the macrophages are inactive and not in the process of ingesting foreign materials, one faces difficulty in distinguishing them from fibroblasts. In the case of a pulpal inflammation these cells exhibit granules and vacuoles in their cytoplasm, and their nuclei increase in size and exhibit a prominent nucleolus. Their presence is disclosed by intravital dyes such as toluidin blue. These cells are usually associated with small blood vessels and capillaries. Ultrastructurally the macrophage exhibits a rounded outline with short, blunt processes (Fig. 6.12). Invaginations of the plasma membrane are noted, as are mitochondria, rough-surfaced endoplasmic reticulum, free ribosomes, and also a moderately dense nucleus. The distinguishing feature of macrophages is aggregates of vesicles, or phagosomes, which contain phagocytized dense irregular bodies (Fig. 6.12).

Dendritic cells, were found in close relation to and in contact with the cell membranes of the endothelial cell. These cells express macrophage related antigens (CD14 and CD68) and were identified by their immunopositivity to HLA-DR monoclonal antibodies. These cells are similar to Langerhans cells. They present the antigen to the T cells. Some of these cells formed a reticular network in the connective tissue. In deciduous teeth these dendritic cells were shown to be closely associated with odontoblasts. Their dendritic process sometimes extended into the dentinal tubules and made contact with the odontoblastic process. Their numbers were found to increase in areas affected by caries, attrition or restorative procedures. These suggest that they have an important role to play in immunosurveillance. In view of their close association with odontoblasts, it is suggested that these cells may have some regulatory function on the odontoblast. Immunocompetent cells present in deciduous teeth increased in number during shedding.

Both *lymphocytes* and *eosinophils* are found extravascularly in the normal pulp (Fig. 6.13), but during inflammation they increase noticeably in number. Most of the lymphocytes present in the pulp are T lymphocytes. *Mast cells* are also seen along vessels in the inflamed pulp. They have a round nucleus and contain many dark-staining granules in the cytoplasm, and their number increases during inflammation.

The *plasma cells* are seen during inflammation of the pulp (Fig. 6.14). With the light microscope the plasma cell nucleus appears small and concentric in the cytoplasm. The chromatin of the nucleus is adherent to the nuclear membrane and gives the cell a cartwheel appearance. The cytoplasm of this cell is basophilic with a light-stained Golgi zone adjacent to the nucleus. Under the electron microscope these cells have a densely packed, rough-surfaced endoplasmic reticulum. Both immature and mature cells may be found.

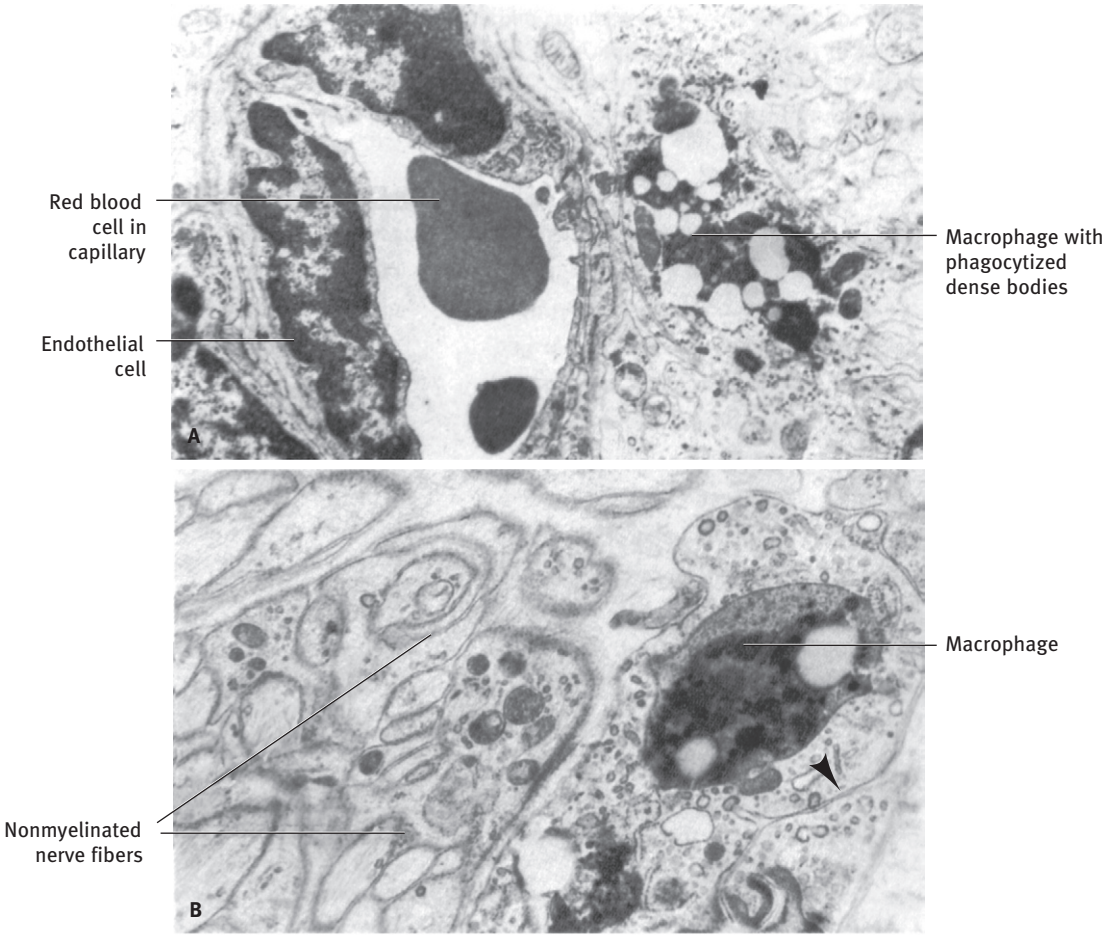

Figure 6.12 (**A**) This histiocyte or macrophage is located adjacent to capillary in peripheral pulp. Characteristic aggregation of vesicles, vacuoles, and phagocytized dense bodies is seen to right of capillary wall. (**B**) Multivesiculated body characteristic of macrophage. Note typical invagination of cell plasma membrane (arrow). This cell is located adjacent to group of nonmyelinated nerve fibers seen on left.

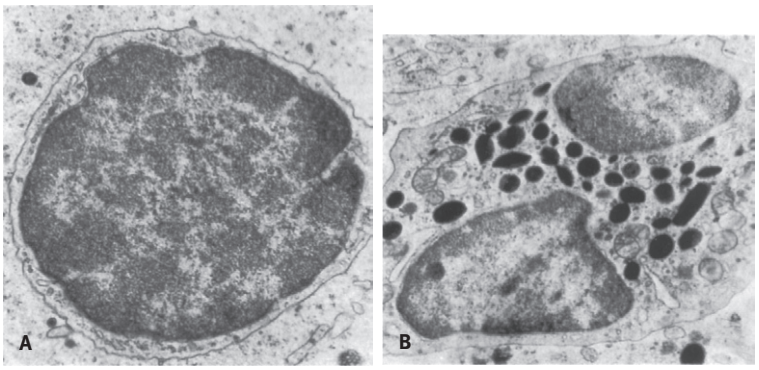

Figure 6.13 (**A**) Small lymphocyte located in pulp. Cytoplasm forms narrow rim around large oval-to-round nucleus. (**B**) Eosinophil in extravascular location in pulp organ. Nucleus is polymorphic, and granules in cytoplasm are characteristically banded.

The mature type exhibits a typical small eccentric nucleus and more abundant cytoplasm (Fig. 6.14). The plasma cells function in the production of antibodies (Table 6.3).

Pulpal stem cells

Among the numerous stem cells that have been identified from dental tissues and characterized, those from the pulpal tissues include dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHED). Stem cells are found in higher concentration in coronal pulp than in radicular pulp.

Pulpal stem cells express cytokeratin 18 and 19, indicating a potential for odontoblast differentiation and dentin repair at sites of injury. A comparative study of bone marrow and dental pulp stem cells indicate they

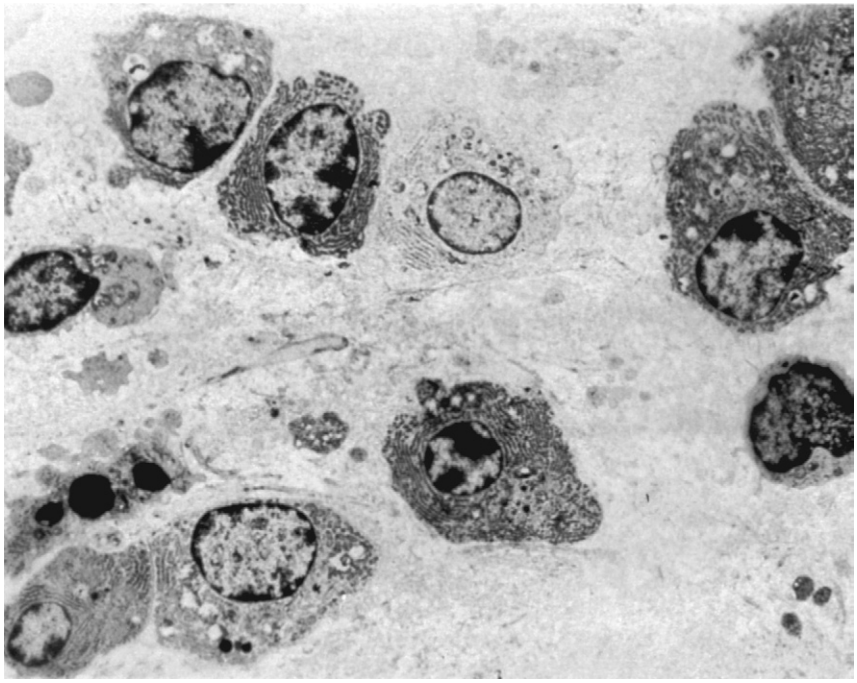

Figure 6.14 Cluster of plasma cells in pulp with early caries pulpitis. Observe dense peripheral nuclear chromatin and cytoplasm with cisternae of rough endoplasmic reticulum (*Courtesy* C Torneck, University of Toronto Dental School).

Table 6.3 Composition of Dental Pulp

Cellular	Extracellular	
	Fibres	Ground Substance
FIBROBLAST/ FIBROCYTE	**Collagen**	**Gag's (glycosaminoglycans):**
Odontoblast	type i	chondroitin A, chondroitin B
Undifferentiated mesenchymal cell	Type iii	hyaluronan
Defense cells	Fibrillin	**Proteoglycans:**
neutrophil		syndecan, versican
basophil		**Glycoproteins:**
eosinophil		laminin,tenascin ,fibronectin, integrins
lymphocyte		
blood monocyte		
macrophage		
dendritic cell		
plasma cell		
Pulpal stem cells		

are influenced by different regulatory mechanisms to engage in bone and dentin formation respectively. Dentine sialoprotein, a marker for dentine synthesis has been observed in dental pulp stem cell transplants, while in bone marrow stem cell transplants expression of fibroblast growth factor (FGF) and matrix metalloproteinase (MMP-9) have been seen. Numerous growth factors including transforming growth factor (TGF), bone morphogenetic protein (BMP-2) and dentin matrix protein 1 (DMP1) are capable of inducing proliferation and differentiation of DPSC's. DMP 1 has been shown to induce formation of dental pulp like tissue in vivo.

Pulpal stem cells are pluripotential having the capacity for angiogenic, chondrogenic, osteogenic, adipogenic and neurogenic differentiation, in some cases exceeding that of bone marrow stem cells. The pulpal tissues of exfoliated deciduous teeth and permanent third molars may serve as a suitable source of stem cells for future stem cell based therapies as they are found to be viable after cryopreservation. The application of DPSCs in regenerative dentistry and medicine (regeneration of bone and neural tissues) holds great promise.

Blood vessels

The pulp organ is extensively vascularized. It is known that the blood vessels of both the pulp and the periodontium arise from the inferior or superior alveolar artery and also drain by the same veins in both the mandibular and maxillary regions. The communication of the vessels of the pulp with the periodontium, in addition to the apical connections, is further enhanced by connections through the accessory canals. These relationships are of considerable clinical significance in the event of a potential pathologic condition in either the periodontium or the pulp, because the infection has a potential to spread through the accessory and apical canals. Although branches of the alveolar arteries supply both the tooth and its supporting tissues, those periodontal vessels entering the pulp change their structure from the branches to the periodontium and become considerably thinner walled than those surrounding the tooth.

Small arteries and arterioles enter the apical canal and pursue a direct route to the coronal pulp (Fig. 6.15). Along their course they give off numerous branches in the radicular pulp that pass peripherally to form a plexus in the odontogenic region (Fig. 6.16). Pulpal blood flow is more rapid than in most areas of the body. This is perhaps attributable to the fact that the pulpal pressure is among the highest of body tissues. The flow of blood in arterioles is 0.3 to 1 mm per second, in venules approximately 0.15

mm per second, and in capillaries about 0.08 mm per second. The largest arteries in the human pulp are 50 to 100 μm in diameter, thus equaling in size arterioles found in most areas of the body. These vessels possess three layers. The first, the tunica intima, consists of squamous or cuboid endothelial cells surrounded by a closely associated basal lamina. Where the endothelial cells contact, they appear overlapped to varying degrees. The second layer, the tunica media, is approximately 5 μm thick and consists of one to three layers of smooth muscle cells (Figs 6.17 and 6.18). A basal lamina surrounds and passes between these muscle cells and separates the muscle cell layer from the intima. Occasionally the endothelial cell wall is in contact with the muscle cells. This is termed a myoendothelial junction. The third and outer layer, the tunica adventitia, is made up of a few collagen fibers forming a loose network around the larger arteries. This layer becomes more conspicuous in vessels in older pulps. Arterioles with diameters of 20 to 30 μm with one or occasionally two layers of smooth muscle cells are common throughout the coronal pulp (Fig. 6.17). The tunica adventitia blends with the fibers of the surrounding intercellular tissue. Terminal arterioles with diameters of 10 to 15 μm appear peripherally in the pulp. The endothelial cells of these vessels contain numerous micropinocytotic vesicles, which function in transendothelial fluid movement. A single layer of smooth muscle cells surrounds these small vessels. Occasionally a fibroblast or pericyte lies on the surface of these vessels. Pericytes are capillary-associated fibroblasts. They are present partially encircling the capillaries. They have contractile properties and they are capable of reducing the size of the capillary lumen. Their nuclei can be distinguished as round or slightly oval bodies closely associated with the outer surface of the terminal arterioles or precapillaries (Figs 6.18 and 6.19). Some authors call the smaller diameter arterioles 'precapillaries.' They are slightly larger than the terminal capillaries and exhibit a complete or incomplete single layer of muscle cells surrounding the endothelial lining. These range in size from 8 to 12 μm.

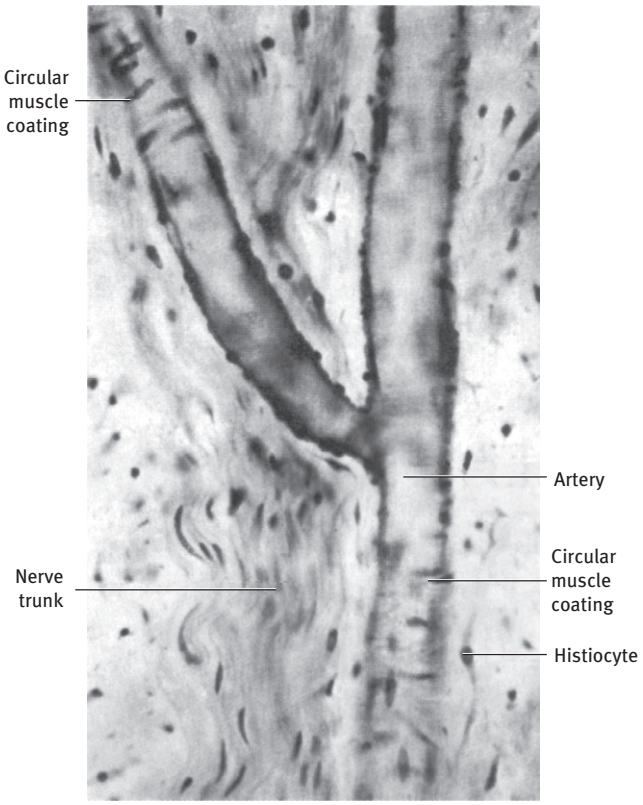

Figure 6.15 Branching artery and nerve trunk in the pulp.

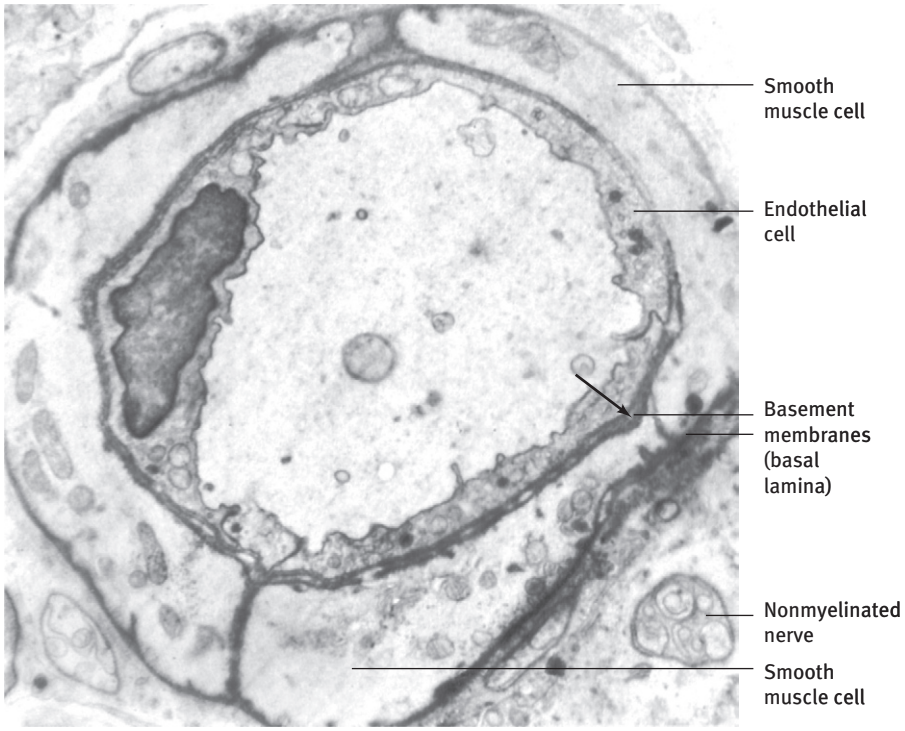

Figure 6.16 Small arteriole near central pulp exhibiting relatively thick layer of muscle cells. Dense basement membrane interspersed between endothelial and muscle cells (arrow).

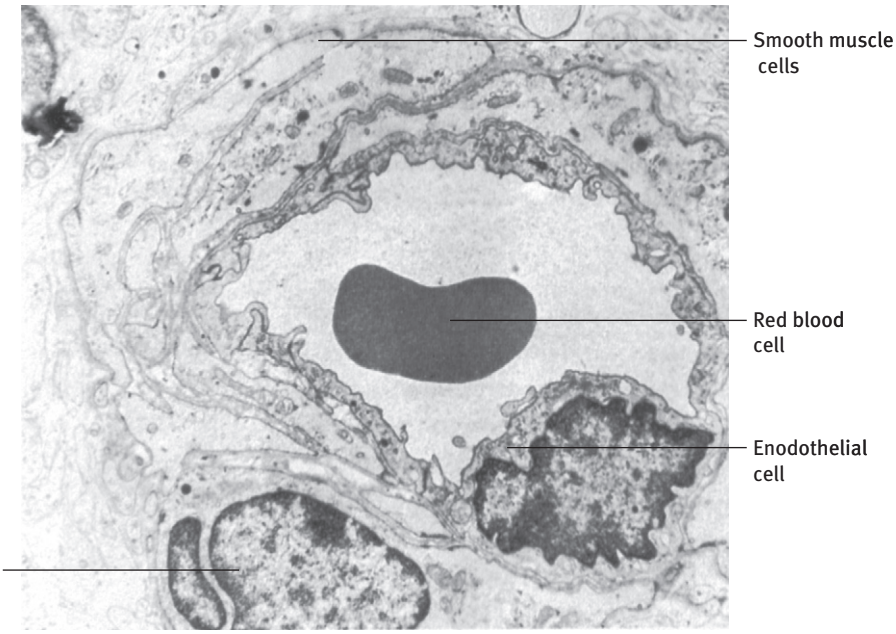

Figure 6.17 Peripheral pulp and small arteriole or precapillary exhibiting two thin layers of smooth muscle cells surrounding the endothelial cell lining of vessel. Nucleus at bottom left of figure belongs to a pericyte.

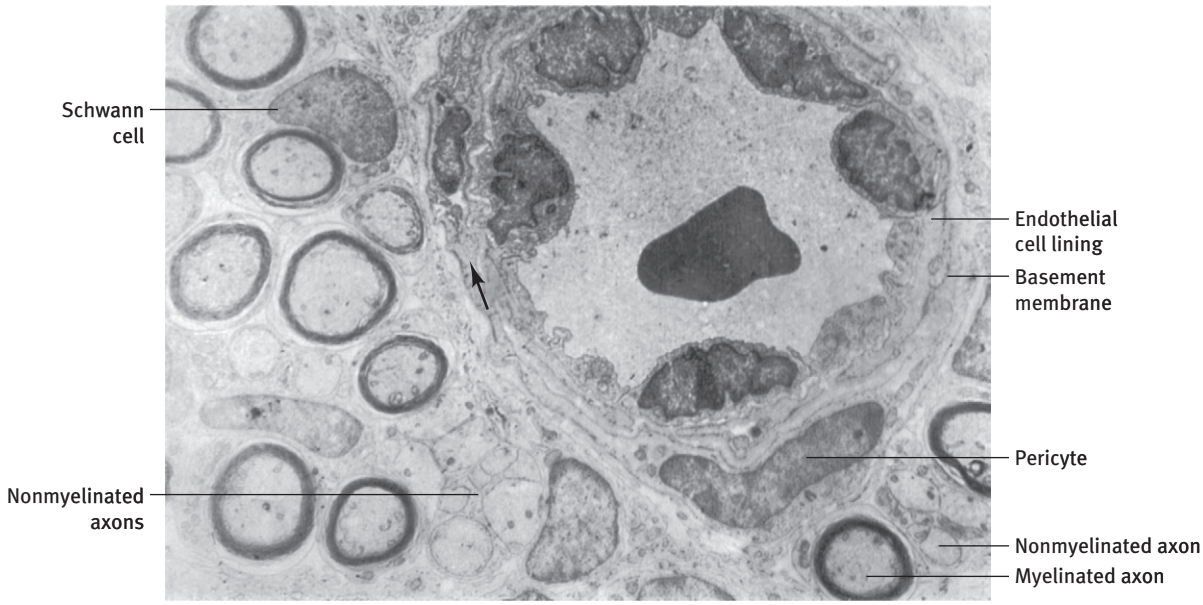

Figure 6.18 Area near subodontoblastic plexus showing both myelinated and nonmyelinated axons adjacent to large capillary or precapillary. Endothelial cell lining is surrounded by basement membrane (arrow) and pericytes.

Veins and venules that are larger than the arteries also appear in the central region of the root pulp. They measure 100 to 150 µm in diameter, and their walls appear less regular than those of the arteries because of bends and irregularities along their course. The microscopic appearance of the veins is similar to that of the arteries except that they exhibit much thinner walls in relation to the size of the lumen. The endothelial cells appear more flattened, and their cytoplasm does not project into the lumen. Fewer intracytoplasmic filaments appear in these cells than in the arterioles. The tunica media consists of a single layer or two of thin smooth muscle cells that wrap around the endothelial cells and appear discontinuous or absent in the smaller venules. The basement membranes of these vessels are thin and less distinct than those of arterioles. The adventitia is lacking or appears as fibroblasts and fibers are continuous with the surrounding pulp tissue. Occasionally two venous loops will be seen connected by an anastomosing branch (Fig. 6.20). Both venous-venous anastomosis and arteriole-venous anastomosis occur in the pulp. The arteriole-venous shunts may have an important role in regulation of pulpal blood flow. Frequently arteriole or precapillary loops with capillaries are found underlying the odontogenic zone in the coronal pulp (Fig. 6.21).

Blood capillaries, which appear as endothelium-lined tubes, are 8 to 10 µm in diameter. The nuclei of these cells may be lobulated and have cytoplasmic projections into the luminal surface. The terminal network of capillaries in the coronal pulp appears nearly perpendicular to the main trunks. The vascular network passes among the odontoblasts and underlies them as well. A few peripheral capillaries found among the odontoblasts have fenestrations in the endothelial cells. These pores are located in the thin part of the capillary wall and are spanned only by the thin diaphragm of contacting inner and outer plasma membranes of endothelial cells (Fig. 6.22). These fenestrated capillaries are assumed to be involved in rapid transport of metabolites at a time when the odontoblasts are active in the process of dentinal matrix formation and its subsequent calcification. Both fenestrated and continuous terminal capillaries are found in the odontogenic region. During active dentinogenesis capillaries appear among the odontoblasts adjacent to the predentin (Fig. 6.16). Later, after the teeth have reached occlusion and dentinogenesis slows down, these vessels usually retreat to a subodontoblastic position (Table 6.4).

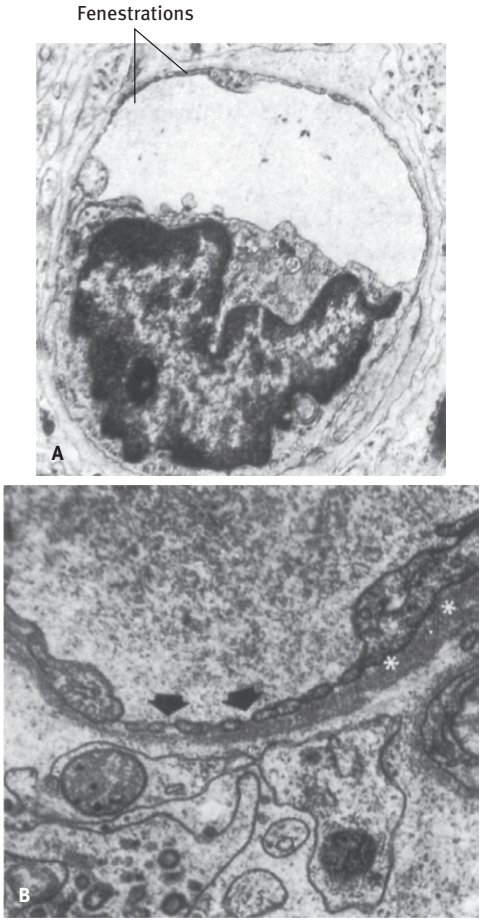

Figure 6.19 (**A**) Terminal capillary loops located among odontoblasts may be fenestrated. These capillaries have both thick and thin segments in their walls. (**B**) Endothelial cell wall bridges pores (arrows) and is supported only by basement membrane (**).

Lymph vessels

Lymph capillaries are described as endothelium-lined tubes that join thin-walled lymph venules or veins in the central pulp. The lymphatic capillaries have thin walls. Cellular projections arise from the endothelial cells. The cells contain multivesicular structures, Weibel-Palade bodies and paracrystalline inclusions. The lymphatic vessels were more numerous in the central part of the pulp than in the peripheral areas. The larger vessels have an irregular-shaped lumen composed of endothelial cells surrounded by an incomplete layer of pericytes or smooth muscle cells or both. Further, the lymph vessels differ from venules in that their walls and basement membrane show discontinuities, with the absence of RBCs but with the presence of lymphocytes in the lumen. In inflamed pulps, due to increased interstitial fluid pressure, gap junction develops between the endothelial cells of the dilated lymph capillaries. Lymph vessels draining the pulp and periodontal ligament have a common outlet. Those draining the anterior teeth pass to the submental lymph nodes; those of the posterior teeth pass to the submandibular and deep cervical lymph nodes.

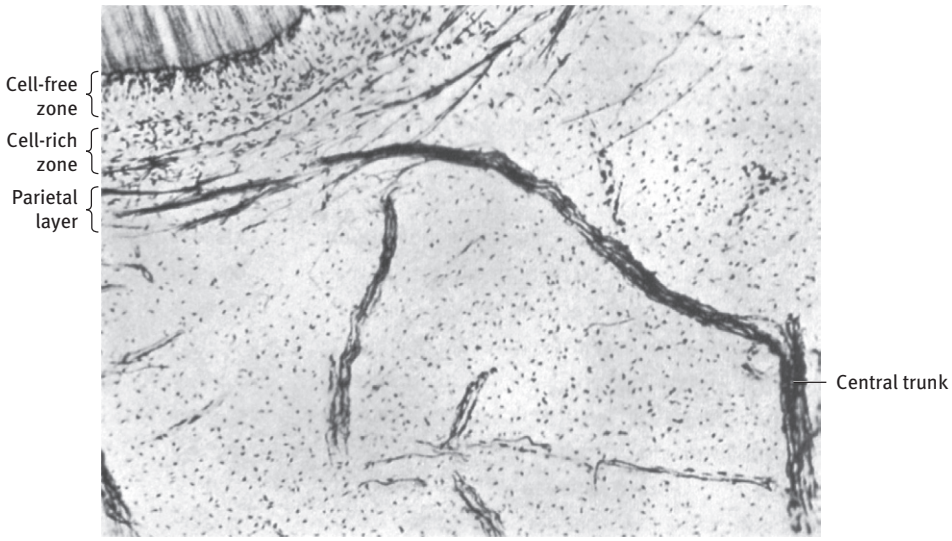

Figure 6.20 Major nerve trunks branch in pulp and pass to parietal layer, which lies adjacent to cell-rich zone. Cell-rich zone curves upward to right.

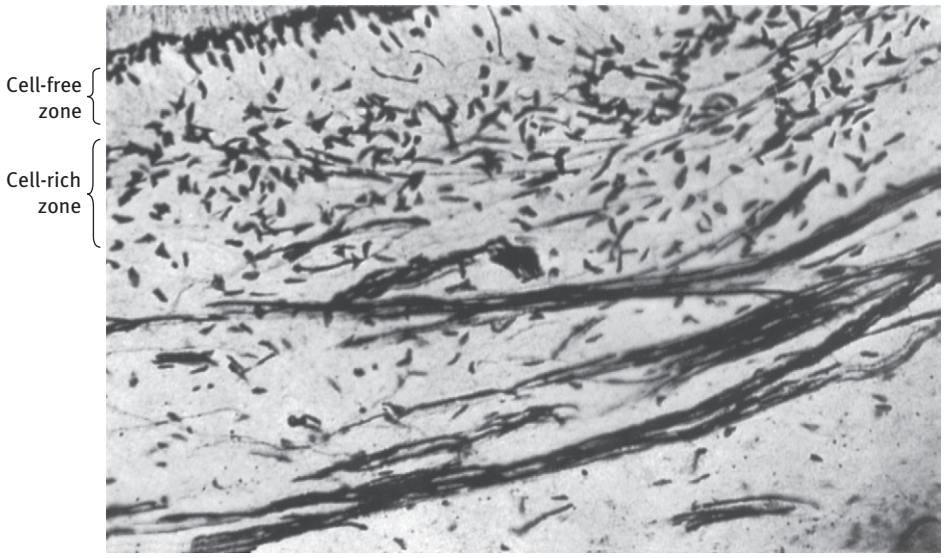

Figure 6.21 Parietal layer of nerves is composed of myelinated nerve fibers. Cell-rich zone curves upward to right.

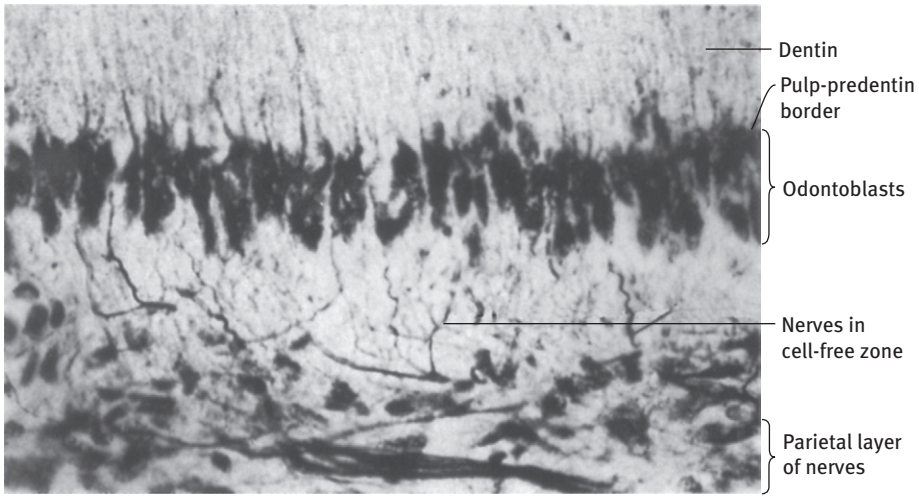

Figure 6.22 Terminal nerve endings located among odontoblasts. These arise from subjacent parietal layer.

Table 6.4 Microcirculation of Pulp

Arteries and arterioles thin walled
Pulpal arteries are as big as arterioles elsewhere
Do not branch in radicular pulp
Subodontoblastic plexus of capillaries seen
Presence of arteriole venous anastomosis
Pericytes in relation to smaller arterioles control blood flow
Sympathetic nerves also control blood flow
Higher capillary pressure
Rapid blood flow & fenestrated capillaries facilitate rapid metabolite transport
Veins and venules thin walled
Lymphatics follow course of blood vessels

Nerves

The abundant nerve supply in the pulp follows the distribution of the blood vessels. The majority of the nerves that enter the pulp are nonmyelinated. Many of these gain a myelin sheath later in life. The nonmyelinated nerves are found in close association with the blood vessels of the pulp and many are sympathetic in nature. They have terminals on the muscle cells of the larger vessels and function in vasoconstriction (Fig. 6.17). Thick nerve bundles enter the apical foramen and pass along the radicular pulp to the coronal pulp where their fibers separate and radiate peripherally to the parietal layer of nerves (Fig. 6.23). The number of fibers in these bundles varies greatly, from as few as 150 to more than 1200. The larger fibers range between 5 and 13 µm, although the majority are smaller than 4 µm. The perineurium and the epineurium of the pulpal nerves are thin. The large myelinated fibers mediate the sensation of pain that may be caused by external stimuli. The peripheral axons form a network of nerves located adjacent to the cell-rich zone. This is termed the parietal layer of nerves, also known as the plexus of Raschkow (Figs 6.23, 6.24). Both myelinated axons, ranging from 2 to 5 µm in diameter, and minute nonmyelinated fibers of approximately 200 to 1600 µm (2000 to 16,000 Å) in size make up this layer of nerves. The parietal layer

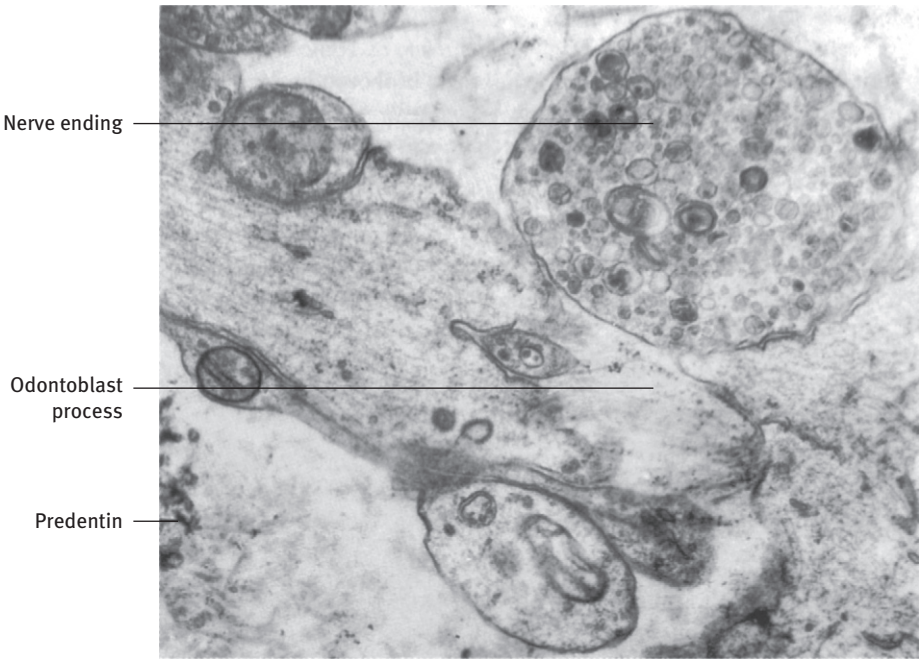

Figure 6.23 Vesiculated nerve endings in predentin in zone adjacent to odontoblast process.

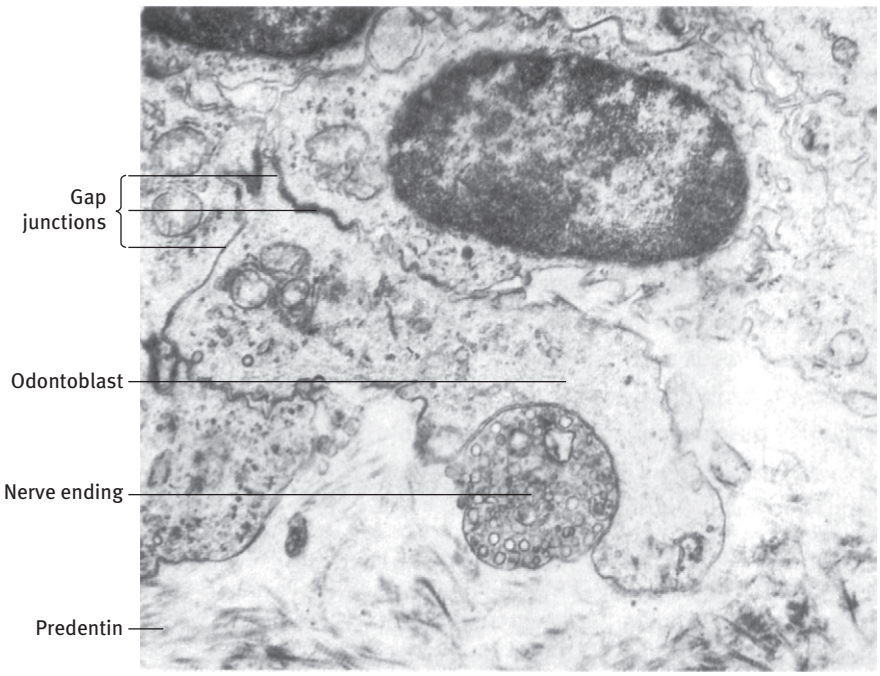

Figure 6.24 Vesiculated nerve ending partially surrounded by an odontoblast process located adjacent to predentin. Note the uniform cleft-like space between the nerve ending and the odontoblast process. Gap junction appears between odontoblasts.

develops gradually, becoming prominent when root formation is complete.

Nerve endings

The mature deciduous teeth is well innervated, especially the coronal pulp, has many nerve endings terminating in or near odontoblast layer, with a few penetrating into the dentin. Nerve axons from the parietal zone pass through the cell-rich and cell-free zones and either terminate among or pass between the odontoblasts to terminate adjacent to the odontoblast processes at the pulp–predentin border or in the dentinal tubules (Fig. 6.25). Nerve terminals consisting of round or oval enlargements of the terminal filaments contain microvesicles, small, dark, granular bodies, and mitochondria (Fig. 6.26). These terminals are very close to the odontoblast plasma membrane, separated only by a 20 μm (200 Å) cleft (Fig. 6.27). Many of these indent the odontoblast surface and exhibit a special relationship to these cells. Most of the nerve endings located among the odontoblasts are believed to be sensory

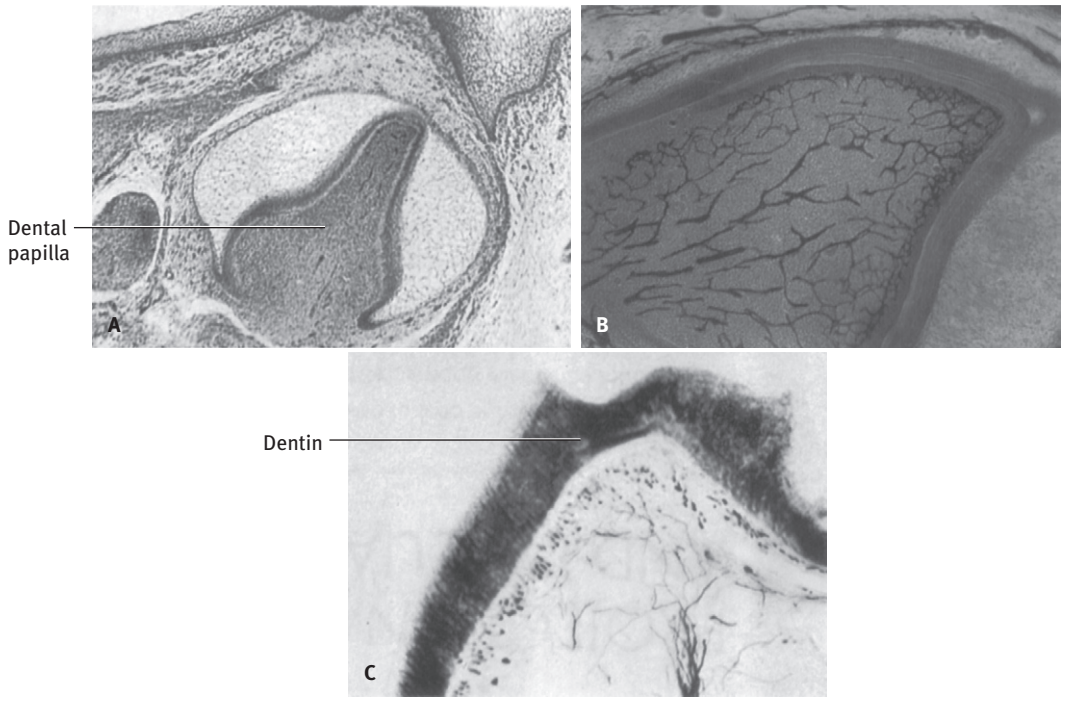

Figure 6.25 (**A**) Young tooth bud exhibiting highly cellular dental papilla. Compare dense cell population to that of adjacent connective tissue. (**B**) Young tooth with blood vessels injected with India ink to demonstrate extent of vascularity of pulp. Large vessels located centrally and smaller ones peripherally among odontoblasts. Pulp surrounded by dentin and enamel. (**C**) Young tooth stained with silver to demonstrate neural elements. Myelinated nerves appear in pulp horn only after considerable amount of dentin has been laid down.

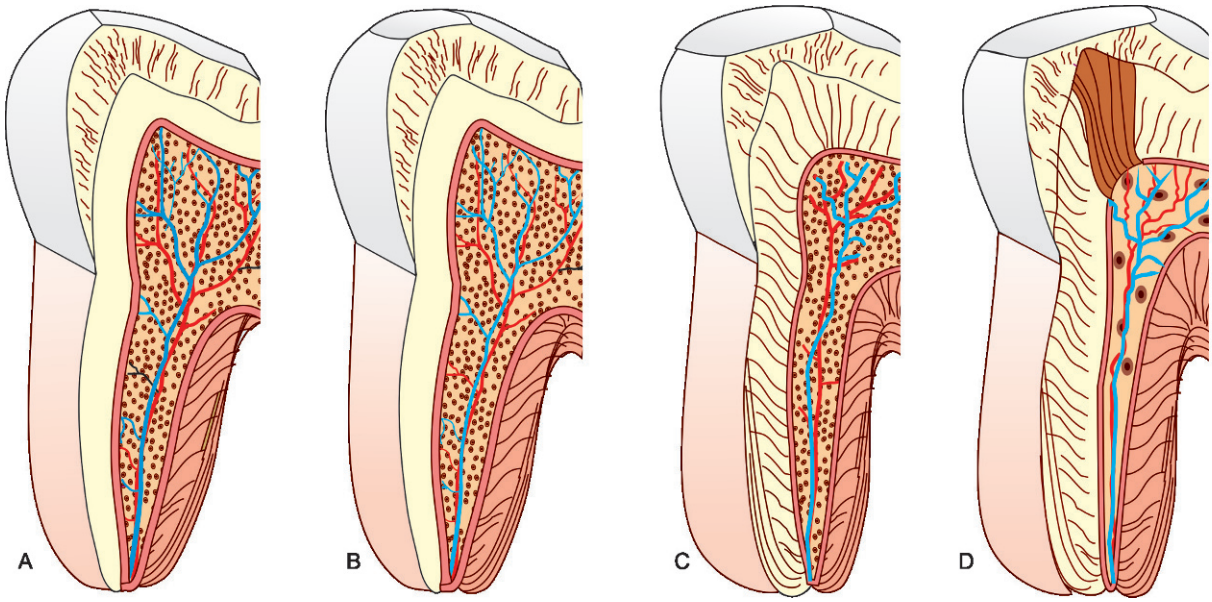

Figure 6.26 These four diagrams depict pulp organ throughout life. Observe first the decrease in size of pulp organ. (**A**) to (**D**) Dentin is formed circumpulpally but especially in bifurcation zone. Note decrease in cells and increase in fibrous tissue. Blood vessels (white) organize early into odontoblastic plexus and later are more prominent in subodontoblastic zone, indicating decrease in active dentinogenesis. Observe sparse number of nerves in young pulp, organization of parietal layer of nerves. They are less prominent in aging pulp. Reparative dentin and pulp stones are apparent in oldest pulp, (**D**).

receptors. Some sympathetic endings are found in this location as well. Whether they have some function relative to the capillaries or the odontoblast in dentinogenesis is not known. The nerve axons found among the odontoblasts and in the cell-free and cell-rich zones are nonmyelinated but are enclosed in a Schwann cell covering. It is presumed that these fibers lost their myelin sheath as they passed peripherally from the parietal zone. More nerve fibers and endings are found in the pulp horns than in other peripheral areas of the coronal pulp (Fig. 6.34 C).

Recently a great deal of information has been reported regarding the types of potential neurotransmitters that

Figure 6.27 Mild pulp response with loss of odontoblast identity and inflammatory cells obliterating cell-free zone.

are present in the nerves of the dental pulp. Substances such as substance P, 5-hydroxytryptamine, vasoactive intestinal peptide, somatostatin, and prostaglandins, as well as acetylcholine and norepinephrine have been found throughout the pulp. The majority of these putative transmitters have been shown to affect vascular tone and subsequently modify the excitability of the nerve endings. Some of the neuropeptides, like calcitonin gene related peptide (CGRP) and substance P are potent vasodilators, while others like norepinephrine and neuropeptide Y are vasoconstrictors. Some neuropeptides like substance P act as nociceptive transmitter, in that they help to transmit pain sensation, while others like somatostatin act against them. Further, it has been suggested that these changes in vascular tone can also affect the incremental growth of dentin.

It is a feature unique to dentin receptors that environmental stimuli always elicit pain as a response. Sensory response in the pulp cannot differentiate between heat, touch, pressure, or chemicals. This is because the pulp organs lack those types of receptors that specifically distinguish these other stimuli (Table 6.5).

Table 6.5 Nerves of the Pulp

Nerves follow the course of blood vessels
Very little branching in radicular pulp
Myelinated nerves lose myelin sheath and form plexus: plexus of Raschkow
Nerve fibers terminate adjacent to odontoblast or in dentinal tubules
Only free nerve endings in pulp: therefore only pain sensation is felt
Myelinated/ fast conducting: 'a' delta fibers mediate sharp pain
Nonmyelinated/ slow conducting: 'c' fibers mediate dull pain
Sympathetic fibers end in blood vessels to control blood flow

FUNCTIONS

Inductive

The primary role of the pulp anlage is to interact with the oral epithelial cells, which leads to differentiation of the dental lamina and enamel organ formation. The pulp anlage also interacts with the developing enamel organ as it determines a particular type of tooth.

Formative

The pulp organ cells produce the dentin that surrounds and protects the pulp. The pulpal odontoblasts develop the organic matrix and function in its calcification. Through the development of the odontoblast processes, dentin is formed along the tubule wall as well as at the pulp–predentin front.

Nutritive

The pulp nourishes the dentin through the odontoblasts and their processes and by means of the blood vascular system of the pulp.

Protective

The sensory nerves in the tooth respond with pain to all stimuli such as heat, cold, pressure, operative cutting procedures, and chemical agents. The nerves also initiate reflexes that control circulation in the pulp. This sympathetic function is a reflex, providing stimulation to visceral motor fibers terminating on the muscles of the blood vessels.

Defensive or reparative

The pulp is an organ with remarkable reparative abilities. It responds to irritation, whether mechanical, thermal, chemical, or bacterial, by producing reparative dentin and mineralizing any affected dentinal tubules. The changes in the odontoblast, subodontoblastic layer and type of tertiary dentin formation varies with the extent of caries exposing the dentin (open/closed

Table 6.6 Functions of Pulp

Function	Mode of Action
Induction	Differentiation of dental lamina & dental organ Determination of tooth morphology
Formative	Production of dentin
Nutritive	Nourishment of dentin
Protective/ sensory	Immune cells, pain perception
Defensive/ reparative	Production of reparative dentin

lesion), its progression (active/slowly progressive lesion). The reparative dentin was found to be more atubular in closed/active lesions and more tubular in open/slowly progressive lesions.

After injury to the mature tooth, the fate of the odontoblast can vary according to the intensity of the injury. Milder injury can result in functional activity leading to focal secretion of a reactionary dentin matrix, called regeneration, while greater injury can lead to odontoblast cell death. Induction of differentiation of a new generation of odontoblast-like cells can then lead to reparative dentinogenesis.

Both the reparative dentin created in the pulp and the calcification of the tubules (sclerosis) are attempts to wall off the pulp from the source of irritation. Also, the pulp may become inflamed due to bacterial infection or by cutting action and placement of an irritating restorative material. The pulp has macrophages, lymphocytes, neutrophils, monocytes, and plasma and mast cells, all of which aid in the process of repair of the pulp. Although the rigid dentinal wall has to be considered as a protection of the pulp, it also endangers its existence under certain conditions. During inflammation of the pulp, hyperemia and edema may lead to the accumulation of excess fluid outside the capillaries. An imbalance of this type, limited by the unyielding enclosure, can lead to pressure on apical vessels and ischemia, resulting in necrosis of the pulp. In most cases, if the inflammation is not too severe, however, the pulp will heal since it has excellent regenerative properties (Table 6.6).

DIFFERENCES IN PRIMARY AND PERMANENT PULP TISSUES

Primary pulp

The primary pulp (pulp of deciduous teeth) functions for a shorter period of time than do the permanent pulps. The average length of time a primary pulp functions in the oral cavity is only about 8.3 years. This amount of time may be divided into three time periods—that of pulp organ growth, which takes place during the time the crown and roots are developing; that period of time after the root is completed until root resorption begins, which is termed the time of pulp maturation; and finally the period of pulp regression, which is the time from beginning root resorption until the time of exfoliation. Let us consider the average time of pulp life based on figures for the entire primary dentition. These three periods (growth, maturation, and regression) are not of equal lengths. Tooth eruption to root completion is about 1 year (11.85 months), and the time of root completion to beginning root loss (based on completion of the permanent crown) is 45.3 months, or 3 years, 9 months. Finally, the time of pulp regression based on the beginning of root resorption to exfoliation is 3 years, 6 months. The amount of time the primary pulp is undergoing changes relative to growth based on both the prenatal crown formation and the postnatal root completion is about 4 years, 2 months, 11 months of which are involved in crown completion from the time of beginning of crown calcification to its completion. The period of time the primary radicular pulp is regressing is based on the time from when the permanent crown is completed till the time of permanent tooth eruption. In some cases, root loss commences before the root is entirely complete. The maximum life of the primary pulp including both prenatal and postnatal times of development and the period of regression is approximately 9.6 years.

Permanent pulp

During crown formation the pulps of primary and permanent teeth are morphologically nearly identical. In the permanent teeth this is a process requiring about 5 years. During this time the tissues are highly cellular, exhibiting a high mitotic rate especially in the cervical region. The young differentiating odontoblasts exhibit few organelles until dentin formation begins; then they rapidly change into protein-synthesizing cells. Both the primary and the permanent pulps are highly vascularized; however, the primary teeth never attain the extent of neural development that occurs in the permanent teeth. This is caused in part by the loss of neural elements during the root-resorption period. The greater the extent of root resorption, the greater the degenerative changes seen in the primary pulps. The architecture of the primary and permanent pulps is similar in appearance to the cell-free and cell-rich zones, parietal layer, and the large nerve trunks and vessels in the central pulp.

The periods of development for the pulps of the permanent teeth are, as might be expected, longer than those required for completion of the same processes in the primary teeth. As mentioned above, crown completion, based on the time during which the crown is completing formation and calcification, averages 5 years, 5 months. From the time of crown completion to eruption the time in both arches averages 3 years, 6 months. The time from eruption to root completion is 3 years, 11 months. Thus the pulp of the permanent teeth undergoes development for about 12 years, 4 months (based on the time from beginning prenatal crown calcification to root completion). This is in contrast to the 4 years, 2 months it takes in the primary teeth. Furthermore, the permanent roots take over twice as long to reach completion (7 years, 5 months) compared to those of the primary pulps (average 3 years, 3 months).

The period of pulp aging is much accelerated in the primary teeth and occupies the time from root completion to exfoliation, or about 7 years, 5 months. Aging of the pulp in the permanent teeth, on the other hand, requires much of the adult life span.

Finally, one should note in passing that for both the primary and permanent teeth the maxillary arches require slightly longer to complete each process of development than do the mandibular arches.

REGRESSIVE CHANGES (AGING)

The age changes in the dental pulp are dealt in Chapter 17 on Age Changes in Oral Tissues. Hence the age change in dental pulp is briefly summarized in this chapter. The age changes in the pulp are decrease in cellularity, increase of collagen fibres and their aggragation into bundles, decrease in vascularity and appearance of calcifications. The calcifications may be diffuse calcifications or nodular calcifications, termed as pulp stones or denticles. Pulp stones may lie free in the pulp, attached to dentinal wall or embedded in it. If pulp stones has the structure of dentin, it is called true denticles, if not, false denticles.

DEVELOPMENT

The tooth pulp is initially called the dental papilla. This tissue is designated as 'pulp' only after dentin forms around it. The dental papilla controls early tooth formation. In the earliest stages of tooth development it is the area of the proliferating future papilla that causes the oral epithelium to invaginate and form the enamel organs. The enamel organs then enlarge to enclose the dental papillae in their central portions (Fig. 6.28A). The dental papilla may play a role to determine the forming enamel organ is to be an incisor or a molar. Recent information indicates that the epithelium may have that information. At the location of the future incisor, the development of the dental pulp begins at about the 8th week of embryonic life in the human. Soon thereafter the more posterior tooth organs begin differentiating. The cell density of the dental papilla is great because of proliferation of the cells within it (Fig. 6.28A). The young dental papilla is highly vascularized, and a well-organized network of vessels appears by the time dentin formation begins (Fig. 6.28B). Capillaries crowd among the odontoblasts during this period of active dentinogenesis. The cells of the dental papilla appear as undifferentiated mesenchymal cells. Gradually these cells differentiate into stellate-shaped fibroblasts. After the inner and enamel organ cells differentiate into ameloblasts, the odontoblasts then differentiate from the peripheral cells of the dental papilla and dentin production begins. As this occurs, the tissue is no longer called dental papilla but is now designated the pulp organ.

Axons of developing nerves reach the jaws and form terminals near sites of odontogenesis before tooth formation starts. Nerve fibers were first seen in the dental follicle in the 11th week of intrauterine life. In the 18th week the nerve fibers were observed in the dental papilla. At that time the first layers of enamel and dentin were being formed. At about 24th week the nerve fibers reach the subodontoblastic region. Subsequently, nerve fibers increase in number and those accompanying blood vessels form neurovascular bundles in the central portion of the developing pulp. During the fetal period no subodontoblastic plexuses or nerve fibers in the predentin or in the dentin were observed. Few large myelinated nerves are found in the pulp until the dentin of the crown is well advanced. At that time nerves reach the odontogenic zone in the pulp horns. The sympathetic nerves, however, follow the blood vessels into the dental papilla as the pulp begins to organize. During development, dental pulp cells produce nerve growth factor and semaphorin 7A and brain derived glial cell line derived neurotrophic factor all of which help to innervate the pulp. Growth factors like neurotrophin and neurturin were shown not be involved in this process.

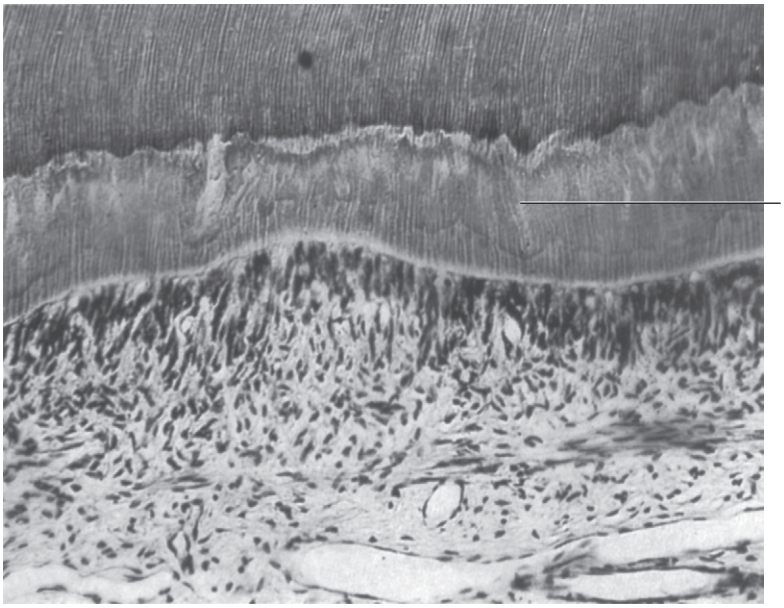

Figure 6.28 Moderate cell response with formation of reparative dentin underlying cavity. Note viable odontoblasts have deposited tubular, reparative dentin.

CLINICAL CONSIDERATIONS

Pulpal inflammation or *pulpitis* is a response of the traumatized pulp, with trauma being a result of a bacterial infection as in dental caries or physical trauma to the tooth structure. Pulpal inflammation in milder forms could result in *focal reversible pulpitis* and may progress if left unchecked to acute and chronic forms of pulpitis. Well vascularized pulpal tissues may at times in carious molar teeth of young adults and children with open apex exhibit a form of hyperplasia, seen clinically from an exposed pulp chamber as a protruding red mass of granulation tissue called *pulp polyp* or *chronic hyperplastic pulpitis*. This condition requires endodontic therapy or extraction of the tooth. Inflammation within the pulp may also sometimes result in a condition called *internal resorption* or *pink tooth*. The outward resorption of dentinal walls by osteodentinoclasts (odontoclasts) results in the pulpal tissue appearing pink through the thin translucent enamel, hence the term pink tooth. This condition may require endodontic therapy. Pulpal infection can spread apically into the periodontal ligament causing granulomas, abscesses and cysts.

For all operative procedures the shape of the pulp chamber and its extensions into the cusps, the pulpal horns, is important to remember. The wide pulp chamber in the tooth of a young person will make a deep cavity preparation hazardous, and it should be avoided, if possible. In some instances of developmental disturbances the pulpal horns project high into the cusps, and the exposure of a pulp can occur when it is least anticipated. Sometimes a radiograph will help to determine the size of a pulp chamber and the extent of the pulpal horns.

If opening a pulp chamber for treatment becomes necessary, its size and variation in shape must be taken into consideration. With advancing age, the pulp chamber becomes smaller (Fig. 6.29), and because of excessive dentin formation at the roof and floor of the chamber, it is sometimes difficult to locate the root canals. In such cases it is advisable when one opens the pulp chamber to advance toward the distal root in the lower molar and toward the lingual root in the upper molar. In this region one is most likely to find the opening of the pulp canal without risk of perforating the floor of the pulp chamber. In the anterior teeth the coronal part of the pulp chamber may be filled with secondary dentin; thus locating the root canal is made difficult. Pulp stones lying at the opening of the root canal may cause considerable difficulty when an attempt is made to locate the canals.

The shape of the apical foramen and its location may play an important part in the treatment of root canals. When the apical foramen is narrowed by cementum, it is more readily located because further progress of the broach will be stopped at the foramen. If the apical opening is at the side of the apex, as shown in Figure 6.2B, not even radiographs will reveal the true length of the root canal, and this may lead to misjudgment of the length of the canal and the root canal filling.

Since accessory canals are rarely seen in radiographs, they are not treated in root canal therapy. In any event it would be mechanically difficult or impossible to reach them. Fortunately, however, the majority of them do not affect the success of endodontic therapy.

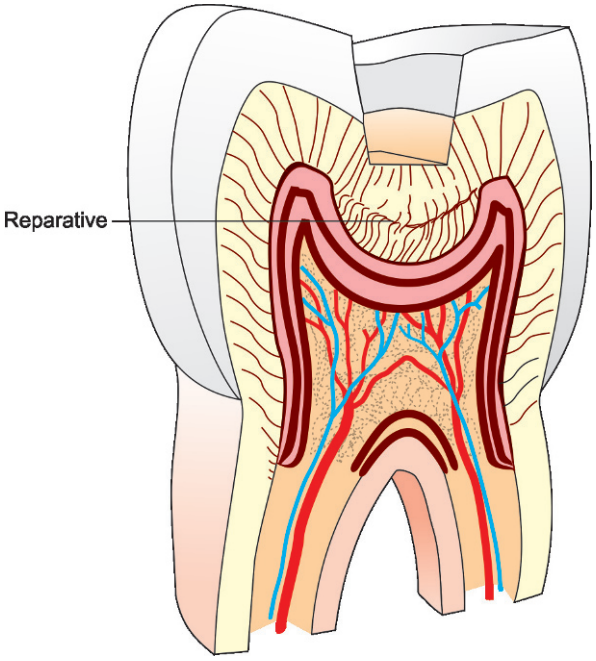

Figure 6.29 Diagram of reparative function of pulp organ to cavity preparation and subsequent restoration. Reparative dentin is limited to zone of stimulation.

When accessory canals are located near the coronal part of the root or in the bifurcation area (Fig. 6.3B), a deep periodontal pocket may cause inflammation of the dental pulp. Thus periodontal disease can have a profound influence on pulp integrity. Conversely, a necrotic pulp can cause spread of disease to the periodontium through an accessory canal. It is recognized that pulpal and periodontal disease may spread by their common blood supply.

Pulp capping is successful, especially in noninfected or minimally infected, accidentally exposed pulps in individuals of any age. In these instances dentin is formed at the site of the exposure; thus a dentin barrier or bridge is developed and the pulp retains vitality. Dentin bridge forms an effective continuous barrier only if operative debris and pulp capping material particles are removed.

All operative procedures cause an initial response in the pulp, which is dependent on the severity of the insult. The pulp is highly responsive to stimuli. Even a slight stimulus will cause inflammatory cell infiltration (Fig. 6.30). A severe reaction is characterized by increased inflammatory cell infiltration adjacent to the cavity site, hyperemia, or localized abscesses. Hemorrhage may be present, and the odontoblast layer is either destroyed or greatly disrupted. It is of interest that most compounds containing calcium hydroxide readily induce reparative dentin underlying a cavity (Fig. 6.31). Most restorative materials also induce reparative dentin formations (Fig. 6.32). Usually the closer a restoration is to the pulp the greater will be the pulp response. Though the high pH of calcium hydroxide is bactericidal and promotes tertiary dentin formation, it has unstable physical properties in that particles of calcium hydroxide get into pulp causing pulpal inflammation. Newer composite resins used as pulp capping agents showed

better sealing properties than the earlier composites and calcium hydroxide. Therefore the bacterial leakage is less compared to calcium hydroxide leading to a better dentin bridge formation.

More than calcium hydroxide, enamel matrix derivative was shown to be more capable of promoting reparative process in the wounded pulp. Mineral trioxide aggregate (MTA) has also been shown to be more effective than calcium hydroxide as pulp capping agents. Inflammation, hyperemia and necrosis were less but more odontoblasts and thicker dentin bridge formation was seen with MTA.

In future, incorporation of bioactive molecules like bone morphogenetic protein, TGF-β1 or purified dentin protein fractions in pulp capping materials, use of tissue cultured dentin and stem cells to produce dentin, may radically alter the present treatment approaches.

The thickness of remaining dentin was shown to be important factor in maintaining the vitality of pulp. A minimum thickness of 5mm or greater has a powerful influence on pulp vitality but little effect on reparative dentin formation and no effect on the intensity of inflammation. The number of vital odontoblast remaining after cavity preparation is a critical factor, apart from patient's age which determines the ability of the pulp to form reparative dentin. Pain may be the only symptom in pulpitis and all other cardinal signs of inflammation like rubor (redness), calor (heat), or tumor (swelling) will not be appreciated clinically because pulp is situated deep within the tooth and surrounded by hard tissues of the tooth. Since the pulp contains only free nerve endings all forms of sensory stimuli like touch, pressure or temperature to the pulp result in causing pain sensation only.

Pulpal pain worsens with the degree of inflammation. Stimuli causing pain act through large diameter A-δ or smaller diameter C-fibers. A-δ fibers are fast conducting myelinated fibers and evoke a sharp pain, while nonmyelinated C-fibers are slow conducting fibers and produce a dull pain on stimulation.

Changes occur in tissue fluid pressure in normal and inflamed pulps, and this largely determines whether pulp necrosis occurs or not. Tissue pressure is the hydrostatic pressure of the interstitial fluid surrounding the pulpal cells. It increases due to increase in blood flow and due to increased interstitial fluid; occurring as a result of inflammation. This will cause increase in lymph flow and increased absorption of fluid into the capillaries in the uninflamed area. This will help in transport of fluid from the pulp and thereby reduces the tissue fluid pressure to normal. Increased tissue pressure will promote outward flow of dentinal fluid through the exposed dentinal tubule. This serves to protect the pulp against entry of harmful substances. If the compensatory mechanisms fail to reduce the tissue fluid pressure, a sustained increase in the pressure occurs, and this will compress the blood vessels causing ischemia and necrosis.

In response to orthodontic forces, the pulp shows cell damage, inflammation, vasodilatation and healing all of which are associated with increased vascularity due to release of angiogenic growth factors.

Since dehydration causes pulpal damage, operative procedures producing this condition should be avoided. When filling materials contain harmful chemicals (e.g. acid in silicate cements and monomer in the composites), an appropriate cavity liner should be used prior to the insertion of restorations. Pulp has to be protected from damage due to heat transmission especially by metallic restorations by the use of bases.

A vital pulp is essential to good dentition. Although modern endodontic procedures can prolong the usefulness of a tooth, a nonvital tooth becomes brittle and is subject to fractures. Therefore, every precaution should be taken to preserve the vitality of a pulp.

In clinical practice, instruments called vitalometers, which test the reaction of the pulp to electrical stimuli, or thermal stimuli (heat and cold) are often used to test the 'vitality' of the pulp. These methods provide information about the status of the nerves supplying the pulpal tissue and therefore check the 'sensitivity' of the pulp and not its 'vitality.' The vitality of the pulp depends on its blood supply, and one can have teeth with damaged nerve but normal blood supply (as in cases of traumatized teeth). Such pulps do not respond to electrical or thermal stimuli but are completely viable in every respect.

Laser Doppler flowmetry, an electro-optical technique used in the recording of pulpal blood flow, has been found to be reliable in assessing the vitality of traumatized teeth. Also, transmitted-light photoplethysmography, which has been used to detect blood flow in young permanent teeth, may be of use in the assessment of pulp vitality.

The preservation of a healthy pulp during operative procedures and successful management in cases of disease are two of the most important challenges to the clinical dentist.

SUMMARY

The pulp is a loose connective tissue occupying the pulp chamber in the crown and root canal in the root. Pulp communicates with the periodontal ligament through the apical foramen and through accessory foramina.

Zones of the Pulp

Pulp can be divided into different zones; the odontogenic zone close to the pulp–dentin border, the cell-free zone of Weil beneath it, and the parietal zone in the remaining area.

Cells of the Pulp

Odontoblasts

The odontoblasts present in the, odontogenic zone varies in size, shape, and arrangement. In the coronal pulp they are columnar in shape and show a pseudostratified arrangement with an average diameter of 7.2 μm and 25 to 40 μm in length, becoming flatter and are arranged in a single layer in the root. Odontoblasts have a basal polarized nucleus and

contact the adjacent cells focally with junctional complexes. The odontoblast morphology and its organelles vary with functional activity of the cell. In the active stage as during the formation of primary dentin formation, the cell is elongated with all the organelles required for protein synthesis. In the resting stage, the cell is stubby with fewer organelles. They are terminally differentiated so they have to be replaced by undifferentiated mesenchymal cells when they die. The cytoplasmic process extending from the apical cytoplasm is usually devoid of organelles and extends to about 2/3rd of the lengths of the dentinal tubules. The cell-free zone contains subodontoblastic plexus of nerves and vessels.

Fibroblasts and Collagen Fibers of the Pulp

Pulp consists of fibroblasts, defense cells like histiocytes, plasma cells and pluripotent undifferentiated mesenchymal cells, and stem cells. The fibroblasts are the most numerous of the pulpal cells. They are star shaped and their process communicates with each other. They form and degrade collagen fibers and the ground substance. Pulp consists of loosely arranged type I fine collagen fibers. Their length varies from 10 to 100nm.

Defence Cells

The histiocyte is an irregularly shaped cell and appear similar to fibroblast. They are stained by vital dyes like toluidin blue. Ultrastructurally they show vesicles containing phagocytosed bodies.

Dendritic cells are antigen presenting cells found in close relation to or contact with their processes to odontoblast or endothelial cells.

The plasma cells are seen only during pulpal inflammation. They are oval shaped cells with eccentric nucleus. They produce antibodies.

Pulpal stem cells

These are pluripotent cells replacing injured odontoblast and produce dentin. They can be induced to proliferate and differentiate by numerous growth factors like transforming growth factor. Pulp of exfoliated deciduous teeth and third molars are a good source of pulpal stem cells and they are used in regeneration of dentin, bone, and neural tissues.

Pulpal Blood Vessels and Circulation

The blood vessels are mainly arterioles of smaller size and thinner walls than elsewhere, the capillaries have fenestrations and there are arteriovenous communications. Blood flow in pulp is higher than in most areas of the body, in capillaries is high— it is1 mm 0.08mm per second. The circulation in pulp facilitates rapid transport of metabolites. Pericytes are cells with contractile properties and are seen on the surface of smaller arterioles. Blood vessels and nerves enter and leave through apical foramen. Blood vessels in the pulp communicate with the vessels in the periodontal ligament through main and accessory canals. Lymph vessels also said to follow the course of blood vessels. Lymph vessels draining anterior teeth drain into submental lymph nodes and those from posterior teeth drain into submandibular lymph nodes.

Nerves of the Pulp

The nerves are of two types—the unmyelinated parasympathetic nerves which are unbranched and end in blood vessels to control the blood flow and the myelinated nerves and somatic nerves which lose their myelin sheath before they branch and form plexus in the cell-free zone. This plexus is often referred to as plexus of Raschkow. Some of these extend to end below the odontoblast and form synapse while others go up to predentin and loop backward while very few travel within the dentin tubules spiraling around the odontoblastic process. Since the pulp contains only free nerve endings all forms of sensory stimuli result in pain sensation.

Function of Pulp

The function of pulp is to produce dentin (formative function), nourish dentin (nutritive function), elicite pain to protect the tooth(protective function) and to repair injured dentin or arrest caries progression by forming reparative dentin (reparative function). In early odontogenesis the pulp anlage interacts with oral epithelial cells to cause differentiation of enamel organ and dental lamina.

Development of Pulp and Differences in Primary and Permanent Teeth Pulp Tissues

The pulp is formed from dental papilla. After the peripheral cells of dental papilla differentiate into odontoblast and produce dentin, the rest of dental papilla becomes pulp. The earliest pulp of deciduous teeth develops by 8th week of embryonic life. The developing pulp is very cellular and very vascular. Nerves appear later (18th week), reach subodontoblastic region by 24th week, the plexuses formation occurring still later.

The pulp of primary (deciduous) teeth functions for a shorter time than pulp of permanent teeth (about 8.3 years). During this period the pulp grows till root completion in about 1 year, it matures in about 3 year, 9 months and later regresses from the time of root resorption to exfoliation in about 3 year 6 months. In permanent teeth as the periods of crown completion (5.5years), the period between crown completion and eruption (about 3.6 years) and from eruption to root completion period (about 3 year, 11 months) are all longer; the pulp growth and development are slower than the deciduous teeth. It is due to the shorter life span of growth and maturity of deciduous pulp, the extent of neural development is less than in permanent teeth.

Age Changes in the Pulp

The age changes in the pulp include decreased cellularity, increase in fibers with bundle formation, degeneration of nerves and calcifications. Pulp arterioles are end arteries and as pulp circulation is not collateral, inflammation of pulp results in necrosis.

REVIEW QUESTIONS

1. Describe the cells of the pulp.
2. What are the types of stem cells present in the pulp and their potential applications?
3. Describe the vasculature of the pulp.
4. What does cell-free zone contain?
5. List out the differences between coronal and radicular pulp.
6. Why all types of sensory stimuli to the pulp is felt as pain?
7. What are the functions of pulp?
8. What are the peculiarities of pulpal inflammation?

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7 Cementum

CHAPTER CONTENTS

Cementum is the mineralized dental tissue covering the anatomic roots of human teeth. It was first demonstrated microscopically in 1835 by two pupils of Purkinje. It begins at the cervical portion of the tooth at the cementoenamel junction and continues to the apex. Cementum furnishes a medium for the attachment of collagen fibers that bind the tooth to surrounding structures. It is a specialized connective tissue that shares some physical, chemical, and structural characteristics with compact bone. Unlike bone, however, human cementum is avascular and noninnervated.

Cementum thickness varies in different teeth and in the same teeth in different regions. Cementum is thinnest at the cementoenamel junction (20 to 50 μm) and thickest toward the apex (150 to 200 μm). The apical foramen is surrounded by cementum. Sometimes cementum extends to the inner wall of the dentin for a short distance, and so a lining of the root canal is formed. Like dentin, cementum can form throughout the life of a tooth.

PHYSICAL CHARACTERISTICS

The hardness of fully mineralized cementum is less than that of dentin. Cementum is light yellow in color and can be distinguished from enamel by its lack of luster and its darker hue. Cementum is somewhat lighter in color than dentin. The difference in color, however, is slight, and under clinical conditions it is not possible to distinguish cementum from dentin based on color alone. Under some experimental conditions cementum has been shown to be permeable to a variety of materials.

CHEMICAL COMPOSITION

On a dry weight basis, cementum from fully formed permanent teeth contains about 45 to 50% inorganic substances and 50 to 55% organic material and water. The inorganic portion consists mainly of calcium and phosphate in the form of hydroxyapatite. Numerous trace elements are found in cementum in varying amounts. It is of interest that cementum has the highest fluoride content of all the mineralized tissues.

The organic portion of cementum consists primarily of type I collagen and protein polysaccharides (proteoglycans). The other types of collagen seen are types III, V, VI and XII. Amino acid analyses of collagen obtained from the cementum of human teeth indicate close similarities to the collagens of dentin and alveolar bone.

The noncollagenous proteins play important roles in matrix deposition, initiation and control of mineralization and matrix remodeling. Many noncollagenous proteins are common to both cementum and bone. Bone sialoprotein (BSP) and osteopontin are two such major proteins that fill up the large interfibrillar spaces. Osteopontin, which is present in cementum in lesser amounts than in bone, regulate mineralization. Cementum derived attachment protein (CAP) is an adhesion molecule unique to cementum. It helps in the attachment of mesenchymal cells to the extracellular matrix. Cemental types vary in the distribution of noncollagenous proteins.

Bone and cementum show similar types of proteoglycans. Chondroitin sulfate, heparan sulfate, hyaluronate, keratan sulfates–fibromodulin and lumican, versican, biglycan and osteoadherin are present in both the tissues.

The matrix of bone and cementum shows many similar growth factors, which control the activities of many cell types. In cementum, like bone, TGFβ, various BMPs, FGF, IGF, EGF and platelet derived growth factor (PDGF) are seen. However, a cementum derived growth factor is seen exclusively in cementum. As in bone, osteoprotegerin (OPG) and RANKL, are detected in periodontal ligament cells during shedding, suggesting that these are involved in resorption of cementum, similar to that of bone. OPG interferes with the binding of RANKL to RANK receptor in cementoblast and helps in the regulation of cemental resorption.

CEMENTOGENESIS

The internal and external enamel epithelium proliferate downwards as a double layered sheet of flat epithelial cells, called the root sheath of ***Hertwig***. This root sheath induces the cells of the dental papilla to differentiate into odontoblasts. These cells secrete the organic matrix of first formed root predentin consisting of ground substance and collagen fibrils. As the odontoblasts retreat inwards, they do not leave behind the odontoblastic process in these first few layers of dentin. Hence, this layer is structure less and is called '***Hyaline layer***'.

Subsequently, breaks occur in the epithelial root sheath allowing the newly formed dentin to come in direct contact with the cells of dental follicle (Fig. 7.1). Cells derived from this connective tissue are called cementoblast. They are responsible for the formation of cementum (Fig. 7.2).

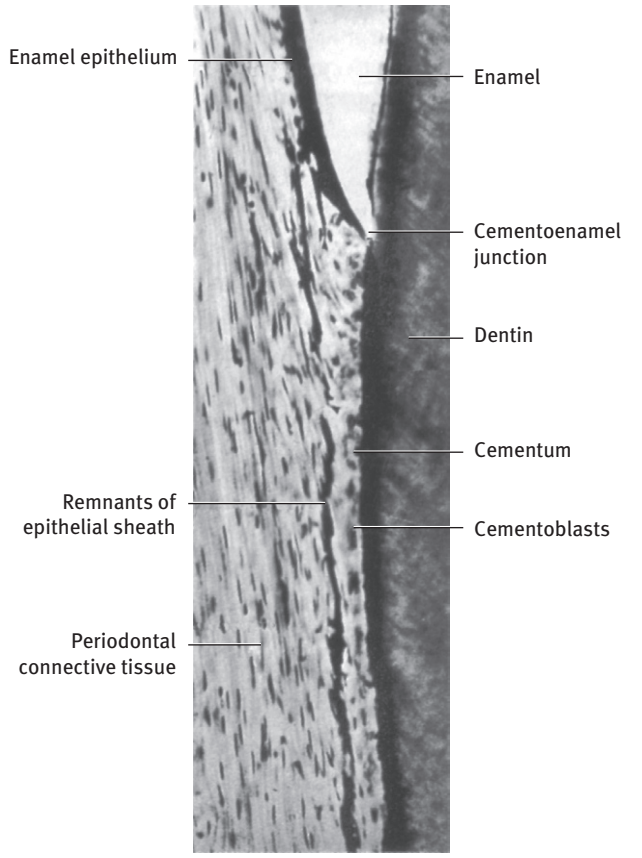


Figure 7.2 Epithelial sheath is broken and separated from root surface by connective tissue.

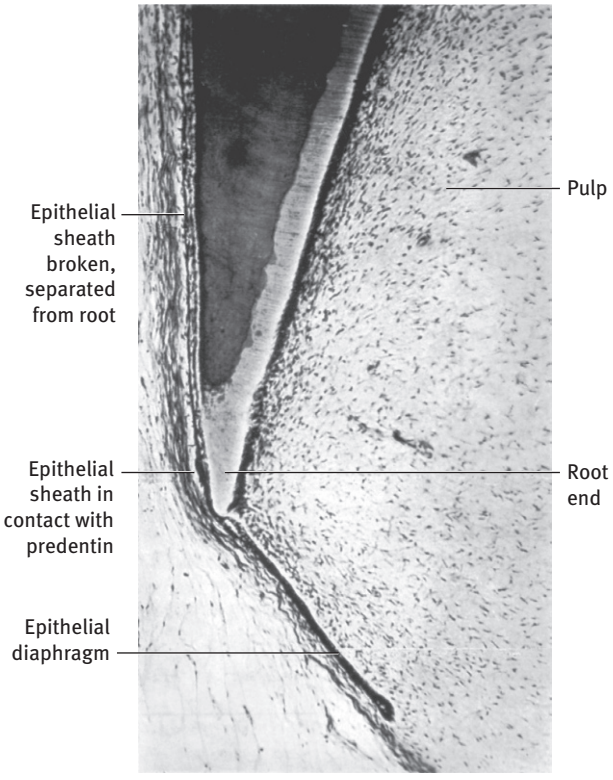


Figure 7.1 Hertwig's epithelial root sheath at end of forming root. At side of root, sheath is broken up, and cementum formation begins (From Gottlieb B: J Periodontol 13:13, 1942).

At the ultrastructural level, breakdown of Hertwig's epithelial root sheath involves degeneration or loss of its basal lamina on the cemental side. At the deep surface, these fibrils intermingle with those of hyaline layer. Loss of continuity of the basal lamina is soon followed by the appearance of collagen fibrils and cementoblasts between epithelial cells of the root sheath. Some sheath cells migrate away from the dentin toward the dental sac, whereas others remain near the developing tooth and ultimately are incorporated into the cementum. Sheath cells that migrate toward the dental sac become the epithelial rests of Malassez found in the periodontal ligament of fully developed teeth.

Cementoblasts

Cementoblasts synthesize collagen and protein polysaccharides, which make up the organic matrix of cementum. At the superficial surface, the collagen fibrils produced by the cementoblast form a fibrous fringe perpendicular to periodontal space. These cells retreat and intermingle with the fibroblasts of the periodontal ligament. These cells have numerous mitochondria, a well-formed Golgi apparatus, and large amounts of granular endoplasmic reticulum. These ultrastructural features are not unique to cementoblasts and can be observed in other cells actively producing proteins and polysaccharides.

Mineralization of the first formed dentin of the hyaline layer occurs within matrix vesicles. But the mineralization does not occur at outermost surface of hyaline layer, but within it. From this initial center, mineralization spreads towards pulp and towards periodontal ligament. Outermost part of the hyaline layer thus undergoes delayed mineralization. The delayed mineralization front of the hyaline layer spreads outwards, until it is fully mineralized and continues into the fibrous fringe secreted by the cementoblast. Thus, the first few layers of acellular cementum are attached to dentin. The fibers in the periodontal ligament are parallel to the root surface at this stage. But in the later stages, the principal collagen fibers of the periodontal ligament become continuous with the fibrous fringe of cementum.

Once the periodontal ligament fibers get attached to the fibrous fringe from acellular cementum, the cementum is classified as ***Acellular extrinsic fiber cementum***. In permanent teeth, the attachment occurs only after the tooth has erupted into the oral cavity (only 2/3rd of the root would have been formed by that time).

Thus, the acellular cementum lining the root before this time may be considered as ***Acellular intrinsic fiber cementum.***

Incremental lines called as lines of ***Salter*** are seen in cementum as during the process of cementogenesis, there are periods of rest and periods of activity. The periods of rests are associated with these lines. The lines are closer in acellular cementum as this cementum is formed slowly.

Epithelial cell rests may be entrapped in the cementum near cementodentinal junction. That part of the cementum is called ***Intermediate cementum***. It usually occurs in the apical half of roots of molar teeth.

After the formation of acellular cementum, a less mineralized cementum is formed called ***Cellular cementum***. The cementoblast secretes the collagen fibers and ground substance which form the ***Intrinsic*** fibers of cellular cementum. These fibers are parallel to the root surface and do not extend into the periodontal ligament. Some cementoblasts get entrapped and are called ***Cementocytes***. Increased rate of cementum formation results in cells getting incorporated into the matrix and a layer of cementoid being present on the surface. These cementocytes are present in spaces called lacunae. The cementocytes in the deeper layers are nonviable as the distance from the surface increases and diffusion of nutrients decreases. The incremental lines in the cellular cementum are widely spaced because of the increased rate of formation. Cellular cementum has no supportive role as no fibers of periodontal ligament are inserted into it.

DSP (Dentin sialoprotein), fibronectin, tenascin and ***prostaglandins (versican, decorin and biglycan)*** are present in cellular cementum, as many prostaglandins are located at the periphery of the lacunae.

The mineralization of cementum matrix may be controlled by hydroxyapatite crystals in adjacent dentin, periodontal ligament fibroblasts rich in ***alkaline phosphatase*** and noncollagenous proteins in the matrix like ***BSP (Bone sialoprotein***). Mineralization is very slow and occurs in a linear fashion. Hence there is no cementoid seen on the surface of acellular cementum. Mineralization of the deeper layer of precementum of cellular cementum occurs in the linear manner, but overall this type of cementum is less mineralized than acellular cementum.

At the apical and furcation areas, the cellular intrinsic fiber cementum alternates with acellular extrinsic fiber cementum to form ***Cellular mixed stratified cementum***.

In the ***Cellular mixed fiber cementum***, the normal cellular intrinsic fiber cementum gives attachment to extrinsic fibers from the periodontal ligament.

The ***Acellular afibrillar cementum*** is formed when there is premature loss of the reduced enamel epithelium protecting the newly formed enamel at the cervical region. It is deposited as a thin layer on the enamel at the cervical margin of the tooth.

Cementoblasts are identified by 3H thymidine labeling. Apart from the inner cells of the dental follicle, cementoblasts are also reported to differentiate from HERS. The cementoblasts that is derived from dental follicle has a similar morphology and phenotype to osteoblasts and is shown to be involved in the formation of cellular intrinsic fiber cementum (CIFC). It has also been suggested that this type of cementoblast and the osteoblast may have a common precursor in the dental follicle. The cementoblasts which are reported to be derived from HERS have a similar morphology to fibroblast and a different phenotype than osteoblasts and are shown to be involved in the formation of acellular extrinsic fiber cementum (AEFC). These two types of cementoblast precursors differ in the expression of surface receptors and their reaction to signaling molecules or differentiating factors. E11 antibody strongly reacts with CIFC forming cementoblasts but not with cementoblasts forming AEFC, showing that the cementoblasts forming cellular cementum and acellular cementum are different. Cementoblasts forming CIFC, express receptors for parathormone (PTH)/PTHrp (parathormone receptor protein) which have a regulatory role in cementogenesis. Cementoblasts forming AEFC do not show these receptors. Extracellular matrix proteins osteopontin and osteocalcin were expressed by cementoblasts producing cellular cementum suggesting osteoblast like phenotype, while cementoblasts producing acellular cementum express only osteopontin suggesting a partial osteoblastic phenotype. Osteonectin synthesized by the cementoblasts producing both acellular and cellular cementum is associated with collagen formation, hence it is seen not only during cementogenesis but also during dentino- and osteogenesis.

Many factors are involved in the differentiation of cementoblast. They are growth factors belonging to the TGF-β family including BMP, transcription factor core binding factor alpha 1 (Cbfα 1) and signaling molecule epidermal growth factor (EGF).

The Hertwig's epithelial root sheath undergoes epithelial mesenchymal transition under the induction of ***TGFβ1*** and develop into cementum forming cells.

BMPs are members of ***TGF-β*** family. ***BMPs 2, 4,*** and ***7*** are known to promote differentiation of pre-osteoblasts and precursors of cementoblasts. ***BMP3*** is said to play a role in cementum formation as this has been found selectively in root lining cells. ***BMP3*** plays a role in differentiation of follicle cells along cementoblast pathway.

Cbfα1 is found to be expressed in periodontal ligament cells, dental follicle cells, cementoblasts, odontoblasts and ameloblasts. It may be involved in cementoblast differentiation.

EGF (Epidermal growth factor) receptor downregulates signal transduction from ligands such as TGF-α and EGF to control cell differentiation.

Prostaglandin E (2) and F (2) alpha enhance differentiation of cementoblasts by activating protein kinase signaling pathway.

FGF (Fibroblast growth factor) is shown to promote cell proliferation, its migration and angiogenesis.

CAP (Cementum attachment protein) helps in the attachment of selected cells to the newly forming tissue. It is located in the matrix of mature cementum and in cementoblasts.

Osteopontin *and* ***Bone sialoprotein (BSP)*** are expressed by cells along root surface, the cementoblasts during early stages of root development. They contain cell adhesion molecule RGD and promote adhesion of selected cells onto newly forming root.

Laminin has been identified on dentin surface at the initiation of cementum formation. This may have a role in attracting cementoblast like cells to the root surface.

Recently ***dentin noncollagenous proteins*** have been identified which can stimulate dental follicle cells to differentiate into cementoblast lineages.

Studies suggest that ***IGF*** and ***PDGF*** have a regulatory function in cementoblast differentiation. These molecules promote cementum formation by altering cell cycle activities.

In the differentiation of the cementoblasts, growth factors belonging to the TGF-β family including BMP, transcription factor core binding factor alpha 1 (Cbfα 1) and signaling molecule epidermal growth factor (EGF) are involved. EGF receptor downregulates signals transduction from ligands such as TGF-α and EGF to control cell differentiation. Prostaglandins E(2) and F(2α) enhance differentiation of cementoblast by activating protein kinase signaling pathway. FGF was shown to promote cementoblast proliferation, its migration and angiogenesis. CAP, BSP and osteopontin help in the attachment of selected cells to the newly forming tissue (Box 7.1).

The development of cellular cementum can be divided into two stages: an early stage in which the extrinsic Sharpey's fibers were few and intrinsic fibers were randomly arranged, and a later stage in which the Sharpey's fibers were thicker and the intrinsic fibers appeared to encircle them. Since the matrix secretion occurs from different regions of the cementoblast (multipolar mode of secretion) and the faster rate of formation are believed to be responsible for cementoblast entrapment in its own matrix leading to the formation of cellular cementum.

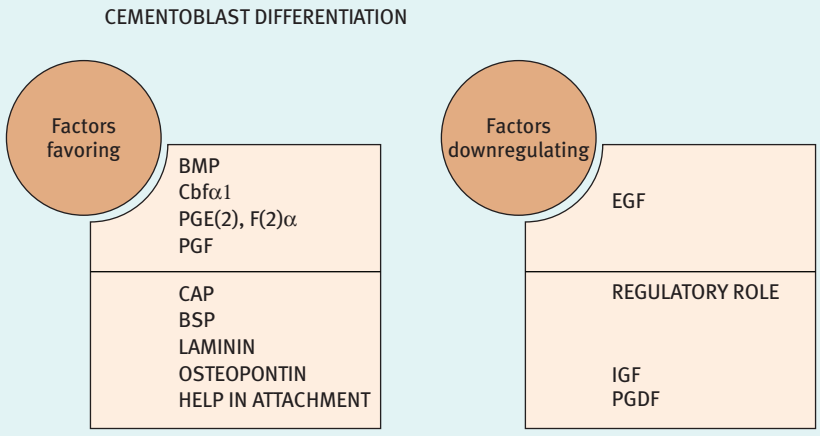
The epithelial cell rests showed a close relationship with cementoblasts during acellular cementum formation but not with cellular cementum formation suggesting that it has a role to play in acellular cementum formation.

The development of acellular cementum is associated with secretion of enamel matrix proteins (EMP) by HERS and is preceded by mineralization of the first layer of dentin adjacent to the root. HERS is also shown to secrete cementum related proteins like BSP, osteopontin and fibrillar collagen. The presence of EMP in cemental matrix is disputed. Epithelial mesenchymal reaction similar to that occurring in the crown is suggested leading to the development of cementoblasts. Enamel proteins including, amelogenin, parathyroid hormone receptor protein and certain basement membrane constituents are reported to be involved in the epithelial mesenchymal reactions.

However, the role of HERS in cementum formation is disputed. In situ hybridization studies and immunological studies localized enamel proteins and amelogenin mRNAs to enamel and were found to be absent in ameloblasts adjacent to root margin. Moreover amelogenin antibodies did not crossreact with cementum protein extracts.

After some cementum matrix has been laid down, its mineralization begins. The uncalcified matrix is

Box 7.1 Cementoblast Differentiation



called cementoid. Calcium and phosphate ions present in tissue fluids are deposited into the matrix and are arranged as unit cells of hydroxyapatite. Mineralization of cementoid is a highly ordered event and not the random precipitation of ions into an organic matrix.

Gla proteins osteocalcin and osteonectin act as nucleators for mineralization, due to their strong affinity for calcium, BSP (bone sialoprotein) and alkaline phosphatase promote mineralization while osteopontin regulates growth of apatite crystals.

The major proteoglycan located exclusively in the nonmineralized cementum was keratan sulfates–lumican and fibromodulin. This finding suggests that they have a regulatory role in cementum mineralization.

Insulin like growth factor (IGF) present in developing and matured cementum monitors mineralization and controls cell differentiation.

Cementoid Tissue

Under normal conditions growth of cementum is a rhythmic process, and as a new layer of cementoid is formed, the old one calcifies. A thin layer of cementoid can usually be observed on the cemental surface (Fig. 7.3). This cementoid tissue is lined by cementoblasts. Connective tissue fibers from the periodontal ligament pass between the cementoblasts into the cementum. These fibers are embedded in the cementum and serve to attach the tooth to surrounding bone. Their embedded portions are known as Sharpey's fibers (Fig. 7.4). Each Sharpey's fiber is composed of numerous collagen fibrils that pass well into the cementum (Fig. 7.5). Cementoid tissue is not observed in AEFC.

STRUCTURE

Light microscopic observations reveal two basic types of cementum, hence they are usually classified on the basis of presence of cementocytes (cellular cementum) or its absence (acellular cementum). It can also be classified on the basis of the type of fibers (intrinsic/extrinsic fibers) presence or their absence (afibrillar cementum).

The intrinsic fibers produced by the cementoblast are smaller (1–2 microns in diameter) and are usually oriented parallel to the surface. The extrinsic fibers produced by periodontal ligament fibroblast are larger bundles (5–7 microns in diameter) and are usually oriented perpendicular to the surface. Thus by combining the types of fibers—extrinsic and intrinsic fibers and the presence or absence of cementocytes, different types of cementum have been described.

Acellular extrinsic fiber cementum (AEFC), *cellular intrinsic fiber cementum* (CIFC), and *acellular afibrillar cementum* are usually described. The acellular extrinsic fiber cementum is regarded as *primary cementum,* because it forms first. The cellular cementum are also known as *secondary cementum,* because it forms later than primary cementum. In addition, *acelllular intrinsic fiber, cellular and acellular mixed fiber cementum* (containing both intrinsic and extrinsic fibers) and *cellular mixed stratified cementum* (containing alternating layers of acellular extrinsic fiber cementum and cellular intrinsic fiber cementum), have been described.

Cementum of different types differ from one another in their location, structure, function, rate of formation, biochemical composition and degree of mineralization.

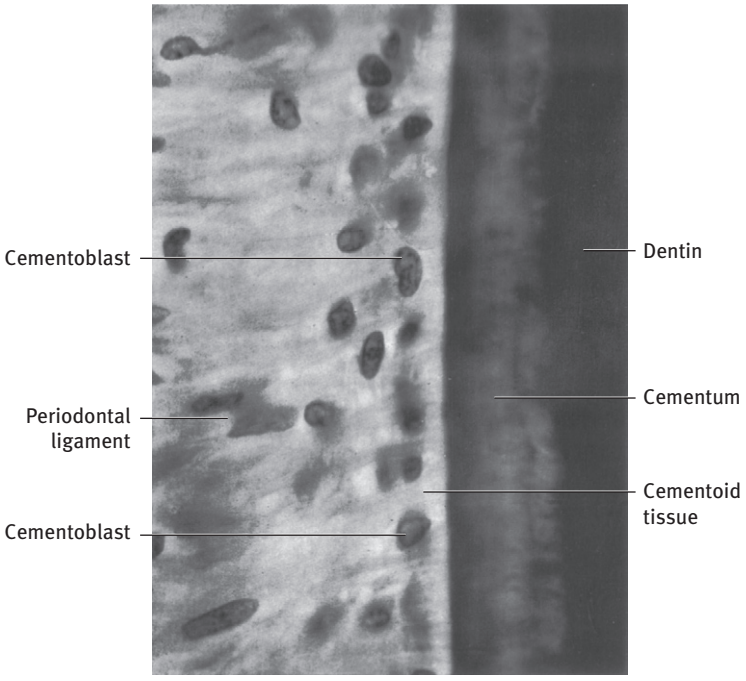


Figure 7.3 Cementoid tissue on surface of calcified cementum. Cementoblasts between fibers.

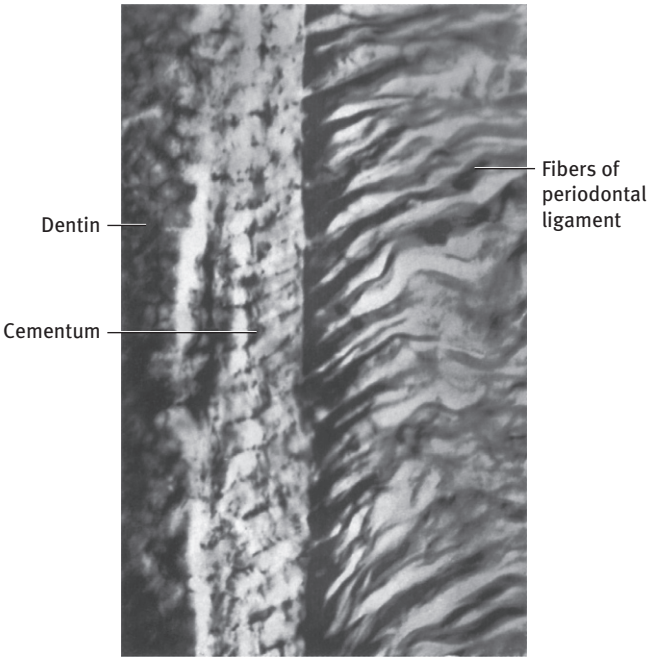


Figure 7.4 Fibers of periodontal ligament continue into surface layer of cementum as Sharpey's fibers.

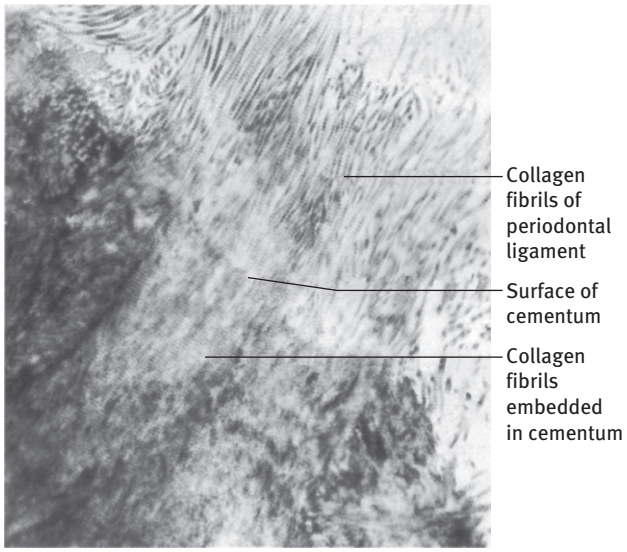


Figure 7.5 Collagen fibrils from periodontal ligament continue into cementum. Numerous collagen fibrils embedded in cementum are collectively referred to as Sharpey's fibers (Decalcified human molar; electron micrograph; ×17,000).

Acellular afibrillar cementum (AAC) The AAC is a mineralized ground substance containing no cells and is devoid of extrinsic and intrinsic collagen fibers. This type of cementum is seen chiefly as coronal cementum, with a thickness of 1 to 15 μm.

Acellular Extrinsic Fiber Cementum

The AEFC extends from cervical margin to apical 1/3rd. It is the only type of cementum seen in single rooted teeth. In decalcified specimens of cementum, collagen fibrils make up the bulk of the organic portion of the tissue. Interspersed between some collagen fibrils are electron-dense reticular areas, which probably represent protein polysaccharide materials of the ground substance (Fig. 7.6). The extrinsic fibers are seen perpendicular to surface of cementum and they are known as *Sharpey's fibers*. In some areas, however, relatively discrete bundles of collagen fibrils can be seen, particularly in tangential sections (Fig. 7.7). These extrinsic collagen fibers are mineralized except for their inner cores. This is particularly true in a zone 10 to 50 μm wide near the cementodentinal junction where unmineralized areas about 1 to 5 μm in diameter are seen. When cementum

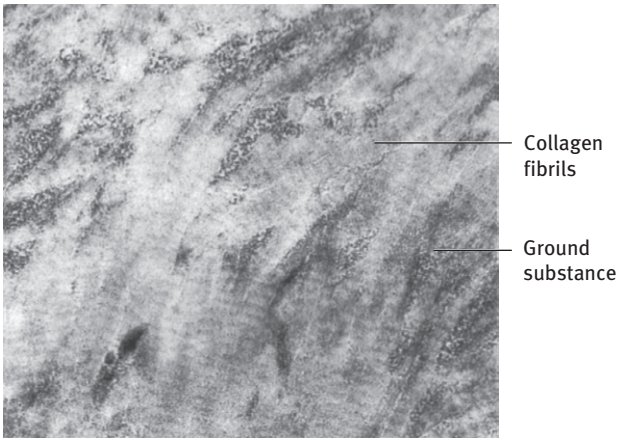


Figure 7.6 Electron micrograph of human cementum showing ground substance interspersed between collagen fibrils (Decalcified specimen; ×42,000).

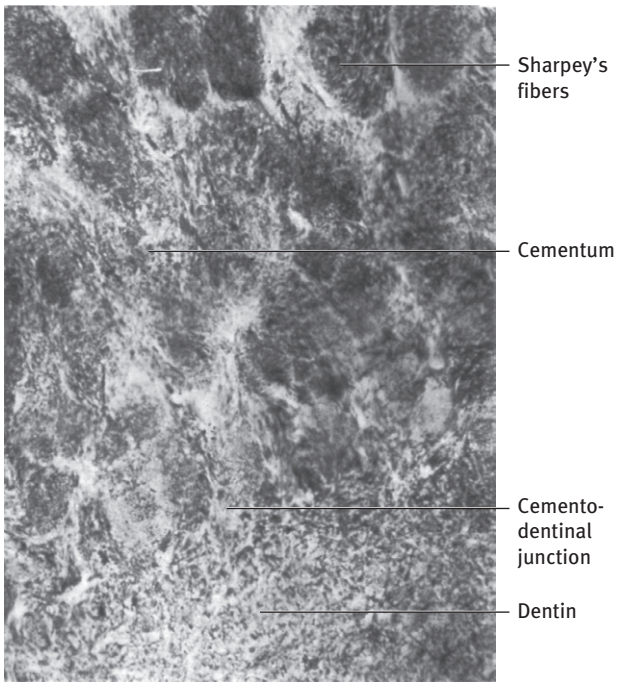


Figure 7.7 Ultrastructural view of cementodentinal junction of human incisor. In this tangential section, Sharpey's fibers are visible as discrete bundles of collagen fibrils (Decalcified specimen; electron micrograph; ×5000).

remains relatively thin, Sharpey's fibers cross the entire thickness of the cementum. With further apposition of cementum, a larger part of the fibers is incorporated in the cementum. The attachment proper is confined to the most superficial or recently formed layer of cementum (Fig. 7.4). The noncollagenous proteins fill up the space between the extrinsic fibers. Since this type of cementum forms slowly and regularly the incremental lines are placed parallel to the surface and closer together than in cellular cementum. Cementoid, the unmineralized matrix seen on the surface of cellular cementum is not observed in AEFC.

The main function of this type of cementum is anchorage especially in single rooted teeth.

Cellular Cementum

The cellular cementum is also known as secondary cementum as this is formed later than the AEFC. The cellular cementum found in the apical third is mainly of two types—the cellular mixed fiber cementum which forms the bulk of secondary cementum and occupies the apical interradicular regions and the CIFC which is present in the middle and apical third. These types are mainly involved in the adaptation and repair of cementum. Since the secondary cementum is formed rapidly the incremental lines are placed further apart than in AEFC.

Cellular intrinsic fiber cementum (CIFC)

This cementum contains cells but has no extrinsic fibers. The fibers present are intrinsic fibers which are secreted by the cementoblasts. It is formed on the root surface and in cases of repair.

Cellular mixed fiber cementum (CMFC)

The cellular mixed fiber cementum is a variant formed at a faster rate with less mineralized fibers. The collagen fibers of CMFC are derived from the periodontal ligament fibroblasts and the cementoblasts. These intrinsic and extrinsic fibers form an intricate pattern running between each other at almost right angles and different orientations, though the number of intrinsic fibers is comparatively less than the extrinsic fibers. The fiber bundles originating from the fibroblast are ovoid or round with 5–7 μm diameter and the fibers originating from the cementoblasts are much more delicate and smaller measuring 1–2 μm in diameter.

Cellular mixed stratified cementum (CMSC)

In this type of cementum the cellular intrinsic fiber cementum alternates with acellular extrinsic fiber cementum. It is formed by cementoblasts and fibroblasts. It appears primarily on apical third of the root and furcation areas. The thickness varies from 100–1000 μm.

The cells incorporated into cellular cementum, cementocytes, are similar to osteocytes. They lie in spaces designated as lacunae. A typical cementocyte has numerous cell processes, or canaliculi, radiating from its cell body. These processes may branch, and they frequently anastomose with those of a neighboring cell. Most of the processes are directed toward the periodontal surface of the cementum. The full extent of these processes does not show up in routinely prepared histologic sections. They are best viewed in mineralized ground sections (Fig. 7.8). The cytoplasm of cementocytes in deeper layers of cementum contains few organelles, the endoplasmic reticulum appears dilated, and mitochondria are sparse. These characteristics indicate that cementocytes are either degenerating or are marginally active cells. At a depth of 60 μm or more cementocytes show definite signs of degeneration such as cytoplasmic clumping and vesiculation. At the light microscopic level, lacunae in the deeper layers of cementum appear to be empty, suggesting complete degeneration of cementocytes located in these areas (Fig. 7.9).

Differences between Cementocytes and Osteocytes

Though the cementocytes resemble the osteocytes, there are a few important differences. The lacunae of cementocytes varies from being ovoid or tubular, but the osteocytic lacunae is invariably oval. The canaliculi are less complicated and sparse, with the majority of them facing the periodontal ligament when compared to osteocytes, whose canaliculi are radiating, more dense and arranged in a complex network. However in both, the cytoplasmic processes are connected with the cells lining the surface. Immunocytochemical studies show that cementocytes are immunopositive for fibromodulin and lumican, but not osteocytes.

Differences between AEFC and Cellular Intrinsic Fiber Cementum (CIFC)

AEFC is a unique tissue and it differs from CIFC in many ways. Apart from the differences in structure, the

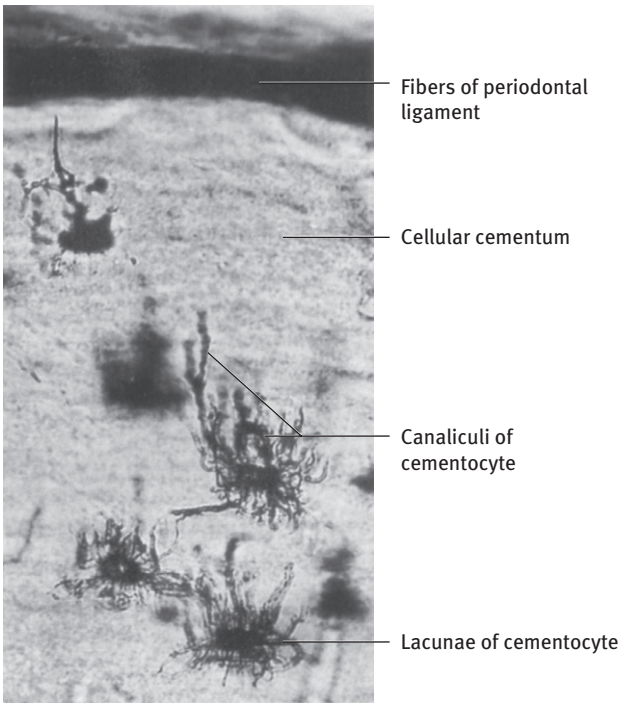


Figure 7.8 Cellular cementum from human premolar. Note lacunae of spider like cementocytes with numerous canaliculi or cell processes (Ground section; ×480).

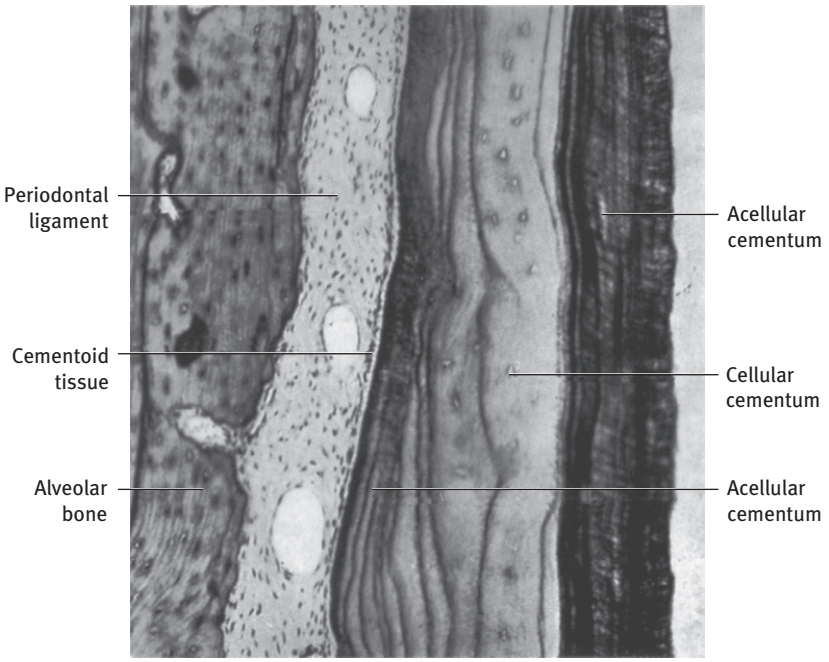


Figure 7.9 Cellular cementum on surface of acellular cementum and again covered by acellular cementum (incremental lines). Lacunae of cellular cementum appear empty, indicating degeneration of cementocytes.

cementoblasts forming AIFC are derived from HERS, its matrix composition and the factors regulating its formation are different.

CIFC responds to local factors and to growth hormone more profoundly than AIFC.

Tenascin, fibronectin and osteocalcin are absent in AEFC, but present in CIFC. In CIFC, fibromodulin, lumican, versican, decorin and biglycan are seen in relation to lacunae and canaliculi. As these structures are absent in AEFC, these proteoglycans are not seen.

Growth factors like TGF-β and IGF are not expressed in AEFC.

Cementoblasts forming CIFC Cexpress receptors for PTH/ PTHrp which have a regulatory role in cementogenesis but not cementoblast forming AEFC. Matrix vesicles are involved in the mineralization of CIFC but not in AEFC.

Radiographic microanalysis showed that there are differences in composition between acellular extrinsic fiber cementum and cellular intrinsic fiber cementum with respect to calcium, magnesium and phosphorus. In CIFC, the Mg/Ca ratio was highest in areas where Ca/P ratio was lower. In AEFC, the Mg/Ca ratio was lower than Ca/P ratio.

The general differences between acellular and cellular cementum is outlined in Table 7.1 and the specific differences between AIFC and CIFC are summarized in Table 7.2.

Both acellular and cellular cementum are separated by incremental lines into layers, which indicate periodic formation (Figs 7.9 and 7.10). Incremental lines can be seen best in decalcified specimens prepared for light microscopic observation. They are difficult to identify at the ultrastructural level. The incremental lines seen in

Table 7.1 Differences between Acellular Cementum and Cellular Cementum

Acellular Cementum	Cellular Cementum
Embedded cementocytes are absent	Embedded cementocytes are present
Deposition rate is slower	Deposition rate is faster
It is the first formed layer	Formed after acellular cementum
Width is more or less constant	Width can be highly variable
Found more at cervical third of tooth	Mainly seen at apical third and interradicular area though a thin layer is present all over the root
Also called as primary cementum	Also called as secondary cementum
Sharpey's fibers are well mineralized	Sharpey's fibers are partially mineralized
Incremental lines are regular and closely placed	Incremental lines are irregular and placed wide apart with variable thickness between them

Table 7.2 Differences between AEFC and CIFC

Acellular Extrinsic Fiber Cementum	Cellular Intrinsic Fiber Cementum
Located from cervical to apical third	Located in apical third and furcations
Formed earlier—primary cementum	Formed later and during repair—secondary cementum
Noncollagenous proteins—tenascin, fibronectin, osteocalcin absent	These noncollagenous proteins are present
Growth factors TGFβ and IGF not seen	These growth factors are seen
Proteoglycans, versican, decorin, biglycan and lumican were not identified in the matrix	These proteoglycans are seen in the matrix
Cementoid is usually absent	Cementoid seen on the surface
Contains only extrinsic fibers of the periodontal ligament formed by fibroblast	Contains only intrinsic fibers produced by cementoblast
Probably the only type of cementum in single rooted teeth	May be absent in single rooted teeth
Main function is anchorage	Main function is adaptation and repair
Slow formation	Rapid formation
Incremental lines therefore closer together	Incremental lines therefore further apart
Cementocytes not seen	Cementocytes, viable to varying degrees and depths seen
Cementoblast suggested to be derived from HERS	Cementoblast suggested to be derived from inner cells of dental follicle. Phenotypic similarity to osteoblast suggested
Cementoblasts do not express parathormone receptor	Cementoblasts express parathormone receptor

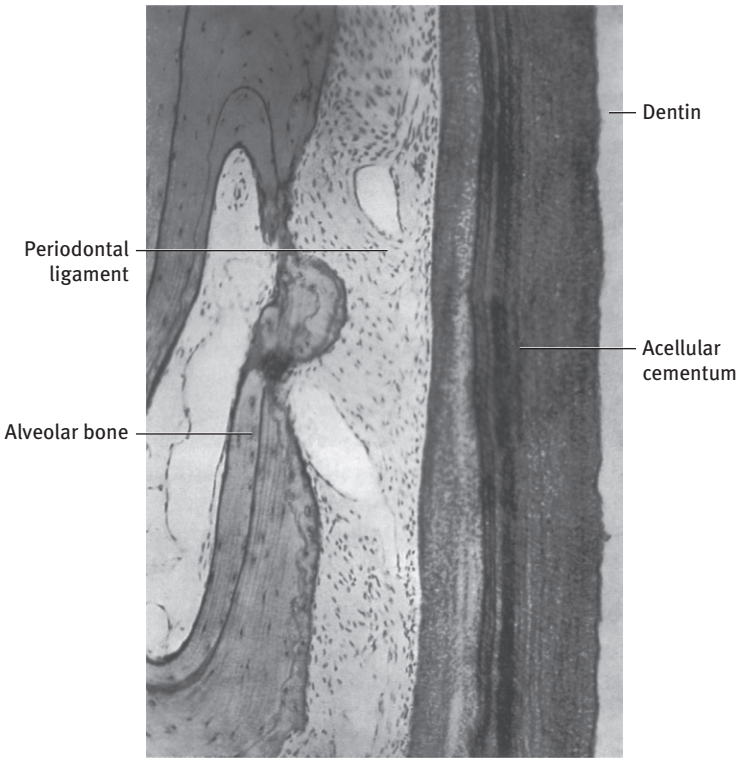


Figure 7.10 Incremental lines in acellular cementum.

ground sections (100 microns) are not seen in semi thin sections (1–2 microns).

Also ultrastructural observations by different electron microscopic methods could not explain any variations in structure that could be attributed to the incremental lines observed in ground sections.

Histochemical studies indicate that incremental lines are highly mineralized areas with less collagen and more ground substance than other portions of the cementum. Incremental lines counted in ground sections using differential interference microscope are of value in age estimation.

The location of acellular and cellular cementum is not definite. As a general rule, however, acellular cementum usually predominates on the coronal half of the root, whereas cellular cementum is more frequent on the

apical half. Layers of acellular and cellular cementum may alternate in almost any pattern. Acellular cementum may occasionally be found on the surface of cellular cementum (Fig. 7.9). Cellular cementum is frequently formed on the surface of acellular cementum (Fig. 7.9), but it may comprise the entire thickness of apical cementum (Fig. 7.11). It is always thickest around the apex and, by its growth, contributes to the length of the root (Fig. 7.12).

Extensive variations in the surface topography of cementum can be observed with the scanning electron microscope. Resting cemental surfaces, where mineralization is more or less complete, exhibit low, rounded projections corresponding to the centers of Sharpey's fibers. Cemental surfaces with actively mineralizing fronts have numerous small openings that correspond to sites where individual Sharpey's fibers enter the tooth (Fig. 7.13). These openings represent unmineralized cores of the fibers. Numerous resorption bays and irregular ridges of cellular cementum are also frequently observed on root surfaces (Fig. 7.14). The Sharpey's fibers in acellular cementum are fully mineralized, whereas in cellular cementum are generally mineralized partially around the periphery of the fibers.

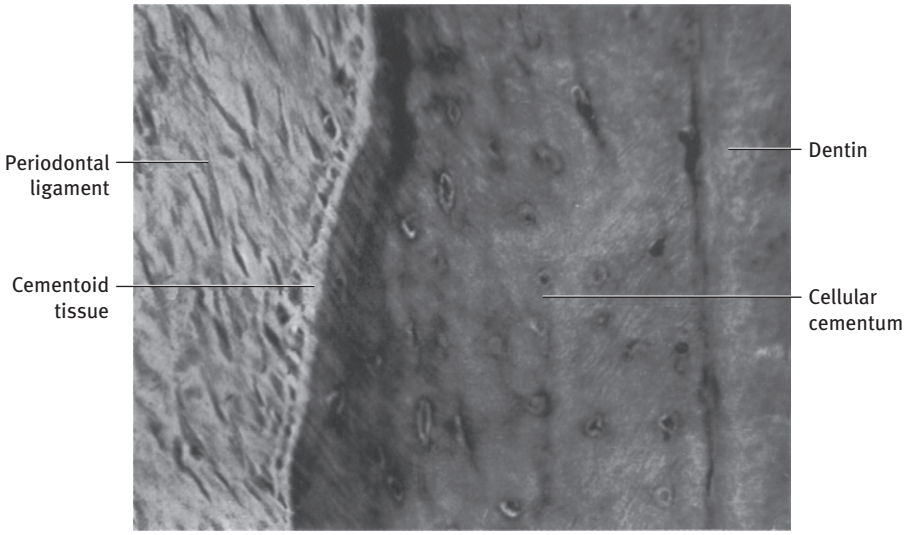


Figure 7.11 Cellular cementum forming entire thickness of apical cementum (From Orban B: Dental histology and embryology, Philadelphia, 1929, P Blakiston's Son & Co).

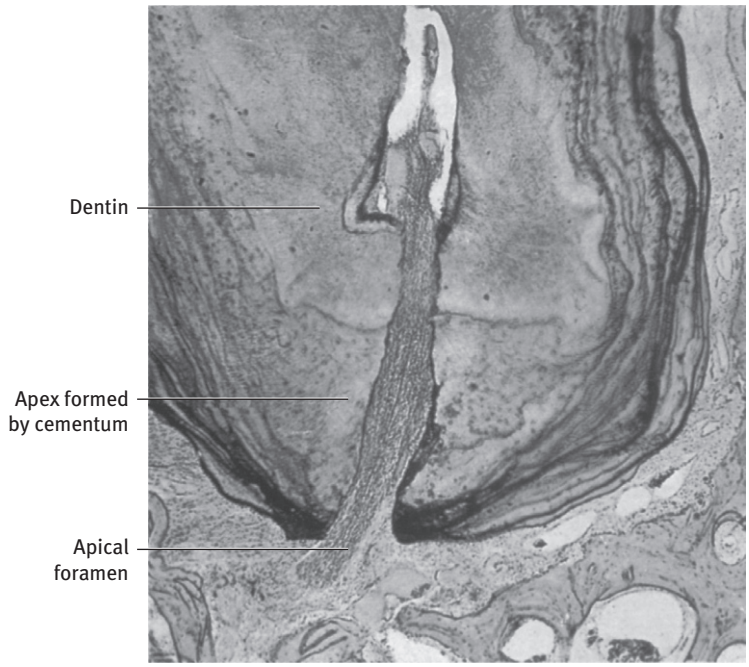


Figure 7.12 Cementum thickest at apex, contributing to length of root.

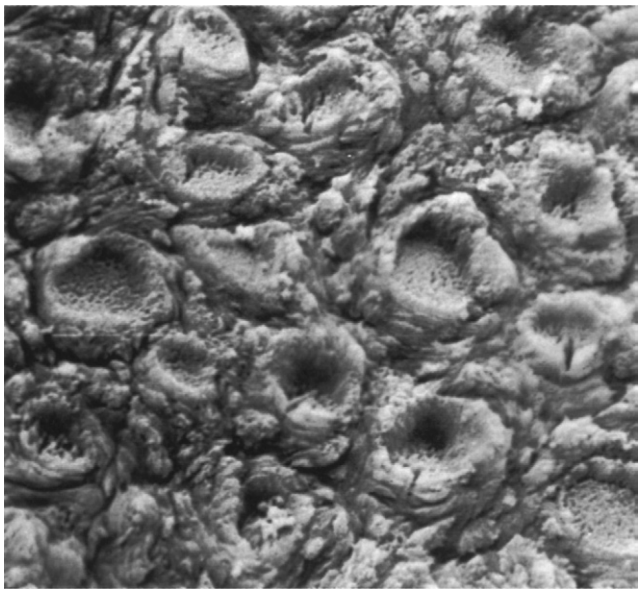


Figure 7.13 Scanning electron micrograph of cemental surface of human molar with actively mineralizing front. Peripheral portions of Sharpey's fibers are more mineralized than their centers (An organic preparation; approximately ×1500) (From Jones SJ and Boyde A: Z Zellforsch 130:318, 1972).

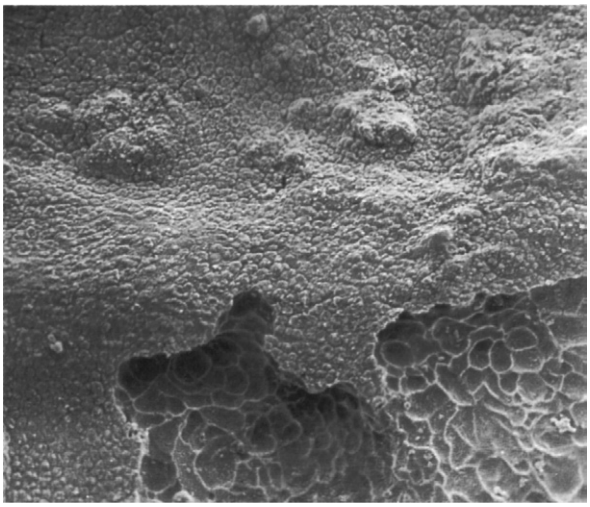


Figure 7.14 Scanning electron micrograph of cemental surface of human molar showing numerous projections of Sharpey's fibers. Note large multiloculate resorption bay at bottom of field (An organic preparation; ×250) (From Jones SJ and Boyde A: Z Zellforsch 130:318, 1972).

CEMENTODENTINAL JUNCTION

The dentin surface upon which cementum is deposited is relatively smooth in permanent teeth. The cementodentinal junction in deciduous teeth, however, is sometimes scalloped. The attachment of cementum to dentin in either case is quite firm, although the nature of this attachment is not fully understood.

The interface between cementum and dentin is clearly visible in decalcified and stained histologic sections using the light microscope (Figs. 7.9 and 7.10). In such preparations cementum usually stains more intensely than does dentin. When observed with the electron microscope, the cementodentinal junction is not as distinct as when observed with the light microscope. A narrow interface zone between the two tissues, however, can be detected with the electron microscope. In decalcified preparations, cementum is more electron dense than dentin, and some of its collagen fibrils are arranged in relatively distinct bundles, whereas those of dentin are arranged somewhat haphazardly (Fig. 7.7). The CD junction is a wide zone containing large quantities of collagen associated with glycosaminoglycans like chondroitin sulfate and dermatan sulfate resulting in increased water content which contributes to the stiffness. This reduction in its mechanical property, helps it to redistribute occlusal loads to the alveolar bone.

The cemental fibers intermingle with the dentinal fibers at the CD junction more in cellular cementum than in acellular cementum and aids in attachment of cementum to the dentin but the presence of proteoglycans is the major factor in this attachment.

Since collagen fibrils of cementum and dentin intertwine at their interface in a very complex fashion, it is not possible to precisely determine which fibrils are of dentinal and which are of cemental origin.

Sometimes dentin is separated from cementum by a zone known as the *intermediate cementum layer*, which does not exhibit characteristic features of either dentin or cementum (Fig. 7.15). As it appears hyaline (structureless), it

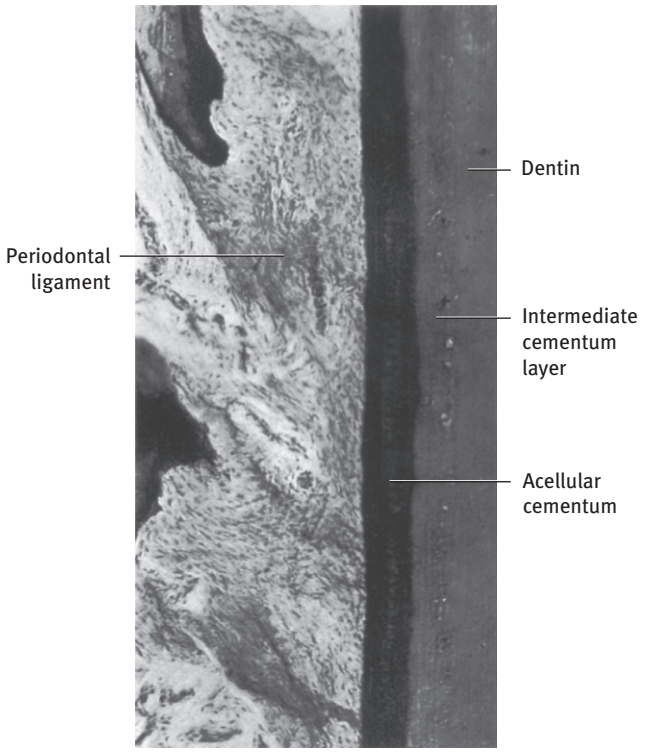


Figure 7.15 Intermediate layer of cementum. Scanning electron micrograph of cemental surface of human molar with actively mineralizing front. Peripheral portions of Sharpey's fibers are more mineralized than their centers (An organic preparation; approximately ×1500) (From Jones SJ and Boyde A: Z Zellforsch 130:318, 1972).

is also known as hyaline layer. This layer is predominately seen in the apical two thirds of roots of molars and premolars and is only rarely observed in incisors or deciduous teeth. It is believed that this layer represents areas where cells of Hertwig's epithelial sheath become trapped in a rapidly deposited dentin or cementum matrix. The exact nature of intermediate layer is a subject of controversy. The intermediate cementum layer is considered to be of dentinal origin. It contains no tubules but wide spaces which are thought to be enlarged terminals of dentinal tubules. It appears very similar to aprismatic enamel, as it is an amorphous layer of noncollagenous material devoid of odontoblasts and cementoblasts. Sometimes it is a continuous layer. Sometimes it is found only in isolated areas. The probable function might be to seal the sensitive root dentin.

CEMENTOENAMEL JUNCTION

The relation between cementum and enamel at the cervical region of teeth is variable. In approximately 30% of all teeth, cementum meets the cervical end of enamel in a relatively sharp line (Fig. 7.16A). In about 10% of the teeth, enamel and cementum do not meet. Presumably this occurs when enamel epithelium in the cervical portion of the root is delayed in its separation from dentin. In such cases there is no cementoenamel junction. Instead, a zone of the root is devoid of cementum and is, for a time, covered by reduced enamel epithelium.

In approximately 60% of the teeth, cementum overlaps the cervical end of enamel for a short distance (Fig. 7.16B). This occurs when the enamel epithelium degenerates at its cervical termination, permitting connective tissue to come in direct contact with the enamel surface. Electron microscopic evidence indicates that when connective tissue cells, probably cementoblasts, come in contact with enamel they produce a laminated, electron-dense, reticular material termed afibrillar cementum. Afibrillar cementum is so named because it does not possess collagen fibrils with a 64 nm (640 Å) periodicity. If such afibrillar cementum remains in contact with connective tissue cells for a long enough time, fibrillar cementum with characteristic collagen fibrils may subsequently be deposited on its surface; thus the thickness of cementum that overlies enamel increases.

Recent observations by optical microscope showed the presence of the fourth type of CE junction; the enamel overlapping the cementum.

In deciduous teeth the types of CE junction was different from that of permanent teeth in that enamel and cementum meeting edge to edge was the most common type, followed by cementum overlapping the enamel. Enamel overlapping cementum and failure of enamel and cementum to meet were observed rarely. The recent scanning electron microscopic studies have shown that CEJ may exhibit all of these patterns in teeth of an individual.

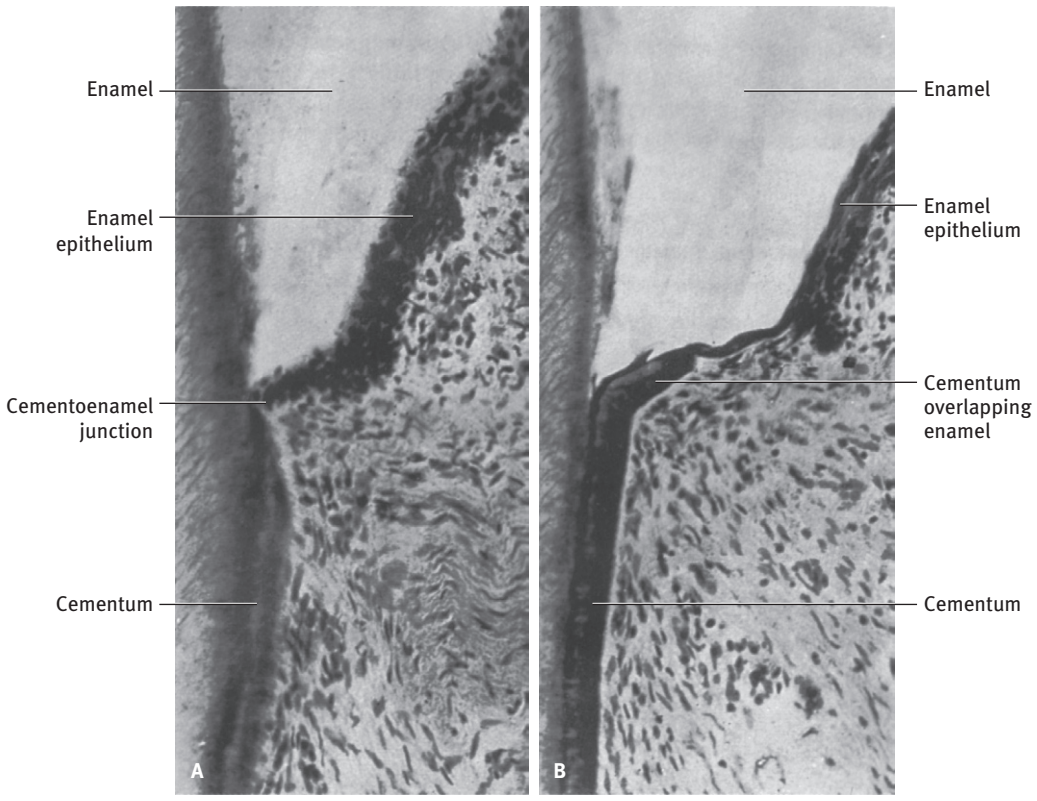


Figure 7.16 Variations at cementoenamel junction. (**A**) Cementum and enamel meet in sharp line. (**B**) Cementum overlaps enamel.

FUNCTIONS

Though different functions have been attributed to the different types of cementum, it should be understood that the cementum functions as a single unit. The functions of cementum, however can be described under the following headings.

Anchorage

The primary function of cementum is to furnish a medium for the attachment of collagen fibers that bind the tooth to alveolar bone. Since collagen fibers of the periodontal ligament cannot be incorporated into dentin, a connective tissue attachment to the tooth is impossible without cementum. This is dramatically demonstrated in some cases of hypophosphatasia, a rare hereditary disease in which loosening and premature loss of anterior deciduous teeth occurs. The exfoliated teeth are characterized by an almost total absence of cementum.

Adaptation

Cementum may also be viewed as the tissue that makes functional adaptation of teeth possible. For example, deposition of cementum in an apical area can compensate for loss of tooth substance from occlusal wear. The continuous deposition of cementum is of considerable functional importance. In contrast to the alternating resorption and new formation of bone, cementum is not resorbed under normal conditions. As the most superficial layer of cementum ages, a new layer of cementum must be deposited to keep the attachment apparatus intact.

Repair

Cementum serves as the major reparative tissue for root surfaces. Damage to roots such as fractures and resorptions can be repaired by the deposition of new cementum. Cementum formed during repair resembles cellular cementum because it forms faster but it has a wider cementoid zone and the apatite crystals are smaller. If the repair takes place slowly, it cannot be differentiated from primary cementum.

HYPERCEMENTOSIS

Hypercementosis is an abnormal thickening of cementum. It may be diffuse or circumscribed. It may affect all teeth of the dentition, be confined to a single tooth, or even affect only parts of one tooth.

In localized hypertrophy a spur or prong like extension of cementum may be formed (Fig. 7.17). This condition frequently is found in teeth that are exposed to great stress. The prong like extensions of cementum provides a larger surface area for the attaching fibers; thus a firmer anchorage of the tooth to the surrounding alveolar bone is assured.

Localized hypercementosis may sometimes be observed in areas in which enamel drops have developed on the dentin. The hyperplastic cementum covering the enamel drops (Fig. 7.18) occasionally is irregular and sometimes contains round bodies that may be calcified epithelial rests. The same type of embedded calcified round bodies frequently are found in localized areas of hyperplastic cementum (Fig. 7.19). Such knob like projections are designated as *excementoses*. They too develop around degenerated epithelial rests.

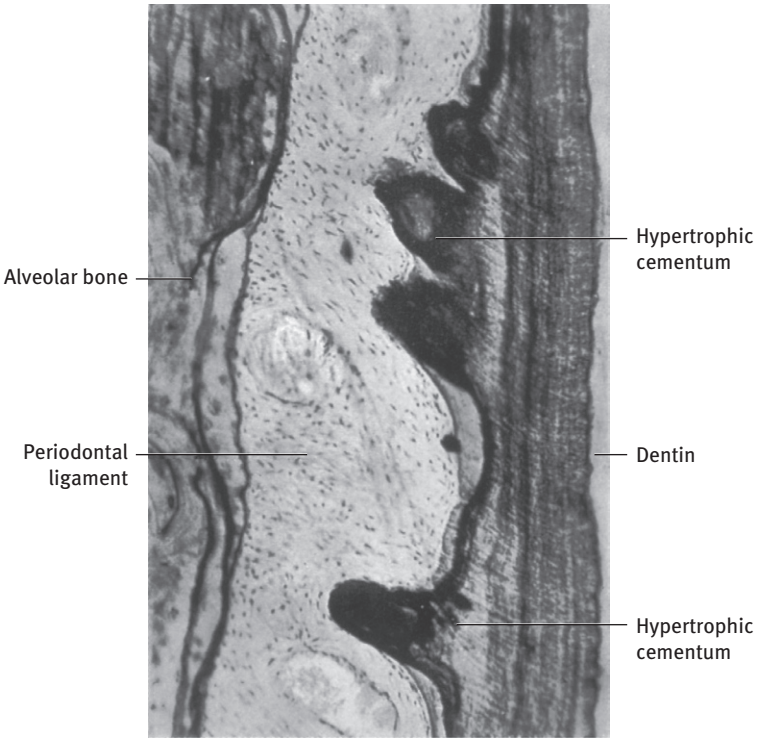


Figure 7.17 Prong like excementoses.

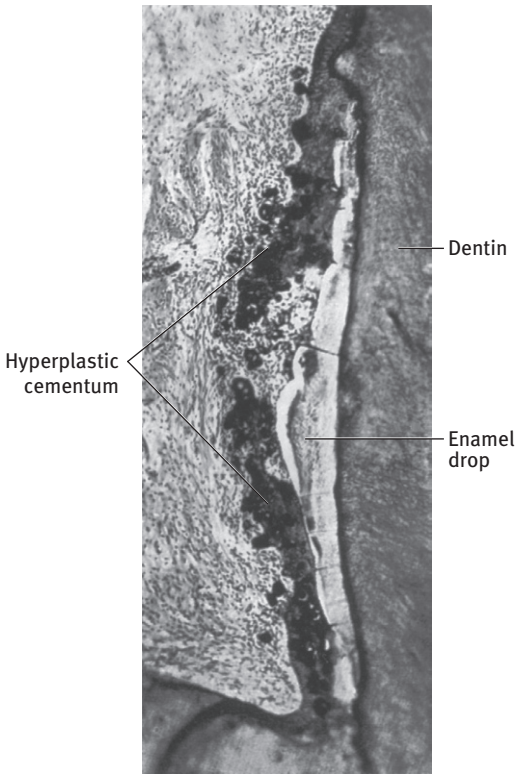


Figure 7.18 Irregular hyperplasia of cementum on surface of enamel drop.

Extensive deposition of cementum is occasionally associated with chronic periapical inflammation. The excessive deposition is circumscribed and surrounds the root like a cuff.

A thickening of cementum is often observed on teeth that are not in function. It may extend around the entire root of the nonfunctioning teeth or may be localized in small areas. Hypercementosis of cementum in nonfunctioning teeth is characterized by a reduction in the number of Sharpey's fibers embedded in the root.

The cementum is thicker around the apex of all teeth and in the furcation of multirooted teeth than on other areas of the root. This thickening is found in embedded and in newly erupted teeth.

In some cases an irregular overgrowth of cementum can be found, with spike like extensions and calcification of Sharpey's fibers and accompanied by numerous cementicles. This type of hypercementosis can occasionally be observed on many teeth of the same dentition and is, at least in some cases, the sequela of injuries to the cementum (Fig. 7.20).

Cemental hyperplasia can reach almost three fold to the range of 200–215 μm thickness with progressing age. Hypercementosis is associated with a large number of neoplastic and non-neoplastic diseases. Generalized thickening is seen in Paget's disease. Localized forms can be seen in benign cementoblastoma, florid cemento-osseous dysplasia, acromegaly, calcinosis and some forms of arthritis.

Hypoplasia or aplasia of cementum is of rare occurrence. It is associated with hypophosphatasia.

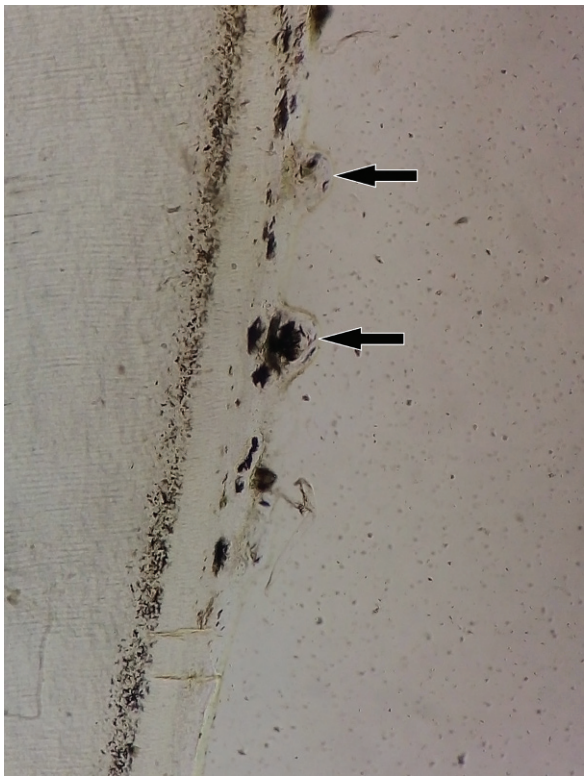


Figure 7.19 Ground section of tooth showing excementoses (arrowed). Excementoses are knob like projections of cementum.

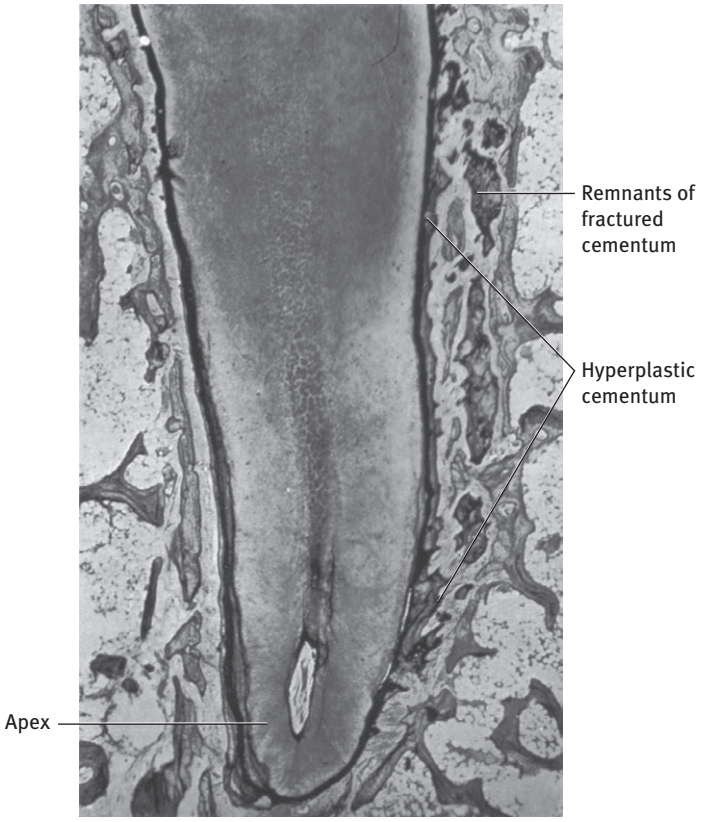


Figure 7.20 Extensive spike like hyperplasia of cementum formed during healing of cemental tear.

CLINICAL CONSIDERATIONS

Cementum is more resistant to resorption than bone, and it is for this reason that orthodontic tooth movement is possible. When a tooth is moved by means of an orthodontic appliance, bone is resorbed on the side of the pressure, and new bone is formed on the side of tension. On the side toward which the tooth is moved, pressure is equal on the surfaces of bone and cementum. Resorption of bone as well as of cementum may be anticipated. However, in careful orthodontic treatment, cementum resorption is minimal or absent, but bone resorption leads to tooth migration.

The difference in the resistance of bone and cementum to pressure may be caused by the fact that bone is richly vascularized, whereas cementum is avascular. Thus degenerative processes are much more easily effected by interference with circulation in bone, whereas cementum with its slow metabolism (as in other avascular tissues) is not damaged by a pressure equal to that exerted on bone.

Cementum resorption can occur after trauma or excessive occlusal forces. In severe cases cementum resorption may continue into the dentin. After resorption has ceased, the damage usually is repaired, either by formation of acellular (Fig. 7.21A) or cellular (Fig. 7.21B) cementum or by alternate formation of both (Fig. 7.21C). In most cases of repair there is a tendency to re-establish the former outline of the root surface. This is called *anatomic repair*. However, if only a thin layer of cementum is deposited on the surface of a deep resorption, the root outline is not reconstructed, and a bay like recess remains. In such areas sometimes the periodontal space is restored to its normal width by formation of a bony projection so that a proper functional relationship will result. The outline of the alveolar bone in these cases follows that of the root surface (Fig. 7.22). In contrast to anatomic repair, this change is called *functional repair*.

If teeth are subjected to a severe blow, fragments of cementum may be severed from the dentin. The tear occurs frequently at the cementodentinal junction, but it may also be in the cementum or dentin.

Transverse fractures of the root may occur after trauma, and these may heal by formation of new cementum.

Frequently, hypercementosis of cementum is secondary to periapical inflammation or extensive occlusal stress. This is of practical significance because the extraction of such teeth may necessitate the removal of bone. This also applies to extensive excementoses, as shown in Figure 7.19. They can anchor the tooth so tightly to the socket that the jaw or parts of it may be fractured in an attempt to extract the tooth. This possibility indicates the necessity for taking radiographs before any extraction. Small fragments of roots left in the jaw after extraction of teeth may be surrounded by cementum and remain in the jaw without causing any disturbance.

In periodontal pockets, plaque and its by-products can cause numerous alterations in the physical, chemical, and structural characteristics of cementum. The surface of pathologically exposed cementum becomes hypermineralized because of the incorporation of calcium, phosphorus, and fluoride from the oral environment. At the light-microscopic level no major structural changes occur on the surface of exposed cementum. However, at the ultrastructural level there is a loss or decrease in the cross-striations of collagen near the surface (Fig. 7.23). Endotoxin originating from plaque can be recovered from exposed cementum, but it is not known if the distribution of the cementum-bound endotoxin is limited to the cemental surface (adsorbed) or if it penetrates into deeper portions of the root (absorbed). However a recent study showed that the lipopolysaccharide produced by the bacteria was confined only to the superficial layers and was associated with microbial colonization.

Alterations in exposed cementum are of particular interest to the periodontal therapist since it is believed that they may interfere with healing during periodontal therapy. Consequently, in periodontal therapy, various procedures (mechanical and chemical) have been proposed that are intended to remove this altered cemental surface, so that periodontal regeneration may occur. Commercially available enamel matrix related proteins were

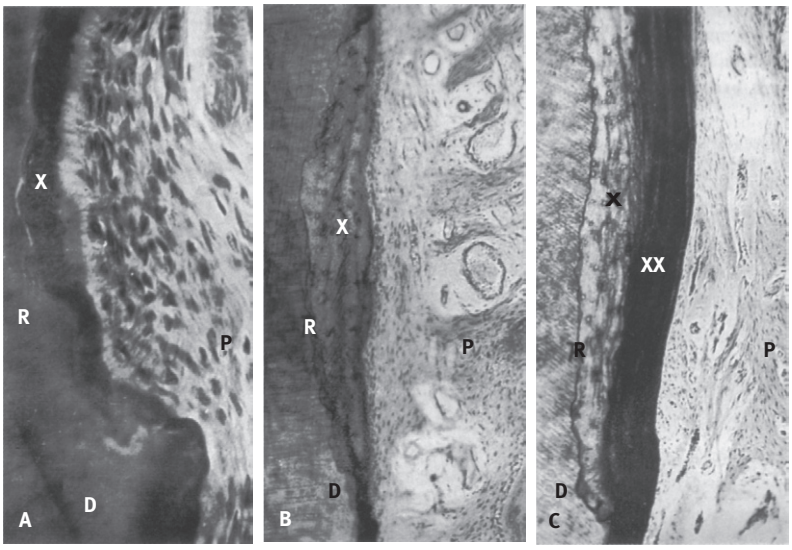


Figure 7.21 Repair of resorbed cementum. (**A**) Repair by acellular cementum, (X). (**B**) Repair by cellular cementum, (X). (**C**) Repair first by cellular, (X), and later by acellular, (XX), cementum. D, Dentin. R, Line of resorption. P, Periodontal ligament.

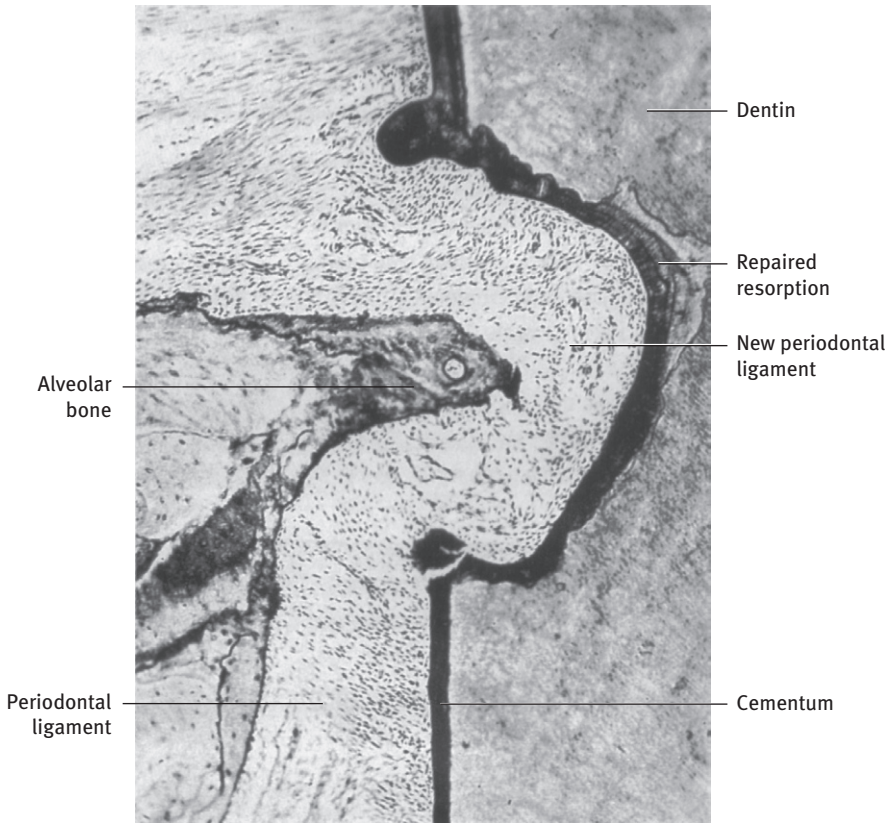


Figure 7.22 Functional repair of cementum resorption by bone apposition. Normal width of periodontal ligament re-established.

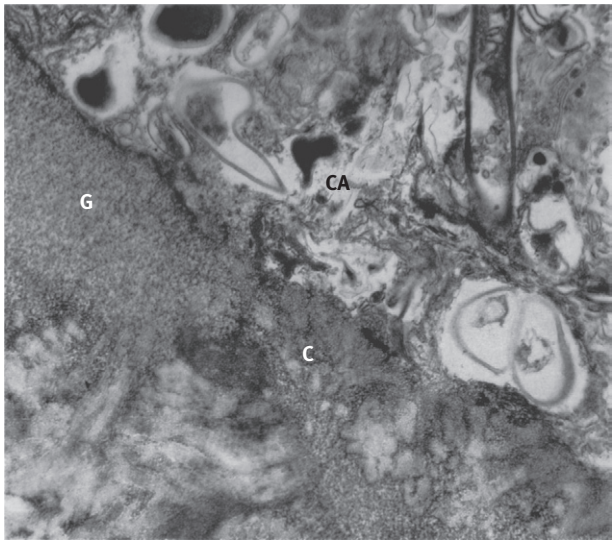


Figure 7.23 Electron micrograph of exposed cemental surface from tooth with periodontal disease. Collagen fibrils at cemental surface (C) have lost their cross-striations or have been replaced by finely granular electron dense material (G). Cell envelopes of bacteria can be observed in calculus (CA) on cemental surface (Decalcified specimen; ×26,000) (From Armitage GC: Periodont Abs 25:60, 1977).

coated on cleaned and planed root surfaces to help in new cementum deposition and regeneration of periodontal ligament. However, the newly formed cementum is not acellular extrinsic fiber type, but it is cellular, has less fibers per unit area and its bonding with the existing cementum is weak. The basis for this therapy is that the enamel matrix proteins formed by HERS, will trigger differentiation of cementoblasts producing AEFC.

Since bone and cementum have similarities in formation, in the factors controlling the growth and their composition, some diseases affecting bone like Paget's disease and hypophosphatasia affect cementum as well.

Variation in thickness of cementum was observed in diabetics. It was found to be thicker in type 2 diabetes than in normal subjects.

Abnormal cemental deposition can sometimes lead to fusion of bone and cementum. This is called ankylosis of the tooth. These teeth will not show post eruptive movements.

Cemental caries can be seen on exposed surfaces of cementum associated with gingival recession of older individuals.

Teeth are one of the most resistant tissues to degrade and degenerate, hence counting of the incremental lines, in cementum for determination of age of an individual is an accepted procedure in forensic dentistry.

SUMMARY

Cementum is a specialized, mineralized, avascular connective tissue covering the anatomical roots of human teeth. It is continuous with the periodontal ligament on its outer surface and to dentin on its deep surface. Cementum is pale yellow in color and softer and more permeable than dentin. The thickness of cementum is approximately 10–15 μm cervically and 200 μm apically.

Inorganic and Organic Constituents

The inorganic component is made up of 45–50% by volume and is in the form of hydroxyapatite crystals. The organic matrix is approximately 33% followed by water which is approximately 22%. The organic matrix is made up of type I collagen, followed by type III, V, VI and XII in small quantities. Several noncollagenous proteins like bone sialoprotein, osteopontin, dentin sialoprotein, fibronectin, tenascin are also present. Cementum derived attachment protein, an adhesion molecule, is present only in cementum.

Cementogenesis

All the types of cementum are produced by cementoblasts which are derived either from HERS or from the dental follicle. Those derived from HERS have a different phenotype than those derived from dental follicle whose phenotype is similar to osteoblasts. Cementum formation is preceded by the formation of the HERS followed by deposition of dentin along the inner aspect of this sheath. Later, fenestrations occur in the root sheath, cementoblast derived from HERS come in contact with dentin and lay down ground substance and collagen fibrils forming acellular cementum. Cellular cementum is formed later by cementoblasts which differentiate from the dental follicle. It is deposited on acellular cementum. Cellular cementum is formed rapidly resulting in some cells getting incorporated into it. These entrapped cells are the cementocytes. On the surface of the cementum an uncalcified layer of cementoid is seen. Mineralization of cementum like other hard tissues is a highly ordered event. Some remaining HERS cells migrate towards the follicle and become the cell rests of Malassez.

Cementoblast's differentiation is regulated by BMPs, cbfa1, prostaglandins, osteopontin and BSP, FGF, IGF and PDGF. The factors effecting the mineralization of cementum are osteocalcin, osteopontin, BSP, proteoglycans, alkaline phosphatase and IGF.

Structure (Types of Cementum)

Structurally cementum are classified based on the presence of cementocytes—cellular cementum or its absence—acellular cementum. The extrinsic fibers are the Sharpey's fibers which are the embedded portions of the principal fibers of the periodontal ligament. Fibers produced by cementoblasts are the intrinsic fibers. Based on the presence and absence of fibers and their origin, cementum are classified as extrinsic fiber cementum, intrinsic fiber cementum, mixed fiber cementum (containing both types of fibers), afibrillar cementum and mixed stratified cementum. Combining these two features of cells and fibers, the different types of cementum are acellular afibrillar cementum, acellular intrinsic fiber cementum, acellular extrinsic fiber cementum, cellular intrinsic fiber cementum, acellular and cellular mixed fiber cementum and cellular mixed stratified cementum (containing alternate layers of acellular extrinsic fiber and cellular intrinsic fiber cementum). The acellular extrinsic fiber cementum is known as primary cementum and is seen from cervical margin to apical third.

Cementocytes

The cementocytes are spider like cells and are similar to osteocytes in that they lie in a lacuna and have canaliculi, but unlike it the canaliculi are directed towards periodontal ligament. The cementocytes are mainly seen in apical third and are viable only near the surface.

Cementodentinal Junction

The apical area where cementum joins the internal root canal dentin is known as cementodentinal junction. This junction is smooth in permanent teeth, but sometimes scalloped in deciduous teeth. Sometimes, dentin is separated from cementum by an intermediate cementum layer. This layer has no characteristics either of cementum or dentin.

Incremental Lines

The incremental lines in cementum are called the lines of Salter. These lines are far apart in cellular cementum, and closely placed in acellular cementum.

Cementoenamel Junction

There are three basic types of cementoenamel junction. Cementum meets enamel edge to edge in 30% of the cases, Cementum overlaps enamel in 60% of the cases, and in 10% cementum and enamel do not meet. A fourth type has been identified recently, where enamel overlaps cementum.

Functions

The main function of cementum is to provide a medium for attachment of periodontal ligament fibers. Continuous formation of cementum helps to maintain the width of periodontal ligament

Differences between Cementum and Bone

Though cementum resembles bone there are a number of features which differentiate it from bone. Cementum is avascular. It shows resistance to resorption than bone, thus making orthodontic movement possible. It is deposited slowly and continuously throughout life and does not undergo remodeling. It does not have a lamellar appearance or marrow spaces histologically. It is not innervated. Cementum derived attachment protein (CAP) promotes attachment of mesenchymal cells to extracellular matrix, and this may be a marker to differentiate bone and cementum.

Repair of Cementum

Injury to cementum results in repair, one in which cementum forms to establish the former root outline called anatomical repair or in functional repair where cementum formation is less but bony projections compensate.

Hypercementosis

Excessive formation of cementum is called hypercementosis. It can be localized or generalized and it occurs due to physiological causes like in accelerated eruption of teeth or pathological causes like secondary to chronic periapical infections, Paget's disease.

Rarely reduction in the amount of cementum as seen in hypophosphatasia occurs.

REVIEW QUESTIONS

1. Describe the organic part of cementum.
2. What is the role of Hertwig's epithelial root sheath in the development of cementum?
3. What are the factors regulating cementogenesis?
4. Describe the types of cementoenamel junction.
5. What is the basis of classification of cementum? What are the different types of cementum?
6. Tabulate differences between acellular and cellular cementum.
7. Tabulate differences between acelluar extrinsic fiber cementum and cellular intrinsic fiber cementum.
8. Describe the cementocytes. How do they differ from osteocytes?
9. What is hypercementosis? What are the different types? Describe the causes of hypercementosis.
10. What are extrinsic fibers and intrinsic fibers in cementum?
11. Describe the cementodentinal junction.
12. How does cementum differ from bone?
13. What is functional repair and anatomical repair?

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Periodontal Ligament

8

CHAPTER CONTENTS

The periodontium is a connective tissue organ, covered by epithelium that attaches the teeth to the bones of the jaws and provides a continually adapting apparatus for support of the teeth during function. It comprises of *cementum, periodontal ligament,* bone lining the tooth socket (*alveolar bone*) and that part of the gingiva facing the tooth (*dentogingival junction*). The periodontium is attached to the dentin of the root by cementum and to the bone of the jaws by alveolar bone. The periodontal ligament occupies the *periodontal space,* which is located between the cementum and the periodontal surface of the alveolar bone, and extends coronally to the most apical part of the lamina propria of the gingiva. At the apical foramen, it is continuous with the dental pulp. Collagen fibers of the periodontal ligament are embedded in cementum and alveolar bone so that the ligament provides soft-tissue continuity between the mineralized connective tissues of the periodontium (Figs 8.1 and 8.2).

Periodontal ligament ranges in width from 0.15 to 0.38 mm. It is thinnest around the middle third of the root, with an hour glass appearance. It shows a progressive decrease in thickness with age. The periodontal thickness

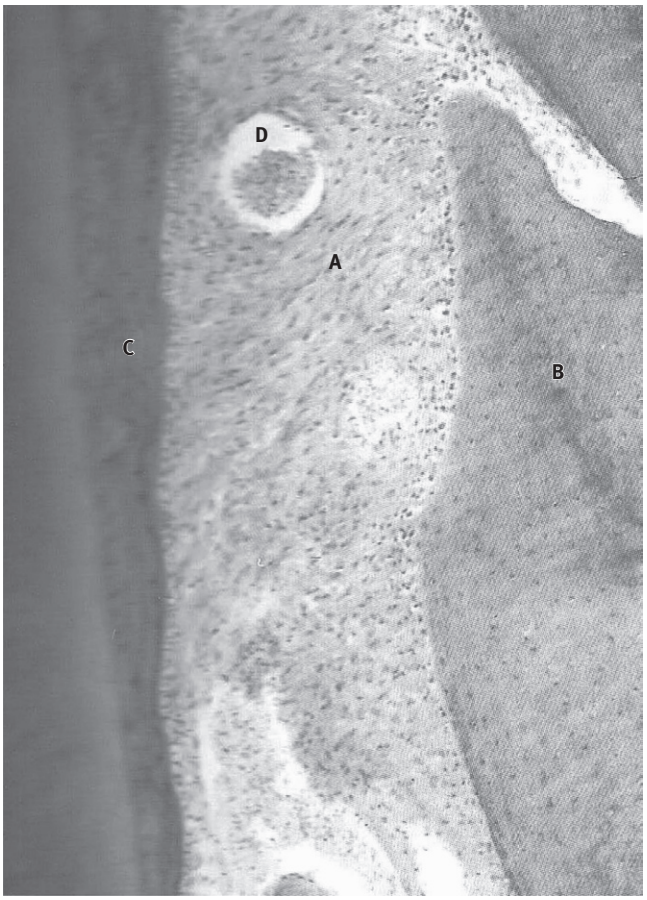

Figure 8.1 Photomicrograph of periodontal ligament (A) between alveolar bone (B) and cementum (C) with fibroblast arranged perpendicular to tooth surface in the alveolar crest region and obliquely arranged apically (D) is a blood vessel magnification (×70).

measures about 0.21 mm in the young adult, 0.18 mm in mature adult, and 0.15 mm in older adult.

The ligament appears, as the periodontal space of 0.4 to 1.5 mm on radiographs, a radiolucent area between the radiopaque lamina dura of the alveolar bone proper and the radiopaque cementum. The periodontal spaces of the permanent teeth are said to be narrower than those of deciduous teeth.

The periodontal ligament is a fibrous connective tissue that is noticeably cellular (Fig. 8.1) and contains numerous blood vessels. All connective tissues, the periodontal ligament included, comprise cells as well as extracellular matrix consisting of fibers and ground substance. The majority of the fibers of the periodontal ligament are collagen, and the matrix is composed of a variety of macromolecules, the basic constituents of which are proteins and polysaccharides. It is important to remember that the extracellular matrix is produced and can be removed by the cells of the connective tissue.

The periodontal ligament has a number of functions, which include attachment and support, nutrition, synthesis and resorption, and proprioception. Over the years it has been described by a number of terms. Among them are desmodont, gomphosis, pericementum, dental periosteum, alveolodental ligament, and periodontal membrane. 'Periodontal membrane' and 'periodontal ligament' are the terms that are most commonly used. Neither term describes the structure and its functions adequately. It is neither a typical membrane nor a typical ligament. How-ever, because it is a complex soft connective tissue providing continuity between two mineralized connective tissues, the term 'periodontal ligament' appears to be the more appropriate.

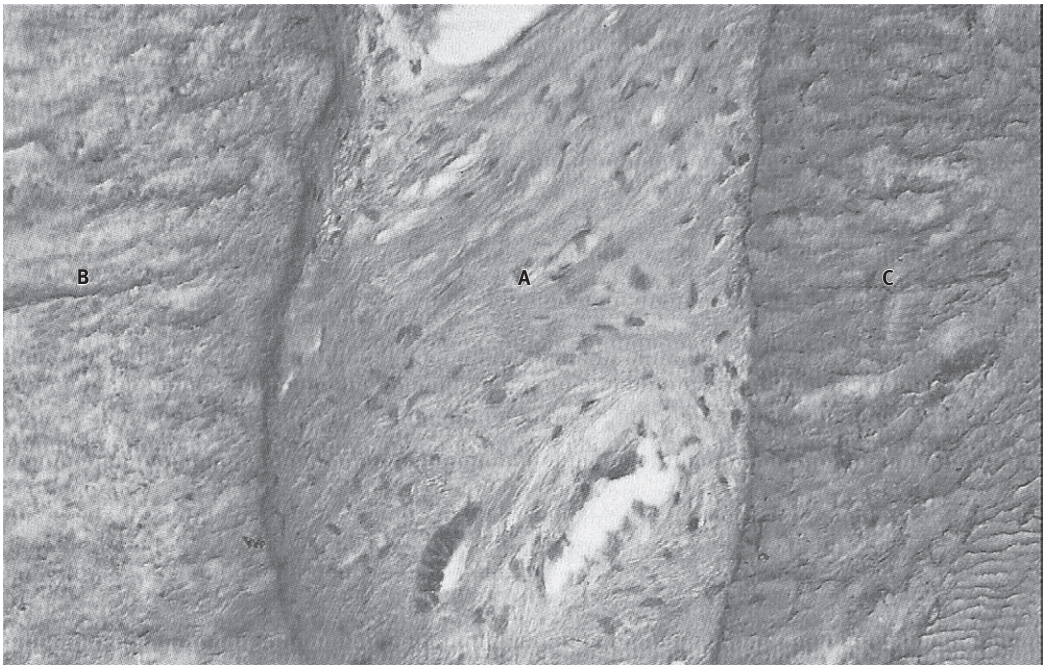

Figure 8.2 Transverse section of periodontal ligament (A) showing its relation to alveolar bone (B) and cementum (C) H&E stain (×140).

DEVELOPMENT

The development of the periodontal ligament begins with root formation prior to tooth eruption. The continuous proliferation of the internal and external enamel epithelium forms the cervical loop of the tooth bud. This sheath of epithelial cells grows apically, in the form of *Hertwig's epithelial root sheath*, between the dental papilla and the dental follicle.

At this stage, the sheath forms a circumferential structure encompassing dental papilla separating it externally from dental follicle cells. The dental follicle cells located between the alveolar bone and the epithelial root sheath are composed of two subpopulations, *mesenchymal cells* of the *dental follicle proper* and the *perifollicular mesenchyme*. The mesenchymal cells of the perifollicular mesenchyme bounded by the dental follicle proper (Fig. 8.3) and the developing alveolar bone are stellate shaped, small and randomly oriented. The cells are more widely separated compared to cells of the dental follicle proper. These cells contain a euchromatic nucleus, very little cytoplasm, that contains a small number of short cisternae of rough endoplasmic reticulum, mitochondria, free ribosomes and an inactive Golgi area. These cells also have long and thin cytoplasmic processes that connect with those from neighboring cells.

As the root formation continues, cells in the perifollicular area, gain their polarity and the cellular volume

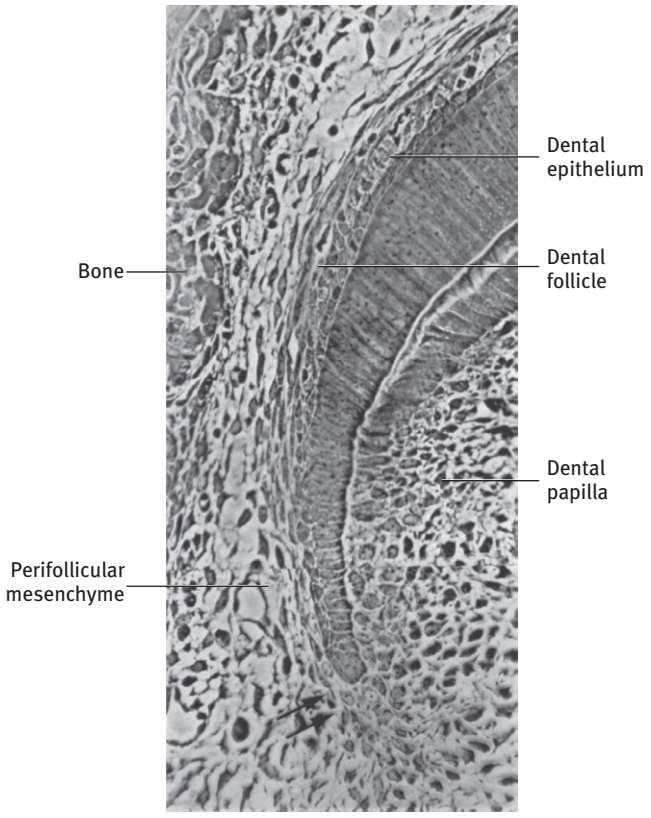

Figure 8.3 Montage phase-contrast photomicrograph of first molar tooth germ of 1-day-old mouse showing the dental follicle, which is continuous with the dental papilla around the cervical loop (arrows) (×450) (From Freeman E and Ten Cate AR: J Periodontol 42:387, 1971).

and synthetic activity increase. These cells become elongated and contain increased amounts of rough endoplasmic reticulum, mitochondria and an active Golgi complex. As a result, they actively synthesize and deposit collagen fibrils and glycoproteins in the developing periodontal ligament.

The developing periodontal ligament and mature periodontal ligament contain undifferentiated stem cells that retain the potential to differentiate into osteoblasts, cementoblasts and fibroblasts. Studies suggest that, stem cells occupy perivascular sites in the periodontal ligament and in adjacent endosteal spaces. It remains to be clarified whether, osteoblasts, cementoblasts and fibroblasts originate from a common ancestor or from a specific line of progenitor cells.

Development of the Principal Fibers

Immediately before tooth eruption, and for sometime thereafter, active fibroblasts adjacent to cementum of the coronal third of the root, appear to become aligned in an oblique direction to the long axis of the tooth. Soon thereafter, the first collagen fiber bundles of the ligament become discernible. These are the precursors of the *alveolar crest fiber bundle* group. Upon eruption of the tooth into the oral cavity, only the *alveolar crest fibers* of the periodontal ligament are discernible histologically.

Further apically, organized fiber groups are not seen. But, examination of the root surface at higher magnification, reveals fine brush like fibers extending from the cementum. Later, similar fibers are observed on the adjacent osseous surface of the developing alveolar process. Both set of fibers, cemental and alveolar, continue to elongate towards each other, ultimately to meet, intertwine and fuse, as covalent bonding and crosslinking of individual collagen molecular units occur.

By the time of first occlusal contact of the tooth with its antagonist, the principal fibers around the coronal third of the root, the *horizontal* group are almost completely developed. The *oblique fibers* in the middle third of the root are still being formed. As eruption continues, and definite occlusion is established, there is a progressive apical maturation of *oblique fiber bundles*. With the formation of the apical fiber group, the definitive periodontal ligament architecture is established. The formation of fiber bundles destined to become the principal fiber groups of the gingival ligament antecede developmentally any of the fiber groups of the periodontal ligament. Type VI collagen has been found to be absent from the ligament during the main axial eruptive phase, but is present when the tooth has fully erupted. Type XII collagen also appears only after the tooth has erupted.

Development of cells

Prior to root formation, the cells of the follicle show very few organelles. With the onset of root formation, the organelles in the cell increase, collagen and ground substance formation begins and fills the extracellular spaces. Stem cells which give rise to cementoblasts, osteoblasts and fibroblasts are seen in a perivascular location. Osteoclasts appear at the alveolar bone surface allowing remodeling of bone in association with tooth eruption.

Periodontal Ligament Collagen Fiber Attachment to the Root Surface

Prior to the onset of cementogenesis, the dental follicle proper cells nearest to the root sheath are aligned parallel to the epithelial cells. Collagen bundles that lie parallel to the root sheath are partly enveloped in cytoplasmic grooves formed by dental follicle proper cells. Cytoplasmic microtubules and collagen secretory granules are oriented in the same direction as the extracellular collagen fibers. With onset of disruption of the root sheath, dental follicle proper cells assume an elongated profile with polarity towards the dentin matrix. The cells appear to move towards the dentin in the spaces created by disruption of the root sheath. During shifting of dental follicle proper cells, the collagen bundles that were initially parallel to the root sheath are reorganized, so that they come to lie in the lateral intercellular spaces between the dental follicle proper cells, oriented perpendicular to the root surface. Collagen fibrils secreted from these leading edge processes intermingle with the dentin matrix collagen.

PERIODONTAL LIGAMENT HOMEOSTASIS

A remarkable capacity of the periodontal ligament is that it maintains its width more or less overtime despite the fact, that it is squeezed in between two hard tissues.

Studies indicate that the population of cells, within the periodontal ligament, both during development and regeneration, secrete molecules that can regulate the extent of mineralization and prevent the fusion of tooth root with surrounding bone (ankylosis).

Various molecules have been proposed, which play a role in maintaining an unmineralized periodontal ligament.

Msx2 prevents the osteogenic differentiation of periodontal ligament fibroblasts, by repressing *Runx2* (*runt related transcription factor 2*), also known as cbfa1 (*core binding factor alpha 1*) transcriptional activity.

The balance between the activities of *bone sialoprotein* and *osteopontin* also contributes towards maintaining an unmineralized periodontal ligament region.

Matrix 'Gla' protein, an inhibitor of mineralization, is also present in the periodontal tissues. Studies suggest that, it may play a role in preserving the width of the ligament.

It has also been suggested that, *RGD-cementum attachment protein*, a collagen associated protein, may play a role in maintaining the unmineralized state of the periodontal ligament.

TGF-ß isoforms synthesized by periodontal ligament cells can induce mitogenic effects, but studies have shown that, these isoforms can also downregulate osteoblastic differentiation of periodontal ligament cells.

Prostaglandins, which are also produced by periodontal ligament cells can inhibit mineralized bone nodule formation and prevent mineralization by periodontal ligament cells *in vitro*.

The periodontal ligament also has the capacity to adapt to functional changes. When the functional demand increases, the width of the periodontal ligament can increase by as much as 50% and the fiber bundles also increase in thickness. Conversely, a reduction in function, leads to narrowing of the ligament and a decrease in number and thickness of the fiber bundles.

Since, the periodontal ligament is not made up of single strands of straight collagen fibers, but consists of a complex meshwork, remodeling does not occur at all the sites synchronously. Unlike the bulk removal of collagen that is effected by extracellular matrix metalloproteinases, collagen phagocytosis enables periodontal fibroblasts to remove collagen fibrils at specific sites.

CELLS

The principal cells of the healthy, functioning periodontal ligament are concerned with the synthesis and resorption of alveolar bone and the fibrous connective tissue of the ligament and cementum. The cells of the periodontal ligament may be divided into:

1. Synthetic cells
   Fibroblasts
   Osteoblasts
   Cementoblasts
2. Resorptive cells
   Osteoclasts
   Fibroblasts
   Cementoclasts
3. Progenitor cells
4. Epithelial rests of Malassez
5. Defense cells
   Mast cells
   Macrophages
   Eosinophils

Synthetic Cells

There are certain general cytologic criteria that distinguish all cells that are synthesizing proteins for secretion (e.g. extracellular substance of connective tissue), and these criteria can be applied equally to osteoblasts, cementoblasts, and fibroblasts. For a cell to produce protein, it must, among other activities, transcribe ribonucleic acid (RNA), synthesize ribosomes in the nucleolus and transport them to the cytoplasm, and increase its complement of rough endoplasmic reticulum (RER) and Golgi membranes for translation and transport of the protein. It must also have the means to produce an adequate supply of energy. Each of these functional activities is reflected morphologically when synthetically active tissues are viewed in the electron and light microscopes. Increased transcription of RNA and production of ribosomes is reflected by a large open-faced or vesicular nucleus containing prominent nucleoli. The development of large quantities of RER covered by ribosomes is readily recognized in the electron microscope and is reflected by hematoxyphilia of the cytoplasm when the cell is seen in the light microscope after staining with hematoxylin and eosin. The hematoxyphilia is the result of interaction of the RNA with the acid hematein in the stain. The Golgi saccules and vesicles are also readily seen in the electron microscope but are not stained by acid hematein and so, in the light microscope, they are

seen in appropriate sections as a clear, unstained area in the otherwise hematoxyphilic cytoplasm. The increased requirement for energy is reflected in the electron microscope by the presence of relatively large numbers of mitochondria. Accommodation of all these organelles in the cell requires a large amount of cytoplasm. Thus a cell that is actively secreting extracellular substance will be seen in the light microscope to exhibit a large, open-faced or vesicular nucleus with prominent nucleoli and to have abundant cytoplasm that tends to be hematoxyphilic, with, if the plane of section is favorable, a clear area representing the Golgi membranes. Cells with the morphology described above, if found at the periodontal surface of the alveolar bone, are active osteoblasts; if lying in the body of the soft connective tissue, are active fibroblasts; and, if found at cementum, are active cementoblasts. These cells all have, in addition to the features described above, the particular characteristics of osteoblasts, fibroblasts, and cementoblasts.

Synthetic cells in all stages of activity are present in the periodontal ligament, and this is reflected directly by the degree to which the characteristics described above are developed in each cell. Cells having a paucity of cytoplasm (i.e. cytoplasm that virtually cannot be distinguished in the light microscope) with very few organelles and a close-faced nucleus are also found in the ligament.

Osteoblasts

The osteoblasts covering the periodontal surface of the alveolar bone constitute a modified endosteum and not a periosteum. A periosteum can be recognized by the fact that it comprises at least two distinct layers, an inner cellular or cambium layer and an outer fibrous layer. A cellular layer, but not an outer fibrous layer, is present on the periodontal surface of the alveolar bone. The surface of the bone lining the dental socket must therefore be regarded as an interior surface of bone, akin to that lining medullary cavities, and not an external surface, which would be covered by periosteum. The surface of the bone is covered largely by osteoblasts in various stages of differentiation (Figs 8.1, 8.4) as well as by occasional osteoclasts. Collagen fibers of the ligament that penetrate the alveolar bone intervene between the cells (Fig. 8.5).

Osteoblasts are bone forming cells lining the tooth socket. These cells are cuboidal in shape, with a prominent round nucleus placed at the basal end of the cell. Rough endoplasmic reticulum, mitochondria and vesicles are abundant in active cells. The cells appear basophilic due to the presence of abundant rough endoplasmic reticulum. Golgi material appears more localized and extensive and is indicated by a pale juxtanuclear area. Microfilaments are prominent beneath the cell membrane at the secreting surface. The cells contact one another through desmosomes and tight junctions. Osteoblasts are also in contact with underlying osteocytes through cytoplasmic processes.

Fibroblast

The *fibroblast* is the predominant cell in the periodontal ligament. These fibroblasts origin in part from the ectomesenchyme of investing layer of dental papilla and from the dental follicle. These cells are different from cells in other connective tissues in a number of respects.

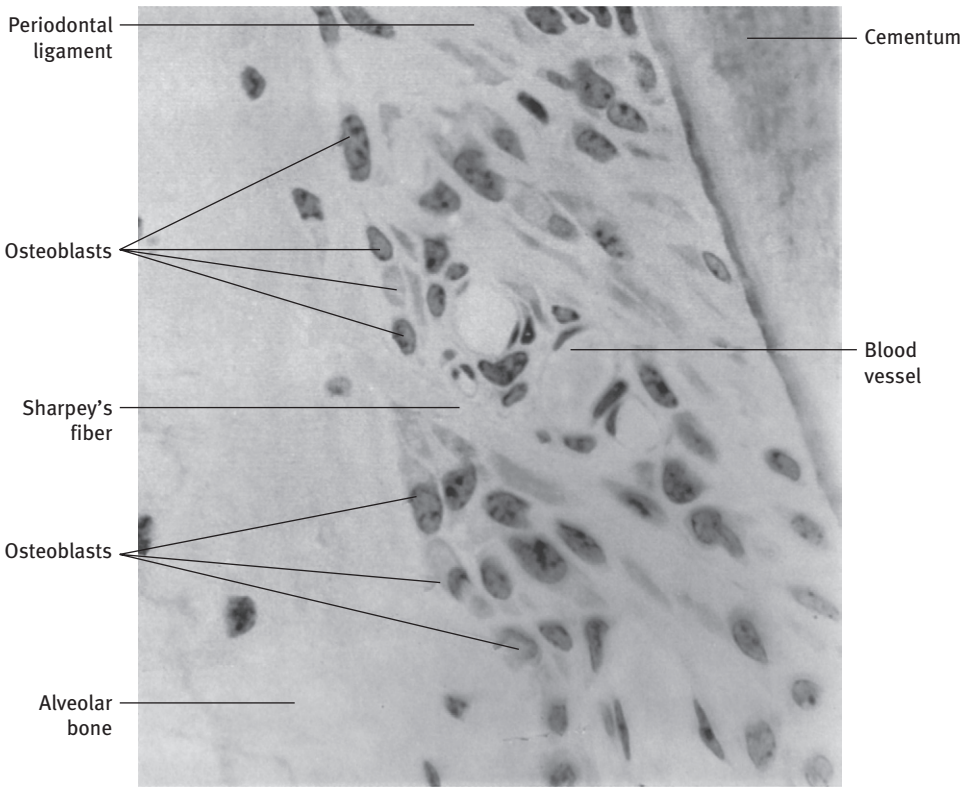

Figure 8.4 Section, 1 μm thick, of mouse molar periodontal ligament. Note osteoblasts lining periodontal surface of alveolar bone, some of which exhibit a negative image of the Golgi complex (Hematoxylin and eosin; ×1100).

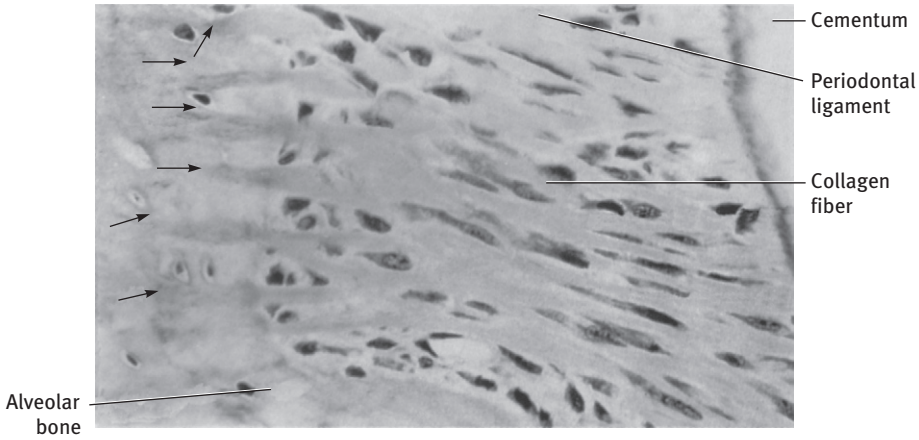

Figure 8.5 Section, 1 μm thick, of mouse molar periodontal ligament. Collagen fibers from ligament that pass between osteoblasts to penetrate alveolar bone as Sharpey's fibers are shown by arrows (Hematoxylin and eosin; ×1000).

For example, the rapid degradation of collagen by fibroblast phagocytosis is the basis for the very fast turnover of collagen in the periodontal ligament.

It is also believed that the periodontal ligament contains a variety of fibroblast cell populations with different functional characteristics. For example, fibroblasts on the bone side of the ligament show abundant alkaline phosphatase activity than those on the tooth side.

Developmental differences may also exist. It has been demonstrated that, the fibroblasts near cementum are derived from ectomesenchymal cells of the investing layer of dental papilla, while fibroblasts near alveolar bone are derived from perivascular mesenchyme.

These fibroblasts are regularly distributed throughout the ligament, and are oriented with their long axis parallel to the direction of collagen fibrils.

Fibroblasts are fusiform and arranged parallel to the tooth surface on examination of the ligament sectioned only in the longitudinal plane. If the ligament is sectioned both transversely and longitudinally, cells take the form of a flattened irregular disk.

The fibroblasts are large cells with extensive cytoplasm and abundant organelles, associated with protein synthesis and secretion. The nucleus occupies a large volume of the cell and contains one or more prominent nucleoli. During development and initial formation of the periodontal ligament, the fibroblasts appear very active with extensive network of rough endoplasmic reticulum, well developed Golgi apparatus and abundant secretory granules containing type I collagen molecules.

The cells also develop long and thin cytoplasmic extensions that form three-dimensional veils that compartmentalize collagen fibrils into fibers. These cells also have well developed cytoskeleton and show contacts of adherens and gap junction types.

At all levels of the cell, intimate connections are established between the plasma membrane and individual collagen fibrils.

Golgi complex contains several Golgi stacks, comprised of cisternae and terminal saccules (Fig. 8.6). Each Golgi stack is made up of five cisternae, about 2 μm in length, terminating at each end in an expanded saccule. Immature cisternae at the cis surface of Golgi complex are dilated. The saccules associated to these

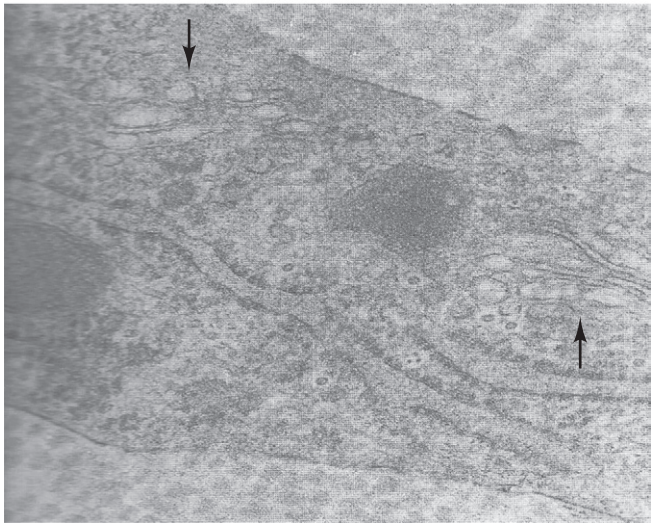

Figure 8.6 Electron micrograph showing Golgi saccules (arrow) within fibroblast. Bar = 0.75 μm.

cisternae contain fine loosely arranged filaments. The cisternae of trans-surface contain dense material and their associated saccules contain rod-like structures with globular terminal elements. These saccules are released to form presecretory granules that quickly associate to microtubules.

Proline is incorporated into collagen polypeptides in rough endoplasmic reticulum. The newly synthesized procollagen molecules are assembled inside Golgi vesicles and are secreted within secretory granules associated with microtubules.

The overlapping of procollagen molecules gives the collagen fibers their characteristic banding (Fig. 8.7).

The fibroblasts of periodontal ligament have cilia. The cilium is different from those in other cell types, as it has only nine tubule doublets. These cilia may be associated with control of the cell cycle or inhibition of centriolar activity. These cells produce growth factors and cytokines such as IGF-1, BMPs, PDGF and IL-1. TGF-ß stimulates the synthesis of collagen and inhibits the synthesis of collagenase.

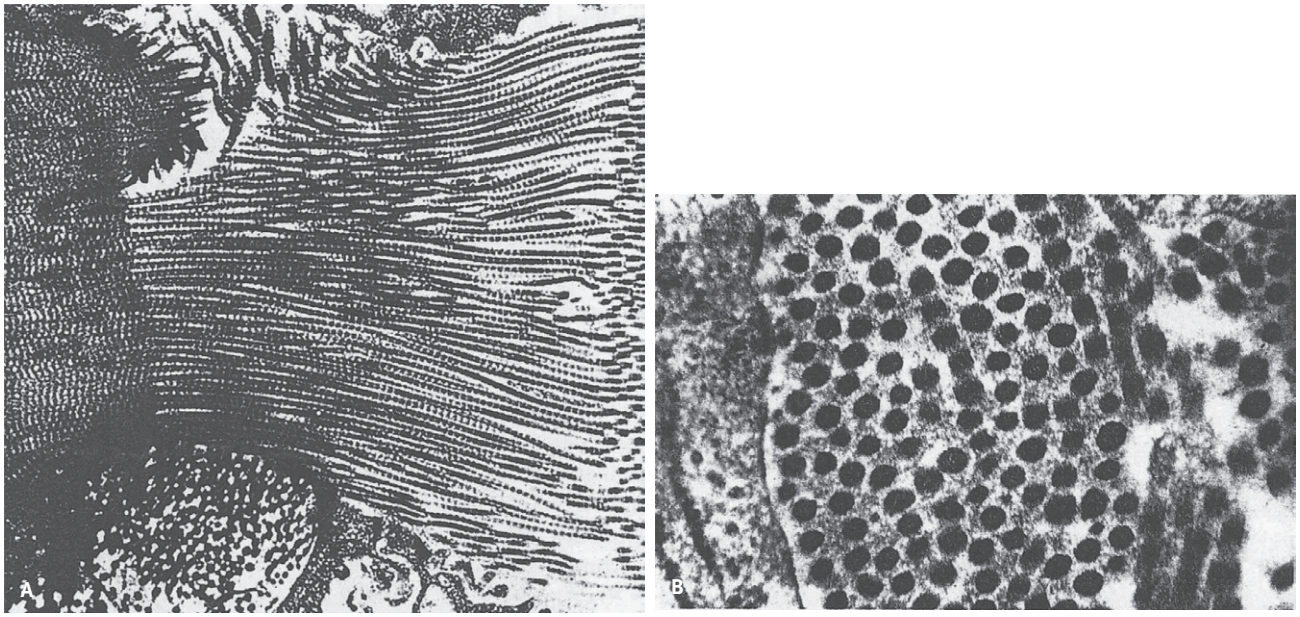

Figure 8.7 Transmission electron micrograph shows collagen fiber (**A**) in longitudinal section with characteristic banding and (**B**) in cross-section (**A** ×16,000, **B** ×100,000).

Fibroblast-matrix adhesion and traction

Fibroblasts attach to the substratum of the extracellular matrix through surface receptors for collagen and fibronectin. The cell membrane integrin α5ß1 attaches to RGD sequence (arginine-glycine-aspartic acid) of fibronectin. Fibronectin molecules can polymerize to form pericellular matrices. Assembly is initiated by binding soluble fibronectin molecules to cell surface integrin receptors (α5ß1 and α5ß3). Integrins are a collection of cell surface proteins that mediate binding of cells to extracellular matrix proteins and to one another. They consist of an α subunit that is linked noncovalently to a ß subunit. The extracellular portion of ß subunit contains arginine-glycine-aspartate (RGD) binding region near **amino terminus**.

The cytoplasmic domain of integrin receptor attaches to the peripheral cytoplasmic protein, talin, which in turn interacts with a protein called vinculin. Conformational changes in vinculin cause it to bind to actin microfilaments in the cortical cytoplasm, thereby completing a molecular bridge between the cell's contractile apparatus and fibronectin in the extracellular matrix.

With the binding of fibronectin to collagen fibrils, the molecular linkage extends from cytoplasmic contractile apparatus to extracellular collagen fiber network establishing a mechanism for exerting traction on the collagen fibers. This cell to matrix contact enables the extracellular matrix to exert an effect on cell shape and behavior. Tension in the extracellular matrix is transmitted to fibroblast integrin receptors, leading to signaling events that alter the activity of the cell. The presence of actin network also endows the periodontal ligament fibroblasts with a degree of contractility, with which it can exert tractional forces on extracellular matrix.

Functions

The role of the fibroblasts is to produce the structural connective tissue proteins, collagen and elastin, as well as proteoglycans, glycoproteins and glycosaminoglycans that comprise the periodontal ligament ground substance. These cells also secrete an active collagenase and a family of enzymes collectively known as matrix metalloproteinases (MMPs).

The fibroblasts of periodontal ligament are characterized by rapid turnover of extracellular matrix in particular collagen. The fibroblasts are responsible for the formation and remodeling of the periodontal ligament fibers, and a signaling system to maintain the width of the periodontal ligament and thickness across the soft tissue boundary defined by this ligament. The cells maintain the width, by preventing encroachment of bone and cementum into periodontal space. The fibroblasts lie within collagen fibers and their shape is governed by the surrounding matrix.

Fibroblasts of periodontal ligament generate an organizational pattern, as they have the ability to both synthesize and shape the proteins of the extracellular matrix, in which collagen fibrils form bundles, that insert into tooth and bone as Sharpey's fibers.

Differences between periodontal ligament fibroblasts and gingival fibroblasts

Periodontal ligament fibroblasts are ectomesenchymal in origin whereas gingival fibroblasts are mesodermal in origin.

Expression of alkaline phosphatase and cyclic AMP is more in periodontal ligament fibroblasts. Gingival fibroblasts are less proliferative

Periodontal ligament fibroblasts can generate a force for tooth eruption as they are motile and contractile. Fibroblasts in PDL are capable of collagen degradation also.

Cementoblasts

The distribution on the tooth surface of variously differentiated cementoblasts is similar to the distribution of osteoblasts on the bone surface. These cells line the surface of

cementum, but are not regularly arranged as osteoblasts. These cells are often indistinguishable from periodontal fibroblasts apart from their location adjacent to cementum surface.

Cementoblasts are almost cuboidal with a large vesicular nucleus, with one or more nucleoli and abundant cytoplasm. All the organelles required for protein synthesis and secretion are present. Cementoblasts have abundant mitochondria and less amounts of rough endoplasmic reticulum than periodontal ligament fibroblasts.

Cells actively depositing cellular cementum exhibit abundant basophilic cytoplasm and cytoplasmic processes and the nuclei are folded and irregularly shaped. Cells depositing acellular cementum do not have prominent cytoplasmic processes. Cell membrane shows gap junctions and desmosomes and has receptors for growth hormone and epidermal growth factor (Table 8.1).

Resorptive Cells

Osteoclasts

Osteoclasts are cells that resorb bone and tend to be large and multinucleated (Fig. 8.8) but can also be small and mononuclear. Multinucleated osteoclasts are formed by fusion of precursor cells similar to circulating monocytes. These characteristic multinucleated cells usually exhibit an eosinophilic cytoplasm and are easily recognizable. When viewed in the light microscope, the cells may sometimes appear to occupy bays in bone (Howship's lacunae) or surround the end of a bone spicule. In the electron microscope their cytoplasm is seen to exhibit numerous mitochondria and lysosomes, abundant Golgi saccules, and free ribosomes but little RER. The part of the plasma membrane lying adjacent to bone that is being resorbed is raised into characteristic folds and is termed the *ruffled* or *striated border*. The ruffled border is separated from the rest of the plasma membrane by a zone of specialized membrane that is closely applied to the bone, the underlying cytoplasm of which tends to be devoid of organelles and has been called the *clear zone* (Fig. 8.9). The area of bone that is sealed off by the ruffled border is exposed to a highly acidic pH by virtue of the active pumping of protons by the osteoclast into this environment. The bone related to the ruffled border undergoes resorption (Fig. 8.10). Osteoclasts are seen regularly in normal functioning periodontal ligament in which the cells play a part in the

Table 8.1 Synthetic Cells

Name of the Cell	Origin	Light Microscopy	Electron Microscopy	Functions
Osteoblasts - line bone surface	Primitive mesenchymal stem cells	• Cuboidal in shape, with a prominent round nucleus at the basal end of the cell	• **Active cells** –abundant RER (Hence cells basophilic), Mitochondria, vesicles, Golgi apparatus (localized, seen as juxtanuclear area)	• Formation of new bone • Regulation of bone remodeling • Mineralization of osteoid • Secretion of noncollagenous and collagenous proteins (type V collagen, osteonectin, osteopontin, RANKL, osteoprotegerin, proteoglycans, latent proteases and growth factors)
Fibroblast - predominant cell	A. Partly from ectomesenchyme of investing layer of dental papilla and dental follicle B. Fibroblasts near cementum from ectomesenchymal cells of investing layer of dental papilla C. Fibroblasts near alveolar bone from perivascular mesenchyme.	**Active fibroblasts –** large cells, extensive cytoplasm, abundant organelles, nucleus occupies large volume of cell with prominent nucleoli.	• **Active fibroblasts** – large cells with a) extensive network of RER b) well developed Golgi apparatus containing Golgi stacks, each stack made up of 5 cisternae c) Cilia – has 9 tubule doublets	• **Secrete** (a) collagen and elastin (structural connective tissue proteins) (b) proteoglycans, glycoproteins and glycosaminoglycans (ground substance) • Matrix metalloproteinases (MMPs) — enzymes • Formation and remodeling of the PDL fibers • Maintain the width of the PDL fibers and thickness of PDL • Produce growth factors and cytokines (IGF-1, BMPs, PDGF, and IL-1)
Cementoblasts - line surface of cementum	HERS or Dental follicle	• Cuboidal cells with abundant cytoplasm and a large vesicular nucleus with one or more nucleoli	• **Active cells** - All the organelles required for protein synthesis present • Cells depositing cellular cementum – abundant cytoplasmic processes • Abundant mitochondria and less amount of RER compared to PDL fibroblasts	• Lay down cementum which helps in attachment of the tooth to the alveolar bone via the collagen fibers of PDL

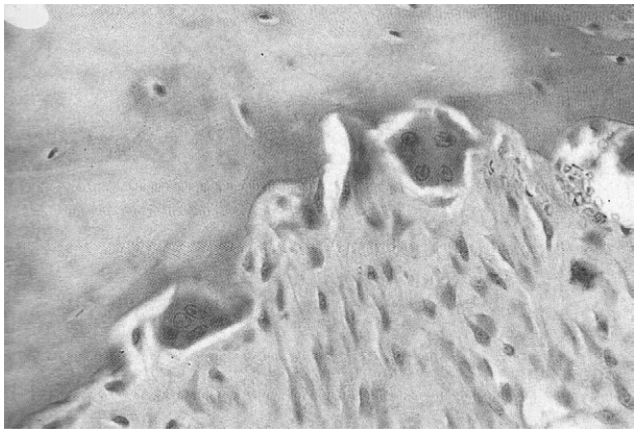

Figure 8.8 A photomicrograph of multinucleated osteoclasts (H&E stain).

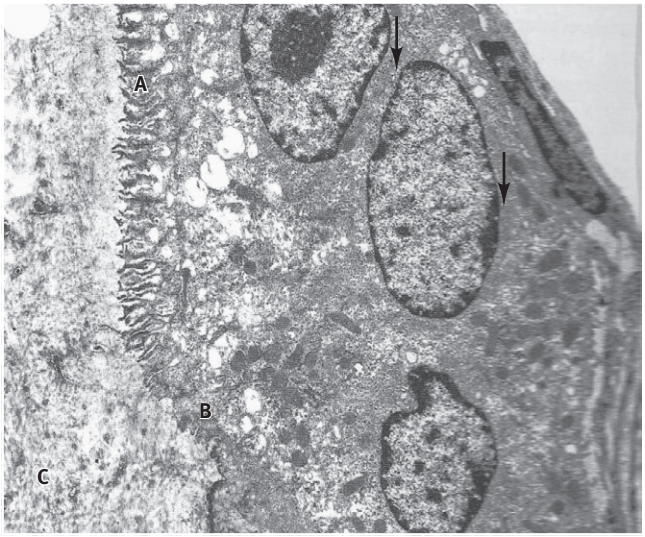

Figure 8.9 Electron micrograph of osteoclast showing abundance of vesicles beneath the brush border (A) lack of villi in the annular zone (B) and Golgi complex in juxtanuclear position (arrow) and little ER. Alveolar bone (C).

removal and deposition of bone that is responsible for its remodeling, a process that allows functional changes in the position of teeth.

Fibroblasts

These cells show rapid degeneration of collagen by fibroblast phagocytosis and that is the basis for fast turnover of collagen in periodontal ligament.

Earlier studies suggested that, collagen degradation was an extracellular event involving the activity of the enzyme collagenase.

Later, studies showed the presence of organelles termed intracellular collagen profiles. These organelles showed banded collagen fibrils within an elongated membrane-bound vacuole. It is thought that these organelles are associated with the degradation of collagen that has been ingested from the extracellular environment.

Some studies suggested that collagen degradation is intracellular in all healthy tissues, where there is controlled turnover and remodeling, and is extracellular in tissues where the changes are pathological or where degradation is rapid and involves a whole tissue simultaneously.

Hence, the degradation of collagen may be expected to include both *intracellular* and *extracellular* events.

Intracellular degradation

In vitro studies have demonstrated that fibroblasts are capable of phagocytosing collagen fibrils from extra-

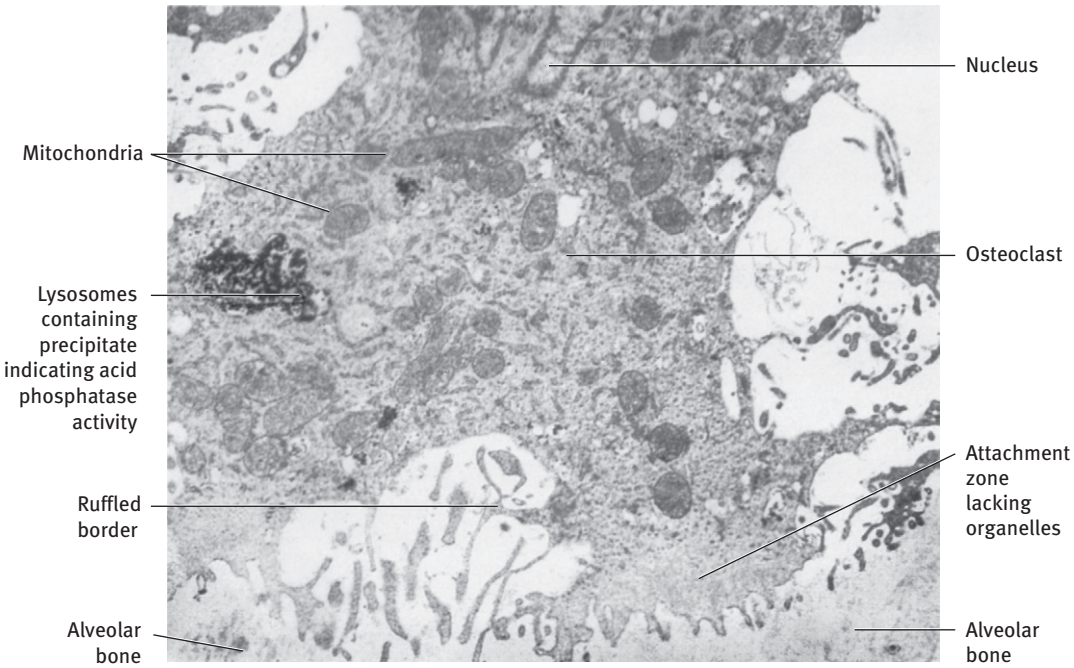

Figure 8.10 Electron micrograph illustrating acid phosphatase activity in lysosomes of osteoclast located on periodontal surface of alveolar bone of mouse molar (× 14,000).

cellular environment and degrading them inside phagolysomal bodies. Cell surface alkaline phosphatase and MMPs may be involved in internalizing a collagen fibril from the extracellular matrix. It has also been reported that collagenase is not involved in the intracellular phase of degradation of collagen fibrils. Lysosomal cysteine proteinases of lysosomal granules are capable of rapid degradation of internal collagen fibrils.

The intracellular digestion of collagen follows a sequence. When a collagen fibril is first phagocytosed by the fibroblast, a banded fibril surrounded by an electron-lucent zone is seen. Subsequently, the banded fibrils are surrounded by an electron-dense zone. At this stage phagosome fuses with primary lysosomes to form a phagolysosome. At the terminal stage, fibrils show indistinct banding and are surrounded by electron dense zone. This suggests that the enzymic degeneration of the fibril has proceeded to a point where the fibril loses its characteristic structure.

The extracellular events in the degradation of collagen involve collagenase (MMP-1) which is thought to cleave the triple helical portion of molecules within the fibril. Together with MMP-IV, it leads to denaturation of collagen under physiological conditions. The rest of the molecule undergoes further proteolysis by MMP-II (gelatinase) and MMP-V. But before any of these collagenase activities can occur, glycoproteins such as fibronectin and proteoglycans on fibril surface, which mask the collagenase binding site must be removed by stromelysin (MMP-III).

Cementoclasts

Cementoclasts resemble osteoclasts and are occasionally found in normal functioning periodontal ligament. This observation is consistent with the knowledge that cementum is not remodeled in the fashion of alveolar bone and periodontal ligament but that it undergoes continual deposition during life. However, resorption of cementum can occur under certain circumstances, and in these instances mononuclear cementoclasts or multinucleated giant cells, often located in Howship's lacunae, are found on the surface of the cementum. The origin of cementoclasts is unknown, but it is conceivable that they arise in the same manner as osteoclasts (Table 8.2).

Progenitor Cells

All connective tissues, including periodontal ligament, contain progenitors for synthetic cells that have the capacity to undergo mitotic division. If they were not present, there would be no cells available to replace differentiated cells dying at the end of their life span or as a result of trauma.

Periodontal regeneration is essentially a re-enactment of the developmental process including morphogenesis, cytodifferentiation, extracellular matrix production and mineralization, These processes support the concept that some mesenchymal stem cells remain within the periodontal ligament and are responsible for tissue homeostasis, serving as a source of renewable progenitor cells generating cementoblasts, osteoblasts and fibroblasts throughout adult life.

Secondly, that progenitor cells are present is evident from the burst of mitoses that occurs after application of pressure to a tooth as in orthodontic therapy or after wounding, maneuvers that stimulate proliferation and differentiation of cells of periodontal ligament.

These progenitor cell populations within the periodontal ligament appear to be in highest concentrations in locations adjacent to blood vessels and also enter the periodontal ligament through penetrations from adjacent endosteal spaces. These cells exhibit some of the classical cytological features of stem cells, including small size, responsiveness to stimulating factors and slow cycle time. Progenitor cells tend to have a small, close-faced nucleus and very little cytoplasm.

Progenitor fibroblasts are small, less polarized and contain less rough endoplasmic reticulum and Golgi saccules. In repair, the migration of new fibroblasts to that site is facilitated by fibrin and fibronectin. Cells of osteoblast subtype can be identified by high levels of alkaline phosphatase. Although, there is still uncertainty surrounding origin of cementoblasts and osteoblasts in the periodontal ligament, evidence suggests that cells of osteoblast subtype develop from perivascular cells in periodontal ligament proper, as well as from progenitor cells arising from adjacent marrow compartments (Fig. 8.11; Table 8.3).

Origin of periodontal stem cells

During embryogenesis, the periodontal ligament is formed by cells residing within the dental follicle. These

Table 8.2 Resorptive Cells

Name of the Cell	Origin	Light Microscopy	Electron Microscopy	Functions
Osteoclasts	Hematopoietic stem cells of monocyte-macrophage lineage	• Large and multinucleated or small and mononuclear • Appear to occupy bays in bone (Howship's lacunae)	• Numerous mitochondria and lysosomes, abundant Golgi saccules, and free ribosomes but little RER	• Plays an important role in bone resorption • Responsible for remodeling
Cementoclasts	Origin of cementoclasts is unknown, but it is conceivable that they arise in the same manner as osteoclasts	• Mononuclear or multinucleated giant cells in Howship's lacunae on surface of cementum	Similar to osteoclasts	Resorption

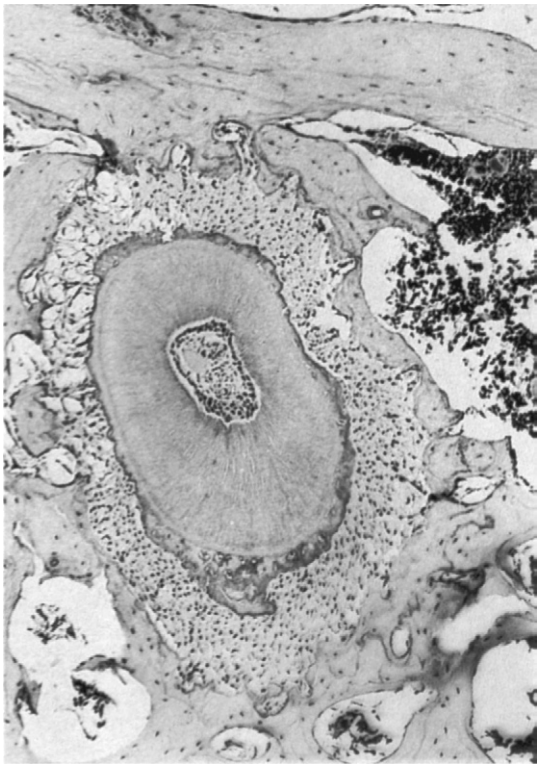

Figure 8.11 Section, 1 μm thick illustrating continuity between endosteal spaces and periodontal ligament in rat mandibular molar (× 160).

Table 8.3 Progenitor Cells

Site and Distribution	Morphology
Progenitor cells appear to be in highest concentration in locations adjacent to blood vessels	Small in size, nucleus close faced and very little cytoplasm

cells are considered to be derived from the ectomesenchyme. The putative stem cell marker STRO-1 used to isolate and purify bone marrow stromal stem cells is also expressed by human periodontal ligament stem cells and dental pulp stem cells. Periodontal ligament stem cells also share a common expression of perivascular cell marker CD146 with bone marrow stromal stem cells. A proportion of these cells also coexpress α smooth muscle actin and/or the pericyte associated antigen 3G5. These observations imply a perivascular origin for these cells.

Of particular significance is failure of the studies to detect hematopoietic markers like CD14, CD45 and CD34 in periodontal stem cells or bone marrow stromal cells. However, many mature mineralized tissue markers including alkaline phosphatase, type I collagen, osteonectin, osteopontin, osteocalcin and bone sialoprotein are expressed by these cells. In addition, these cells have the potential to express a variety of antigens associated with endothelium (CD106), perivascular tissue (α muscle actin, CD146, 3G5) as well as soft connective tissue proteins, type I and type III collagens. But, studies using semiquantitative reverse transcription-polymerase chain reaction, imply that periodontal ligament cells represent a unique population of postnatal stem cells distinct from bone marrow-derived mesenchymal stem cells.

Thus to conclude, within the total fibroblastic colony-forming unit population, there is a mixture of stromal progenitor cells at various stages of development, that are most likely maintained by a minor population of multipotential, mesenchymal stem cells with the capacity for cell renewal (Table 8.4).

Relationship between Cells

The cells of the periodontal ligament form a three-dimensional network, and, in appropriately oriented sections, their processes can be seen to surround the collagen fibers of the extracellular substance. Cells of periodontal ligament associated with bone, fibrous connective tissue, and cementum are not separated from one another, but adjacent cells generally are in contact with their neighbors, usually through their processes (Fig. 8.12). The site of some of the contacts between adjacent cells may be marked by modification of the structure of the contiguous plasma membranes (Figs. 8.13 and 8.14). but the nature of these junctions has not yet been elucidated satisfactorily. Although many appear to be zonula occludens, it is conceivable that they are in fact gap junctions (Fig. 8.13). Gap junctions in other tissues occur between cells that have been found to be in direct communication with one another. It is likely that some form of communication must exist between the cells of the periodontal ligament; to facilitate the homeostatic mechanisms that are known to operate in the periodontal ligament.

Epithelial Rests of Malassez

The periodontal ligament contains epithelial cells that are found close to the cementum (Fig. 8.15). These cells were first described by Malassez in 1884 and are the remnants of the epithelium of Hertwig's epithelial root sheath. At the time of cementum formation, the continuous layer of epithelium that covers the surface of the newly formed dentin breaks into lace-like strands. The epithelial rests persist as a network, strands, islands, or tubule-like structures near and parallel to the surface of the root (Figs 8.16 and 8.17). They lie about 25 μm from the cementum surface. Incross-sections, they appear cluster like. The cluster arrangement appears like a duct with the cells separated from the surrounding connective tissue by a basal lamina. A network of interconnecting strands parallel to the long axis of the root can be seen in tangential or serial sections. These cell rests are abundant in the furcation areas.

These cell rests can be distinguished from fibroblasts in periodontal ligament by the close packing of their cuboidal cells and the deeply stained nucleus. The nucleus is prominent. The cytoplasm is scanty, and shows tonofibrils that insert into the desmosomes found between adjacent cells and into hemidesmosomes between the cells and the basal lamina. Tight junctions are also seen between the cells.

Table 8.4 Markers for Periodontal Ligament Stem Cells

BONE MARROW MARKERS	PERIVASCULAR CELL MARKERS	MATURE MINERALIZED MATRIX MARKERS	CONNECTIVE TISSUE PROTEIN MARKERS
STRO-1	CD146	Alkaline phosphatase	Type I and III collagen
	αSMA	Osteonectin	
	Pericyte associated antigen 3G5	Osteopontin	
	CD106	Osteocalcin	
		Bone sialoproteins	

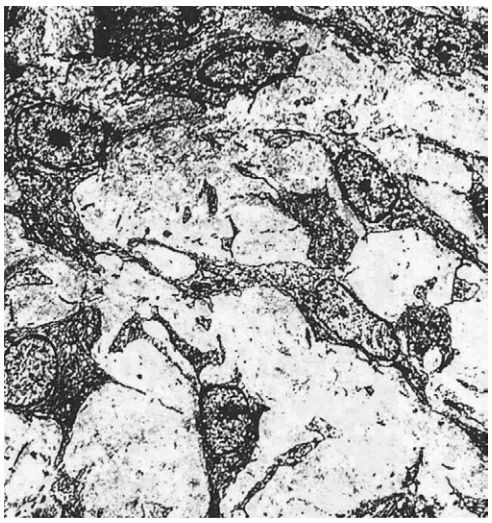

Figure 8.12 Transmission electron micrograph showing close association of principal fiber with fibroblast (× 3000).

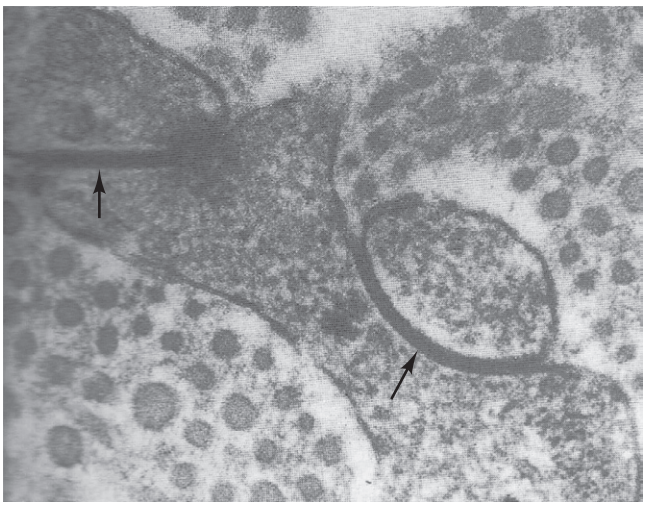

Figure 8.13 Electron micrograph showing gap junctions (arrows) between fibroblastic processes. (Bar = 100 nm).

Mitochondria are distributed throughout the cytoplasm. Rough endoplasmic reticulum and Golgi apparatus are poorly developed, indicating lack of protein synthesis (Fig. 8.18).

Studies have reported that epithelial cell rests attach to and spread rapidly on fibronectin, vitronectin and type I collagen present in the extracellular matrix.

The distribution of these cells varies according to site and age. They are less numerous in older individuals and more numerous in children. Up to the second decade, these cells are most commonly found in apical region, later they are mainly located cervically in the gingiva above the alveolar crest. These cells may proliferate to form cysts and tumors or may also undergo calcification to become *cementicles* (Table 8.5).

Defense Cells

Defense cells include macrophages, mast cells and eosinophils.

Mast cells

The mast cell is a relatively round or oval cell having a diameter of about 12 to 15 µm. Mast cells are often as-

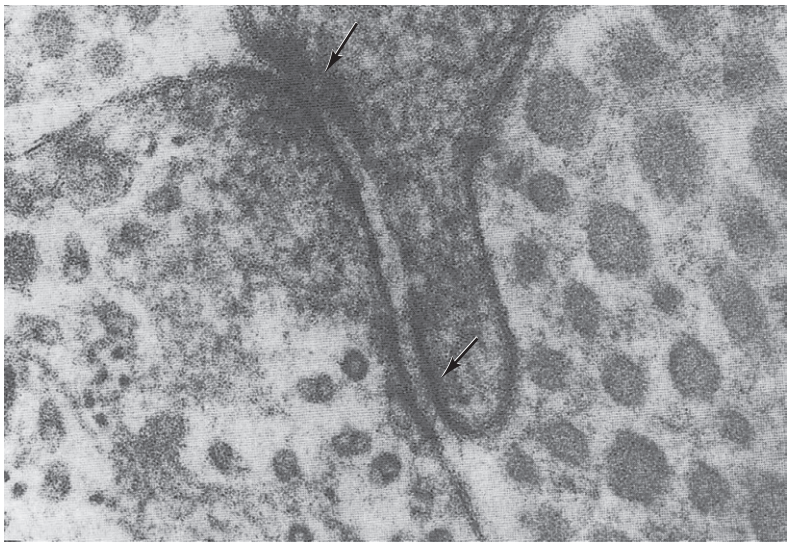

Figure 8.14 Electron micrograph of two desmosomal junction (arrows) between fibroblast. (Bar = 100 nm).

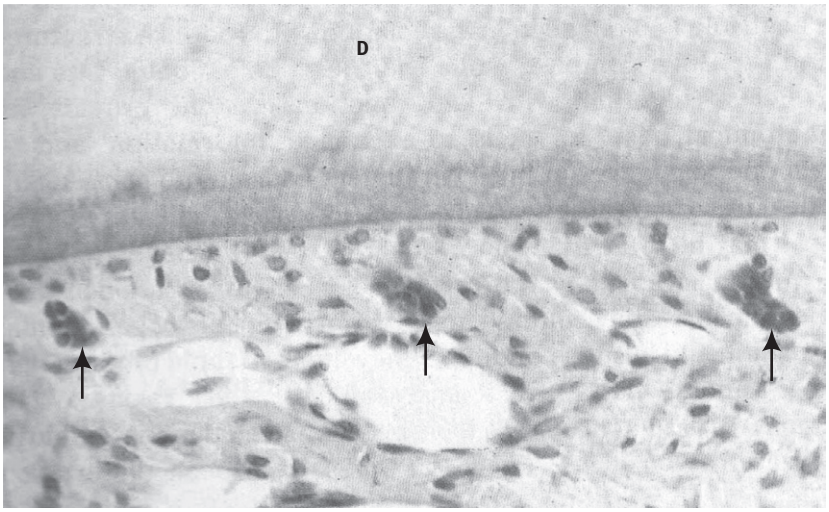

Figure 8.15 Photomicrograph of epithelial rests (arrow). D = dentin (H&E stain ×200).

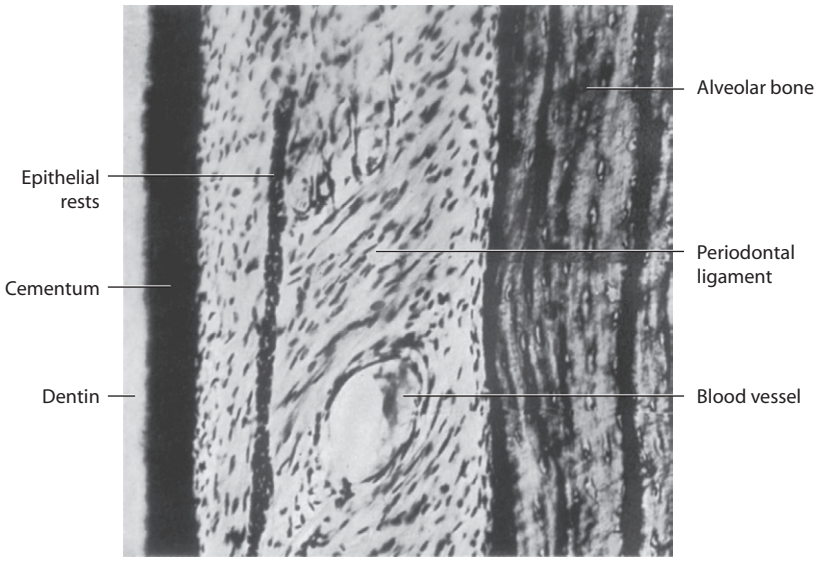

Figure 8.16 Long strand of epithelium in periodontal ligament.

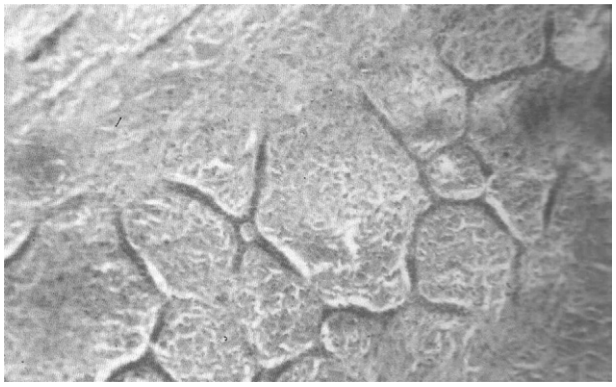

Figure 8.17 Photomicrograph of epithelial cell rests appearing as a network in tangential sections. H&E stain (× 160).

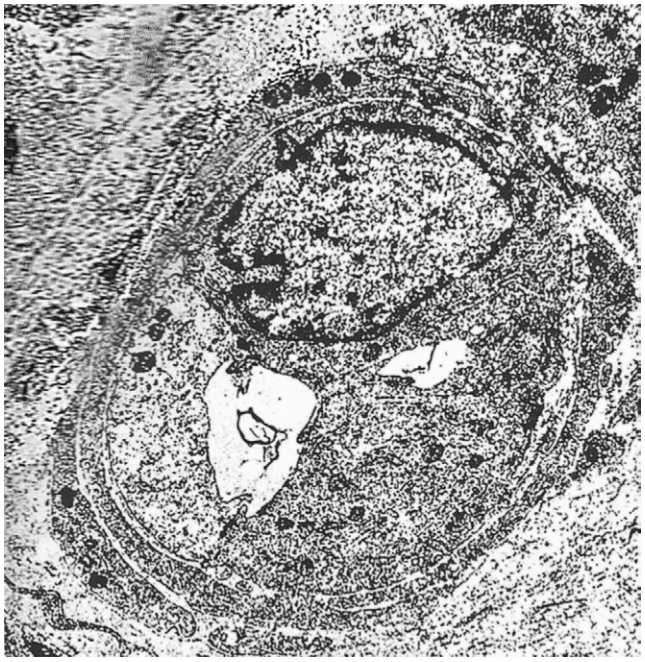

Figure 8.18 Transmission electron micrograph of epithelial cell rests (× 61,000).

sociated with blood vessels. The cells are characterized by numerous cytoplasmic granules, which frequently obscure the small, round nucleus. The granules stain with basic dyes but are most readily demonstrated by virtue of their capacity to stain metachromatically with metachromatic dyes such as azure A. They are also positively stained by the periodic acid-Schiff reaction. The granules are dense, membrane bound vesicles approximately 0.5 to 1 µm in diameter. When the cell is stimulated it degranulates. The granules have been shown to contain heparin and histamine and, in some animals, serotonin. In some preparations, mast cells may be seen to have degranulatedso that many or all of the granules are located outside the cell.

Electron microscopy shows the mast cell cytoplasm containing free ribosomes, short profiles of granular endoplasmic reticulum, few round mitochondria, and a prominent Golgi apparatus (Fig. 8.19).

Mast cell histamine plays a role in the inflammatory reaction, and mast cells have been shown to degranulate in response to antigen–antibody reaction on their surface. Occa-sional mast cells may be seen in the healthy periodontal ligament. The release of histamine into the extracellular environment causes proliferation of endothelial cells and mesenchymal cells. Conse-quently, mast cells may play an important role in regulating endothelial and fibroblast cell populations.

Macrophages

Macrophages are also found in the ligament and are predominantly located adjacent to blood vessels. These defense cells are derived from monocytes and phagocytose particulate matter and invading microorganisms. Resting macrophages can be distinguished from fibroblasts in the electron microscope by the presence of numerous microvilli, lysosomes and their membrane bound vesicles of varying density and paucity of rough endoplasmic reticulum and Golgi complex.

The wandering type of macrophage, probably derived from blood monocytes, has a characteristic ultrastructure that permits it to be readily distinguished from fibroblasts. It has a nucleus, generally of regular contour, which may be horseshoe or kidney shaped and which

Table 8.5 Epithelial Rests of Malassez

Cell Type	Origin	Light Microscopy	Electron Microscopy	Distribution	Clinical Significance
• **Remnants of Hertwig's epithelial root sheath** • Abundant in furcation areas • Parallel to root surface • Lie about 25 µm from the cementum surface	• At the time of cementum formation, the continuous layer of epithelium that covers the surface of newly formed dentin breaks into lace-like strands	• **Cross-section** - Cluster like • **Tangential section**-A network of interconnecting strands parallel to the long axis of the root	• Scanty cytoplasm with tonofibrils that insert into the desmosomes between adjacent cells and hemidesmosomes between the cells and basal lamina • Mitochondria - distributed throughout the cytoplasm • RER, Golgi apparatus-poorly developed	• Older individuals - less numerous • Children - more numerous • Second decade-in apical region • Later- cervically in the gingiva above the alveolar crest	• Proliferate to form cysts and tumors or may also undergo calcification forming cementicles

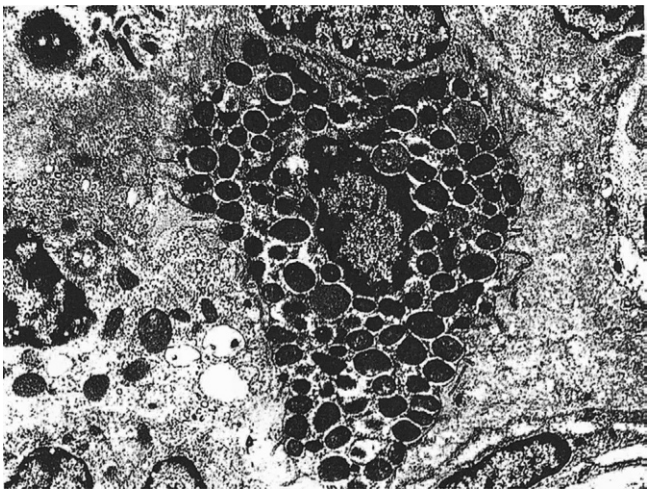

Figure 8.19 Ultrastructure of a mast cell showing numerous membrane bound vesicles (×5000).

exhibits a dense uneven layer of peripheral chromatin. Nucleoli are rarely seen.

Macrophages are readily identified in the electron microscope, and it is apparent that the surface of the cell is generally raised in microvilli and the cytoplasm contains numerous free ribosomes. The rough endoplasmic reticulum is relatively sparse and is adorned with widely spaced polysomes that are composed of only two to four ribosomes each. The Golgi apparatus is not well developed, but the cytoplasm contains numerous lysosomes in which identifiable material may be seen (Figs 8.20 and 8.21).

In the periodontal ligament macrophages may play a dual role: (1) phagocytosing dead cells and (2) secreting growth factors that regulate the proliferation of adjacent fibroblasts. Macrophages also synthesize a range of molecules with important functions, like interferon, prostaglandins and factors that enhance the growth of fibroblasts and endothelial cells.

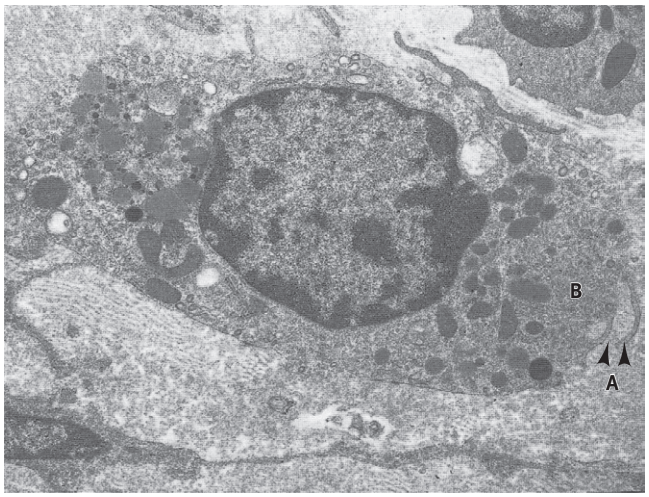

Figure 8.20 Electron micrograph of a macrophage showing many lysosomes and mitochondria and few rough ER. A=microvilli, B=Golgi complex, Bar=1.5 μm.

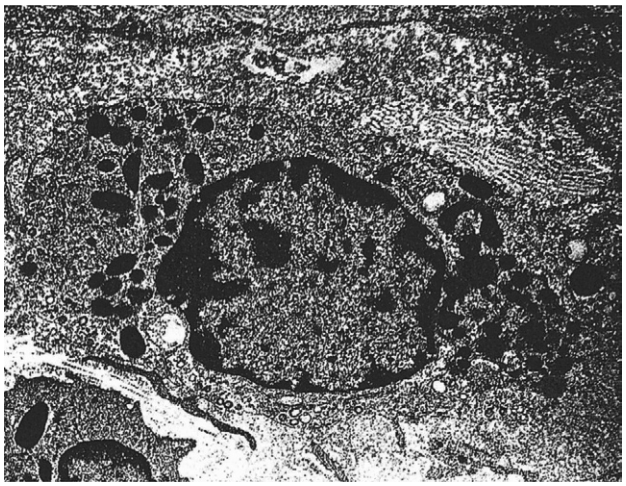

Figure 8.21 Transmission electron micrograph of macrophages (× 6000).

Eosinophils

Eosinophils are occasionally seen in the periodontal ligament. They possess granules that consist of one or more crystalloid structures (Fig. 8.22). The cells are capable of phagocytosis (Table 8.6).

EXTRACELLULAR SUBSTANCE

The extracellular substance of the periodontal ligament comprises the following:

Fibers	Ground Substance
Collagen	Glycosaminoglycans
Elastic-oxytalan	Proteoglycans
Reticular	Glycoproteins
Secondary	
Indifferent fiber plexus	

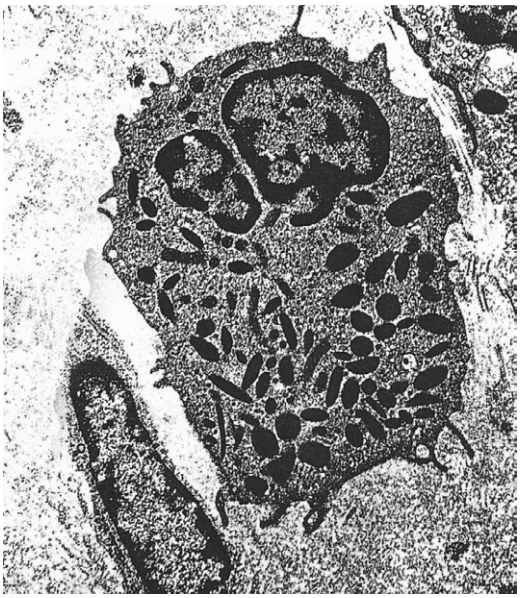

Figure 8.22 Ultrastructure of eosinophil showing characteristic granules (× 2500).

Table 8.6 Defense Cells

Name of the Cell	Origin	Light Microscopy	Electron Microscopy	Functions
Mast cells associated with blood vessels	Hematopoietic stem cell	• Round or oval cell, 12-15 μm in diameter • Numerous cytoplasmic granules - dense, membrane bound vesicles 0.5-1 μm in diameter, containing heparin and histamine and stain with metachromatic dyes	Cytoplasm - free ribosomes, short profiles of granular ER, few round mitochondria and a prominent Golgi apparatus	The release of histamine into the extracellular environment - proliferation of endothelial cells and mesenchymal cells
Macrophages located adjacent to blood vessel	Hematopoietic stem cell	• Nucleus, - horse shoe or kidney shaped with dense and uneven layer of peripheral chromatin • Nucleoli are rarely seen	Resting macrophages - distinguished from fibroblast by the presence of numerous microvili, lysosomes and membrane bound vesicles of varying density and paucity of RER and Golgi complex	Dual role: Phagocytose dead cells Secrete growth factors that regulate the proliferation of adjacent fibroblasts Also synthesize - interferon, prostaglandins and factors that enhance the growth of fibroblasts and endothelial cells
Eosinophils Occasionally seen	Hematopoietic stem cell	8 μm in diameter, nuclei are bilobed, granules with 1 or more crystalloid structures, with bright red staining properties with acidic dyes such as eosin		Phagocytosis

Fibers

The connective tissue fibers are mainly collagenous, but there may be small amounts of oxytalan and reticulin fibers, and in some species, elastin fibers.

Collagen

Collagen is a protein composed of different amino acids; the most important being glycine, proline, hydroxylysine and hydroxyproline. The amount of collagen in a tissue can be determined by its hydroxyproline content.

The collagen is gathered to form bundles approximately 5 μm in diameter. These bundles are termed *principal fibers*. Within each collagen bundle, subunits are present called collagen fibrils.

These fibrils are formed by packing together of individual tropocollagen molecules. The periodontal ligament fibrils are small and the diameter reflects the mechanical demands put upon the connective tissue. Collagen fibrils have transverse striations with a characteristic periodicity of 64 nm. These striations are caused by the overlapping arrangement of the tropocollagen molecules (Fig. 8.7).

The collagen fibril diameters of the mammalian periodontal ligament are small with a mean diameter of 45–55 nm. The small diameter of the fibrils could be due to high rate of collagen turnover or the absence of mature collagen fibrils.

The main types of collagen in the periodontal ligament are type I and type III. More than 70% of periodontal ligament collagen is type *I*.

Type I collagen is uniformly distributed in the ligament. It contains two identical α1(I) chains and a chemically different α2 chain. It is low in hydroxylysine and glycosylated hydroxylysine.

Type III collagen accounts for about 20% of collagen fibers. It consists of three identical α1(III) chains. It is high in hydroxyproline, low in hydroxylysine and contains cysteine. Type III collagen is covalently linked to type I collagen throughout the tissue. It is found at the periphery of Sharpey's fiber attachments into alveolar bone. The function of type III may be involved with collagen turnover, tooth mobility and collagen fibril diameter.

Small amounts of type V and type VI collagens and traces of type IV and type VII collagen are also found in the ligament. Type V collagen coats cell surfaces and other types of collagen. This collagen increases in amount in periodontal inflammatory disease. Type VI is a short chain molecule that ramifies the extracellular matrix, but is not directly associated with major banded collagen fibrils. It may play a role in maintaining the integrity and elasticity of the extracellular matrix. Type IV and type VII are associated with epithelial cell rests and blood vessels. Type IV does not form fibrils, but a structural role in maintaining integrity of the periodontal ligament, by anchoring the elastic system to the vasculature, has been proposed. Type XII collagen is believed to occur within the periodontal ligament, only when the ligament is fully functional.

The principal fiber group is the *alveolodental ligament*, which consists of five fiber groups: alveolar crest, horizontal, oblique, apical and interradicular group in multirooted teeth (Figs. 8.23–8.25).

1. Alveolar crest group Alveolar crest fibers extend obliquely from the cementum just beneath the junctional epithelium to the alveolar crest. Fibers also run from the cementum over the alveolar crest and to the fibrous layer of the periosteum covering the alveolar bone. These

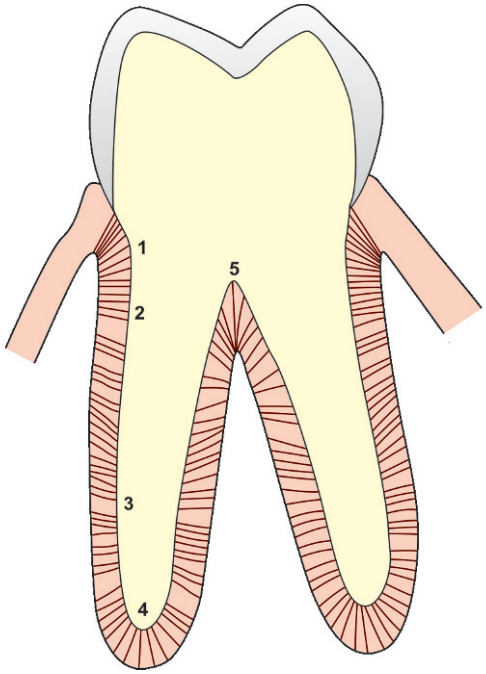

Figure 8.23 Diagrammatic representation of a longitudinal section of tooth in situ showing principal fibers. 1–alveolar crest fibers, 2–horizontal fibers, 3–oblique fibers, 4–apical fibers, 5–interradicular fibers.

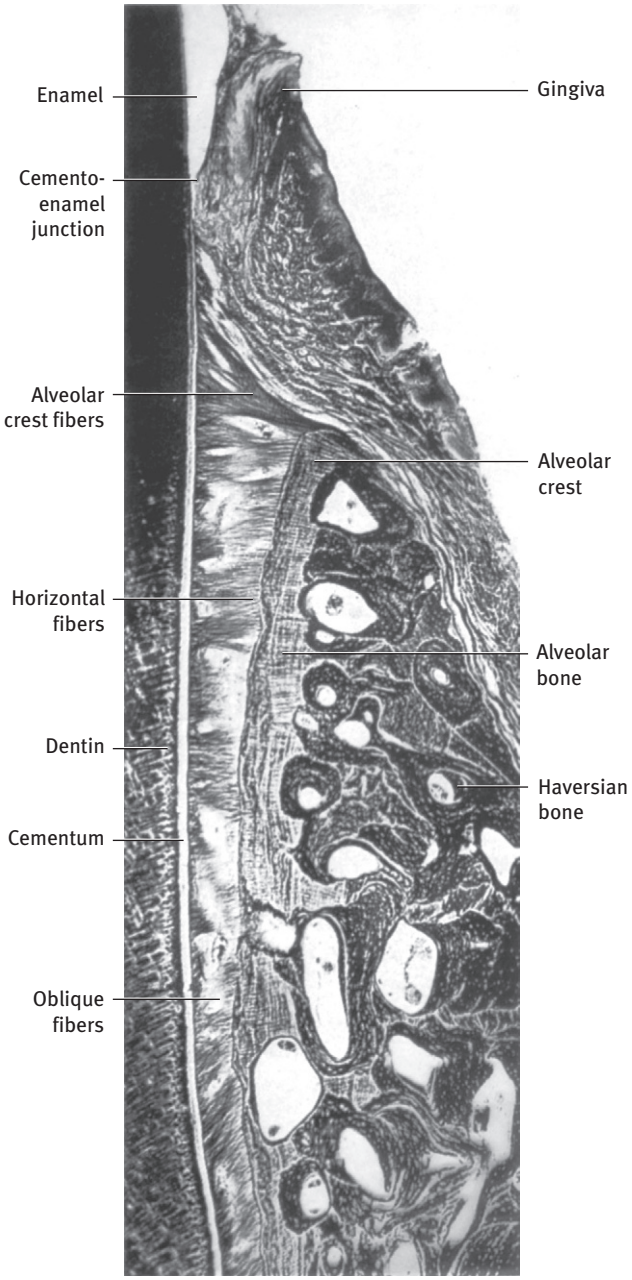

Figure 8.24 Fibers of periodontal ligament.

fibers resist tilting, intrusive, extrusive and rotational forces. Confusion often arises concerning anatomic differentiation of the periodontal alveolar crest group, from an immediately suprajacent gingival fiber group, the *dentoperiosteal* fibers. The collagenous elements of these two anatomic groups, intertwine along their respective courses. Any collagenous elements located apical to the line, joining the height of each interdental bony septum, may be termed periodontal, and those coronal to the line gingival.

2. Horizontal group These fibers run at right angles to the long axis of the tooth from cementum to alveolar bone, and are roughly parallel to the occlusal plane of the arch. They are found immediately apical to the alveolar crest fiber group. These bundles pass from their cemental attachment directly across the periodontal ligament space to become inserted in the alveolar process as Sharpey's fibers. They are limited mostly to the coronal one-fourth of the periodontal ligament space. These fibers resist horizontal and tipping forces.

3. Oblique group Oblique fibers are the most numerous and occupy nearly 2/3rd of the ligament. These fibers are inserted into the alveolar bone at a position coronal to their attachment to cementum, thereby resulting in their oblique orientation within the periodontal space. These fibers resist vertical and intrusive forces.

4. Apical group From the cementum at the root tip, fibers of the apical bundles radiate through the periodontal space to become anchored into the fundus of the bony socket. The apical fibers resist the forces of luxation, may prevent tooth tipping and probably protect delicate blood and lymph vessels and nerves traversing the periodontal ligament space at the root apex. These fibers are not seen on incompletely formed roots.

5. Interradicular group The principal fibers of this group are inserted into the cementum from the crest of interradicular septum in multirooted teeth. These fibers resist tooth tipping, torquing and luxation. These fibers are lost, if age-related gingival recession proceeds to the extent, that the furcation area is exposed. Total loss of these fibers occurs in chronic inflammatory periodontal disease.

Some histologists also consider the *gingival fiber group* to be part of the principal fibers of the periodontal ligament.

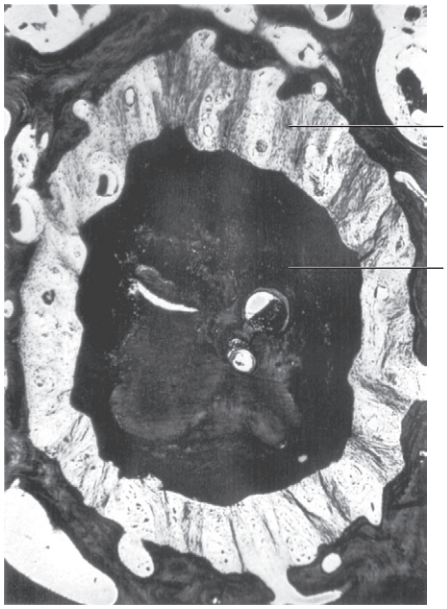

Figure 8.25 Apical fibers of periodontal ligament (From Orban B: Dental histology and embryology. Philadelphia, 1929, P Blakiston's Son & Co).

The gingival fiber groups are found within the lamina propria of the marginal gingiva. These gingival fibers are separate, but adjacent fiber groups, which support the marginal gingival tissues, to maintain their relationship to the tooth (Table 8.7).

Sharpey's Fibers

Collagen fibers are embedded into cementum on one side of the periodontal space and into alveolar bone on the other. The embedded fibers are termed *Sharpey's fibers* (Figs. 8.1, 8.4 and 8.5). Sharpey's fibers are more numerous but smaller at their attachment into cementum than alveolar bone. The mineralized parts of Sharpey's fibers in alveolar bone appear as projecting stubs covered with mineral clusters. The mineralization is at right angles to long axis of fibers, indicating that in function, the fibers are subjected to tensional forces. Sharpey's fibers in primary acellular cementum are mineralized fully, those in cellular cementumand bone are mineralized, partially at their periphery.

Few Sharpey's fibers pass uninterruptedly through the bone of the alveolar process (termed *transalveolar fibers*) to continue as principal fibers of the adjacent periodontal ligament, or they may mingle buccally and lingually with fibers of the periosteum that cover the outer cortical plates of the alveolar process. These fibers pass through the alveolar process, only when the process consists entirely of compact bone and contains no haversian system. Once embedded in either the wall of the alveolus or the tooth, Sharpey's fibers calcify to a certain degree and are associated with an abundance of noncollagenous proteins namely, osteopontin and bone sialoprotein.

Although not a common finding when viewed through conventional light microscopy, transalveolar fibers are readily seen in the high-voltage electron microscope. Conceivably these fibers become entrapped in alveolar bone either during development of the interdental septum or by bone deposition at the alveolar crest. As a result of tooth drift and the resultant bone remodeling, the fibers may be severed, and some may be relinked to periodontal ligament fibers by an unbanded link protein. Transalveolar fibers may serve as a mechanism to connect adjacent teeth.

Intermediate Plexus

Earlier, it was believed that, the principal fibers frequently followed a wavy course from cementum to bone

Table 8.7 Principal Fiber Groups

Type of Fiber	Origin and Insertion	Function
Alveolar crest group	• Extend obliquely from cementum just beneath junctional epithelium to alveolar crest • Also extend from cementum over the alveolar crest to fibrous layer of periosteum covering alveolar bone	Resist tilting, intrusive, extrusive and rotational forces
Horizontal group **limited to coronal one fourth of pdl space**	• Extend at right angles to the long axis of the tooth from cementum to the alveolar bone and parallel to occlusal plane • Gets inserted into alveolar process as Sharpey's fibers	Resist horizontal and tipping force
Oblique group **most numerous and occupy 2/3rd of ligament**	Extend into alveolar bone coronal to their attachment to cementum	Resist vertical and intrusive forces
Apical group **not seen in incompletely formed roots**	Extend from root tip and radiate through the periodontal space into fundus of bony socket	Resist luxation, prevent tooth tipping, protect delicate lymph and blood vessels and nerves traversing the PDL space at the root apex
Interradicular group **fibers lost if furcation area is exposed**	Extend into cementum from the crest of inter–radicular septum of multirooted teeth	Resist tooth tipping, torque and luxation

and are joined in the mid region of the periodontal space, giving rise to a zone of distinct appearance, the so-called *intermediate plexus*. The plexus was also considered to be an area of high metabolic activity, in which splicing and unsplicing of fibers might occur. However, research over the past few years demonstrated that, once cemental fibers meet and fuse with osseous fibers no such plexus remains.

Secondly the entire periodontal ligament is metabolically active, not just the middle or intermediate zone. Studies also indicate that with tooth movement, areas of highest activity are fiber terminals near cementum and bone, not in the middle.

The recent concept is that, fibers cross the entire width of periodontal space, but branch en route and join neighboring fibers to form a complex three-dimensional network. It seems improbable that one single fibril extends the entire dimension of tooth to bone.

However, this plexus has usually been observed in longitudinal sections of continuously growing incisors of rodents and not in cross-sections. Hence, it is an artifact, related to the fact that the collagen fibers in periodontal ligament of continuously growing incisors are arranged in the form of sheets rather than bundles. No intermediate plexus is seen across the periodontal space in teeth of noncontinuous growth.

Some authors have proposed a zone of shear, as a site of remodeling during eruption. Some believe, it lies near the center of periodontal ligament.

A specific type of waviness has been reported in collagenous tissues including the periodontal ligament, called *crimping*. These are best seen under polarizing microscope. The crimp is gradually pulled out when the ligament is subjected to mechanical tension, until it disappears.

Elastic Fibers

There are three types of elastic fibers, which are histochemically and ultrastructurally different. They are, the *mature elastic* fibers, (*elastin) fibers*, the *elaunin fibers*, and the *oxytalan fibers*. Elaunin and oxytalan fibers have been described as immature elastic fibers. *Mature elastic fibers* consist of a microfibrillar component surrounding an amorphous core of elastin protein. Elastin protein contains a high percentage of glycine, proline and hydrophobic residues, with little hydroxyproline and no hydroxylysine. The microfibrillar component is located around the periphery and scattered throughout the amorphous component. These fibers are observed only in walls of afferent blood vessels, where they constitute the elastic laminae of larger arterioles and of arteries of greater caliber.

Elaunin fibers are seen as bundles of microfibrils embedded in a relatively small amount of amorphous elastin. These fibers may be found within the fibers of the gingival ligament. An elastic meshwork has been described in the periodontal ligament as being composed of many elastin lamellae with peripheral oxytalan fibers and elaunin fibers.

Oxytalan fibers are a type of immature elastic fibers. Oxytalan appears to consist of microfibrillar component only.

These fibers can be demonstrated under the light microscope when the section is oxidized strongly before staining with elastin stains. Oxytalan fibers are not susceptible to acid hydrolysis. These fibers are approximately 0.5–2.5 μm in diameter and appear to have elastin and type IV collagen. Under the electron microscope, fibers believed to be oxytalan resemble developing elastic fibers (Fig. 8.26).

The orientation of the oxytalan fibers is quite different from that of the collagen fibers. Instead of running from bone to tooth, they tend to run in an axial direction (Fig. 8.27) one end being embedded in cementum or possibly bone, and the other often in the wall of a blood vessel. In the cervical region, they follow the course of gingival and transseptal fibers. Within the periodontal ligament proper, these fibers are longitudinally oriented, crossing the oblique fibers perpendicularly. In the vicinity of the apex they form a complex network. The function of the oxytalan fibers is unknown, but it has been suggested that they may play a part in supporting the blood vessels of the periodontal ligament. They are thicker and more numerous in teeth that are subjected to high loads, as in abutment teeth for bridges and teeth that are moved orthodontically. Thus, these fibers may have a role in tooth support (Table 8.8).

Reticular Fibers

These are fine immature collagen fibers with argyrophilic staining properties and are related to basement membrane of blood vessels and epithelial cells which lie within the periodontal ligament. These fibers are composed of type III collagen.

Secondary Fibers

These are located between and among the principal fibers. These fibers are relatively non-directional and randomly oriented. They may represent the newly formed collagenous elements that have not yet been incorporated into principal fiber bundles. These fibers appear to traverse the periodontal ligament space coronoapically and are often associated with paths of vasculature and nervous elements.

Indifferent Fiber Plexus

In addition to these fiber types, small collagen fibers associated with the large principal collagen fibers have been described. These fibers run in all directions, forming a plexus called *indifferent fiber plexus*.

But some studies report that this plexus is seen in ground sections examined under scanning electron microscope, but not under transmission electron microscope or light microscope. Hence, some authors consider it to be an artifact produced by preparation.

Ground Substance

Within the periodontal ligament are, blood vessels, lymph vessels, nerves and connective tissue cells interspersed in an extracellular matrix containing collagens and the ground substance. Ground substance has been estimated to contain 70% water and is thought to have a

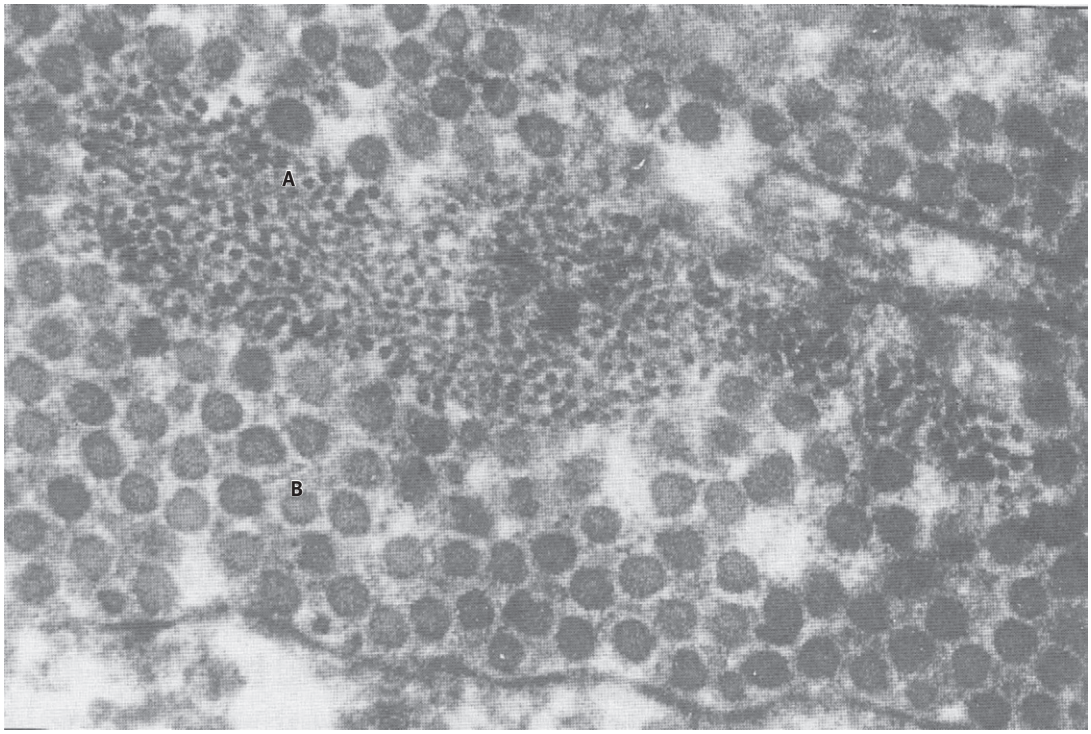

Figure 8.26 Transmission electron micrograph of oxytalan fiber in cross-section (A) surrounded by collagen fiber (B). Note the spaces between them represent the ground substance magnification (× 95,000).

Figure 8.27 Photomicrograph of oxytalan fibers in monkey periodontal ligament (monopersulfate thionine stain ×180).

significant effect on the tooth's ability to withstand stress loads.

Indeed, the ground substance is a gel-like matrix present in every nook and cranny, including the interstices between fibers and between fibrils. It is important to understand that all anabolites reaching the cells from the microcirculation in the ligament and all catabolites passing in the opposite direction must pass through the ground substance. Its integrity is essential, if the cells of the ligament have to function properly. The other functions of ground substance are ion and water binding and exchange, control of collagen fibrillogenesis and fiber orientation and binding of growth factors.

The ground substance consists mainly of glycosaminoglycans, proteoglycans and glycoproteins. All components of the ground substance are presumed to be secreted by fibroblasts. The main type of glycosaminoglycan

Table 8.8 Elastic Fibers

Mature		Immature
Elastic	Elaunin	Oxytalan
Elastin fibers of microfibrillar component surrounding an amorphous core of elastin protein. They are only seen in the walls of afferent blood vessels where they constitute the elastic laminae of larger arterioles and of arteries of greater caliber	Seen as bundles of microfibrils embedded in relatively small amount of amorphopus elastin. Found within fibers of gingival ligament	Consists of microfibrillar component only. 0.5 – 2.5 microns in diameter Fibers run in an axial direction one end being embedded in cementum or possibly bone and the other often in the wall of a blood vessel. Support blood vessels of PDL

is hyaluronan. Dermatan, chondroitin and heparin sulfates may also be found. Hyaluronan occupies a large volume of the periodontal ligament. Most of theglycosaminoglycan is located near the surface of collagen fibrils.

Proteoglycans are compounds containing glycosaminoglycans attached to a protein core. The two main types in the periodontal ligament are dermatan sulfate and proteoglycan containing chondroitin sulfate and dermatan sulfate hybrids. The proteodermatan sulfate proteoglycan is same as decorin and assists in collagen fibrillogenesis and increases the strength of collagen fibrils. The other proteoglycans are similar to biglycan which controls hydration of extracellular matrix of the connective tissue.

Small leucine rich proteoglycans including *fibromodulin* and *perlecan* are present in the periodontal ligament. Another proteoglycan, CD44 has been found to localize to surface of resident fibroblasts. *Syndecan 1 and 2* have been located in both developing periodontal ligament and within ligament of adults.

The predominant glycoprotein in the ground substance is fibronectin. It promotes attachment of cells to the collagen fibrils. It may also be involved in cell migration and orientation. The other glycoproteins seen in the periodontal ligament are tenascin and vitronectin. Tenascin has been found adjacent to alveolar bone and cementum. It has been reported that tenascin may act to transfer the forces of mastication and stresses of tooth support to specific protein structures. Vitronectin has been observed adjacent to elastin fibers.

Other molecules found in the periodontal ligament are osteonectin, laminin and undulin. Undulin has been found principally in the basement membrane of the epithelial cell rests of Malassez.

The composition of the ground substance is said to vary according to the developmental state of the tissue. There is a marked change in the amount of hyaluronate as development proceeds from the dental follicle to the initial periodontal ligament. There is also an increase in the amount of proteoglycans during eruption.

Interstitial Tissue

Interstitial connective tissue is a loose connective tissue that contains blood vessels, lymphatics, nerves and less regularly arranged collagen fibers (Fig. 8.28).

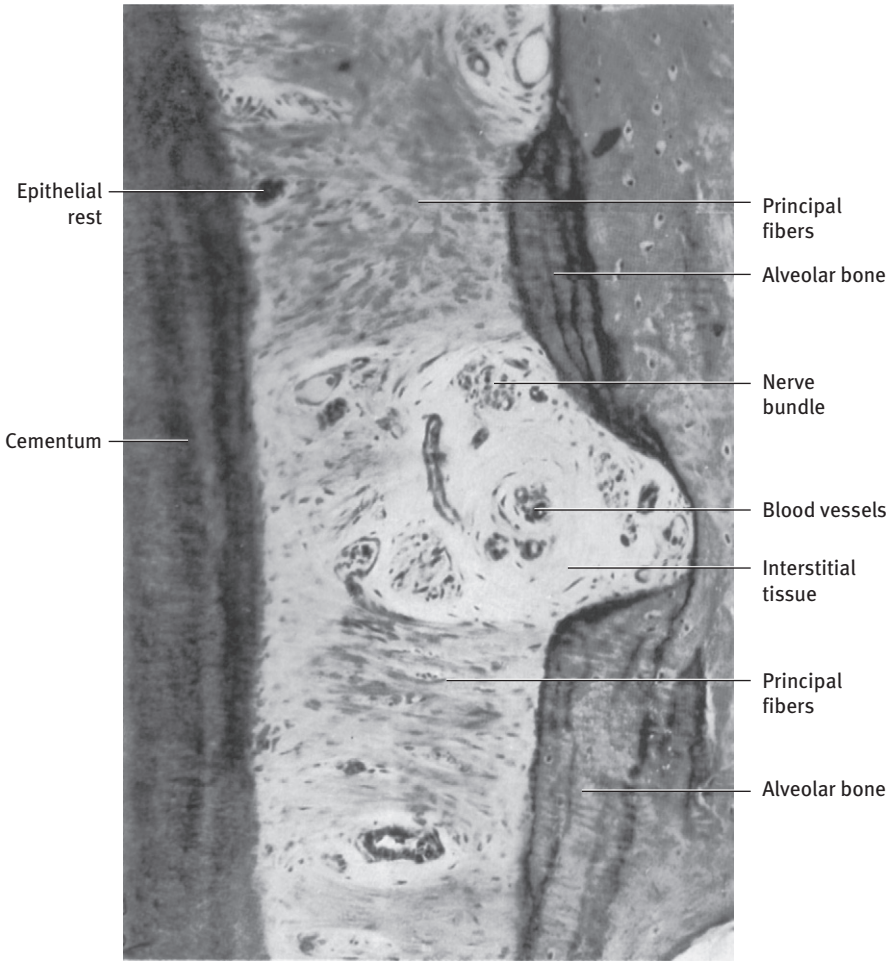

Figure 8.28 Interstitial spaces in periodontal ligament contain loose connective tissue, vessels, and nerves (From Orban B: J Am Dent Assoc 16:405, 1929).

STRUCTURES PRESENT IN CONNECTIVE TISSUE

The following discrete structures are present in the connective tissue of the periodontal ligament:

Blood vessels	Nerves
Lymphatics	Cementicles

Blood vessels

The periodontal ligament is a high fiber density tissue with abundant vascular supply. Generally in a dense fibrous tissue elsewhere in mammals, fewer vascular elements are present but in the PDL a higher or elevated metabolic requirement explains the rich vasculature.

Arterial supply. The blood supply is derived from the inferior and the superior alveolar arteries to the mandible and maxilla respectively and reach the PDL from three sources:

1. Branches in the periodontal ligament from apical vessels that supply the dental pulp.
2. Branches from intra-alveolar vessels. These branches run horizontally, penetrating the alveolar bone to enter the periodontal ligament (Fig. 8.29).
3. Branches from gingival vessels. These enter the periodontal ligament from the coronal direction.

The arterioles and capillaries of the microcirculation ramify in the periodontal ligament, forming a rich network of arcades that is more evident in the half of the periodontal space adjacent to bone than that adjacent to cementum. There is a particularly rich vascular plexus at the apex and in the cervical part of the ligament. The dental arteries gives afferent branches prior to entering the apical foramina which provide a basket like network of vessels in the apical third of the PDL. The interradicular arteries branch into vessels of lesser caliber to emerge from the cribriform plate as perforating arteries and supply the PDL along most of the coronoapical extent including the bifurcation and the trifurcation areas. The interdental artery also exit the bone to supply the middle three-fifth of the PDL though most of the interdental arteries emerge from the crest of the alveolar process and supply the coronal aspect of PDL. Irrespective of their origin all the vessels within the PDL intercommunicate forming an organizing plexus through the periodontal space.

The arterioles in PDL range from a diameter of 15 to 50 µm with an average diameter of 20 µm.

The PDL has some specialized features in the vasculature namely, the presence of large number of fenestrations in the capillaries (Fig. 8.30) and a cervical plexus of capillary loops (Fig. 8.31). In fibrous connective tissue, usually continuous capillaries are seen. Hence fenestration of capillaries in PDL is unusual especially in large numbers (up to $40 \times 10^6/mm^3$ of tissue). The fenestrated capillary beds have an increased capacity for diffusion and filtration. These functions possibly might be related to the high metabolic requirement of PDL because of rate of turnover.

In the area of the gingival crevice a cervical plexus of capillary loops completely encircles the tooth within the connective tissue. The cervicular capillary loops arise from a circular plexus of 1 to 4 intercommunicating vessels of 6–30 µm diameter at the level of junctional epithelium (Fig. 8.31). This plexus is separated from marginally situated loops in the gingiva by a distinct gap. The circular plexus anastomoses with both gingival and PDL vessels. This complex structure of vasculature is not completely understood though it is thought to be related to dentogingival seal. It might also be providing a means for flow reversal and rapid redistribution of blood under varying occlusal loads.

The blood supply increases in the PDL from incisors to molars. The single rooted teeth have more supply in gingival third followed by apical third and the least in middle third. In molars it is equal in middle and apical areas but is greater at the gingival area. These variations might be depicting the functional variations at different sites.

Venous drainage. The venous channels accompany their arterial counterparts. The channels are larger in diameter with mean average of 28 µm. These channels receive blood from the capillary network and also specialized shunts called glomera in the PDL. The specialized shunts provide an arteriovenous anastomosis and

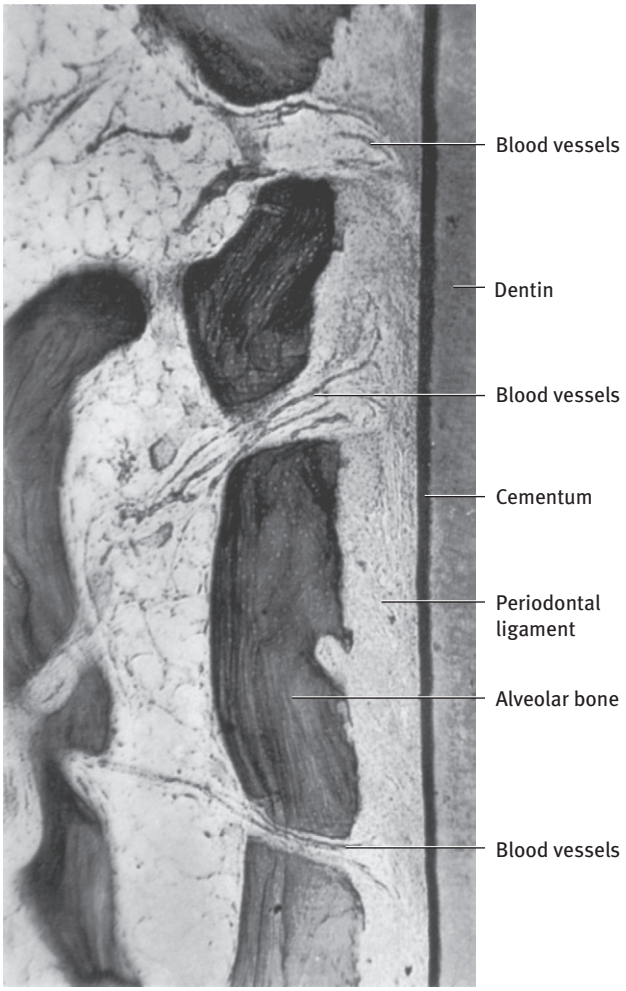

Figure 8.29 Blood vessels enter periodontal ligament through openings in alveolar bone (From Orban B: Dental histology and embryology. Philadelphia, 1929, P Blakiston's Son & Co).

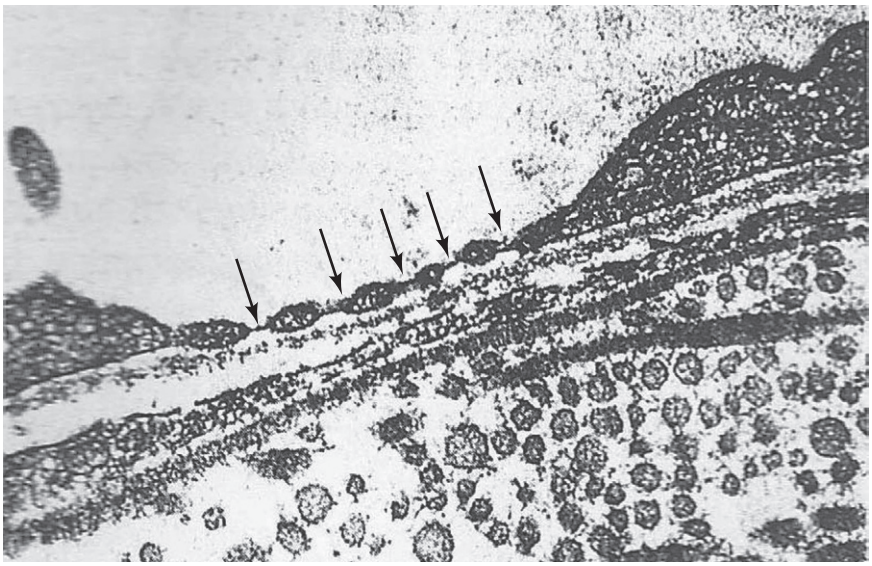

Figure 8.30 Transmission electron micrograph of fenestrated capillaries. Arrowheads indicate fenestrations (× 35,000).

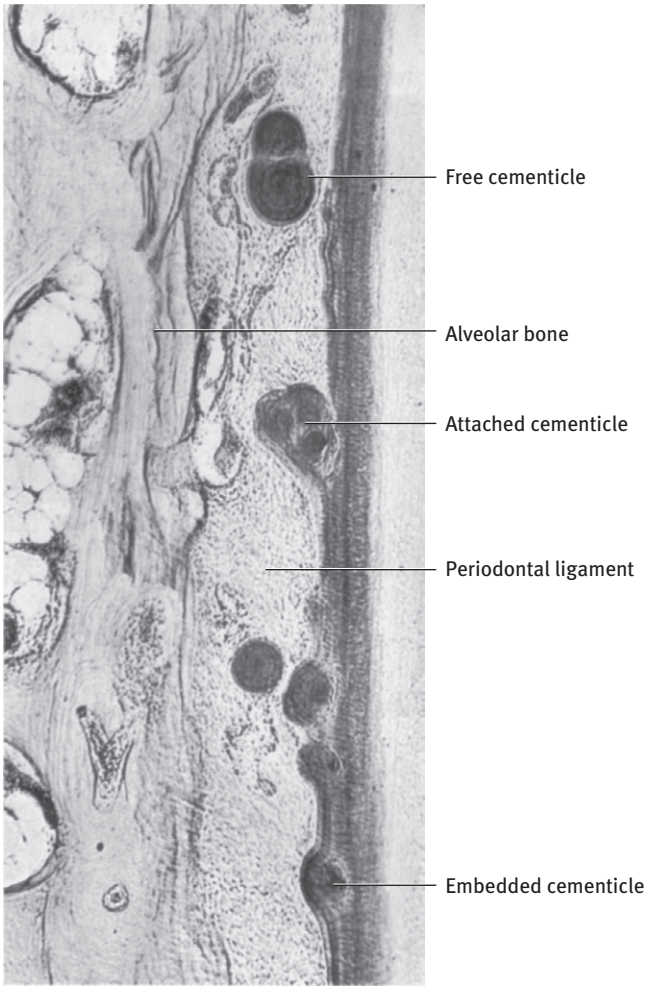

Figure 8.31 Cementicles in periodontal ligament.

drainage bypassing the capillaries. In some parts of the PDL, particularly around the apex a dense venous network is generally seen.

Lymphatic drainage

A network of lymphatic vessels, following the path of the blood vessels, provides the lymph drainage of the periodontal ligament. The flow is from the ligament toward and into the adjacent alveolar bone. The lymphatic vessels are like veins provided with valves. The lymph from the periodontal tissues drains into the lymph nodes of head and neck. The submental nodes drain the labial and lingual gingiva of the mandibular incisors, the submandibular lymph nodes drain the lingual gingiva of the mandibular premolar and the molar region, the third molars are drained directly to jugulodigastric lymph nodes. The maxillary palatal gingiva is drained into the deep cervical lymph nodes. The buccal gingiva of the maxilla drains into the submandibular lymph nodes.

It may course apically through the substance of PDL to arise and pass through the fundus of the socket or may through the cribriform plate. They finally empty into larger channels after pursuing intraosseous path. The flow is via the alveolar lymph channels which are joined by the dental and interradicular lymph channels.

An effective and adequate vasculature and lymphatic architecture is manifested in various developmental and healing process in the PDL.

Nerves

The PDL has functionally two types of nerve fibers: sensory and autonomic. The sensory fibers are associated with nociception and mechanoception, with touch, pressure, pain and proprioceptive sensations. The autonomic fibers are associated with PDL vessels.

All PDL innervations are mediated by the dental branches of alveolar nerves which enter through apical perforation of the tooth socket and perforating branches of interalveolar nerves traversing the bone.

Nerves, which usually are associated with blood vessels, pass through foramina in the alveolar bone, including the apical foramen, to enter the periodontal ligament. In the region of the apex, they run toward the cervix, whereas along the length of the root they branch and run both coronally and apically. The nerve fibers are either of large diameter and myelinated or of small diameter, in which case they may or may not be myelinated. The small fibers appear to end in fine branches throughout the ligament and the large fibers in a variety of endings, for example, knob like, spindle like, and Meissner like, but these seem to vary among the species. The large diameter fibers appear to be concerned with discernment of pressure and the small diameter ones with pain. Some of the unmyelinated small diameter fibers evidently are associated with blood vessels and presumably are autonomic.

Generally the myelinated fibers of PDL are 5–15 μm in diameter and the unmyelinated are 0.5 μm in diameter.

PDL mechanoceptors exhibit directional sensitivity as they respond to a force applied to the crown in a particular direction. Their conduction velocities place them in A α group of fibers. The response characteristics can vary from slowly to rapidly adapting fibers. It is said that 75% of mechanoceptors of PDL have their cell bodies in the trigeminal ganglion and in remaining, 25% cell bodies lie in mesencephalic nucleus.

In the PDL numerous nerve terminals are seen especially in the principal fibers.

Encapsulated pressure receptors and acini form fine pain receptors are seen in greatest numbers which function during mastication.

Cementicles

Calcified bodies called cementicles are sometimes found in the periodontal ligament. These bodies are seen in older individuals, and they may remain free in the connective tissue, they may fuse into large calcified masses, or they may be joined with the cementum (Fig. 8.31). As the cementum thickens with advancing age, it may envelop these bodies. When they are adherent to the cementum, they form excementoses. The origin of these calcified bodies is not established. It is possible that degenerated epithelial cells form the nidus for their calcification.

FUNCTIONS

The periodontal ligament has the following functions:

Supportive	Homeostatic
Sensory	Eruptive
Nutritive	Physical

Supportive

When a tooth is moved in its socket as a result of forces acting on it during mastication or through application of an orthodontic force, part of the periodontal space will be narrowed and the periodontal ligament contained in these areas will be compressed. Other parts of the periodontal space will be widened. The compressed periodontal ligament provides support for the loaded tooth. The collagen fibers in the compressed ligament, in concert with water molecules and other molecules bound to collagen, act as a cushion for the displaced tooth. The pressure of blood in the numerous vessels also provides a hydraulic cushion for the support of the teeth. It has often been suggested that the collagen fibers in the widened parts of the periodontal space are extended to their limit when a force is applied to a tooth and, being non-elastic, prevent the tooth from being moved too far.

Hence it is believed that the PDL behaves as suspensory ligament. Accordingly load on the PDL is dissipated to alveolar bone through the oblique principal fibers of PDL primarily, which is placed in tension and on release of load, an elastic recoil of tissue enables the tooth recovery to its resting position. However tooth mobility, surgical, morphological and biochemical studies provide evidence against the concept that PDL is a suspensory ligament. Physiological tooth mobility studies show the property of hysteresis, which suggests that the tissue has viscoelastic properties. The loaded tooth support system shows elasticity and in the recovery phase to its original position and also phases of movement. The viscoelastic properties might be displayed because of blood being squeezed out of the PDL via cribriform plate in the early intrusive phase, similarly loss of water from proteoglycans and partial disaggregation of large polymers might be seen in slow phases.

The tooth mobility studies with lathyrogens (drugs inhibiting formation of collagen crosslinks) and vasoactive drugs indicate that both PDL collagen fibers and periodontal vasculature are involved in tooth support.

The recent biochemical analysis of the proteoglycans in the PDL shows that the degree of aggregation/disaggregation of the ground substance may have a role in tooth support.

Thus it is evident that collagen fibers, vasculature and ground substance of the PDL are all contributing to the tooth support. Hence the tooth supporting system is not a property of one single component of PDL but a function of the tissue on the whole.

Sensory

The periodontal ligament, through its nerve supply, provides the most efficient proprioceptive mechanism, allowing the organism to detect the application of the most delicate forces to the teeth and very slight displacement of the teeth.

Mechanoprotection protects both supporting structures of the tooth and the substances of the crown from excessive masticatory forces.

The response elements of stromal cells and actin dependant sensory system are involved in the mechanical signal transduction.

To survive a mechanically active environment, cells adapt to variations of applied membrane tension. This adaptation involves sensing increase in the intracellular tension, maintaining contact with extracellular matrix ligands and preventing irreversible membrane disruptions.

The cortical actin assembly regulates stretch-activated cation permeable channel activity and provides a desensitization mechanism for cells exposed to repeated long-term mechanical stimuli. The actin response is cytoprotective. Basically the cortical actin assembly regulates the activity of stretch-activated calcium permeable channels

since sustained force application desensitizes these channels to subsequent force applications.

Recent studies suggest that actin binding protein-280 plays a vital role in mechanoprotection by:

1. Reinforcing the membrane cortex and thereby preventing force induced membrane disruption.
2. Increasing the strength of cytoskeletal links to the extracellular matrix.
3. Desensitizing stretch-activated ion-channel activity.

Nutritive

The ligament contains blood vessels, which provide anabolites and other substances required by the cells of the ligament, by the cementocytes, and presumably by the more superficial osteocytes of the alveolar bone. Experimental extirpation of the ligament results in necrosis of underlying cementocytes. The blood vessels are also concerned with removal of catabolites. Occlusion of blood vessels leads to necrosis of cells in the affected part of the ligament; this occurs when too heavy a force is applied to a tooth in orthodontic therapy.

Homeostatic

It is evident that the cells of the periodontal ligament have the capacity to resorb and synthesize the extracellular substance of the connective tissue of the ligament, alveolar bone, and cementum. It is also evident that these processes are not activated sporadically or haphazardly but function continuously, with varying intensity, throughout the life of the tooth. Alveolar bone appears to be resorbed and replaced (i.e. remodeled) at a rate higher than other bone tissue in the jaws. Furthermore the collagen of the periodontal ligament is turned over at a rate that may be the fastest of all connective tissues in the body, and the cells in the bone half of the ligament may be more active than those on the cementum side. Visual evidence for the high turnover of protein in the periodontal ligament is provided by the numerous silver grains seen in radioautographs of the tissue removed from animals. On the other hand, deposition of cementum by cementoblasts appears to be a slow, continuous process, and resorption is not a regular occurrence.

The mechanisms whereby the cells responsible for these processes of synthesis and resorption are controlled are largely unknown. It is evident that the processes are exquisitely controlled because, under normal conditions of function, the various tissues of the periodontium maintain their integrity and relationship to one another.

The preservation of the PDL width throughout mammalian lifetime is an important measure of PDL homeostasis. Recent studies have given an insight into the function of biological mechanisms that tightly regulate the metabolism of spatial location of the cell populations involved in the formation of bone, cementum, and the PDL. The ability of the PDL cells to synthesize and secrete a wide range of regulatory molecules is an essential component of tissue remodeling and PDL homeostasis. The cytokines and growth factors are locally acting regulators of cell function. The transforming growth factor ß isoforms synthesized by PDL cells can induce mitogenic effect and can also dose dependently downregulate osteoblastic activity and differentiation of PDL cells. Bone formation is also regulated by the prostaglandins and the paracrine factors.

This type of cellular signaling system may be capable of accurately 'measuring' and maintaining the width of PDL which functions under physical forces of mastication. Appropriate regulations of these systems is important since failure of homeostatic mechanism to regulate PDL width may lead to tooth ankylosis and/or root resorption.

If the balance between synthesis and resorption is disturbed, the quality of the tissues will be changed. For example, if an experimental animal is deprived of substances essential for collagen synthesis such as vitamin C or protein, resorption of collagen will continue unabated, but its synthesis and replacement will be markedly reduced. This will result in progressive destruction and loss of extracellular substance of periodontal ligament. This eventually will lead to loss of attachment between bone and tooth, and finally to loss of the tooth such as occurs in scurvy when vitamin C is absent from the diet.

In all areas of the periodontal ligament, there is apparently a continual slow death of cells, which are replaced by new cells that are provided by cell division of progenitor cells in the ligament.

Another aspect of homeostasis relates to function. A periodontal ligament supporting a fully functional tooth exhibits all the structural features described above. However, with loss of function, much of the extracellular substance of the ligament is lost, possibly because of diminished synthesis of substances required to replace structural molecules resorbed during normal turnover, and the width of the periodontal space is subsequently decreased (Fig. 8.32). These changes are accompanied by increased deposition of cementum and a decrease in alveolar bone tissue mass per unit volume. The process is reversible if the tooth is returned to function, but the precise nature of the stimuli that control the changed activity of the cells is unknown.

Eruptive

The cells, vascular elements and extracellular matrix proteins of the PDL function collectively to enable mammalian teeth of limited eruption to adjust their position while remaining firmly attached to the bone socket.

The PDL provides space and acts as a medium for cellular remodeling and hence continued eruption and approximal shift occurs.

Physical

In the periodontal ligament the physical function entails not only protection of vessels and nerves from mechanical forces but also to offer resistance to impact from occlusal forces. The PDL acts by transmission of occlusal forces to the bone by which shock absorption is achieved. As we see in case of tooth support two theories try to explain how the impact of occlusal forces is atraumatically transmitted to the bone. The theories are: the tensional and the viscoelastic system theory. The tensional theory has insufficient evidence to explain the process whereas the viscoelastic system theory is more accepted and aptly explains the phenomena.

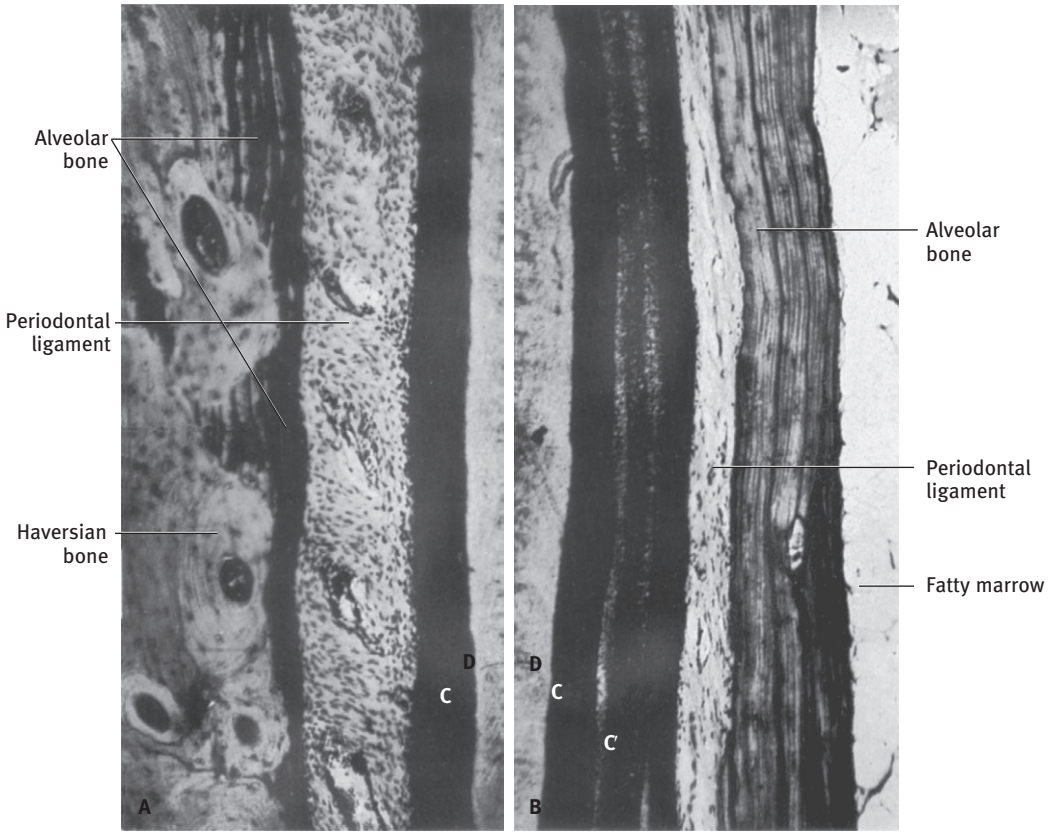

Figure 8.32 Periodontal ligament of a functioning, (**A**) and of a nonfunctioning, (**B**) tooth. In the functioning tooth, the periodontal ligament is wide, and principal fibers are present. Cementum, C is thin. In the nonfunctioning tooth, the periodontal space is narrow, and no principal fiber bundles are seen. Cementum is thick, C and C′. Alveolar bone is lamellated. D Dentin.

AGE CHANGES IN PERIODONTAL LIGAMENT

The age changes in the periodontal ligament are dealt in detail in Chapter 17 on Age Changes in Oral Tissues. However a brief mention of the age changes is outlined in the following paragraph.

With age, the cells decrease in number and the activity in the ligament also decreases. Due to lack of functional stimulation the width of the ligament also decreases.

UNIQUE FEATURES OF PERIODONTAL LIGAMENT

The periodontal tissue is mainly made up of collagen fibers in a proteoglycans stroma and many types of connective tissue cells as in any other soft fibrous connective tissue elsewhere in the body. But it has cells that form and resorb cementum and bone, and the collagen fibers in a specific orientation connecting the two mineralized tissues makes it unique. The tissue hydrostatic pressure is high. The tissue is extremely cellular, with fibroblast showing many intercellular contacts, well innervated with many mechanoreceptors and highly vascular; unlike any other connective tissue in the adult.

However, recently it has been shown that the PDL bears a resemblance to immature, fetal like connective tissues.

The features being, high cellularity, very high rates of turnover and with significant amount of type III collagen. The collagen fibers are also sharp with unimodal size and frequency. It is seen that the collagen fibers show reducible crosslink in collagen dehydro dihydroxy lisinonor leucine. The ground substance of PDL occupies large volume with high content of gluconate rich proteoglycans and glycoproteins tenascin and fibronectin. PDL also shows the presence of pre-elastin fibers like oxytalan.

Thus the PDL has structural, ultrastructural and biochemical features like fetal tissue. This has helped us to understand periodontal inflammatory diseases and for evolving newer treatment modalities.

CLINICAL CONSIDERATIONS

The primary role of the periodontal ligament is to support the tooth in the bony socket. Its thickness varies in different individuals, in different teeth in the same person, and in different locations on the same tooth, as is illustrated in Tables 8.9 and 8.10.

The measurements shown in Tables 8.9 and 8.10 indicate that it is not feasible to refer to an average figure as normal width of the periodontal ligament. Measurements of a large number of ligaments range from 0.15 to 0.38 mm. The fact that the periodontal ligament is thinnest in the middle region of the root seems to indicate that the

Table 8.9 Thickness of Periodontal Ligament of 154 Teeth from 14 Human Jaws*

	Average at Alveolar Crest (mm)	Average at Mid Root (mm)	Average at Apex (mm)	Average for Entire Tooth (mm)
Ages 11–16 (83 teeth from 4 jaws)	0.23	0.17	0.24	0.21
Ages 32–50 (36 teeth from 5 jaws)	0.20	0.14	0.19	0.18
Ages 51–67 (35 teeth from 5 jaws)	0.17	0.12	0.16	0.15

From Coolidge ED: J Am Dent Assoc 24:1260, 1937.
*The table shows that the width of the periodontal ligament decreases with age and that it is wider at the crest and at the apex than at the midroot.

Table 8.10 Comparison of Periodontal Ligament in Different Locations around the Same Tooth (Subject 11 Years of Age)*

	Mesial (mm)	Distal (mm)	Labial (mm)	Lingual (mm)
Upper right central incisor, mesial and labial drift	0.12	0.24	0.12	0.22
Upper left central incisor, no drift	0.21	0.19	0.24	0.24
Upper right lateral incisor, and labial drift	0.27	0.17	0.11	0.15

From Coolidge ED: J Am Dent Assoc 24:1260, 1937
*The table shows the variation in width of the mesial, distal, labial, and lingual sides of the same tooth

fulcrum of physiologic movement is in this region. The thickness of the periodontal ligament seems to be maintained by the functional movements of the tooth. It is thin in functionless and embedded teeth and wide in teeth that are under excessive occlusal stresses (Fig. 8.32).

For the practice of restorative dentistry the importance of these changes in structure is obvious. The supporting tissues of a tooth long out of function are poorly adapted to carry the load suddenly placed on the tooth by a restoration. This applies to bridge abutments, teeth opposing bridges or dentures, and teeth used as anchorage for removable bridges. This may account for the inability of a patient to use a restoration immediately after its placement. Some time must elapse before the supporting tissues become adapted again to the new functional demands. An adjustment period, likewise, must be permitted after orthodontic treatment.

Acute trauma to the periodontal ligament, accidental blows, or rapid mechanical separation may produce pathologic changes such as fractures or resorption of the cementum, tears of fiber bundles, hemorrhage, and necrosis. The adjacent alveolar bone is resorbed, the periodontal ligament is widened, and the tooth becomes loose. When trauma is eliminated, repair usually takes place. Occlusal trauma is always restricted to the intraalveolar tissues and does not cause changes of the gingiva such as recession or pocket formation or gingivitis.

Orthodontic tooth movement depends on resorption and formation of both bone and periodontal ligament. These activities can be stimulated by properly regulated pressure and tension. The stimuli are transmitted through the medium of the periodontal ligament. If the movement of teeth is within physiologic limits (which may vary with the individual), the initial compression of the periodontal ligament on the pressure side is compensated for by bone resorption, whereas on the tension side bone apposition is seen. Application of large forces results in necrosis of periodontal ligament and alveolar bone on the pressure side, and movement of the tooth will occur only after the necrotic bone has been resorbed by osteoclasts located on its endosteal surface.

The periodontal ligament in the periapical area of the tooth is often the site of a pathologic lesion. Inflammatory diseases of the pulp progress to the apical periodontal ligament and replace its fiber bundles with granulation tissue. This lesion, called a periapical granuloma, may contain epithelial cells that undergo proliferation and produce a cyst. The periapical granuloma and the apical cyst are the most common pathologic lesions of the jaws.

Last but not the least, the commonest pathology related to the PDL is chronic inflammatory periodontal disease. The toxins released from the bacteria in the dental plaque and metabolites of the host's defense mechanism destroy the PDL and the adjacent bone very frequently. Periodontal disease results because of periodontic bacteria coupled with specific host inflammatory response. The polymorphic nature of the immune system may explain individual differences in susceptibility to periodontitis and disease progression. Progression of the periodontal disease leads to tooth mobility and further loss of tooth. To repair the existing destruction of PDL can be quite challenging. It involves limiting the disease process and to regenerate the host tissues (PDL and bone) to their original form in such a way that reattachment of PDL to the bone becomes possible.

Various surgical techniques like guided tissue regeneration are being used for correction of PDL destruction. To date in many of these procedures implantation of structural substitutes have included, tube of autografts (cortical/cancellous bone, bone marrow), autografts (demineralized freeze-dried/freeze-dried bone) and alloplastic materials like ceramics, hydroxyapatite, polymers,

and bioglass, etc. But due to various reasons their stability and effectiveness is questioned.

More recently tissue engineering principles used with biological principles like gene therapy, use of biocompatible scaffolds (with growth factors) offer an interesting alternative to existing therapies.

Bioactive molecules such as growth factors, cytokines, bone morphogenic proteins and certain enamel proteins (amelogenin or enamel matrix protein), natural bone mineral have been seen to induct formation of periodontal cells and help in periodontal regeneration.

A better understanding of cell and molecular biology of the developing and regenerating periodontium offers newer avenues to regenerate the PDL. The use of progenitor cells or the mesenchymal stem cells to regenerate the PDL is being thought of. The PDL regeneration is said to be achieved by the activation of the mesenchymal stem cells towards terminal differentiation, tissue repair and degeneration.

Newer options of treatment are made available from time to time yet safeguarding the integrity of the PDL and alveolar bone is still one of the most important challenge.

SUMMARY

The periodontal ligament is a soft, vascular and cellular connective tissue that surrounds the roots of the teeth and joins the root cementum with the socket wall. Coronally, the ligament is continuous with the lamina propria of the gingiva and is demarcated from the gingiva by alveolar crest fibers. The periodontal ligament is derived from the dental follicle.

The shape of the ligament is that of an hour glass and is narrowest in the mid root level. The periodontal ligament space is decreased in non-functional and unerupted teeth and is increased in teeth subjected to heavy occlusal stress.

Periodontal Ligament Homeostasis

Maintenance of the normal width of the periodontal ligament irrespective of tooth position or its movement is termed periodontal ligament homeostasis. Growth factors (cytokines) like fibroblast growth factor, platelet derived growth factor, transforming growth factor, interleukin-1, interferon-γ and matrix metalloproteinases play important stimulatory and inhibitory roles of cells and in degradation of fibers to maintain the normal width of the periodontal ligament. The factors maintaining the periodontal ligament in an unmineralized state are Msx2, bone sialoprotein, osteopontin, proteoglycans and cementum attachment protein.

Collagen Fibers of the Periodontal Ligament

The predominant collagen present in the ligament is type I, followed by type III and small amounts of type V and VI and traces of type IV and VII. The most important elements of the periodontal ligament are cells and principal fibers.

Principal Fibers of the Periodontal Ligament

The principal fibers are collagenous, arranged in wavy bundles in longitudinal sections and are 5 μm in diameter. These principal fibers connect the alveolar bone and the cementum. Five groups can be identified and they are alveolar crest, horizontal, oblique, apical and interradicular. The fibers are named according to their orientation or location. Majority of fibers belong to the oblique group and interradicular fibers are seen only in multirooted teeth.

Sharpey's Fibers

The terminal portions of the principal fibers embedded in cementum and bone are termed Sharpey's fibers. Transalveolar fibers are those Sharpey's fibers that pass through the alveolar bone to the adjacent periodontal ligament.

Other Types of Fibers of the Periodontal Ligament

The ligament also contains immature elastic fibers—the oxytalan and elaunin fibers. Oxytalan fibers run in a perpendicular direction and connect cementum with walls of blood vessels. They probably serve to support the blood vessels.

In addition to the above mentioned fibers, there are small fibers associated with the large principal collagen fibers termed indifferent fiber plexus.

Cells of the Periodontal Ligament

The cells present in the periodontal ligament are the synthetic cells and resorptive cells. The synthetic cells are the fibroblasts, osteoblasts and cementoblasts. The resorptive cells are fibroblasts, osteoclasts and cementoclasts. Fibroblasts are the most numerous cells and are capable of synthesizing and degrading collagen. The turnover rate of collagen is rapid and is probably highest among all the connective tissues. The degradation of collagen may occur both extracellularly and intracellularly. Fibroblasts are in contact with each other through gap junctions. Cementoclasts are rarely seen as cementum resorption is not seen normally. A population of stem cells, which are different, than bone marrow stem cells are present.

Other cells in the periodontal ligament are cell rests of Malassez, defense cells like macrophages, mast cells and eosinophils. Macrophages not only phagocytose dead cells but also secrete growth factors to regulate fibroblast proliferation. Similarly mast cells regulate endothelial and fibroblast proliferation. Progenitor cells are also seen and are capable of undergoing mitotic division. These cells are seen predominantly adjacent to blood vessels.

Ground Substance

The ground substance of the ligament is made up of glycosaminoglycans, glycoproteins and proteoglycans. 70% of the ground substance is water. All anabolites and catabolites pass through the ground substance. The ground substance composition varies with the state of development of tooth.

Remodeling of fibers was believed to occur in the Intermediate plexus. This plexus has been observed only in the longitudinal sections of growing incisors of roots and not in cross-sections. Hence, it is considered to be an artifact as collagen fibers of periodontal ligament in continuously growing incisors are arranged in sheets than in bundles.

Vascularity and Innervation

The periodontal ligament has a rich vascular supply and lymphatic drainage. A high and complex metabolic requirement explains the presence of special features like, cervical and circular plexus along with fenestrated capillaries. An encompassment of both large and small, myelinated and unmyelinated fibers take care of the functional, sensory needs of the periodontal ligament.

Functions

The ligament has many functions. The main function is to support the tooth. Its sensory function involves the detection of even the slightest amount of force applied to the tooth through its proprioceptors. The nutritive function to all the cells of periodontal ligament is through the abundant blood supply. Homeostatic function is to maintain the width of the periodontal ligament irrespective of the direction of tooth movement and position of the tooth. This is done by formation or resorption of bone, formation of cementum, formation and degradation of collagen fibers and the ground substance and detachment and reattachment of collagen fibers to their new locations. Apart from these the periodontal ligament has eruptive and physical functions.

Age Changes

Age changes are seen in the ligament in the form of cementicle formation, reduced cellular and functional activity.

Different forms of periodontitis are the most common set of pathology associated with the ligament. Though common, it is challenging to treat these pathologies. Recent advents in technique and material sciences may yield long-term promising results.

REVIEW QUESTIONS

1. Enumerate the principal fibers of the periodontal ligament. Describe the course and function of each group.
2. Which are the types of collagen seen in the periodontal ligament?
3. Describe briefly the growth factors involved in the normal biology of the periodontium.
4. Describe the synthetic cells in the periodontal ligament and what are their functions?
5. What is the average width of the periodontal ligament? How does it vary? How is the width maintained?
6. Describe the resorptive cells in the ligament and their functions.
7. Write notes on:
   Oxytalan fibers
   Cementicles
   Cell rests of Malassez
   Indifferent fiber plexus
   Intermediate plexus
   Age changes in periodontal ligament.
8. What is the ground substance of the periodontal ligament made up of? What are its functions?
9. Discuss the functions of the periodontal ligament.
10. Describe the special features of the blood vessels of the ligament.

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9 Bone

CHAPTER CONTENTS

Bone is a living tissue, which makes up the body skeleton and is one of the hardest structures of the animal body. Bone possesses a certain degree of toughness and elasticity. It provides shape and support for the body. It also provides site of attachment for tendons and muscles, which are essential for locomotion. It protects the vital organs of the body. Bone serves as a storage site for minerals and provides the medium, the marrow for the development and storage of blood cells.

CLASSIFICATION OF BONES

Bones may be classified according to their shape, mode of development or by their histologic appearance.

Classification Based on Shape

Bones are classified according to their shape as **long**, **short**, **flat** and **irregular bones**.

Long bones

These bones are long and slender. They are longer than they are wider. The long bones include the bones of the arm—humerus, radius, ulna; leg—femur, tibia, fibula; fingers and toes—each phalanx (individual bone of finger or toe, all together the phalanges); palms of hands and soles of feet—metacarpals and metatarsals.

The long bones have a tubular diaphysis or the shaft, which is made of compact bone at the periphery, surrounding a central medullary cavity, which contains yellow marrow. The two bulky ends of the long bone are called the epiphysis, which are made of compact bone at the periphery and spongy bone at the center. The articular surface of the epiphysis is covered with hyaline cartilage, which provides a cushioning effect to the opposing bone ends during joint movements and absorbs stress. ***Epiphyseal line*** is present between the diaphysis and each epiphysis of an adult long bone. This line is a remnant of the ***epiphyseal plate***.

Short bones

These bones are usually cube shaped of nearly equal length and width. They consist of spongy bone which is covered by a thin layer of compact bone. They contain bone marrow, but no marrow cavity. These bones include bones of wrist—carpals and ankle—tarsals.

Flat bones

These bones are thin, flat, curved, with no marrow cavity. Spongy bone is present between upper and lower layer of compact bone. Flat bones include bones of sternum, ribs, scapula, clavicle, and bones that form roof of the skull, parietal, frontal, temporal and occipital.

Irregular bones

These bones have complex shapes, notched or with ridges and are not included in any of the above categories. These bones are primarily made of spongy bone, which is covered with a thin layer of compact bone, with bone marrow, but no marrow cavity. The irregular bones include bones of vertebrae, facial bones (ethmoid, sphenoid) pelvic bones (ischium and pubis), calcareous (heel bone) and mandible.

Sesamoid bones

These bones develop in tendons, where there is considerable pressure, tension or friction. Patella (knee cap) is a good example.

Classification Based on Development

Developmentally, bones are classified as **endochondral bones** and **intramembranous bones**.

Endochondral bones

These bones are formed by replacement of hyaline cartilage with bony tissue. This type of ossification occurs in bones of trunk and extremities.

Intramembranous bones

These bones are formed by replacement of sheet like connective tissue membrane with bony tissue. This ossification occurs in the cranial and facial flat bones of the skull, mandible and clavicles.

Classification Based on Microscopic Structure

Histologically, bones are classified as **mature bone** and **immature bone**.

Mature bone is further classified as compact bone and cancellous bone.

Compact bone (cortical bone) consists of tightly, packed osteons or haversian systems, forming a solid mass. Since the bone mass is arranged in layers, it is also called **lamellar bone** (Fig. 9.1).

Cancellous bone (spongy bone) has a honeycomb appearance, with large marrow cavities and sheets of trabeculae of bone in the form of bars and plates (Fig. 9.2).

Woven or immature bone is the first formed bone with irregularly oriented collagen fibers of varying diameters. This type of bone is not usually seen after birth. However it is seen in the alveolar bone and during healing of fractures. Since this bone forms rapidly, it incorporates many osteocytes (Fig. 9.3).

COMPOSITION OF BONE

Bone is a connective tissue composed of cells, fibers and ground substance. The intercellular substance of bone consists of organic and inorganic substances. The inorganic part of bone is made of bone minerals.

The **mineral** component is composed of hydroxyapatite crystals, with carbonate content and low Ca/P ratio than the pure hydroxyapatite. Small amounts of calcium phosphate are also present. Bone crystals are in the form of thin plates or leaf-like structures. They are packed closely with long axis nearly parallel to collagen fibrils

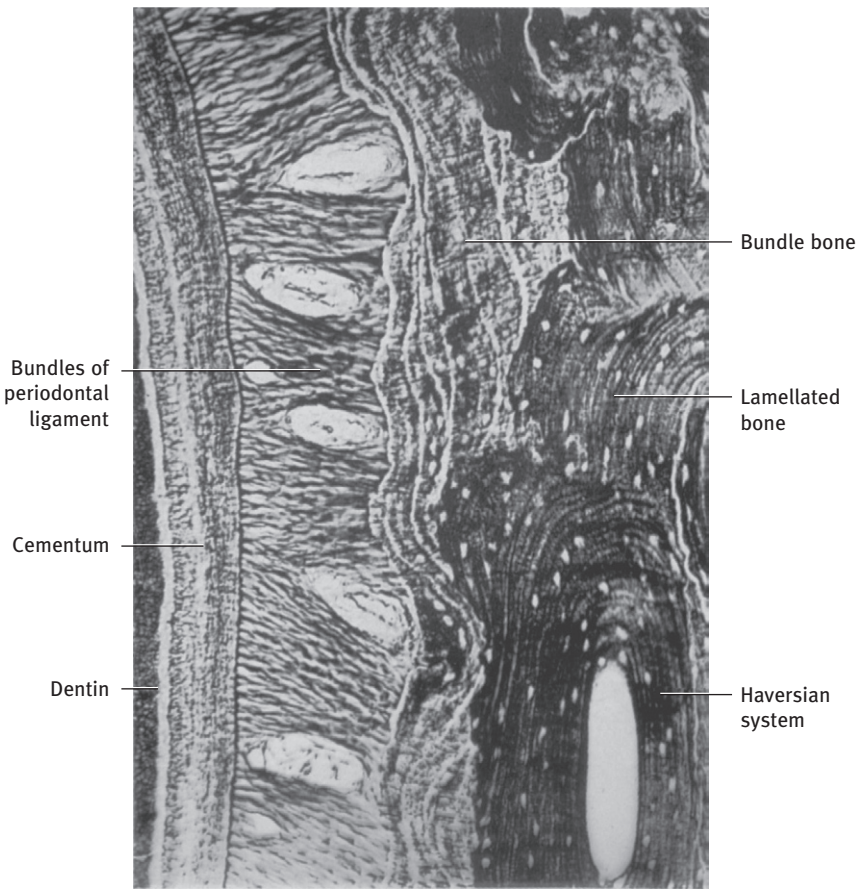


Figure 9.1 Alveolar bone proper consisting of bundle bone and haversian bone on distal alveolar wall. A reversal line separates the two (silver impregnation).

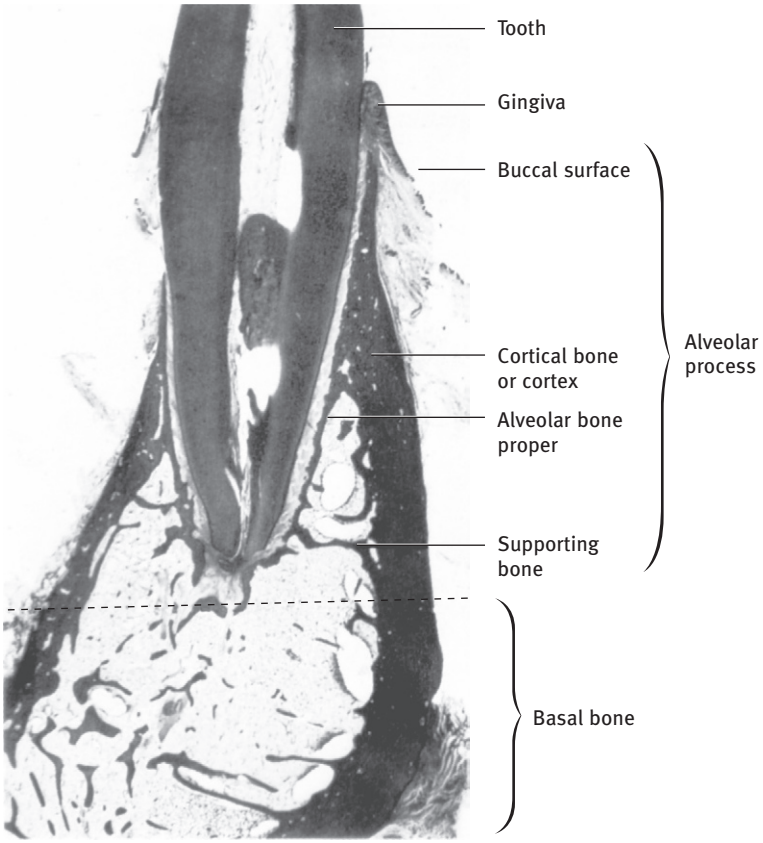


Figure 9.2 Section through mandible showing relationship of tooth to alveolar process and basal bone. Note the supporting bone is made up of spongy (cancellous) bone (From Bhaskar SN: Synopsis of oral histology, St Louis, 1962, The CV Mosby Co).

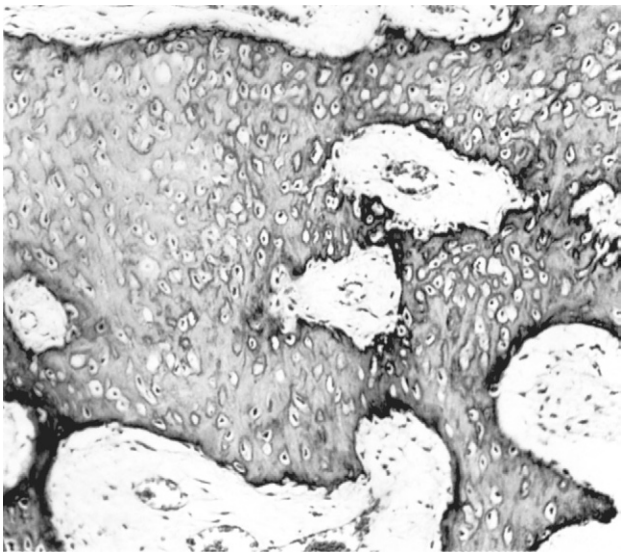


Figure 9.3 Immature bone. Note many osteocytes and absence of lamellae or resting lines (From Bhaskar SN: Synopsis of oral histology, St Louis, 1962, The CV Mosby Co).

axis. The narrow gaps between the crystals contain associated water and organic macromolecules.

The ions present are calcium phosphate, hydroxyl and carbonate. Citrate, magnesium, sodium, potassium, fluoride, iron, zinc, copper, aluminum, lead, strontium, silicon and boron are present in small quantities.

The organic matrix is known as **osteoid** and is made up of collagen and the noncollagenous proteins.

Collagen is the major organic component in mineralized bone tissues. Type I collagen (> 95%) is the principal collagen in mineralized bone and, together with type V collagen (< 5%), forms heterotypic fiber bundles that provide the basic structural integrity of connective tissues. The elasticity of collagen imparts resiliency to the tissue and helps to resist fractures. Alveolar bone contains ***type I, type V, type III*** and ***type XII*** collagen. Sharpey's fibers contain type III collagen with type I collagen. Type XII collagen is also present in alveolar bone and has been found to be expressed under conditions of mechanical strain. Types III and XII collagen fibers are produced by fibroblasts during the formation of the periodontal ligament. Types I, V and XII collagens are expressed by osteoblasts. The pink to red color of bone matrix seen in hematoxylin and eosin sections is due to the substantial collagen content.

In **woven bone**, fibers are interwoven with a great amount of interfibrillar space that is occupied by mineral crystals and associated acidic proteins. In **maturebone**, collagen fibers form organized sheets. The fibers are oriented perpendicular to each other with little interfibrillar space.

Noncollagenous Proteins

Noncollagenous proteins comprise the remaining 10% of the total organic content of bone matrix. Most are ***endogenous proteins*** produced by bone cells, while some like ***albumin*** are derived from other sources such as ***blood***

and become incorporated into bone matrix during osteosynthesis. Some of the important noncollagenous proteins are Osteocalcin (Gla proteins), Osteopontin, bone sialoproteins and Osteonectin. Osteonectin forms 25 % of noncollagenous proteins and is bound to collagen. Bone matrix also contains proteoglycans, of which Biglycan and Decorin are important. It also contains bone morphogenetic proteins (BMPs) and a variety of growth factors, like platelet derived growth factor (PDGF), fibroblast growth factor (FGF) and insulin like growth factors (IGF). Lysyl oxidase and tyrosine rich acidic matrix proteins (TRAMP) are important components of demineralized bone matrix.

BONE HISTOLOGY

Osteoid is an unmineralized bone matrix on the surface, where active bone formation is taking place. It is approximately 5–10 μm before the commencement of mineralization. The mineralizing front is linear. Osteoid contains type I collagen fibers, parallel to the bone surface, embedded in ground substance of proteoglycans, glycoproteins and other proteins.

All mature bones have a dense outer sheet of ***compact bone*** and a central medullary cavity. The cavity is filled with red or yellow bone marrow in living bone. This cavity shows a network of bone trabeculae. ***Trabecular, spongy*** or ***cancellous bone*** are the terms used to describe this network.

The outer aspect of compact bone is surrounded by a condensed fibrocollagen layer, the ***periosteum*** which has two layers: an *outer layer* which is a dense, irregular connective tissue termed *fibrous layer*; and an *inner osteogenic layer*, next to the bone surface consisting of bone cells, their precursors and a rich vascular supply. The periosteum is active during fetal development. It is also important in the repair of fractures.

The inner surfaces of compact and cancellous bone are covered by a thin cellular layer called **endosteum.** In resting adult bone, quiescent osteoblasts and osteoprogenitor cells are present on the endosteal surfaces. These cells act as reservoir of new bone forming cells for remodeling or repair.

At the periosteal and endosteal surfaces, the lamellae are arranged in parallel layers surrounding the bony surface and are called ***circumferential lamellae.***

Deep to the circumferential lamellae, the lamellae are arranged as small concentric layers around a central vascular canal. ***Haversian (vascular) canal*** (about 50μ in diameter) and the ***concentric lamellae*** together is known as the **osteon** or **haversian system** (Fig. 9.4). There may be up to 20 concentric lamellae within each osteon. Osteon is the basic metabolic unit of bone. A cement line of mineralized matrix delineates the haversian system. This cement line contains little or no collagen, and is strongly basophilic, because it has a high content of glycoproteins and proteoglycans. It marks the limit of bone erosion prior to the formation of osteon, and is therefore also known as ***reversal***

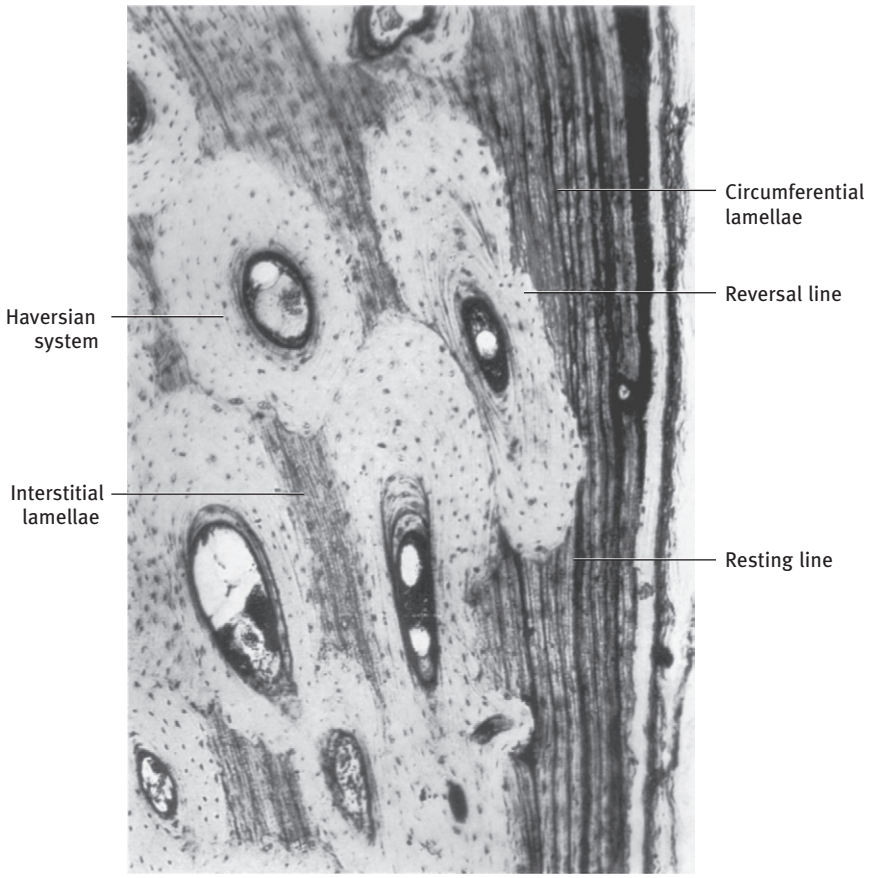


Figure 9.4 Appositional growth of mandible by formation of circumferential lamellae. These are replaced by haversian bone; remnants of circumferential lamellae in the depth persisting as interstitial lamellae.

line (Fig. 9.5). This line appears to be highly irregular as it is formed by the scalloped outline of the Howship's lacunae. This line has to be distinguished from the more regular appearance of the ***resting line***, which denotes the period of rest during the formation of bone (Fig. 9.4). The collagen fibers within each lamella spiral along the length of lamella, but have different orientations to those in adjacent lamella. This pattern is to withstand torsion stresses.

Adjacent haversian canals are interconnected by ***Volkmann's canals***, channels that contain blood vessels, creating a rich vascular network, throughout the compact bone. Osteocytes are present in lacunae, at the junctions of the lamellae. Small canaliculi radiate from lacunae to haversian canal to provide a passage way through the hard matrix. The canaliculi connect all the osteocytes in an osteon together. This connecting system permits nutrients and wastes to be relayed from one osteocyte to the other. The adult bones, between the osteons, contain **interstitial lamellae**, which are remnants of osteons, left behind during remodeling.

Spongy bone

Spongy bone and compact bone have the same cells and intercellular matrix, but differ in the arrangement of components. Spongy bone looks like a poorly organized tissue in contrast to compact bone.

The bony substance consists of large slender spicules called ***trabeculae***. The trabeculae are up to 50 μm thick. The trabeculae are oriented along lines of stress to withstand the forces applied to bone (Fig. 9.2). The marrow spaces are large. The trabeculae surround the marrow spaces from where they derive their nutrition through diffusion.

Hemopoietic Tissue in Bones

Red marrow is present in young bone and yellow marrow in old bone. Red marrow is found within cavities of spongy bone of long bones and diploe of flat bones. In newborn infants, the medullary cavity and all areas of spongy bone contain red bone marrow. Red marrow contains stem cells of both the fibroblast/mesenchymal type and blood cell lineage. Yellow marrow is seen in epiphysis of long bones. In old bone, the marrow is yellow, with loss of hemopoietic potential and increased accumulation of fat cells.

Yellow marrow of the medullary cavity can revert to red marrow, if a person is anemic and needs increased red blood cell production.

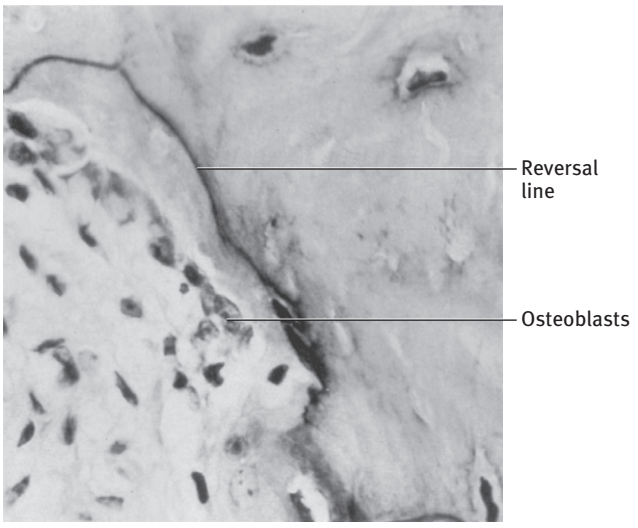


Figure 9.5 Reversal line in bone (From Bhaskar SN: Synopsis of oral histology, St Louis, 1962, The CV Mosby Co).

BONE CELLS

Osteoblasts

Osteoblasts are mononucleated cells responsible for the synthesis and secretion of the macromolecular organic constituents of bone matrix. These cells are derived from osteoprogenitor cells of mesenchymal origin, which are present in the bone marrow and other connective tissues. Periosteum also serves as an important reservoir of osteoblasts, particularly during childhood growth, after skeletal fractures or with bone forming tumors.

Morphology

Osteoblasts are basophilic, plump cuboidal or slightly flattened cells. These cells are found on the forming surfaces of growing or remodeling bone. These cells produce the organic matrix of bone (*Osteoid*) which primarily consists of type I collagen and the balance being noncollagenous proteins (Fig. 9.6).

Osteoblasts exhibit abundant and well developed protein synthetic organelles. The intense cytoplasmic basophilia is due to an abundance of rough endoplasmic reticulum. The procollagen and other organic constituents of bone matrix synthesized by this organelle, enter its lumen and are carried by transfer vesicles to the Golgi complex and assembled within Golgi complex in secretory granules. A pale juxtanuclear area indicates the site of the Golgi complex. These granules release their contents along the surface of cell opposed to forming bone which, assemble extracellularly as fibrils to form osteoid. The presence of the noncollagenous proteins within the secretory collagen granule or in a distinct population of granules is debatable. But, the noncollagenous proteins are also released along the surface of osteoblasts apposed to osteoid and diffuse from osteoblast surface, towards the mineralization front, where they participate in regulating mineral deposition. Nucleus is situated eccentrically in the part of the cell that is farthest away from the adjacent bone surface.

Organic matrix is deposited around the cell bodies and their cytoplasmic processes resulting in the formation of canaliculi. The cytoplasmic processes are not seen in H and E sections, but in other preparations, it is known that, they are in contact with one another and also with processes of osteocytes in lacunae beneath them. The cells contact one another by adherens and gap junctions. These are functionally connected to microfilaments and enzymes associated with intracellular secondary messenger systems. This arrangement provides for intercellular adhesion and cell to cell communication and ensures that osteoblast layer completely covers the osteoid surface and functions in a coordinated manner.

Osteoblasts also contain prominent bundles of actin, myosin and cytoskeletal proteins which are associated with maintenance of cell shape, attachment and motility.

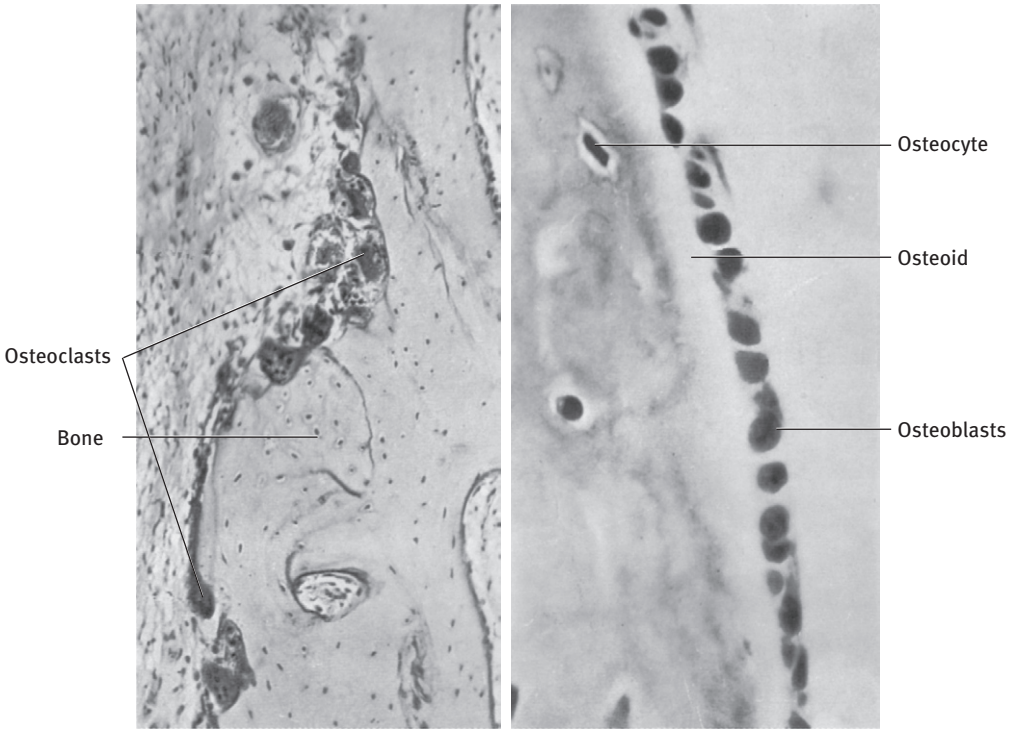


Figure 9.6 Resorption and apposition of bone. Left, osteoclasts in Howship's lacunae. Right, osteoblasts along bone trabecula. Layer of osteoid tissue is a sign of bone formation.

Formation

Osteoblasts are derived from undifferentiated pluripotent mesenchymal stem cells. Osteoprogenitor cells are divided into two types, ***determined (DOPCs)*** and ***inducible*** osteogenic precursor cells ***(IOPCs)***. These DOPCs are present in the bone marrow, endosteum and periosteum and differentiate into osteoblasts under the influence of systemic and bone derived growth factors. The IOPCs represent mesenchymal cells present in other organs and tissues that may differentiate into bone forming cells when stimulated.

The osteoprogenitor cells express transcription factors ***cbfa1/Runx-2*** and osterix which are essential for osteoblast differentiation. cbfa1 is a member of runt related family of transcription factors. It triggers the expression of BSP, osteopontin, osteocalcin and type I collagen. Osterix, a zinc finger-containing transcription factor, is similar to Runx-2.

Functions

The main function of osteoblast is the formation of new bone via synthesis of various proteins and polysaccharides. Other functions include the regulation of bone remodeling and mineral metabolism. Osteoblasts also play a significant role in the mineralization of osteoid. Osteoblasts secrete type I collagen which is widely distributed and not unique to osteoblasts whereas, osteocalcin and cbfa-1 (osteoblast specific transcription factor) are specific to cells of osteoblast lineage. These provide useful markers of osteoblast phenotype. Osteoblasts also secrete small amounts of type V collagen, osteonectin, osteopontin, RANKL, osteoprotegerin, proteoglycans, latent proteases and growth factors including bone morphogenetic proteins. Osteoblasts exhibit high levels of alkaline phosphatase on outer surface of plasma membrane which is used as a cytochemical marker to distinguish preosteoblasts from fibroblasts. Total alkaline phosphatase activity has been recognized as a reliable indicator of osteoblast function. Osteoblasts express receptors for various hormones including PTH, vitamin D_3, estrogen and glucocorticoids, which are involved in the regulation of osteoblast differentiation. The osteoblasts recognize the resorptive signal and transmit it to the osteoclast.

RANKL is a membrane bound TNF related factor, that is expressed by osteoblasts/stromal cells. The presence of RANKL is vital in osteoclast differentiation.

Cbfa1 expressed by osteoprogenitor cells, regulates the expression of OPG (osteoprotegerin) which is a potent inhibitor of osteoclast formation and function (Tables 9.1, 9.2).

Bone lining cells

Once osteoblasts have completed their function, they are either entrapped in the bone matrix and become osteocytes or remain on the surface as ***lining cells***. Osteoblasts flatten, when bone is not forming and extend along the bone surface and hence the name. These cells contain very few organelles but, retain gap junctions with osteocytes, while retaining its vitality.

But 50–70% of osteoblasts present at the remodeling site cannot be accounted for after enumeration of lining cells and osteocytes. It has been proposed that missing osteoblasts die by apoptosis. Growth factors and cytokines produced in bone microenvironment influence this process. Tumor necrosis factor (TNF) promotes apoptosis. ***TGF-β*** and ***IL-6*** have anti-apoptotic effects. ***Glucocorticoids*** and ***estrogen*** withdrawal promote apoptosis in osteoblasts and osteocytes.

Osteocytes

Osteoblasts produce the extracellular matrix, osteoid. As the osteoblasts form the bone matrix, they get entrapped

Table 9.1 Factors Favoring Formation of Osteoblasts

Transcription factors
Differentiation
- Wnt/β-catenin pathway
- Cbfa1 (core -binding factor α1)
- Osterix

Regulatory
- Runx-2

Local and systemic factors
(can act in a paracrine/ autocrine way and regulate the activity of specific transcription factors)
Bone morphogenetic proteins (BMPs)
BMP-2, BMP-4, BMP-6
Cell growth factors
Fibroblast growth factor (FGF), Insulin–like growth factor (IGF 1 and 2), transforming growth factor β, platelet derived growth factor and vascular endothelial growth factor
Hormones
PTH, Insulin, Growth hormone and Vitamin D_3.
Glucocorticoids(in vitro)
cytokine – IL 6

Table 9.2 Factors Limiting Formation of Osteoblasts

TNFα
Prolonged treatment with glucocorticoids (in vivo)

within the matrix they secrete, and are called ***osteocytes***. The number of osteoblasts that become osteocytes, depends on the rapidity of bone formation. ***Embryonic (woven)*** bone and ***repair*** bone, show more osteocytes, than lamellar bone, as they are formed rapidly. There are approximately ten times more osteocytes than osteoblasts in an individual bone. The average half life of human osteocytes is approximately 25 years. The life span of osteocytes exceeds that of active osteoblasts, which is estimated to be only three months in human bones.

During the preparation of ground sections, the osteocytes are lost, but the spaces are filled with debris and appear black, when viewed under the microscope, using transmitted light.

Within the bone matrix, the osteocyte reduces in size, creating a space around it, called the ***osteocytic lacuna***. Under the electron microscope, it has been observed that, a thin layer of uncalcified tissue, lines the lacuna. The lacunae can appear ovoid or flattened. Narrow extensions of these lacunae form channels called ***canaliculi***. ***Osteocytic processes*** are present within these canaliculi. Canaliculi do not usually extend through and beyond the reversal line surrounding an osteon, and so do not communicate with neighboring systems. These processes contain bundles of microfilaments and some smooth endoplasmic reticulum. At the distal end, these processes contact the processes of adjacent cells, i.e. other osteocytes through gap junctions. They also maintain contact with osteoblasts and bone lining cells on the surface.

The canaliculi penetrate the bone matrix and permit diffusion of nutrients, gases and waste products between osteocytes and blood vessels. Osteocytes also sense the changes in environment and send signals that affect response of other cells involved in bone remodeling. This interconnecting system maintains the bone integrity and bone vitality. Failure of the interconnecting system between osteocytes and osteoblasts leads to sclerosis and death of bone.

Mature inactive osteocytes possess an ellipsoid cell body with long axis parallel to the surrounding bony lamellae. The nucleus is oval with a narrow rim of faintly basophilic cytoplasm. The cell has very few organelles, but contains sufficient rough endoplasmic reticulum and large Golgi region, to suggest that these cells are capable of keeping the bone matrix in a state of good repair. Osteocytes secrete a few matrix proteins. Lysosomes have also been described in the older osteocytes.

Old osteocytes retract their processes from the canaliculi, and when dead, their lacunae and canaliculi may get plugged with debris. The death of the osteocytes leads to resorption of the matrix by osteoclasts.

Transformation of osteoblasts into osteocytes. At the end of bone forming phase, osteoblasts can have one of four different fates:

(a) Become embedded in the bone as osteocytes
(b) Transform into inactive osteoblasts and become bone lining cells
(c) Undergo apoptosis
(d) Transdifferentiate into cells that deposit chondroid or chondroid bone.

The transformation process is proposed to involve three cells, preosteoblasts which differentiate into osteoblasts, and these osteoblasts which become trapped as osteocytes.

Preosteoblasts are less cuboidal in shape and are located at a distance from the bone surface, do not deposit bone matrix, but can still divide. These cells produce type I collagen precursor molecules, which later assemble into collagen fibrils after post transitional modification.

Preosteoblasts differentiate into active bone matrix secreting osteoblasts, which are cuboidal in shape, and ultimately deposit the bone matrix.

As the bone matrix deposition continues, osteoblasts become embedded in the secretory product, the osteoid. Cells at this early stage of osteoblast to osteocyte differentiation are called large osteocytes. These cells are large with a well developed Golgi apparatus for collagen storage.

On mineralization of the osteoid, there is a reduction in the endoplasmic reticulum and Golgi apparatus in the osteocyte, suggesting a decrease in protein synthesis.

Four schemes have been proposed to explain how an osteoblast could get trapped within bone matrix.

– Osteoblasts are unpolarized and lay down bone in all directions, i.e. the cells become trapped in their own secretions.
– Individual osteoblasts are polarized, but those within same generation are polarized differently to those in adjacent layers. As a result, bone is deposited in all directions and osteoblasts become trapped.
– Osteoblasts of each generation are polarized in the same direction. One generation buries the preceding one in bone matrix.
– Within one generation, some osteoblasts slow down rate of bone deposition or stop laying down bone, so that they become trapped by the secretion of their neighboring cells.

It has also been proposed that osteoblasts are highly polarized and function as a unit to lay down bone

synchronously. All cells move away from osteogenic front as bone matrix is deposited, ultimately resulting in acellular bone.

Decision to transform into osteocyte, during bone formation, processes on the vascular surface of the osteocytes continue to grow to enable osteocytes to remain in contact with active osteoblast layer and to modulate their activity. When these vascular facing processes stop growing, they produce a signal that induces the recruitment of these osteoblasts with which they are losing contact. The committed osteoblasts are then transformed into osteoblastic osteocytes. The signal to stop growing a vascular process, may be issued by the osteoblasts, with which they have contact or it may be due to the gradual reduction in the vascular supply to the osteocyte, as new layers of bone are laid down on the osteogenic front.

Several factors have been reported to modulate osteoblast function and are involved in controlling the decision to transform into an osteocyte.

- The transcription factors Runx-2 and osterix are important for osteoblast differentiation.
- Leptin is a gene product synthesized by adipocytes but may serve an important signal to modulate osteoblast function. It has also been shown that leptin protects cells from apoptosis and facilitates transformation from osteoblast to preosteocyte.
- TGF-β related signaling mechanism can increase the propensity of osteoblast to mature into an osteocyte and decrease the duration of its productive functioning by shortening its life span.

Two different mechanisms of transformation have been proposed:

- Stationary osteoblasts transform into osteocytes by self-burial
- Dynamic osteoblasts are selected to transform into osteocytes by secretory activity of neighboring osteoblasts.

But, there is not only one mechanism for transforming osteoblasts into osteocytes, since different mechanisms exist in different bones, different types of bone formation, different positions within a bone, and different vertebrate species.

Osteoclasts

The word 'osteoclast' is derived from the Greek words for 'bone and broken'. Osteoclast is a type of bone cell that removes bone tissue by removing the mineralized matrix of bone.

Morphology

Osteoclasts lie in resorption bays called Howship's lacunae. Osteoclast is a large cell approximately 40–100 μm in diameter with 15 to 20 closely packed nuclei. Osteoclasts with many nuclei resorb more bone than osteoclasts with few nuclei (Fig. 9.6). The different nuclei are proposed to be of different ages and there is evidence of apoptosis. These cells are variable in shape due to their motility. The cytoplasm of the osteoclast shows acid phosphatase containing vesicles and vacuoles. The presence of acid phosphatase distinguishes the osteoclast from other multinucleated giant cells. Mitochondria are extensive and distributed throughout the cytoplasm, except below the ruffled border. Rough endoplasmic reticulum is relatively sparse for the size of the cell. Golgi complex is extensive and arranged in stacks. The cytoplasm also contains microtubules, which transport vesicles between Golgi stacks and ruffled membrane. Cathepsin containing vesicles and vacuoles are present close to the ruffled border indicating resorptive activity of these cells.

Formation of osteoclast

Osteoclasts are derived from hemopoietic cells of monocyte macrophage lineage. The earliest identifiable hematopoietic precursor that can form osteoclast is the granulocyte-macrophage colony forming unit (CFU-GM). The early precursor cells proliferate and differentiate to form post mitotic committed precursor cells. These committed precursors then differentiate and fuse to form immature multinucleated giant cells. These are activated to form bone resorbing osteoclasts. The differentiation into osteoclasts is through a mechanism involving cell–cell interaction with osteoblast stromal cells. The formation of osteoclast requires the presence of ***RANK ligand (receptor activator of nuclear factor κB)*** and ***M-CSF (macrophage colony stimulating factor).*** These two membrane bound proteins are produced by neighboring stromal cells and osteoblasts. These react with RANK to produce fully functional osteoclast. The interaction between RANK and RANKL is opposed by Osteoprotegerin (OPG), secreted by osteoblasts, leading to inhibition of osteoclast differentiation and thus help in regulation of osteoclast formation (Flowchart 9.1).

Regulation of osteoclast activity

Many factors both local and systemic may act alone or in conjunction with other factors to promote the formation of osteoclast or its activity. These factors are outlined in Table 9.3.

BONE FORMATION

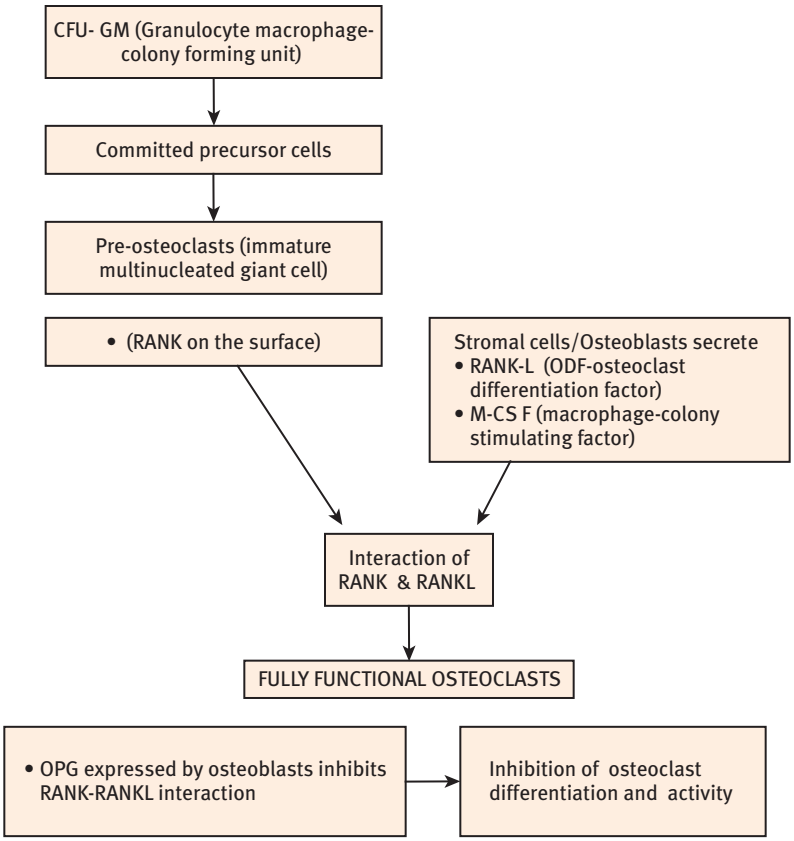
Intramembranous Ossification

Intramembranous ossification is the direct formation of bone within highly vascular sheets of condensed primitive mesenchyme. This process occurs in the flat bones of the skull and clavicles. It begins approximately towards the end of second month of gestation. The sequence of events is as follows.

Formation of bone matrix within the fibrous membrane

At the site where a bone will develop, there is initially loose mesenchyme, which appears as widely separated, pale staining, stellate cells with interconnecting cytoplasmic processes.

Then a center of osteogenesis develops in association with capillaries that grow into the mesenchyme. These mesenchymal cells in the center become round and basophilic with thick interconnecting cytoplasmic processes. These cells differentiate into osteoblasts and secrete the organic bone matrix. Once surrounded by bone matrix, these cells are called osteocytes. The matrix



Flowchart 9.1 Formation of osteoclasts.

Table 9.3 Factors Regulating Osteoclast Formation and Activity

Factors Favoring Formation	Factors Limiting Formation
Transcription factors -NF-κβ **Local and systemic factors** (can act in a paracrine and autocrine way and regulate the activity of specific transcription factors) (a) **hematopoietic factors** • M-CSF • GM-CSF (b) **cytokines**- IL- 1 ,IL-6 ,IL-8 ,IL- 11 and TNF-α (c) **hormones** • **Vitamin D_3** (transiently at high doses, in vivo) • **Pth** (at chronic exposure) • **PGE_2** (chronic exposure and higher concentrations) • **Glucocorticoids** (pharmacological in vivo)	**Differentiation** • OPG • OCIL **Local or systemic factors** (can act in a paracrine and autocrine way and regulate the activity of specific transcription factors) a.) **Growth factors**- TGF-β. IGF-I and IGF-II b.) **Hormones-** • **Glucocorticoids** (physiological concentration in vitro), • **Pth** (physiological concentration) • **PGE_2** (lower concentration, systemic administration) • **Calcitonin** • **Estrogen** c.) **Cytokines**- IL- 4, IL- 10, IL-12, IL- 13, IL-18 IFN-γ d.) **Pharmacological**- bisphosphonates

soon begins to calcify. The earliest crystals appear in association with extracellular matrix vesicles produced by osteoblasts. Crystal formation subsequently extends into collagen fibrils in the surrounding matrix. Even after calcification begins, the osteocytes obtain nutrients and oxygen by diffusion along bone canaliculi.

Formation of woven bone

The first small mass of newly formed bone matrix is an irregular shaped spicule. The bony spicules gradually lengthen into longer anastomosing structures called trabeculae. The trabeculae extend in a radial pattern leading to an anastomosing network of trabeculae characteristic of ***spongy bone***. The spicules and trabeculae are easily recognized in hematoxylin and eosin sections, because the matrix stains a bright pink color, and they are also covered with large rounded osteoblasts that have intensely basophilic cytoplasm. These trabeculae enclose local blood vessels. This early membrane bone is termed **woven** bone.

At this stage, few mesenchymal cells remain undifferentiated. But, before these cells disappear, they leave a layer of flat cells called osteogenic cells on trabeculae

which do not have osteoblasts. In richly vascular areas, these osteogenic cells give rise to osteoblasts that form bone matrix. In areas, with no capillary blood supply, they form chondroblasts which lay down cartilage.

Appositional growth mechanism and formation of compact bone plates

The new layers of bone matrix are deposited on pre-existing bone surfaces. The osteogenic cells on the surface of spicules and trabeculae are always in a superficial position repeating the process again and again. This is ***appositional growth***, which results in build up of bone tissue one layer at a time. Every generation of osteoblasts produce their own canaliculi. Hence, all the new osteocytes remain linked through canaliculi to bone surface above and to osteocytes below. As the trabeculae increase in width due to appositional growth, neighboring capillaries are incorporated to provide nutrition to osteocytes in deeper layers. New bone is deposited on some surfaces and resorbed at other sites leading to remodeling of trabeculae. This remodeling maintains shape and size of bone throughout life.

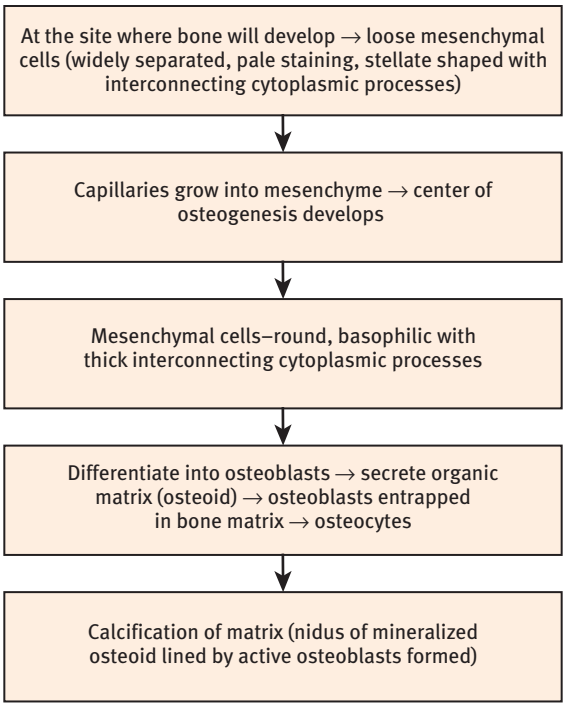
Continued appositional growth and remodeling of trabeculae converts cancellous bone to compact bone. Cancellous bone is in the central part of bones as the trabeculae do not increase in size. The vascular tissue in cancellous bone differentiates into red marrow.

Formation of osteon

As layers of bone tissue build up by apposition, the trabeculae thicken and the soft tissue spaces get narrowed. This process converts cancellous bone to compact bone. As cancellous bone gets converted to compact bone, a number of narrow canals are formed lined by osteogenic cells. These canals enclose vessels that were present in soft tissue spaces of cancellous network. The consecutive lamellae of bone become added to the bony walls of spaces in cancellous bone, which is called ***osteon*** or ***haversian system***. These osteons are called ***primitive osteons*** as they are short, compared to those in long bones.

While these changes occur, external to the woven bone, there is condensation of vascular mesenchyme called the ***periosteum***.

The mechanism of intramembranous ossification involves bone morphogenetic proteins ***(BMPs)*** and activation of transcription factor called ***cbfa1***. BMPs activate ***cbfa1 gene*** in mesenchymal cells. The ***cbfa1 transcription factor*** transforms mesenchymal cells into osteoblasts. It is believed that the proteins activate genes for osteocalcin, osteopontin and other bone specific extracellular matrix proteins (Flowchart 9.2).



Flowchart 9.2 Intramembranous bone formation.

Differences Between Immature Bone and Mature Bone (Woven Bone and Lamellar Bone)

Woven bone is an immature bone characterized by ***intertwined collagen fibers*** oriented in many directions, hence the name. In ***lamellar*** bone, a distinctive, orderly arrangement is seen, which is the result of repeated addition of uniform lamellae to bony surfaces during appositional growth. The direction of collagen fibrils in any given lamella lies at right angles to that of the fibrils in the adjacent lamella.

In ***woven bone***, there is a ***great*** amount of ***interfibrillar*** space that is occupied by mineral crystals and acidic proteins. In ***mature bone***, the ***interfibrillar space*** is ***less***.

The matrix of ***woven*** bone in hematoxylin and eosin section is tinged with ***blue*** indicating that it has higher ***proteoglycan*** content. Lamellar bone shows comparatively uniform ***acidophilic*** staining of the matrix.

Rates of ***deposition*** and ***mineralization*** are ***faster*** for ***woven*** bone than ***lamellar*** bone. Hence, woven bone shows higher proportion of ***osteocytes*** than ***lamellar*** bone.

Woven bone is enriched in BAG-75 (bone acidic glycoprotein- 75) and BSP (bone sialoprotein). Lamellar bone is enriched in osteocalcin. Osteopontin and type 1 collagen are seen in both woven and lamellar bone matrices.

Mineral density is lower and water content higher in woven bone matrix.

Woven bone can be ***entirely removed*** by ***osteoclasts***, whereas only a ***portion*** of ***lamellar*** matrix of a given bone is resorbed at one time.

Matrix vesicles participate in initiation of mineralization of ***woven bone***, whereas ***collagen*** mediated mechanism is operative in calcification of ***lamellar*** bone.

In woven bone, the **osteocytes** are isodiametric whereas in lamellar bone, osteocytes are flattened and oblate with their short axis parallel to the thickness of the lamellae (Table 9.4).

Intracartilaginous (Endochondral) Bone Formation

This type of ossification involves the replacement of a cartilaginous model by bone, and occurs at the extremities of all long bones, vertebrae, ribs, articular extremity of the mandible and base of the skull. The process involves the following steps.

Table 9.4 Differences between Woven Bone and Lamellar Bone

Woven Bone	Lamellar Bone
Immature	Mature
Collagen fibers intertwined	Collagen fibers orderly
Interfibrillar space more	Interfibrillar space less
Hematoxyphilic matrix	Eosinophilic matrix
Formation and mineralization faster	Formation and mineralization slower
Mineral density lower, water content higher	Mineral density higher, water content lower
Mineralization-matrix vesicles play a role	Mineralization- collagen mediated
Osteoclast can remove woven bone totally	Osteoclast can remove portions of lamellar bone at a time

Formation of a cartilaginous model

At the site where a limb will later emerge, the embryo shows outgrowth of mesoderm covered by ectoderm. The mesenchymal cells in this site condense, differentiate into chondroblasts and form the cartilage matrix, resulting in the development of a ***hyaline cartilage model***. This process begins late in the second month of development. The model is surrounded by a ***perichondrium***, made up of an ***inner chondrogenic*** layer and an ***outer fibrous layer***. No osteoblasts are produced by the cells in the chondrogenic layer, because differentiation is taking place in an avascular environment. Fibroblasts in fibrous layer produce collagen and a dense fibrous covering is formed.

The ***growth*** of the cartilage model is by ***interstitial*** and ***appositional*** growth. Increase in the length is by ***interstitialgrowth***, due to repeated division of chondrocytes, along with production of additional matrix by the daughter cells. Widening of the model is due to further addition of matrix to its periphery by new chondroblasts, derived from the chondrogenic layer of the perichondrium. This is called ***appositional growth.*** In case of long bones as the differentiation of cartilage cells moves towards the metaphysis, the cells organize into longitudinal columns which are subdivided into three zones:

1. Zone of proliferation. The cells are small and flat, and constitute a source of new cells.

2. Zone of hypertrophy and maturation. This is the broadest zone. The chondrocytes hypertrophy, and in the early stages secrete type II collagen. As hypertrophy proceeds, proteoglycans are secreted. The increased cell size and increased cell secretion lead to an increase in the size of the cartilaginous model. As the chondrocytes reach maximum size, they secrete type X collagen and noncollagenous proteins. Subsequently, there is partial breakdown of proteoglycans, creating a matrix environment receptive for mineral deposition.

3. Zone of provisional mineralization. Matrix mineralization begins in the zone of mineralization by formation of matrix vesicles. These membrane bound vesicles bud off from the cell and form independent units in the longitudinal septa of the cartilage.

Formation of bone collar

The capillaries grow into the perichondrium that surrounds midsection of the model. The cells in the inner layer of the perichondrium differentiate into osteoblasts in a vascular environment and form a thin collar of bone matrix around the mid region of the model. At this stage, ***perichondrium*** is referred to as ***periosteum*** as the differentiation of cells from the inner layer of the perichondrium is giving rise to bone. Vascularization of the middle of the cartilage occurs, and chondroclasts resorb most of the mineralized cartilage matrix. The bone collar holds together the shaft, which has been weakened by disintegration of the cartilage. Hence, more space is created for vascular ingrowth.

Formation of periosteal bud

Periosteal capillaries accompanied by osteogenic cells invade the calcified cartilage in the middle of the model and supply its interior. The osteogenic cells and the vessels comprise a structure called the ***periosteal bud.*** The periosteal capillaries grow into the cartilage model and initiate development of a primary ossification center. Osteogenic cells in the periosteal bud give rise to osteoblasts that deposit bone matrix on the residual calcified cartilage. This results in the formation of cancellous bone that has remnants of calcified cartilage. This is the ***mixed spicule.*** The network of mixed spicules is called ***primary spongiosa***. The calcified cartilage in the trabeculae in hematoxylin and eosin sections stains pale blue to mauve, whereas bone matrix appears bright pink to red.

Formation of medullary cavity

As the primary ossification center enlarges, spreading proximally and distally, osteoclasts break down the newly formed spongy bone and open up a medullary cavity in the center of the shaft. Hematopoietic stem cells enter the medullary cavity giving rise to myeloid tissue.

The two ends of the developing bone are at this stage still composed entirely of cartilage. The ***midsection*** of the bone becomes the ***diaphysis*** and the ***cartilaginous ends*** of bone become the ***epiphysis.*** Hence, the primary center of ossification is the ***diaphyseal center of ossification.***

Formation of secondary ossification center

At birth, most of the long bones have a bony diaphysis surrounding remnants of spongy bone, a widening medullary cavity, and two cartilaginous epiphysis. Shortly before or after birth, ***secondary ossification centers*** appear in one or both epiphysis. Initially chondrocytes in the middle of the epiphysis hypertrophy and mature, and the matrix partitions between their lacunae calcify.

Periosteal buds carry mesenchymal cells and blood vessels and process is same as that occurring in a primary ossification center, except that the spongy bone in the interior is retained and no medullary cavity forms in the epiphysis. The ossification spreads from secondary center in all directions. Eventually, the cartilage in the middle of epiphysis gradually gets replaced by cancellous bone. When secondary ossification is complete, hyaline cartilage remains at two places—on the articular surface as ***articular cartilage*** and at the junction of the diaphysis and epiphysis, where it forms the ***epiphyseal plates.***

This plate continues to form new cartilage, which is replaced by bone, a process that increases the length of the bone. Long bones have one or two secondary ossification centers. Short bones have one ossification center.

The union of primary and secondary ossification center is called ***epiphyseal line.***

Long bones development depends on endochondral bone formation, which requires balance between hypertrophic cartilage (HC) formation and its ossification. Dysregulation of this process may result in skeletal dysplasia and heterotopic ossification. Endochondral ossification requires the precise orchestration of HC, vascularization, extracellular matrix remodeling, and the recruitment of osteoclasts and osteoblasts. Matrix metalloproteinase-9 (MMP-9), vascular endothelial growth factor (VEGF) and osteoclasts have all been shown to regulate endochondral ossification.

Calcification is the process of deposition of insoluble calcium salts in a tissue.

Mechanism of calcification. It is not entirely clear if the first formed solid phase is amorphous or crystalline. It is widely conceded that the first formed solid phase is amorphous. This initial phase is subsequently transformed to hydroxyapatite. Amorphous calcium phosphate appears as microscopic spheres 30–100 nm in diameter, comprising randomly packed apatite crystals each of about 0.95 nm diameter.

Under normal conditions, there is insufficient concentration of available calcium and phosphate ions in blood and tissue fluid, for calcium phosphate to crystallize or precipitate spontaneously. The critical factor is the local (Ca^{2+}) x. (Pi) ion product. Pi is the total free inorganic orthophosphate. When factors operate locally, to raise this ion product, calcium phosphate separates out in the solid phase and undergoes solid phase transition to a number of crystalline arrangements. Once the microcrystals have begun to form, they continue to grow and also catalyse further crystallization of calcium phosphate even at sites where the Ca^{2+}. Pi product does not exceed the plasma level (Flowchart 9.3).

Theories of calcification

Traditionally, calcification has been treated from the point of view of precipitation dynamics, with initially the only ions needed being calcium and phosphate. The extracellular fluid is supersaturated with respect to the basic calcium phosphate, yet the mineralization is not a widespread phenomenon. Thus for precipitation to occur, the conditions in the osteoid matrix must be in some way especially favorable to this process. Interplay of various factors may contribute to conditions that may favor calcification.

A) Nucleation theory Neumann and Neumann (1953) put forward the theory of epitactic nucleation based on the concept of seeding or epitaxy. A nucleus is formed, probably in relation to collagen, effective in aggregating calcium and phosphate ions. The hydroxyapatite crystals then grow spontaneously by addition of these from the saturated surrounding fluids. Acceptance of this theory led to many attempts to determine the nature and distribution of nucleation sites.

a) ***Ground substance.*** The components of ground substance, mainly sulfated glycosaminoglycans and proteoglycans, have been suggested for this function. Qualitative and quantitative changes of proteoglycans in the cartilaginous matrix prior to mineralization have been reported, thus supporting this phenomenon.

b) ***Collagen.*** The *initial mineral deposits* in the organic matrix of bone appear to occur at discrete sites in, or on the collagen fibrils.

The dimensions of water filled pore space in the matrix, particularly within the collagen macromolecular structure are crucial in allowing both the ingress of ions, the formation of ion clusters and the aggregation of such clusters to form nuclei from which crystal growth can continue. As mineralization progresses, axial periodicity of the collagen is first accentuated; this suggests that growth follows a specific fibril controlled pattern. Apart from collagen, other tissues closely associated with collagen also serve this function, e.g. phosphoproteins, including osteonectin. Phosphoproteins induce apatite formation, but are more involved in controlling the shape, size and orientation of bone crystals.

c) ***Mitochondria*** in osteoblasts play an important indirect role in the calcification process. Mitochondria are the earliest storage sites of calcium and phosphate in the form of amorphous calcium phosphate. This stored mineral is made available extracellularly, liberated directly due to cell destruction, or released indirectly in the form of constituent ions. This then supports the growth of crystals forming extracellularly, in association with matrix vesicles.

B) Role of matrix vesicles The crystals have been found to be formed in association with matrix vesicles. Matrix vesicles are small ***membrane bound*** structures, 25 to 250 nm in diameter, lying free in the matrix, where calcification is known to be underway. These are rounded outgrowths of cell membrane that bud from osteoblasts, chondrocytes and odontoblasts. The vesicles are rich in phospholipids, especially phosphatidyl serine, a lipid with high affinity for calcium ions. Vesicles also contain annexins. Annexins in the vesicles form a calcium channel, thus incorporating the ion within the matrix vesicles. Matrix vesicles ***accumulate*** Ca^{2+} and their membranes furnish binding sites for the ***nucleation of hydroxyapatite crystals.***

The mineralization of bone requires the presence of extracellular matrix vesicles, since the first step of mineralization is initiated inside these organelles. They serve as a site for Ca^{2+} and Pi accumulation by creating a specific environment where deposition of initial amorphous mineral complexes (nucleation) occurs and where hydroxyapatite (HA) is produced. The needle like crystals form on the inner surface of vesicle membrane.

Calcification

The extracellular matrix contains sufficiently high levels of Ca^{2+} and Pi concentrations to sustain the nucleation process and to propagate mineralization. Ion channels and transporters present in vesicle membrane are responsible for Ca^{2+} and Pi uptake into these organelles. The hydroxyapatite crystals are released into the extracellular matrix after reaching a certain thickness. The release of the crystals is proposed to be triggered by phospholipases leading to tissue calcification which affects the membrane fluidity. The released crystals serve as a template for the formation of crystalline arrays.

Their essential role in the initiation of calcification remains disputed and it is difficult to exclude the problem of artifactual precipitation of crystals in vesicles

FORMATION OF CARTILAGINOUS MODEL
At the site where the bone will develop → condensation of mesenchymal cells → in avascular condition → Chondroblast lay down cartilage matrix → surrounded by perichondrium → followed by mineralization of matrix

GROWTH OF CARTILAGE MODEL
- Increase in length → Interstitial growth
- Increase in thickness → Appositional growth

FORMATION OF PERIOSTEUM
- In the middle of diaphysis → capillaries grow into the perichondrium→perichondrium is referred to as periosteum

FORMATION OF BONE COLLAR
- Cells in the inner layer of perichondrium (periosteum) → differentiate into osteoblasts thin collar of bone matrix formed → around mid region of model.

CALCIFICATION OF MATRIX
- Chondrocytes grow → secrete alkaline phosphatase → calcification of matrix → eventually blood supply cut off → no nutrient → death of chondrocytes → cavitation of cartilage matrix

FORMATION OF PERIOSTEAL BUD
- Periosteal capillaries and osteogenic cells invade mid region of model → development of primary ossification center

FORMATION OF MIXED SPICULE
- Osteogenic cells in periosteal bud → differentiate into osteoblasts → deposit bone matrix on residual calcified cartilage

FORMATION OF MEDULLARY CAVITY
- Osteoclasts break down newly formed spongy bone → formation of medullary cavity
- At this stage → 2 ends of developing bone → composed of cartilage (epiphysis)

FORMATION OF SECONDARY OSSIFICATION CENTER (In one or both epiphysis)
- Formation of bone same as in primary ossification center.
- No medullary cavity formation in epiphysis
- Hyaline cartilage at 2 places, i.e. on articular surface (articular cartilage) and at junction of epiphysis and diaphysis (epiphyseal plate).

Flowchart 9.3 Endochondral bone formation.

during specimen preparation for electron microscopy. They have not always been found in mineralizing fronts in osteoid of more mature bone and some have failed to find calcium tightly bound to matrix vesicles when using rapid freezing and freeze substitution of tissue to preserve ultrastructure.

C) Alkaline phosphatase is the enzyme which participates in the process of calcification. This enzyme ***hydrolyses*** a broad range of ***organic phosphate*** containing substrates and ***increases*** the ***local inorganic phosphate*** concentration. This enzyme ***resides*** in ***matrix vesicles*** budding from cell membranes of chondrocytes, osteoblasts and odontoblasts. Alkaline phosphatase hydrolyses PPi, which is an inhibitor of hydroxyapatite formation and also provides inorganic phosphates for the formation of hydroxyapatite crystals. It is used as a marker of active tissue ***mineralization***.

Inhibitors of calcification

Collagen and other potential nucleating agents occur in tissues that do not calcify. The probable reason is that, the collagen molecules are packed closely together in soft tissues than in bone, which impede phosphate ion access to intrafibrillary nucleation sites. Pyrophosphate, diphosphonates or adenosine triphosphate can delay or prevent the transformation of amorphous calcium phosphate to hydroxyapatite. Other potential inhibitors include citrate, magnesium, and proteins like albumin. Components of the bone matrix may act locally and inhibit mineralization, e.g. proteoglycans.

BONE RESORPTION

Bone resorption is the removal of mineral and organic components of extracellular matrix of bone under the action of osteolytic cells, of which the most important is the osteoclast.

Sequence of events of bone resorption

The first phase involves the formation of osteoclast progenitors in the hematopoietic tissues, followed by their vascular dissemination and the generation of resting preosteoclasts and osteoclasts in the bone itself. The second phase consists of activation of osteoclasts at the surface of mineralized bone. Osteoblasts play a major role by retracting, to expose the mineral to the osteoclast and releasing a soluble factor that activates these cells. The third phase involves the activated osteoclasts resorbing the bone.

Alterations in the osteoclast

Immediately before the resorption event, the osteoclasts undergo changes by assuming a polarity of structure and function. These changes facilitate bone resorption. The two distinct alterations are the development of a ruffled border and a sealing zone at the plasma membrane. These changes occur only in the region of the cell that is next to the bone surface. The ruffled border consists of many infoldings of the cell membrane, resulting in finger-like projections of the cytoplasm. Thus, an extensive surface is created well suited for an intensive exchange between the cell and bone.

At the periphery of the ruffled border, the plasma membrane is smooth and apposed closely to the bone surface. The adjacent cytoplasm, devoid of cell organelles contains contractile actin microfilaments, surrounded by two vinculin rings. This region is called the clear (sealing) zone. This zone serves to attach the cell very closely to the surface of bone and creates an isolated microenvironment, in which resorption can take place without diffusion of the hydrolytic enzymes produced by the cell into adjacent tissue. When osteoclasts arrive at the resorption site, they use the sealing zone to attach themselves to the bone surface. The attachment of the osteoclast cell membrane to the bone matrix at the sealing zone is due to the presence of cell membrane proteins known as integrins, especially αVβ3. Integrins are a large family of heteromeric cell surface receptors composed of noncovalently bound α and β subunits which interact with extracellular matrix molecules, serum constituents and adhesion molecules of immunoglobulin family. αVβ3, a vitronectin receptor, is expressed by resorbing osteoclasts. These integrins bind to specific amino acid sequences present in proteins of the bone matrix, namely, RGD (Arg-Gly-Asp).

Removal of hydroxyapatite

The initial phase involves the dissolution of the mineral phase by the action of hydrochloric acid (HCl). The protons for the acid arise from the activity of cytoplasmic carbonic anhydrase II, which is synthesized in the osteoclast. The protons are then released across the ruffled border into the resorption zone by an ATP consuming proton pump. This leads to a fall in pH to 2.5–3.0 in the osteoclast resorption space. The *proton pump* is an absolute requirement for normal bone resorption to take place.

Degradation of organic matrix

Organic constituents of bone tissue remain after the dissolution of mineralized component. Next step involves the digestion of organic components of matrix. Proteolytic enzymes are synthesized by osteoclasts, namely, cathepsin-K and MMP-9 (matrix metalloproteinase). The enzymes are synthesized in rough endoplasmic reticulum, transported to Golgi complexes and moved to the ruffled border in transport vesicles, and the contents of the same are released into sealed compartment, creating extracellular lysosomes. As a result, a visible depression or Howship's lacunae is excavated into the bone.

Cathepsin-K is the most important enzyme in bone resorption. It is a collagenolytic papain like cysteine protease expressed in osteoclasts. *In vivo* studies, have shown that, activation of cathepsin-K occurs intracellularly before secretion into lacunae and onset of bone resorption. The processing of procathepsin-K to mature cathepsin-K occurs as osteoclast approaches bone. Cathepsin-K degrades major amount of type I collagen and other noncollagenous proteins, which have been demineralized by the acidic environment of the resorptive zone.

MMP-9 (collagenase B) is believed to be required for osteoclast migration. MMP-13 is proposed to be involved in bone resorption and osteoclast differentiation.

Removal of degradation products from lacunae

Once liberated from bone, the free organic and nonorganic particles of bone matrix are taken in or endocytosed from the resorption lacunae, across the ruffled border, into the osteoclast. These are then packed in membrane bound vesicles within cytoplasm of osteoclast. These vesicles and their contents pass across the cell and fuse with FSD (functional secretory domain), a specialized region of the basal membrane. Then the vesicles are released by exocytosis. The changes in the cytoplasm framework in the cell and presence of clusters of matrix fragments in the region directly outside the cell next to FSD, indicate that matrix fragments have been expelled from the cell by exocytosis into extracellular space away from bone.

Following resorption, osteoclasts undergo apoptosis, which provides a mechanism for limiting resorptive activity. Factors like TGF-β and estrogen promote apoptosis. PTH and IL-1, act as suppressors prolonging osteoclast activity (Flowchart 9.4).

Differences between the Resorbed and Unresorbed Surfaces

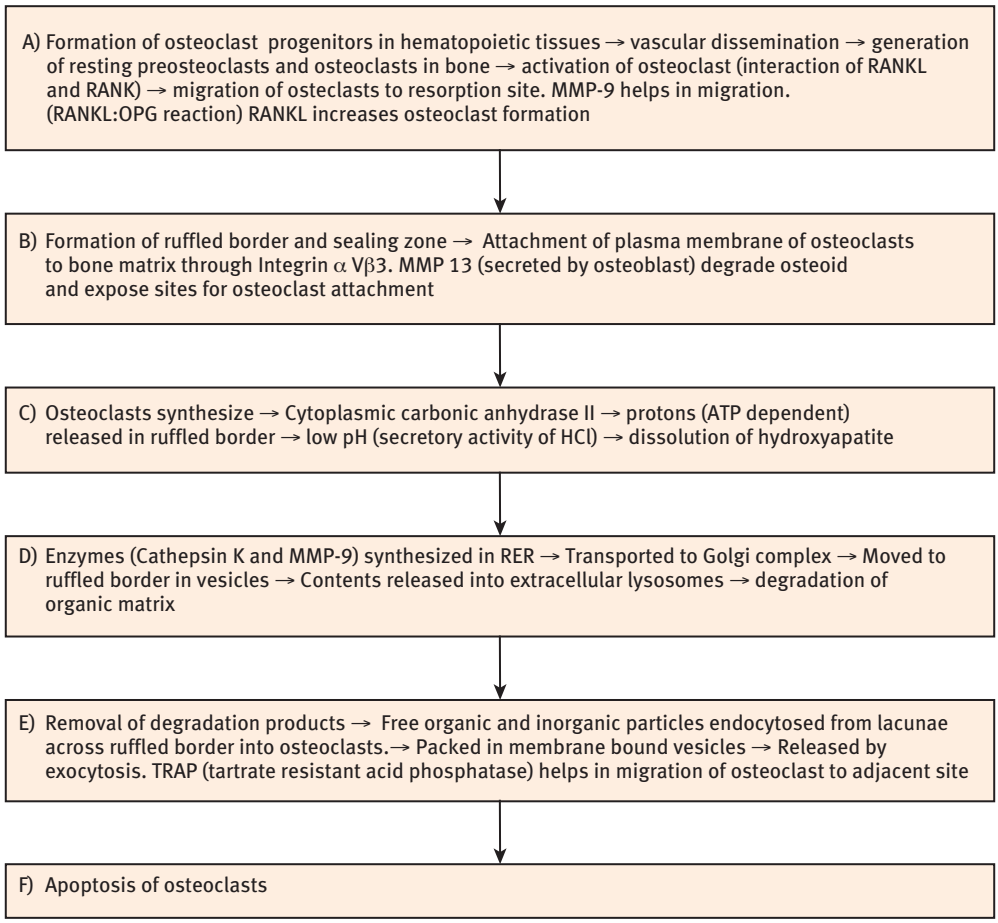
The resorbing surface is scalloped and exhibits scattered osteoclasts unlike osteoblasts, which line the bone surface.

Osteoclasts lie in the Howship's lacunae or resorption bays, whereas osteoblasts are present on the bone surface (Fig. 9.6).

The side of the osteoclast cell adjacent to bone contains few nuclei than the opposite side. Osteoblast is a mononucleated cell on the bone surface.

Role of TRAP in Bone Resorption

Tartrate resistant acid phosphatase (TRAP) is synthesized as a latent inactive proenzyme. The molecule is cleaved by an enzyme—cysteine proteinases converting it



Flowchart 9.4 Bone resorption—sequence of events.

into an active form. This active enzyme plays a role in bone resorption inside and outside the osteoclast cell.

Extracellular role of TRAP

TRAP accumulates extracellularly in bone matrix, immediately adjacent to ruffled border of resorbing osteoclasts. Osteopontin, bone sialoprotein and osteonectin act as substrates for TRAP. Osteopontin is highly expressed at the bone surface, opposite sealing zone of resorbing osteoclasts, and is essential for resorption to take place. Osteopontin enables osteoclasts to adhere to bone surface by binding with integrin Vβ3, which are abundantly present at the sealing zone. TRAP can remove phosphate groups from osteopontin, an event that consequently disrupts adhesion of osteoclasts to the bone. This suggests that the enzyme might regulate osteoclast adhesion to the bone and also enable migration of osteoclasts to adjacent sites of resorption. The ability of TRAP to degrade phosphoproteins in bone by dephosphorylation may illustrate a preliminary stage in the degradation of the bone matrix. TRAP can hydrolyse and liberate pyrophosphate from bone matrix which is an inhibitor of resorption. This hydrolysis event would enable osteoclasts to begin bone resorption activity.

Intracellular role of TRAP

Intracellularly TRAP has been found to be co-localized with organic products of bone degradation released from bone matrix during resorption and endocytosed into osteoclasts. It has been put forward that TRAP containing vesicles fuse with transcytotic vesicles transporting the matrix degradation products from ruffled border to FSD (functional secretory domain) of *osteoclasts*. In this location, TRAP is secreted out of cells together with matrix degradation products. After this stage, both entities leak into the circulation at a rate that corresponds to the amount of resorption activity being undertaken by the osteoclast.

The enzyme is synthesized to help dispose of the products of bone breakdown within transcytotic vesicles. Along with fragments of bone matrix, it is released into the extracellular environment as an active enzyme by exocytosis at the FSD. TRAP then subsequently leaks into the circulation through the interstitial fluid.

Extracellular fate of TRAP

Once secreted TRAP is exposed to physiological influences present in body fluids. It binds to 2 macroglobulin, a high molecular weight molecule in serum. It has been proposed that, 2 macroglobulin may be a carrier molecule for TRAP, that mediates clearance of enzyme from areas of bone resorption and then the circulation. Ultimately, TRAP has the fate of all circulating enzymes. Its structure becomes compromised, leading to its inactivation as a catalyst. It loses its binuclear iron center, which is then recycled, and the iron free enzyme protein is broken down by proteases in the plasma and

the liver. The fragments that result from these events are eventually metabolized by the liver and/or removed in the urine.

The concentration of osteoclast derived TRAP in serum can be assessed by immunoassay, and has a quantitative and dynamic relation to amount of resorption taking place on a day by day basis.

The immunoassay would provide additional information in the hospital's clinical laboratory for diagnosis and monitoring of bone resorption conditions.

BONE REMODELING

Bone remodeling is performed by clusters of bone resorbing osteoclasts and bone forming osteoblasts arranged within temporary anatomical structures known as **basic multicellular units (BMUs)**. Traversing and encasing the BMU is a canopy of cells that creates a bone-remodeling compartment (BRC).

An active BMU consists of a leading front of bone resorbing osteoclasts. Reversal cells of unclear phenotype follow the osteoclasts, covering the newly exposed bone surface and prepare it for deposition of replacement bone. Osteoblasts occupy the tail portion of the BMU and secrete and deposit unmineralized bone matrix known as osteoid and direct its formation and mineralization into mature lamellar bone. At any given time, the process of bone synthesis and bone breakdown go on simultaneously (Fig. 9.7) and the status of the bone represents the net result of a balance between the two processes. This phenomenon is called 'coupling' of bone resorption and formation.

The main functions of remodeling are to prevent the accumulation of damaged and fatigued bone by regenerating new bone, allow the bone to respond to changes in mechanical forces and to facilitate mineral homeostasis.

Sequence of Events in Bone Remodeling

Bone turnover rates of 30 to 100% per year are common in rapidly growing children. In adulthood, the rate is slow. The turnover rate of trabecular bone and endosteal surface of cortical bone is more than cortical bone turnover. The sequence of events is as follows:

Activation stage. The cells of the osteoblast lineage interact with hematopoietic cells to initiate osteoclast formation. This stage of bone remodeling involves detection of an initiating remodeling signal. This signal can take several forms, like direct mechanical strain on the bone that results in structural damage or hormonal action on bone cells in response to more systemic changes in homeostasis. Daily activity also places mechanical strain on the skeleton and osteocytes probably sense changes in these physical forces and translate them into biological signals that initiate bone remodeling.

Resorption stage. In this stage, osteoblasts respond to signals generated by osteocytes and recruit osteoclast precursors to the remodeling site. In addition to recruitment of osteoclast precursors, osteoblast expression of the master osteoclastogenesis cytokines, M-CSF, RANKL and osteoprotegerin (OPG) is also modulated in response to PTH. OPG expression is reduced and M-CSF and RANKL production increased to **promote osteoclast formation and subsequent activity**. M-CSF promotes proliferation

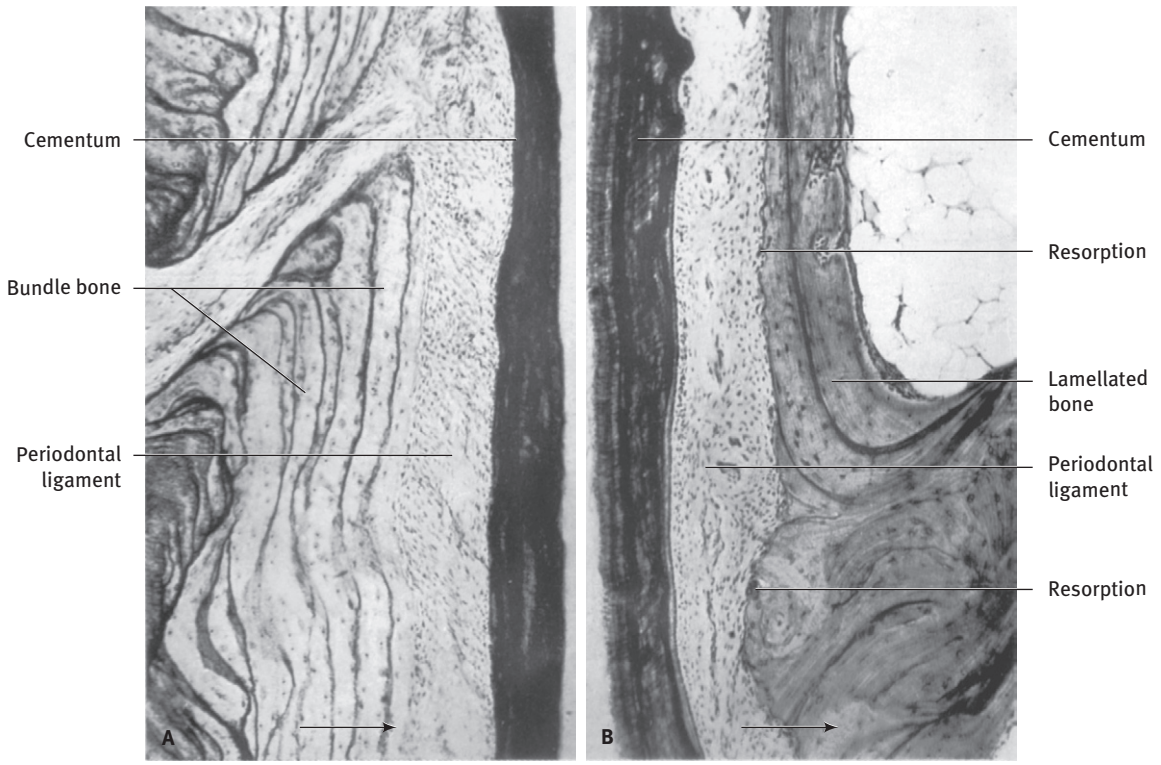


Figure 9.7 Alveolar bone showing remodeling with (**A**) Apposition of bundle bone on distal alveolar wall and (**B**) Resorption of bone on mesial alveolar wall. Mesial drift indicated by arrow (From Weinmann JP: Angle Orthod 11:83, 1941).

and survival of osteoclast precursors and directs spreading, motility and cytoskeletal organization in mature cells. RANKL also promotes proliferation of osteoclast precursors and additionally coordinates the differentiation of osteoclast precursors to multinucleated osteoclasts, promotes resorption activity and prolongs the life of the mature cells. Matrix metalloproteinases (MMPs), including MMP-13, are also secreted from osteoblasts in response to mechanical and endocrine remodeling signals. MMPs degrade the unmineralized osteoid that lines the bone surface and expose RGD adhesion sites within mineralized bone, that are necessary for osteoclast attachment.

The osteoclasts tunnel into surface of bone, which lasts for three weeks. In haversian canals, closest to the surface, osteoclasts travel along a vessel, resorb the haversian lamellae, and a part of circumferential lamellae, and form a resorption tunnel or cutting cone. Resorption tunnels can be distinguished from Haversian canals or Volkmann's canals as they have irregular etched outlines and presence of osteoclasts along the border.

Reversal stage. Resorption phase is followed by the reversal phase, comprising the differentiation of osteoblast precursors and discontinuation of bone resorption with osteoclast apoptosis. Following osteoclast-mediated resorption, the Howship's lacunae remain covered with undigested demineralized collagen matrix. A mononuclear cell of undetermined lineage removes these collagen remnants and prepares the bone surface for subsequent osteoblast-mediated bone formation. This reversal cell is from the osteoblast lineage, based on cell morphology and the positive expression for alkaline phosphatase.

However, the mesenchymal bone lining cells are more ideally equipped to deposit the collagenous matrix that forms along osteopontin-rich cement lines within Howship's lacunae. The final role of the reversal cells may be to receive or produce coupling signals that allow transition from bone resorption to bone formation within the BMU.

In the next stage, activated osteoblasts lay down new bone material, until the resorbed bone is entirely replaced by new one. Once mesenchymal stem cells or early osteoblast progenitors have returned to the resorption lacunae, they differentiate and secrete molecules that ultimately form replacement bone.

Collagen type I is the primary organic component of bone. Noncollagenous proteins, including proteoglycans, Gla-containing proteins (matrix Gla protein and osteocalcin) and lipids comprise the remaining organic material.

These osteoblasts lay down a new set of haversian lamellae, encircling a vessel upon a reversal line. This cement line is a thin layer of glycoproteins comprising bone sialoprotein and osteopontin, that acts as a cohesive mineralized layer between the old bone and new bone to be secreted. The entire area of osteon, where active formation occurs is termed the filling cone. Fragments of lamellae from old bone haversian systems are left behind as interstitial lamellae (Fig. 9.4).

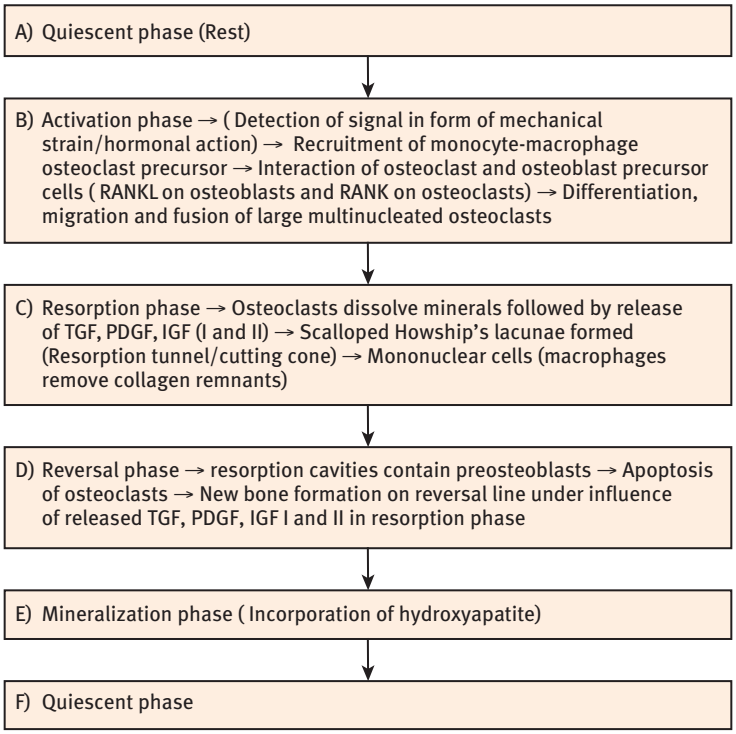
For bone to assume its final form, hydroxyapatite is incorporated into this newly deposited osteoid.

Osteoblasts that become encased in the new bone are transformed into osteocytes. The osteoblasts become quiescent at the end of bone remodeling and form flattened lining cells on the bone surface until a new remodeling cycle is triggered (Flowchart 9.5).

Mediators of Bone Remodeling

Hormones

Parathyroid hormone (PTH) is produced in the parathyroid glands in response to hypocalcemia, stimulating bone resorption. A stimulating role in bone formation



Flowchart 9.5 Bone remodeling—sequence of events.

has also been established through the synthesis of IGF 1 and TGF β. This dual effect of resorption and formation is explained by the fact that the continuous supply of PTH stimulates bone resorption through the synthesis of RANKL on the part of the osteoblastic cells, while at intermittent doses, it would stimulate the formation of bone, associated with an increase of the growth factors and with a decrease in the apoptosis of osteoblasts.

Calcitonin is secreted when blood calcium levels rise. It inhibits bone resorption and promotes calcium salt deposition in bone matrix, effectively reducing blood calcium levels. As blood calcium levels fall, calcitonin release wanes as well. Calcitonin also reduces the number and activity of osteoclasts.

Vitamin D metabolites

The major active metabolite of vitamin D is 1, 25-dihydroxycholecalciferol. This has been shown to affect bone formation and also to cause bone resorption. Its effect on bone resorption appears to be by the differentiation of committed progenitor cells into mature cells. It favors the intestinal absorption of calcium and phosphate and therefore bone mineralization. It is necessary for normal growth of the skeleton.

Estrogen receptors are present on osteoblasts, osteocytes and osteoclasts. They favor bone formation, increasing the number and function of osteoblasts. It has also been proposed that these hormones are believed to increase the levels of OPG, which inhibits resorption.

Growth hormones act directly on the osteoblasts stimulating their activity and increasing the synthesis of collagen, osteocalcin and alkaline phosphatase. Indirect action is produced through an increase in the synthesis of IGF-I and II by osteoblasts, which stimulate the proliferation and differentiation of osteoblasts.

Glucocorticoids at high doses inhibit the synthesis of IGF-1 by osteoblasts and suppress BMP-2 and Cbfa-1 which are essential for the formation of osteoblasts. But, at physiological doses they are believed to have osteogenic capacity favoring osteoblastic differentiation.

Local Factors

IL-1 does not have direct action on the osteoclast, but like PTH acts via the osteoblast. It has a ***direct*** promotional effect on ***osteoclast formation.*** It inhibits the apoptosis of osteoclasts.

TNF-α and TNF-β ***stimulate bone resorption*** *in vitro.* The bone resorbing effect is mediated through osteoblasts. ***TNF*** is also believed to ***inhibit*** bone ***collagen*** and ***noncollagenous protein synthesis.***

Prostaglandins are local pathological mediators of bone destruction, in particular, where there is inflammation.

IGF-I and II increase the number and function of osteoblasts stimulating collagen synthesis. They mediate osteoblast–osteoclast interaction and participate in bone remodeling.

BMPs are highly abundant in bone tissue and participate in the formation of bone and cartilage. They are the most important factors for osteoblast differentiation.

Other growth factors like PDGF, FGF and EGF may also play an important role in physiologic remodeling and an even more important role in remodeling associated with skeletal repair. PDGF stimulates protein synthesis by osteoblasts and also favors bone resorption. FGF is a mitogen for osteoblasts. In bone, EGF has both, a formative and destructive function.

Bacterial products such as lipopolysaccharide, capsular material, lipoteichoic acids and peptidoglycans may act as foreign antigens and induce monocytes, macrophages and then bone cells to produce prostaglandins and cytokines such as IL-1, leading to bone resorption.

Mechanical factors Under muscular action, tension is transmitted to the bone, which is detected by osteocyte network. These osteocytes produce prostaglandins and IGF-1 which stimulate osteoblast activity leading to increased bone formation. Absence of muscular activity accelerates resorption (Table 9.5).

Markers of Bone Turnover

The markers of bone formation are **(*Serum markers*)**

- alkaline phosphatase (total)
- alkaline phosphatase (skeletal isoenzymes)
- osteocalcin
- procollagen I extension peptide

The markers of bone **resorption** are (***Urinary markers***)

- urine calcium
- urinary hydroxy proline
- collagen crosslink fragments (first to be hydrolysed)
- urine N-telopeptide (N terminus of collagen fibrils)
- urine C-telopeptide (C terminus of collagen fibrils)
- urine total pyridinoline
- urine free deoxypyridinoline

Serum markers

- Serum TRAP
- Serum β2 macroglobulin is a marker for high bone remodeling. It plays a role as bone derived growth factor regulating osteoblasts and osteoclasts. It has been proposed as a bone remodeling biological marker in

Table 9.5 Regulatory Factors in Bone Remodeling

SYSTEMIC FACTORS	LOCAL FACTORS
1.) **HORMONES**	a.) Growth Factors
a.) Decrease bone resorption	IGF I & II
Calcitonin	TGF b
Estrogen	FGF
b.) Increase bone resorption	PGDF
PTH	b.) Cytokines
Glucocorticoids	IL- I, IL- 6,IL- 11
Vitamin D(high doses)	PGE_2
c.) Increase bone formation	
Growth hormone	
Vitamin D	
Insulin	
Low dose PTH	
d.) Decrease bone formation	
Glucocorticoids	

high bone turnover conditions, especially vitamin D deficiency and secondary hyperparathyroidism.

Pathologies caused by improper control of remodeling are: osteoporosis, osteopetrosis, malignant bone tumors inflammatory joint diseases, hyperparathyroidism, Paget's disease and hyperthyroidism.

ALVEOLAR BONE

The ***alveolar process*** is defined as that part of the maxilla and the mandible that forms and supports the sockets of the teeth.

Functions of alveolar bone are:

- Houses the roots of teeth.
- Anchors the roots of teeth to the alveoli, which is achieved by the insertion of Sharpey's fibers into the alveolar bone proper.
- Helps to move the teeth for better occlusion.
- Helps to absorb and distribute occlusal forces generated during tooth contact.
- Supplies vessels to periodontal ligament.
- Houses and protects developing permanent teeth, while supporting primary teeth.
- Organizes eruption of primary and permanent teeth.

DEVELOPMENT OF ALVEOLAR PROCESS

Near the end of the second month of fetal life, the maxilla as well as the mandible form a groove that is open towards the surface of the oral cavity (Figs 9.8, 9.9).

Tooth germs develop within the bony structures at late bell stage. Bony septa and bony bridge begin to form and separate the individual tooth germs from one another, keeping individual tooth germs in clearly outlined bony compartments. Along with tooth germs, alveolar nerves and vessels are also present. At this stage, dental follicle surrounds each tooth germ, which is located between tooth germs and its bony compartment. Even prior to root formation, tooth germs within bony compartments show bodily movement in various directions to adjust to growing jaws. This movement causes bony remodeling of bony compartment through bone resorption and bone deposition. The major changes in the alveolar process begin to occur with development of roots and tooth eruption. As roots develop, the alveolar process increases in height. Also, the cells in the dental follicle start to differentiate into periodontal ligament and cementum. At the same time, some cells in the dental follicle differentiate into osteoblasts and form alveolar bone proper.

Hence, an alveolar process in the strict sense of the word develops only during the eruption of the teeth. It is important to realize that, during growth, part of the alveolar process is gradually incorporated into the maxillary or mandibular body while it grows at a fairly rapid rate at its free borders. During the period of rapid growth, a tissue may develop at the alveolar crest that combines characteristics of cartilage and bone. It is called chondroid bone (Fig. 9.10). The alveolar process forms with the development and the eruption of teeth, and conversely, it gradually diminishes in height after the loss of teeth.

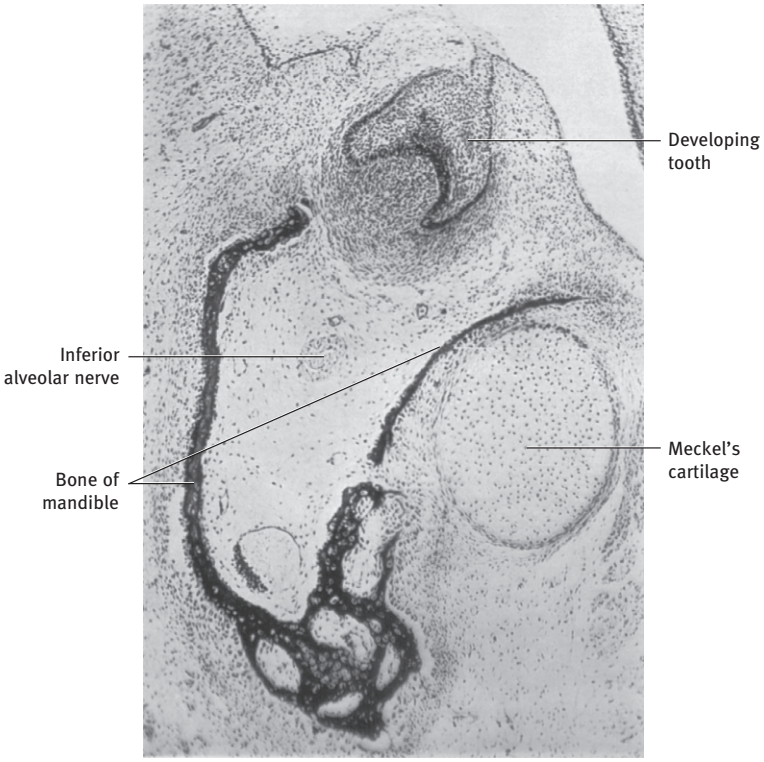


Figure 9.8 Development of mandible as intramembranous bone lateral to Meckel's cartilage (human embryo 45 mm in length).

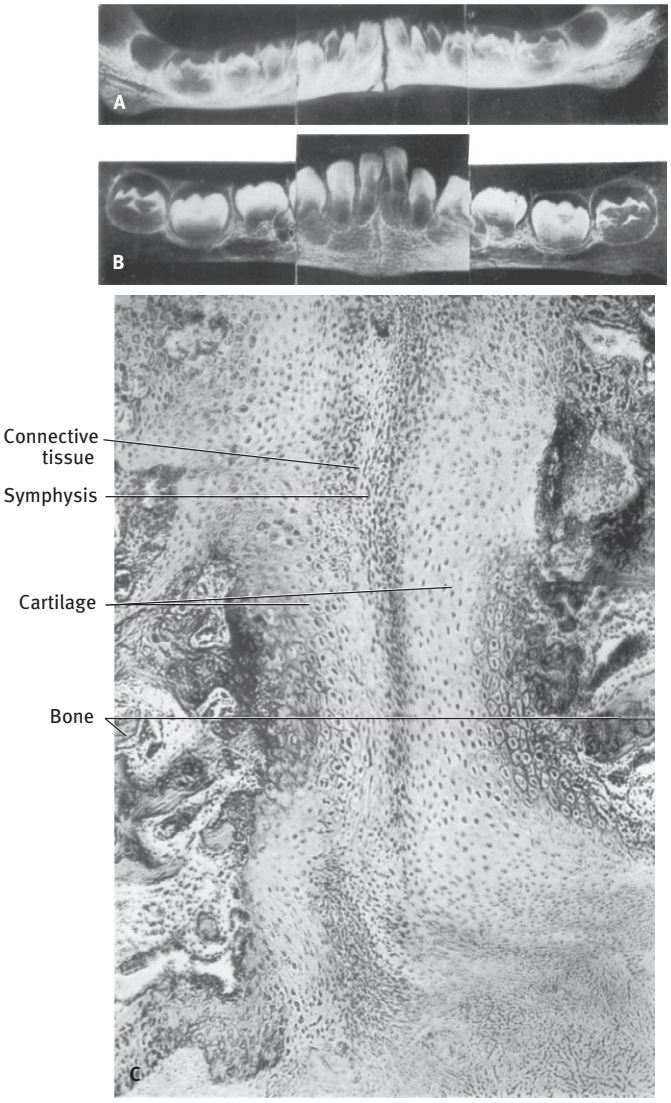


Figure 9.9 Development of mandibular symphysis and formation of bony septa between developing teeth. (**A**) Newborn infant. Symphysis wide open. Mental ossicle (radiograph). (**B**) Child 9 months of age. Symphysis partly closed. Mental ossicles fused to mandible and bony septa between developing teeth seen (radiograph). (**C**) Frontal section through mandibular symphysis of newborn infant. Connective tissue in midline connects plates of cartilage on either side. Cartilage is later replaced by bone.

STRUCTURE OF THE ALVEOLAR BONE

Anatomically, no distinct boundary exists between the body of the maxilla or the mandible and their respective alveolar processes. In some places, the alveolar process is fused with and partly masked by, bone that is not functionally related to the teeth. In the anterior part of the maxilla, the palatine process fuses with the oral plate of the alveolar process. In the posterior part of the mandible, the oblique line is superimposed laterally on the bone of the alveolar process. As a result of its adaptation to function, two parts of the alveolar process can be distinguished, the alveolar bone proper and the supporting alveolar bone.

Alveolar bone proper

The alveolar bone proper consists partly of *lamellated* and partly of *bundle bone* and is about 0.1–0.4 mm thick. It surrounds the root of the tooth and gives attachment to principal fibers of the periodontal ligament.

Lamellated bone

The lamellar bone contains osteons each of which has a blood vessel in a haversian canal. Blood vessel is surrounded by concentric lamellae to form osteon. Some lamellae of the lamellated bone are arranged roughly parallel to the surface of the adjacent marrow spaces, whereas others form haversian systems.

Bundle bone

Bundle bone is that bone in which the principal fibers of the periodontal ligament are anchored. The term *'bundle'* was chosen, because, the bundles of the principal fibers continue into the bone as *Sharpey's* fibers (Fig. 9.11). The bundle bone is characterized by the scarcity of the fibrils in the intercellular substance. These fibrils, more over, are all arranged at right angles to Sharpey's fibers. The bundle bone contains fewer fibrils than does lamellated bone, and therefore it appears dark in routine hematoxylin and eosin stained sections and much lighter in preparations stained with silver than does lamellated bone. These fibers are mineralized at the periphery and have a larger diameter. These fibers are less numerous than the corresponding fiber bundles in the cementum on the opposite side of the periodontal ligament. The collagen adjacent to bone is always less mature than that adjacent to cementum. In some areas, the alveolar bone proper consists mainly of bundle bone. Bundle bone is formed in areas of recent bone apposition. Lines of rest are seen in bundle bone.

Radiographically, it is also referred to as the *lamina dura*, because, of increased radiopacity, which is due to the presence of thick bone without trabeculations, that X-rays must penetrate and not to any increased mineral content.

The *alveolar bone proper*, which forms the inner wall of the socket is perforated by many openings that carry branches of the interalveolar nerves and blood vessels into the periodontal ligament, and it is therefore called the *cribriform plate* (Fig. 9.12). Bone between the teeth is called *interdental septum* and is composed entirely of cribriform plate. The interdental and interradicular septa contain the perforating canals of *Zuckerkandl and Hirschfeld* (nutrient canals) which house the interdental and interradicular arteries, veins, lymph vessels and nerves (Fig. 9.13).

The *supporting alveolar bone* consists of two parts:

(a) Cortical plates
(b) Spongy bone

Cortical plates

Cortical plates consist of compact bone and form the outer and inner plates of the alveolar processes. The cortical plates, continuous with the compact layers of the maxillary and mandibular body, are generally much thinner in the maxilla, than in the mandible. They are thickest in the premolar and molar region of the lower jaw, especially on the buccal side. In the maxilla, the outer cortical plate is perforated by many small openings through which blood and lymph vessels pass.

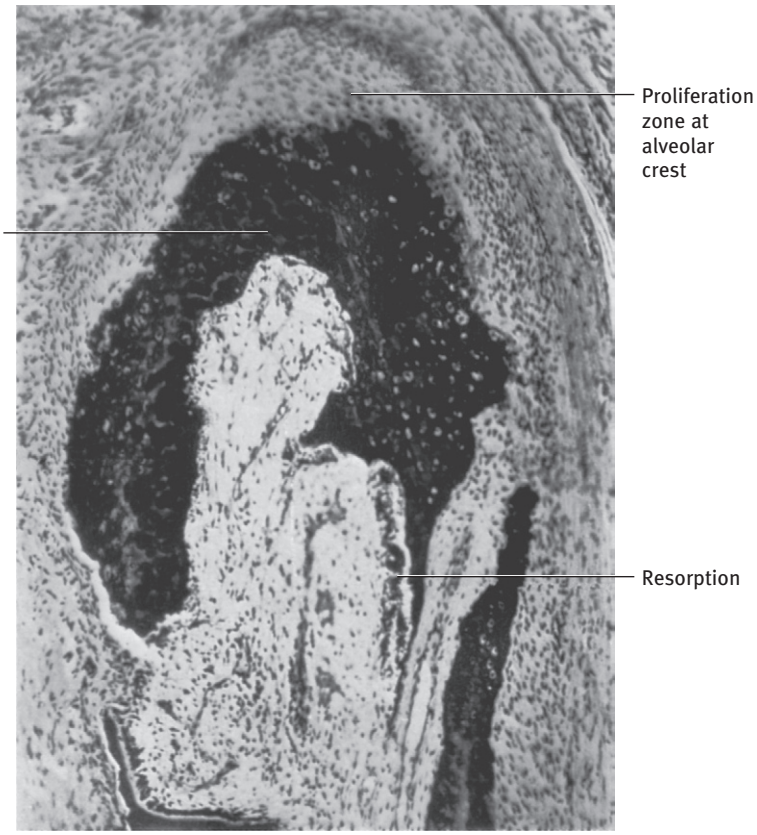


Figure 9.10 Vertical growth of mandible at alveolar crest. Formation of chondroid bone that later is replaced by typical bone.

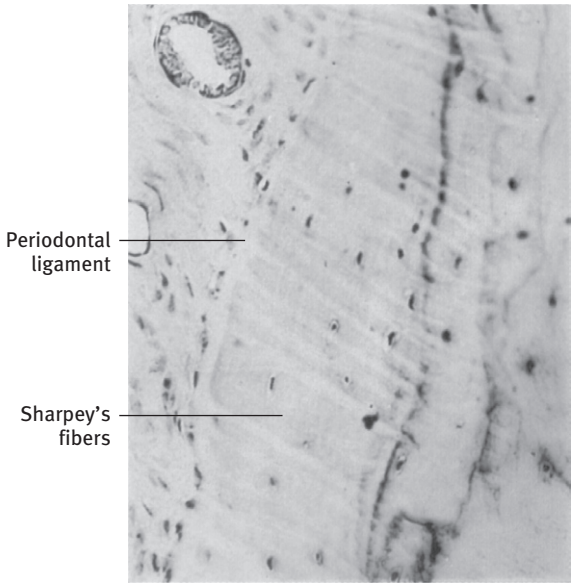


Figure 9.11 Histologic section showing Sharpey's fibers in alveolar bone proper (From Bhaskar SN: Synopsis of oral histology, St Louis, 1962, The CV Mosby Co).

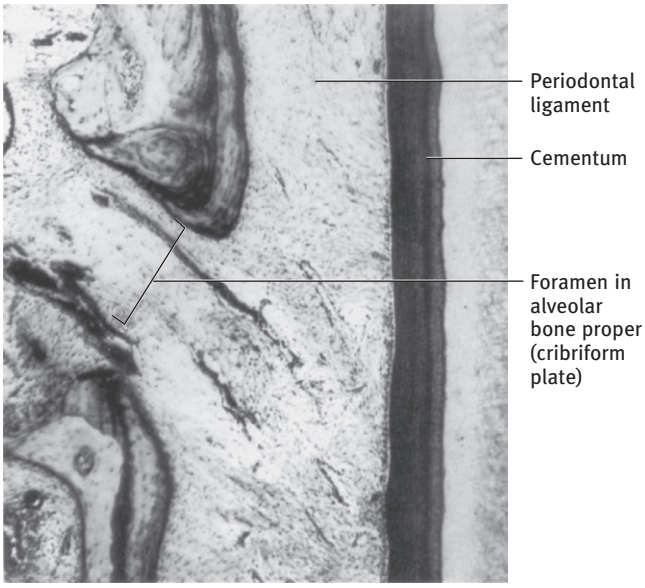


Figure 9.12 Histologic section showing foramen in alveolar bone proper (cribriform plate) (From Bhaskar SN: Synopsis of oral histology, St Louis, 1962, The CV Mosby Co).

In the region of the anterior teeth of both jaws, the supporting bone usually is very thin. No spongy bone is found here, and the cortical plate is fused with the alveolar bone proper (Fig. 9.14). In such areas, notably in the premolar and molar regions of the maxilla, defects of the outer alveolar wall are fairly common. Such defects, where periodontal tissues and covering mucosa fuse, do not impair the firm attachment and function of the tooth.

Bone underlying the gingiva is the cortical plate. Both ***cribriform plate*** and ***cortical plate*** are ***compact bone*** separated by spongy bone.

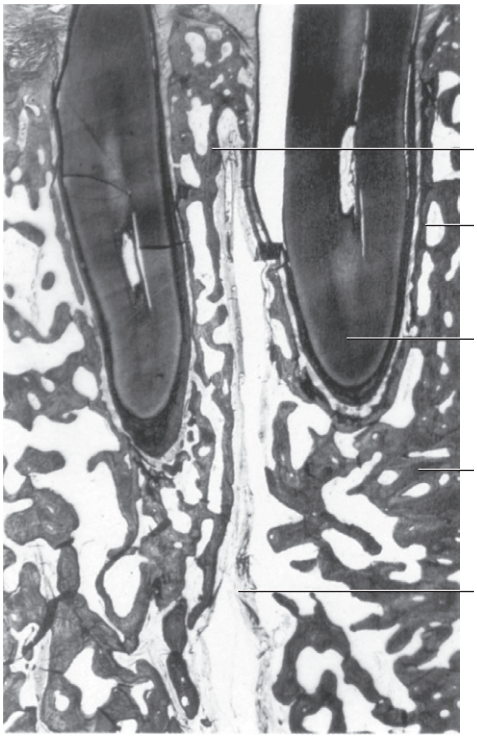


Figure 9.13 Section through jaw showing nutrient canal of Zuckerkandl and Hirschfeld in interdental bony septum (From Bhaskar SN: Synopsis of oral histology, St Louis, 1962, The CV Mosby Co).

Histologically, the cortical plates consist of longitudinal lamellae and haversian systems (Fig. 9.4). In the lower jaw, circumferential or basic lamellae reach from the body of the mandible into the cortical plates.

Spongy bone

Spongy bone fills the area between the cortical plates and the alveolar bone proper. It contains trabeculae of lamellar bone. These are surrounded by marrow that is rich in adipocytes and pluripotent mesenchymal cells.

The trabeculae contain osteocytes in the interior and osteoblasts or osteoclasts on the surface. These trabeculae of the spongy bone buttress the functional forces to which alveolar bone proper is exposed. The cancellous component in maxilla is more than in the mandible. The study of radiographs permits the classification of the spongiosa of the alveolar process into two main types.

In ***type I*** the interdental and interradicular trabeculae are regular and horizontal in a ladder like arrangement (Fig. 9.15).

Type II shows irregularly arranged, numerous, delicate interdental and interradicular trabeculae. Both types show a variation in thickness of trabeculae and size of marrow spaces. The architecture of ***type I*** is seen most often in the mandible and fits well into the general idea of a trajectory pattern of spongy bone. ***Type II***, although evidently functionally satisfactory, lacks a distinct trajectory pattern, which seems to be compensated for by the greater number of trabeculae in any given area. This arrangement is

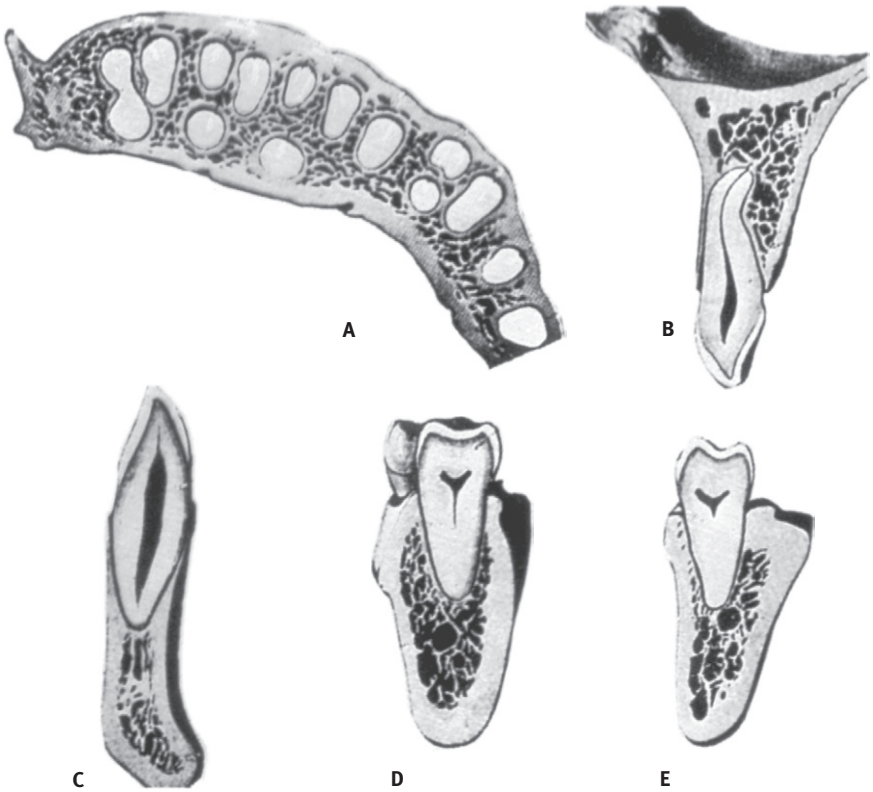


Figure 9.14 Gross relations of alveolar processes. (**A**) Horizontal section through upper alveolar process. (**B**) Labiolingual section through upper lateral incisor. (**C**) Labiolingual section through lower canine. (**D**) Labiolingual section through lower second molar. (**E**) Labiolingual section through lower third molar (From Sicher H and Tandler J: Anatomie für Zahnärzte [Anatomy for dentists], Vienna, 1928, Julius Springer Verlag).

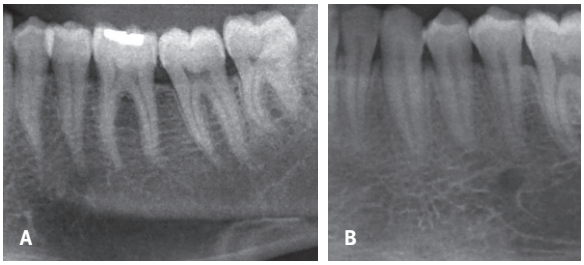


Figure 9.15 Radiograph of the mandible showing types of supporting alveolar bone (spongiosa). (**A**) Type I alveolar spongiosa showing ladder-like arrangement of the trabeculae. (**B**) Type II alveolar spongiosa showing irregularly arranged trabeculae (Courtesy Department of Oral Medicine and Radiology, KSR Institute of Dental Science and Research).

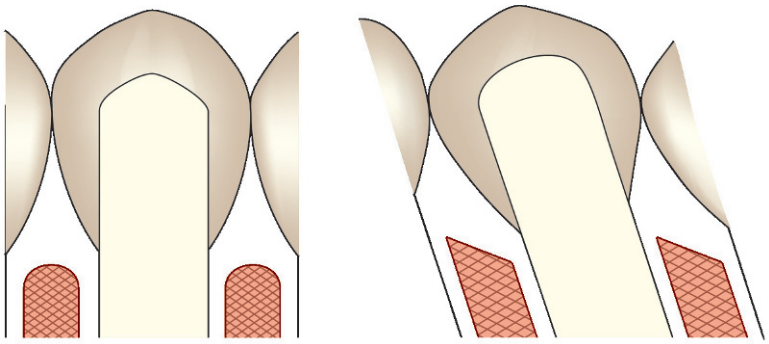


Figure 9.16 Diagram showing relation between cementoenamel junction of adjacent teeth and shape of crests of alveolar septa (From Ritchey B and Orban B: J Periodontol 24:75, 1953).

more common in the maxilla. From the apical part of the socket of lower molars, trabeculae are sometimes seen radiating in a slightly distal direction.

These trabeculae are less prominent in the upper jaw, because of the proximity of the nasal cavity and the maxillary sinus. In the condylar process, in the angle of the mandible, in the maxillary tuberosity, and in other isolated foci, hematopoietic cellular marrow is found.

Crest of the alveolar septa The shape of the outlines of the crest of the alveolar septa in the radiograph is dependent on the position of the adjacent teeth. In a healthy mouth the distance between the cementoenamel junction and the free border of the alveolar bone proper is fairly constant. If the neighboring teeth are inclined, the alveolar crest is oblique. In the majority of individuals the inclination is most pronounced in the premolar and molar regions, with the teeth being tipped mesially. Then the cementoenamel junction of the mesial tooth is situated in a more occlusal plane than that of the distal tooth, and the alveolar crest therefore slopes distally (Fig. 9.16). Cortical bone and alveolar bone meet at the alveolar crest usually 1.5 to 2 mm below the level of the cementoenamel junction on the tooth it surrounds.

INTERNAL RECONSTRUCTION OF ALVEOLAR BONE

Mesial drift and continuous tooth eruption elicit remodeling of alveolar bone proper. During the mesial drift of a tooth, bone is apposed on the distal and resorbed on the mesial alveolar wall (Fig. 9.7). The distal wall is made up almost entirely of bundle bone. However, the osteoclasts in the adjacent marrow spaces remove part of the bundle bone, when it reaches a certain thickness. In its place, lamellated bone is deposited.

On the mesial alveolar wall of a drifting tooth, the sign of active resorption is the presence of Howship's lacunae containing osteoclasts. Bundle bone, however, on this side is always present in some areas but forms merely a thin layer (Fig. 9.17). This is because the mesial drift of a tooth does not occur simply as a bodily movement. Thus resorption does not involve the entire mesial surface of the alveolus at one and the same time. Moreover, periods of resorption alternate with periods of rest and repair. It is during these periods of repair that bundle bone is formed, and detached periodontal fibers are again secured. Islands of bundle bone are separated from the lamellated bone by reversal lines that turn their convexities towards the lamellated bone (Fig. 9.17).

During these changes, compact bone may be replaced by spongy bone or spongy bone may change into compact bone. This type of internal reconstruction of bone can be observed in physiologic mesial drift or in orthodontic mesial or distal movement of teeth. In these movements an interdental septum shows apposition on one surface and resorption on the other. If the alveolar bone proper is thickened by apposition of bundle bone, the interdental marrow spaces widen and advance in the direction of apposition. Conversely, if the plate of the alveolar bone proper is thinned by resorption, apposition of bone occurs on those surfaces that face the marrow spaces. The result is a reconstructive shift of the interdental septum.

Alterations in the structure of the alveolar bone are of great importance in connection with the physiologic eruptive movements of the teeth. These movements are directed mesioocclusally. At the alveolar fundus the

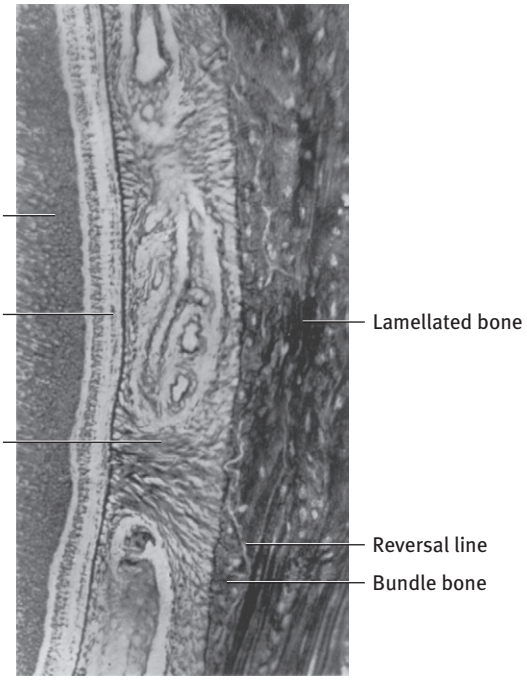


Figure 9.17 Mesial alveolar wall where alveolar bone proper consists mostly of lamellated bone and islands of bundle bone, which anchor principal fibers of periodontal ligament.

continual apposition of bone can be recognized by resting lines separating parallel layers of bundle bone. When the bundle bone has reached a certain thickness, it is resorbed partly from the marrow spaces and then replaced by lamellated bone or spongy trabeculae. The presence of bundle bone indicates the level at which the alveolar fundus was situated previously.

AGE CHANGES

The age changes are described in detail in Chapter 17 on Age Changes in Oral Tissues. However a brief mention of the age changes is given below.

Alveolar bone loss occurs and marrow space increases. Marrow shows more adipose tissue. There is an apparent increase in size of the maxillary sinus.

CLINICAL CONSIDERATIONS

Bone, although one of the hardest tissues of the human body, is biologically a highly plastic tissue. Where bone is covered by a vascularized connective tissue, it is exceedingly sensitive to pressure, whereas tension acts generally as a stimulus to the production of new bone. It is this biologic plasticity that enables the orthodontist to move teeth without disrupting their relations to the alveolar bone. Bone is resorbed on the side of pressure and apposed on the side of tension; thus the entire alveolus is allowed to shift with the tooth. It has been shown that on the pressure side there is an increase in the level of cyclic adenosine monophosphate (cAMP) in cells. This may play some role in bone resorption. At sites of alveolar bone compression, osteoclasts proliferate and initial resorption of the superficial bone takes place. It is believed that, the initial response may involve osteoblasts which can produce collagenolytic enzymes to remove a portion of unmineralized extracellular matrix, thereby, facilitating access of osteoclast precursors to the bone surface. Osteoblastic cells also produce cytokines and chemokines, which can attract monocyte precursors and promote osteoclast differentiation. In quiescent areas, retraction or apoptotic death of bone lining cells will expose the mineralized bone surface to osteoclasts.

At sites of tension, osteoblasts are activated to produce osteoid that subsequently mineralizes to form new bone.

The adaptation of bone to function is quantitative as well as qualitative. Whereas, increase in functional forces leads to formation of new bone, decreased function leads to a decrease in the volume of bone. This can be observed in the supporting bone of teeth that have lost their antagonists. Here the spongy bone around the alveolus shows pronounced rarefaction. The bone trabeculae are less numerous and very thin (Fig. 9.18). The alveolar bone proper, however, is generally well preserved because it continues to receive some stimuli from the tension of the periodontal tissues.

During healing of fractures or extraction wounds, an embryonic type of bone is formed, which only later is replaced by mature bone. The embryonic bone also called immature or coarse fibrillar bone, is characterized, among other aspects, by the greater number, size and irregular arrangement of the osteocytes than are found in mature bone (Fig. 9.3). The greater number of cells and the reduced volume of calcified intercellular substance render this immature bone more radiolucent than mature bone. This explains why bony callus cannot be seen in radiographs at a time when histologic examination of a fracture reveals a well-developed union between the fragments and why a socket after an extraction wound appears to be empty at a time, when it is almost filled with immature bone. The visibility in radiographs lags 2 or 3 weeks behind actual formation of new bone.

The most frequent and harmful change in the alveolar process is that which is associated with periodontal disease. The bone resorption is almost universal, occurs more frequently in posterior teeth, is usually symmetrical, occurs in episodic spurts, is both of the horizontal and vertical type (i.e. occurs from the gingival and tooth side, respectively), and is intimately related to bacterial plaque and pocket formation. It has been shown, for example, endotoxins produced by the gram-negative bacteria of the plaque lead to an increase in cAMP, which increases the osteoclastic activity.

Resorption after tooth loss has been shown to follow a predictable pattern. The labial aspect of the alveolar crest is the principal site of resorption, which reduces first in width and later in height.

The pattern of resorption is different in the maxilla and mandible. The residual alveolar ridge resorbs downward and outward in the mandible, whereas, in the maxilla the resorption is upwards and inwards. Nontraumatic loss of anterior maxillary teeth is followed by a progressive loss of bone mainly from the labial side. In the deciduous dentition, loss of a retained second deciduous molar, which has no succedaneous permanent tooth to replace it, is also associated with bone loss. The cause for resorption of alveolar bone after tooth loss has been assumed to be due to

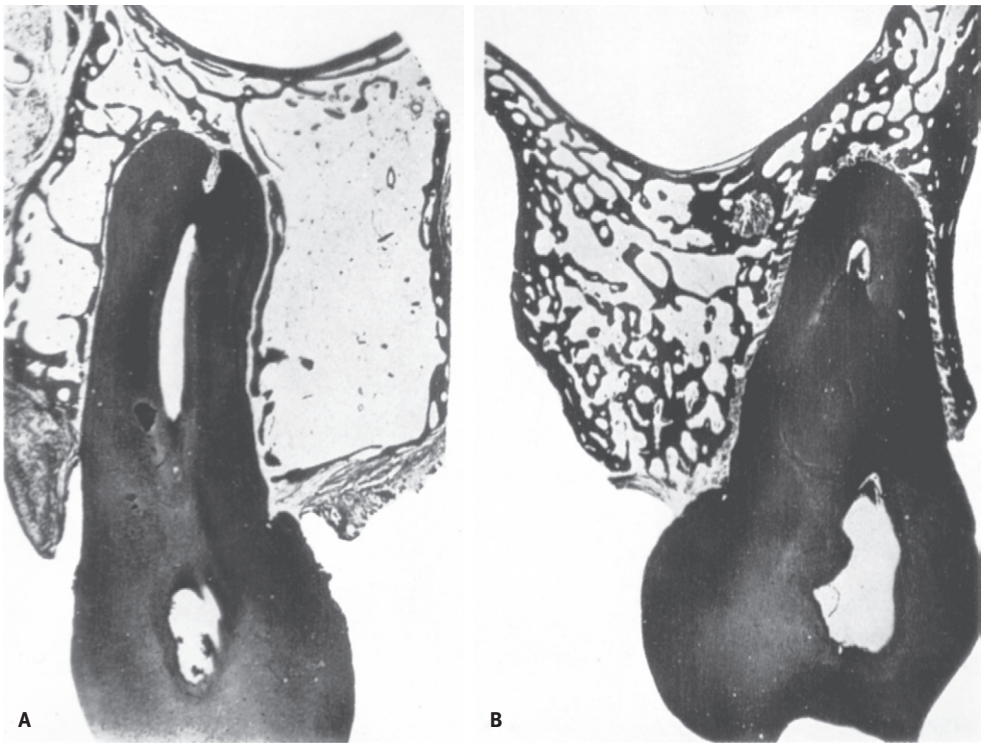


Figure 9.18 Osteoporosis of alveolar process caused by inactivity of tooth that has no antagonist. Labiolingual sections through upper molars of same individual. (**A**) Disappearance of bony trabeculae after loss of function. Plane of mesiobuccal root. Alveolar bone proper remains intact. (**B**) Normal spongy bone in plane of mesiobuccal root of functioning tooth (From Kellner E: Z Stomatol 18:59, 1920).

disuse atrophy, decreased blood supply, localized inflammation or unfavorable prosthesis pressure. Alveolar ridge defects and deformities can also be the result of congenital defects, trauma, periodontal disease or surgical ablation, as in the case of tumor surgery.

Lamina dura is an important diagnostic landmark in determining health of the periapical tissues. Loss of density usually means infections, inflammation and resorption of bone socket.

THERAPEUTIC CONSIDERATIONS

Traditional treatment methods for promoting bone healing primarily utilize bone grafts or synthetic materials to fill the defects and provide structural support.

Bone grafting to stimulate bone deposition has been used in periodontal surgery since the 1970s. It involves a surgical procedure to place bone or bone substitute material into a bone defect with the objective of producing new bone and possibly the regeneration of periodontal ligament and cementum.

Autografts utilize the patient's bone, which can be obtained from intraoral or extraoral sites. They are the best materials for bone grafting, are very well accepted by the body and may produce the fastest rate of bone growth; however, there is the potential risk of additional discomfort and a secondary procedure. With autografts, the patient is assured of protection from disease transmission and/or immune reaction.

Allografts are obtained from another human source, typically highly processed bone powder from human cadavers. The age and health of the donor can affect the rate of bone regeneration. The risk of disease transmission and/or rejection is handled by processing and quality control. The allografts are freeze-dried at ultra-low temperatures and dried under high vacuum. They are available either demineralized or non-demineralized. Unlike synthetic bone, which only provides scaffolding for osteoconduction, allografts include growth factors which are also osteoinductive. Allografts induce bone growth and provide an environment that increases the body's regenerative process.

Xenografts are obtained from animal sources; usually cows and/or pigs. They include processed animal bone or growth proteins. Again, the risk of disease transmission and/or rejection is reduced by processing.

The drawbacks of using bone grafts include a lack of viable bone tissue available for autografts and allografts transplantation and potential disease transmission with allografts and xenografts.

In cases where bone grafts from human or animal sources are not feasible, synthetic graft materials (alloplasts) are used.

Synthetic bone grafting materials

Examples of synthetic materials include natural and synthetic hydroxyapatites, ceramics, calcium carbonate (natural coral), silicon-containing glasses, and synthetic polymers.

Synthetic materials carry no risk of disease transmission or immune system rejection. They help create an environment that facilitates the body's regenerative process.

A guided tissue regeneration approach has evolved which is an epithelial exclusionary technique. This technique facilitates the cells of the periodontal ligament to differentiate

into osteoblasts, cementoblasts and fibroblasts resulting in regeneration of the lost periodontium. It involves use of membranes, nonresorbable and/or resorbable, which prevent the downgrowth of the epithelium thereby promoting regeneration. Example of a resorbable material is collagen and nonresorbable material is ePTFE (expanded polytetrafluoroethylene) membrane.

Biologically mediated strategies

Biologically mediated strategies include materials, such as enamel matrix proteins, that can be premixed with vehicle solution. They are intended as an adjunct to periodontal surgery for topical application onto exposed root surfaces or bone to treat intrabony defects and furcations due to moderate or severe periodontitis. Enamel matrix protein leave only a resorbable protein matrix on the root surface, which makes bone more likely to regenerate. These bone morphogenetic proteins help to initiate a cascade of events leading to the differentiation of progenitor cells into phenotypes involved in periodontal regeneration.

Bone tissue engineering has emerged as a new therapeutic alternative to promote bone healing. This approach aims to regenerate or repair bone tissue with various combinations of polymeric scaffolds, cells and inductive factors into a system that actively stimulates tissue formation.

Scaffolds are of two types:

- Which simply guide and support bone regeneration—Osteoconductive
- Which actively stimulate bone regeneration via the delivery of inductive factors—Osteoinductive.

These scaffolds are a porous composite material composed mainly of hydroxyapatite in a collagen I matrix. These materials promote osteoblasts and osteoprogenitor cell attachment and differentiation to enhance bone tissue formation.

Other materials used for bone tissue engineering include synthetic, biocompatible and biodegradable polymers such as poly (lactide-co-glycolide)

- For filling small defects, injectable polymers such as alginate or poly(ethylene glycol) are preferred.

Cells. Osteoblasts and osteoprogenitor cells have been incorporated into various scaffolds to enhance bone repair. These cells must be obtained from patient or donor. Stem cells derived from bone marrow periosteum, adipose tissue, skeletal muscle or baby teeth can be induced to differentiate into diverse cell types such as muscle, nerve, cartilage, bone and fat.

Direct or gene therapy approaches to the delivery of osteoinductive factors are also promising approaches for bone regeneration.

Controlled release of proteins from a polymeric scaffold allows localized sustained protein delivery and may be a more effective means to enhance bone formation.

Delivery of BMP-2 from absorbable collagen sponge induces bone formation and heals bony defects. BMP-2 can also be delivered from synthetic materials such as poly(ethylene glycol) hydrogels to promote repair of bone defects. Other than BMP-2, sustained release of FGF-2 and IGF-1 can also accelerate bone formation.

SUMMARY

Bone is a mineralized connective tissue with a relatively flexible character and compressive strength. The property of plasticity allows it to be remodeled according to the functional demands placed on it.

Classification of Bones

Bones are classified as long, short, flat and irregular based on the shape. They are also termed mature, immature, compact and cancellous depending on the microscopic structure. Based on their development bones are classified as endochondral and intramembranous.

Inorganic and Organic Constituents of Bone

The mineral component of bone is predominantly made up of hydroxyapatite crystals. The organic component is predominantly made up of type I collagen followed by type V collagen and noncollagenous proteins. Osteocalcin, osteopontin, bone sialoprotein and osteonectin are the predominant noncollagenous proteins present in the bone. The matrix also contains proteases and a variety of cytokines.

Histology of Bone

All bones are made up of an outer compact bone and central medullary cavity. The cavity has red or yellow bone marrow and a network of bony trabeculae, i.e. spongy bone. The outer part of compact bone is surrounded by periosteum. The inner surface of compact and cancellous bone is covered by endosteum.

Osteon is the basic metabolic unit of bone, which is made up of a central haversian canal surrounded by concentric lamellae. Circumferential lamellae are present at the periosteal and endosteal surfaces in parallel layers. Adjacent haversian systems are connected by Volkmann's canals. Regularly appearing resting lines, which denote the rest period, and irregularly appearing reversal lines denoting the junction between bone resorption and bone formation is seen.

Bone Cells

The cells in bone are the osteoblasts, osteoclasts and osteocytes.

Osteoblasts

Osteoblasts are plump cuboidal cells having all the organelles for protein synthesis. Osteoblasts secrete the organic components of bone matrix. cbfa1 is a transcriptional factor essential for osteoblast differentiation. The activity of osteoblasts is regulated by hormones like PTH, vitamin D_3, growth hormone and insulin. The other factors are BMPs, IGF-1 and 2, FGF, TGF-β and PDGF.

Osteocytes

After completion of the function, osteoblasts remain on the surface as lining cells or get entrapped within the matrix, to become the osteocytes. Osteocytes have an interconnecting system through canaliculi with the overlying osteoblasts and neighboring osteocytes, thus maintaining the vitality and integrity of bone.

Osteoclasts

Osteoclasts are the bone resorbing cells derived from hemopoietic cells of monocyte–macrophage lineage. They appear as multinucleated giant cells and occupy cavities on the surface of bone called Howship's lacunae. They develop a ruffled border and area devoid of organelles and containing smooth plasma membrane called sealing zone close to the resorbing surface of bone. RANKL and M-CSF secreted by osteoblasts and stromal cells are required for the formation of osteoclast. Interaction between RANKL and RANK on the surface of osteoclast precursors is necessary for the differentiation of the cell.

Factors regulating osteoclast activity are estrogen, PTH, vitamin D_3 and calcitonin. The other factors are interleukins, bisphosphonates and prostaglandins.

Bone Formation

Bone formation is intramembranous or endochondral. In intramembranous formation, bone is directly formed within a vascular, fibrous membrane. Endochondral bone formation is preceded by the formation of a hyaline cartilaginous model that is replaced by bone.

Intramembranous Bone Formation

In the intramembranous bone formation bony spicules that form initially grow and unite to form trabeculae, which extend in a radial direction enclosing blood vessels. This immature bone is called woven bone. It has intertwined collagen fibers and a lower mineral density compared to mature bone called lamellar bone; which has an orderly arrangement of collagen fibers and a higher mineral content.

Endochondral Bone Formation

In the endochondral bone formation the cartilage grows by interstitial and appositional growth. Capillaries grow into the cartilage and the inner cellular layer of the perichondrium differentiates into osteoblast forming bone. The bone along with blood vessels called periosteal bud form primary ossification center. The medullary cavity is produced by osteoclastic resorption and secondary ossification centers containing cartilage remains at the growing ends of bone or the epiphyses.

Mineralization of Bone

Various factors contribute to conditions that may favor calcification. Theories that have been put forward to explain the mechanism of calcification are Nucleation theory, Matrix vesicle theory and Alkaline phosphatase theory. Matrix vesicles, which accumulate calcium, lay down apatite in relation to collagen fibrils, phosphoproteins like osteonectin help in this process.

Bone Resorption

Bone resorption is brought about by osteoclasts and TRAP plays an important role in the resorptive mechanism. Hydrochloric acid released by the osteoclast dissolves the apatite and the organic matrix is removed by proteolytic enzymes like cathepsin-K. The free organic and inorganic particles are endocytosed by osteoclast packed into membrane bound vesicles and released by exocytosis. TRAP helps in regulation of osteoclast adhesion to bone, its migration to adjacent site and also fuse with the resorbed material within osteoclast for its subsequent disposal.

Bone Remodeling

In order to maintain stability and integrity of bone, it constantly undergoes remodeling. About 10% of bone material is renewed each year. This process is brought about by osteoclasts and osteoblasts. Osteoclasts resorb bone by tunneling through it. These are called cutting cone. After its activity ceases, osteoblasts lay down bone forming new haversian systems called filling cone. PTH, vitamin D, growth factors, bacterial products like lipopolysaccharides are the important mediators of bone remodeling.

Markers for Bone Turnover

The important markers for bone turnover include serum alkaline phosphates for bone formation, urinary calcium and hydroxyproline for bone resorption, and serum β2 microglobulin for bone remodeling

Alveolar Bone

Alveolar process is a part of the maxilla and mandible that forms and supports the sockets of the teeth. It is classified as alveolar bone proper and supporting alveolar bone.

Alveolar Bone Proper

The alveolar bone proper forms the inner wall of the socket. The alveolar bone proper is made up of lamellated and bundle bone. Into the bundle bone bundles of Sharpey's fibers are attached and continue into it. The alveolar bone proper is also called cribriform plate because many vessels and nerves penetrate it. Radiographically, it is also called lamina dura because it is an area of increased radiodensity due to increased bone thickness.

Supporting Alveolar Bone

The supporting alveolar bone consists of cortical plates and spongy bone. The arrangements of trabeculae in the spongy bone are of two types: a regularly arranged type I and an irregularly arranged type II.

Alveolar Bone Formation and Resorption

Due to continuous eruption of teeth called physiological mesial drift alveolar bone undergoes internal reconstruction; bone forms on the distal side and resorbs on the mesial side. This plasticity of bone also enables orthodontic movement possible. Bone is resorbed on the side of pressure and deposited on the side of tension. In the healing of fractures, a bony callus is formed which is an embryonic type of bone with less amounts of calcified intercellular substance.

Alveolar process is resorbed in periodontal disease and is both of horizontal and vertical type. Resorption after tooth loss is downward and outward in the mandible, but in the maxilla it is upwards and inwards.

Alveolar bone is dependant on the presence of teeth for its development. In the absence of teeth, alveolar bone is poorly developed.

Therapeutic Considerations

Bone grafts or synthetic materials are used to fill the bony defects and provide structural support.

In guided tissue regeneration, materials like collagen are used to support natural bone formation processes. The success of GTR technique is not always predictable and this approach is limited in its application.

Enamel matrix proteins have been used as an adjunct to periodontal surgery for topical application onto exposed root surfaces or to treat intrabony defects.

Bone tissue engineering is a multidisciplinary field with a potential to replace tissues lost as a result of trauma, cancer surgery or organ dysfunction.

REVIEW QUESTIONS

1. How are the bones classified?
2. Which are the noncollagenous proteins in bone?
3. Describe the structure of an osteon.
4. Describe the structure and functions of osteoblasts.
5. What are the factors regulating osteoblast and osteoclast formation?
6. How does osteoblasts influence the formation of osteoclast?
7. Describe the structure of osteoclast.
8. How does an osteoblast get transformed into an osteocyte?
9. What is appositional growth?
10. What is a mixed spicule?
11. How is the secondary ossification center formed in endochondral bone formation?
12. Tabulate the differences between woven bone and lamellar bone.
13. Describe the process of bone resorption.
14. Discuss the factors that initiate mineralization.
15. Explain the action of mediators of bone remodeling.
16. Enumerate the serum markers of bone remodeling.
17. Write notes on:
    Bundle bone
    Lamina dura
    Cribriform plate.
18. Describe the structure of the alveolar bone.
19. How is the integrity and vitality of alveolar bone maintained?

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10 Oral Mucous Membrane

CHAPTER CONTENTS

Body cavities that communicate with the external surface are lined by mucous membranes, which are coated by serous and mucous secretions. The surface of the oral cavity is a mucous membrane. The oral mucosa is continuous with the skin of the lip through the vermilion border. Posteriorly it is continuous with the mucosa of the pharynx. Its structure varies in an apparent adaptation to function in different regions of the oral cavity. Areas involved in the mastication of food, such as the gingiva and the hard palate, have a much different structure than does the floor of the mouth or the mucosa of the cheek.

CLASSIFICATION OF ORAL MUCOSA

The classification based on these functional criteria, divides the oral mucosa into three major types:

1. Masticatory mucosa (gingiva and hard palate)
2. Lining or reflecting mucosa (lip, cheek, vestibular fornix, alveolar mucosa, floor of mouth and soft palate)
3. Specialized mucosa (dorsum of the tongue and taste buds)

The masticatory mucosa is bound to bone and does not stretch. It bears forces generated when food is chewed. The lining mucosa is not equally exposed to such forces. However, it covers the musculature and is distensible, adapting itself to the contraction and relaxation of cheeks, lips, and tongue and to movements of the mandible produced by the muscles of mastication. It makes up all the surfaces of the mouth except for the dorsum of the tongue and the masticatory mucosa.

The specialized (sensory) mucosa is so called because it bears the taste buds, which have a sensory function.

These will be discussed in detail in subsequent paragraphs. Two areas with a slightly different structure—the dentogingival junction (the attachment of the gingiva to the tooth) and the red zone or vermilion border of the lips will also be discussed (Table 10.1).

FUNCTIONS OF ORAL MUCOSA

The oral mucosa has many important functions.

Defense

The integrity of the oral epithelium is an effective barrier for the entry of the microorganisms. It should be noted that the oral cavity being an 'ideal incubator', harbors a wide variety of organisms. These commensal organisms become pathogenic if the host defense is compromised. Infection occurs if the epithelial integrity is broken down resulting in bacterial invasion or if their toxins are allowed to seep through the epithelium. The oral mucosa is impermeable to bacterial toxins. It also secretes antibodies and has an efficient humoral and cell mediated immunity.

Lubrication

The secretion of salivary glands keeps the oral cavity moist and thus prevents the mucosa from drying and cracking thereby ensuring an intact oral epithelium. A moist oral cavity helps in speech, mastication, swallowing and in the perception of taste.

Sensory

The oral mucosa is sensitive to touch, pressure, pain and temperature. The sensitivity of these sensations vary in different regions. Generally these are better appreciated in the anterior part of the mouth. The sensation of taste is a unique sensation, felt only in the anterior 2/3rd of the dorsum of the tongue. Swallowing, gagging, retching and salivating reflexes are initiated by receptors in the oral mucosa. Touch sensations in the soft palate result in gag reflex.

Table 10.1 Classification of Oral Mucosa

A. BASED ON FUNCTION
1. Masticatory mucosa
2. Lining mucosa
3. Specialized mucosa

B. BASED ON TYPE OF EPITHELIUM
1. Keratinized
2. Nonkeratinized

Protection

The oral mucosa protects the deeper tissues from mechanical forces resulting from mastication and from abrasive nature of foodstuffs.

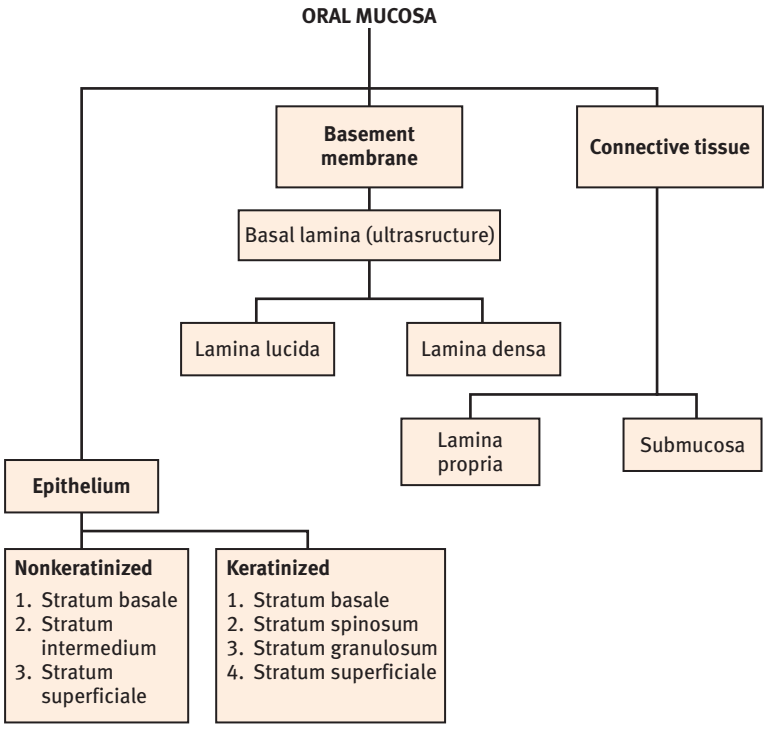
DEFINITIONS AND GENERAL CONSIDERATIONS (Flowchart 10.1)

The oral mucosa consists of epithelium and connective tissue termed lamina propria. The oral mucosa is attached by a loose connective tissue termed submucosa to the underlying structure which may be bone or muscle (Fig. 10.1). The oral mucosal epithelium is of stratified squamous type.

Comparison of Oral Mucosa with Skin and Intestinal Mucosa

Thus, the structure of the oral mucous membrane resembles the skin in many ways. It is also composed of two layers, epithelium and connective tissue. These layers are analogous to the epidermal and dermal layers of the skin. It is also similar to intestinal mucosa in that it has an epithelium, lamina propria, and is attached by the submucosa to the underlying muscles. However, an intervening layer of smooth muscles and elastic fibers termed muscularis mucosae is present between lamina propria and submucosa in the intestinal mucosa (Fig. 10.2). The epithelium of the skin is always orthokeratinized, but the oral mucosal epithelium depending on the region; may be nonkeratinized and if keratinized, the keratinization may be ortho or parakeratinized. Unlike skin, oral epithelium lacks a layer, termed stratum lucidum. The content of dermis and lamina propria differ considerably. Skin appendages; namely the hair follicles, sebaceous glands and sweat glands are not seen in the lamina propria. Likewise, salivary glands are not seen in the dermis. The two layers, epithelium and connective tissue form an interface that is folded into corrugations. Papillae of connective tissue protrude toward the epithelium (Fig. 10.2) carrying blood vessels and nerves. Although some of the nerves actually pass into it, the epithelium does not contain blood vessels. The epithelium, in turn, is formed into ridges that protrude toward the lamina propria. These ridges interdigitate with the papillae and are called epithelial ridges. When the tissue is sectioned for microscope, these ridges look like pegs as they alternate with the papillae, forming a serpentine interface. At one time, the epithelial ridges were mistakenly called epithelial pegs.

The two tissues are intimately connected. At their junction there are two different structures with very similar names, the basal lamina and the basement membrane. The basal lamina is evident at the electron microscopic level and is epithelial in origin (Fig. 10.3). The basement membrane is evident at the light microscopic level.



Flowchart 10.1 Structure of oral mucosa.

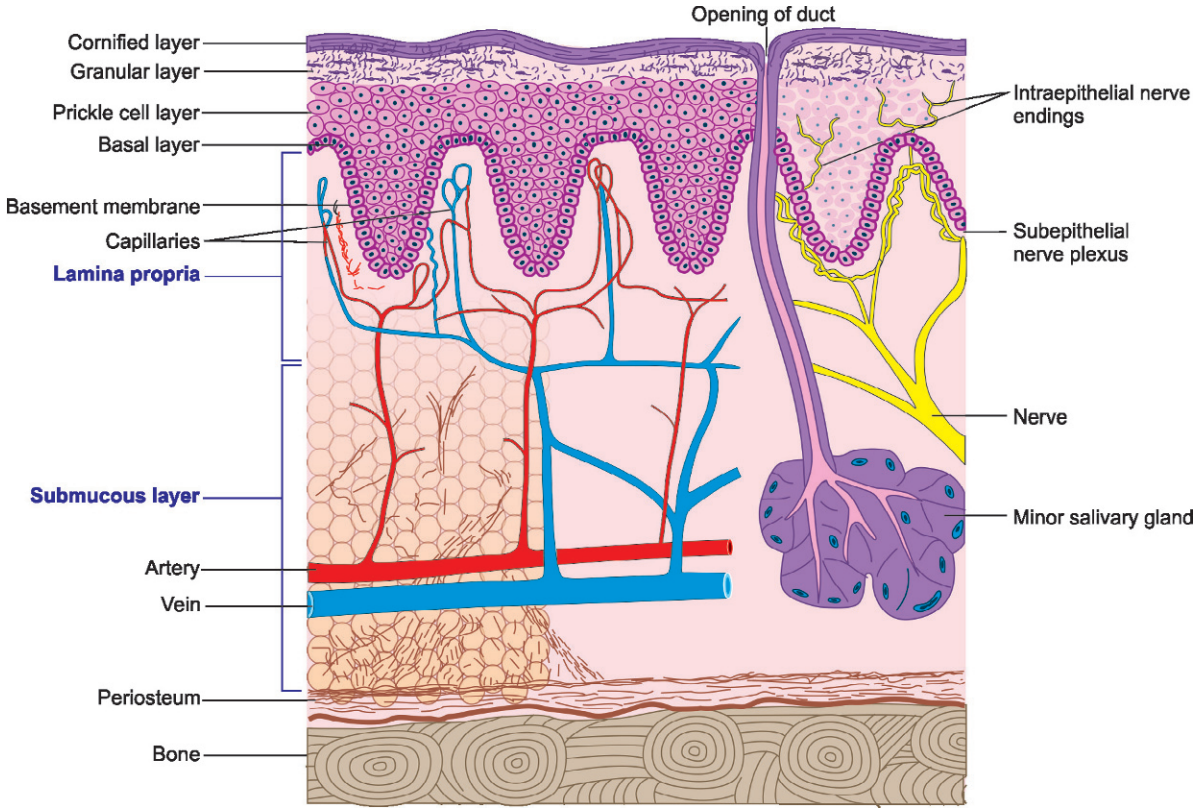


Figure 10.1 Diagram of oral mucous membrane (epithelium, lamina propria, and submucosa).

Basement Membrane

The interface between the connective tissue and the epithelium in light microscope appears thick and it includes the reticular fibers. It is a zone that is 1 to 4 μm wide and is relatively cell free. This zone stains positively with the periodic acid-Schiff method, indicating that it contains neutral mucopolysaccharides (glycosaminoglycans). Ultrastructurally, basement membrane is called basal lamina, but it is not just a membrane but it is a basal complex consisting of lamina and fibers. The basal

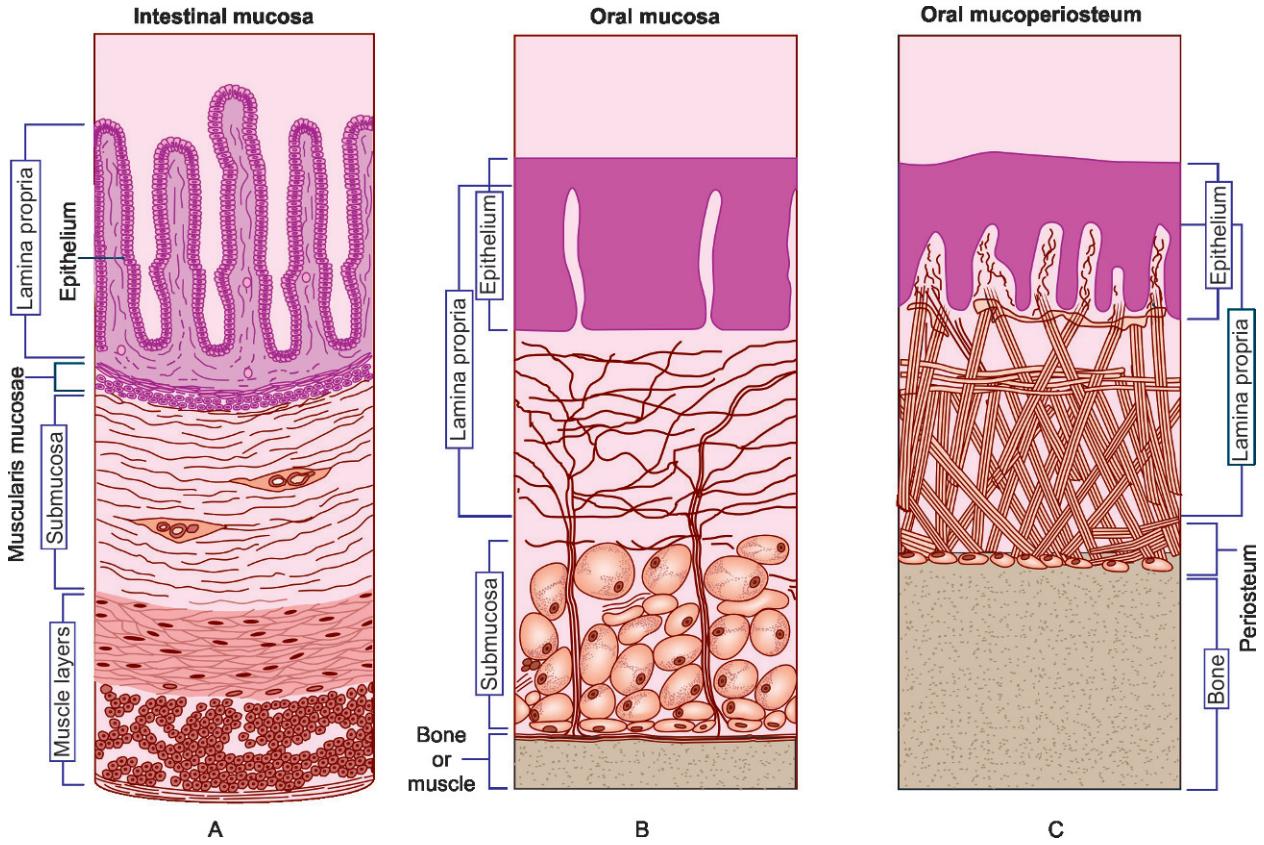


Figure 10.2 Diagrammatic representation of **(A)** Intestinal mucosa. **(B)** Oral lining mucosa. **(C)** Oral masticatory mucosa.

lamina is made up of a clear zone (lamina lucida) just below the epithelial cells and a dark zone (lamina densa) beyond the lamina lucida and adjacent to the connective tissue (Fig. 10.3). Anchoring fibrils, which contain type VII collagen, form loops and are inserted into the lamina densa. Collagen fiber of type I and II run through these loops. Lamina densa contains type IV collagen coated with heparin sulfate in chicken wire (net like) configuration. Lamina lucida is a 20–40 nm wide glycoprotein layer and it contains type IV collagen and an antigen bound by the antibody KF-1. The lamina lucida has been shown to contain laminin and bullous pemphigoid antigen. Laminin is a large, triple-chain molecule (Mr = 10^6). Laminin and type IV collagen promote epithelial cell growth. Basement membranes promote differentiation. They also promote peripheral nerve regeneration and growth, and they tend to prevent metastases.

Lamina Propria

The lamina propria may be described as a connective tissue of variable thickness that supports the epithelium. It is divided for descriptive reasons into two parts—papillary and reticular. The papillary portion is between the epithelial ridges and the reticular portion is below it. The reticular layer was thought to contain fine immature argyrophilic (silver staining) reticular fibers. The reticular layer contains net like arrangement of collagen fibers. The presence of reticular fibers in this layer is questioned.

Since there is considerable variation in length and width of the papillae in different areas, the papillary portion is also of variable depth. The two portions are not separate. They are a continuum, but the two terms are used to describe this region in different ways. The reticular zone is always present. The papillary zone may be absent in some areas such as the alveolar mucosa when the papillae are either very short or lacking.

The interlocking arrangement of the connective tissue papillae and the epithelial ridges and the even finer undulations and projections found at the base of each epithelial cell increases the area of contact between the lamina propria and epithelium (Fig. 10.4). This additional area facilitates exchange of material between the epithelium and the blood vessels in the connective tissue.

In oral mucosa, the ground substance in the lamina propria like elsewhere contains glycoproteins and proteoglycans. Hyaluronan, heparan sulfate, versican, decorin, biglycan and syndecan are the important proteoglycans present. Apart from fibroblasts, mast cells and macrophages are present in the lamina propria.

The collagen fibers in the lamina propria are of types I and III. The presence of elastic fibers in the lining mucosa helps to restore tissue form after stretching. Elastic fibers do not form bundles like collagen fibers.

The lamina propria may attach to the periosteum of the alveolar bone, or it may overlay the submucosa, which varies in different regions of the mouth such as the soft palate and floor of the mouth (Table 10.2).

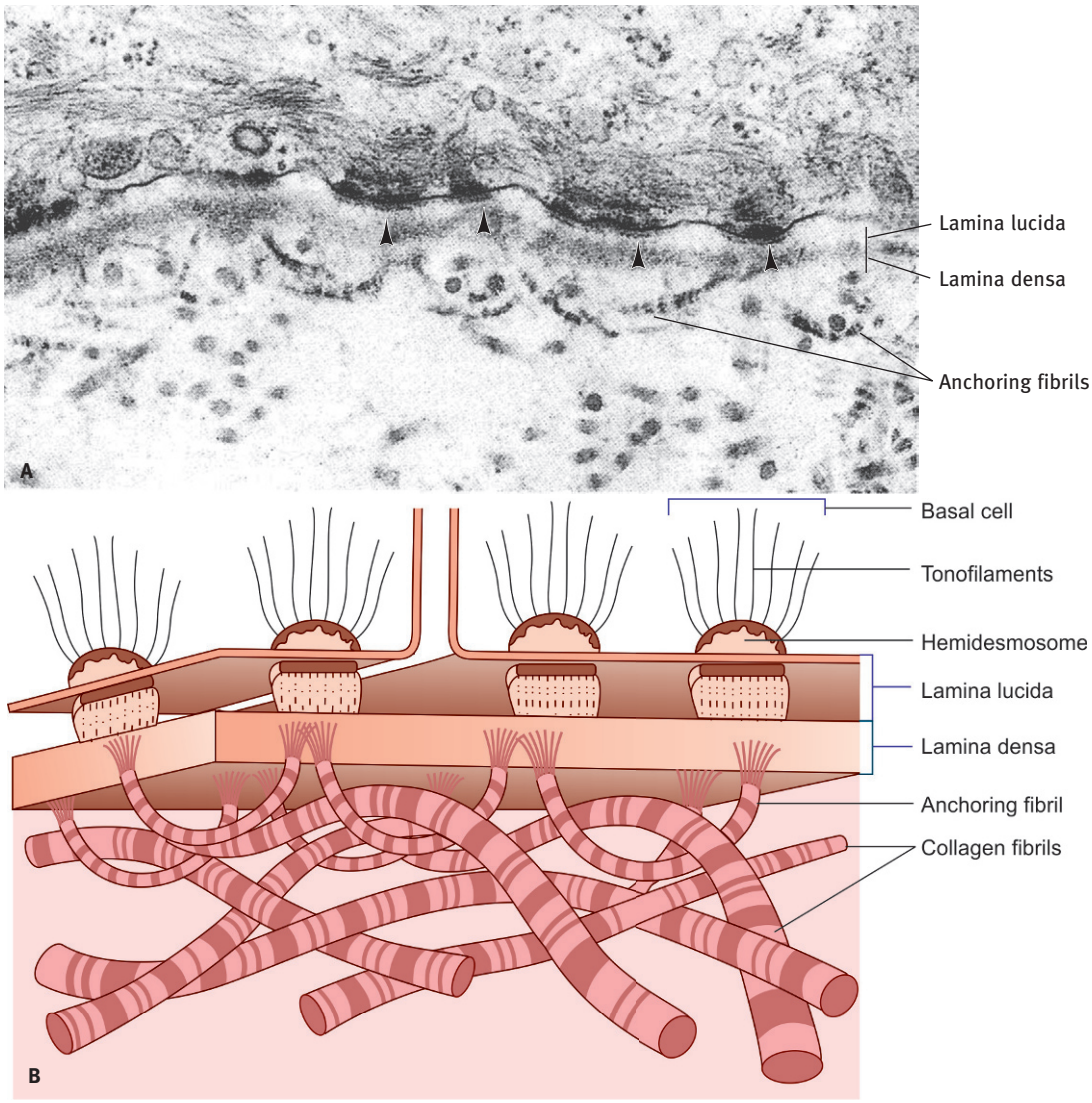


Figure 10.3 Ultrastructure of basal lamina. **(A)** Hemidesmosomes (arrowheads) at the plasma membrane of basal cells. **(B)** Diagrammatic representation of the details of the complex.

Submucosa

The submucosa consists of connective tissue of varying thickness and density. It attaches the mucous membrane to the underlying structures. Whether this attachment is loose or firm depends on the character of the submucosa. Glands, blood vessels, nerves, and also adipose tissue are present in this layer. Compared to skin blood supply to the oral mucosa is profuse and among the various regions blood flow to the gingiva is greatest.

It is in the submucosa that the larger arteries divide into smaller branches, which then enter the lamina propria. Here they again divide to form a subepithelial capillary network in the papillae. The veins originating from the capillary network course back along the path taken by the arteries. The blood vessels are accompanied by a rich network of lymph vessels. The sensory nerves of the mucous membrane tend to be more concentrated toward the anterior part of the mouth (rugae, tip of tongue, etc.). The nerve fibers are myelinated as they traverse the submucosa but lose their myelin sheath before splitting into their end arborizations. Sensory nerve endings of various types are found in the papillae (Fig. 10.5A). Specialized receptors for cold (Krause end bulbs), touch (Meissner's corpuscles) and free nerve endings (pain receptors) are found. The organized mechanoreceptive corpuscles are present in the mucosae of gingiva, cheek, tongue and soft and hard palate. They are elongated or globular in shape, being located in the connective tissue papillae. The capsule is composed of several layers of cytoplasmic extensions of perineural cells. Numerous bundles of collagen fibers are noted at the periphery of the corpuscle. Free nerve endings are surrounded by a thin cytoplasm of Schwann cells. Some of the fibers enter the epithelium, where they terminate between the epithelial cells as free nerve endings (Fig. 10.5B). Nerve endings in the epithelium are often associated with Merkel cells.

The blood vessels are accompanied by nonmyelinated visceral nerve fibers that supply their smooth muscles. Other visceral fibers supply the glands.

In studying any mucous membrane, the following features should be considered: (1) type of covering

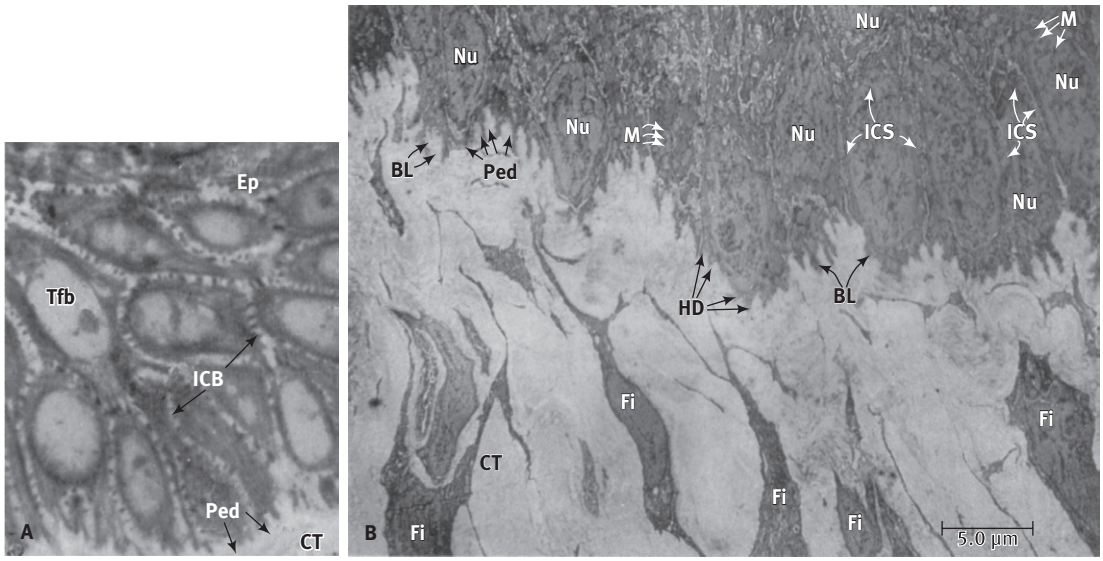


Figure 10.4 **(A)** Photomicrograph of human gingival epithelial cells, Ep. Pedicles, Ped, are present at base of basal cells and extend toward connective tissue, CT. Tonofibrils, Tfb, are evident both in cells and apparently coursing across intercellular bridges, ICB. **(B)** Electron micrograph of rat gingiva. Several basal cells with apparent pedicles, Ped, extending toward connective tissue, CT, but separated from it by basal lamina, BL, which is barely visible. Fibroblasts, Fi, may be noted within connective tissue. Epithelial cells contain prominent nucleus, Nu, and are demarcated from adjacent cells by lighter appearance of intercellular spaces, ICS. Small, round, light areas in epithelial cells are mitochondria, M. Pedicles, Ped, in this electron micrograph are of a much smaller dimension than larger undulations of basal cell surface outlined by arrows at HD. These, in turn, are smaller than ridges shown in Figure 10.4 A (A,× 1400) (From Stern IB: Periodontics 3:224, 1965).

Table 10.2 Contents of the Lamina Propria

1. Ground substance
2. Fibers
   - Collagen Fibers
   - Elastic Fibers
3. Cells
   - Fibroblasts
   - Mast cells
   - Macrophages
   - Inflammatory cells
4. Blood vessels and nerves

epithelium—the degree of keratinization and the thickness of epithelium, (2) structure of the lamina propria, its density and thickness, and the presence or lack of elasticity, (3) the form of junction between the epithelium and lamina propria, and (4) the membrane's fixation to the underlying structures, that is, the submucous layer. Considered as a separate and well-defined layer, submucosa may be present or absent. Looseness or density of its texture determines whether the mucous membrane is movably or immovably attached to the deeper layers. Presence or absence and location of adipose tissue or glands should also be noted (Table 10.3).

STRUCTURE OF THE ORAL EPITHELIUM

The epithelium of the oral mucous membrane is of the stratified squamous variety. It may be keratinized (orthokeratinized or parakeratinized) or nonkeratinized, depending on location. In humans the epithelial tissues of the gingiva and the hard palate (masticatory mucosa) are keratinized (Fig. 10.6A), although in many individuals the gingival epithelium is parakeratinized (Fig. 10.6C). The cheek, faucial, and sublingual tissues are normally nonkeratinized (Fig. 10.6B). Both the keratinized and the nonkeratinized epithelium consists of two groups of cells namely the keratinocytes and the nonkeratinocytes. These will be dealt in detail later in this chapter.

Cytokeratins

Cytokeratins (CK) form the cytoskeleton of all epithelial cells, along with microfilaments and microfibrils. Cytokeratins are seen not only within the cell but also in cell contact areas like desmosomes. They serve to provide mechanical linkages and distribute the forces over a wide area. They function as stress bearing structures within the epithelial cell and are important in maintaining cell shape. Cytokeratins are termed intermediate filaments as their diameter (7–11 nm) is intermediate between the larger microtubules (25 nm) and smaller microfilaments (4–6 nm). The molecular weights of cytokeratins however vary widely from 40 to 200 Kda than microtubules (55 Kda) and microfilaments (25 Kda).

About 20 types of cytokeratin are recognized. They can be classified into two main types: type I (basic cytokeratins) and type II (acidic cytokeratins). They have been assigned numbers: type I (1–8) and type II (9–20). Cytokeratins always occur in pairs of combination of type I with type II. In the absence of its pair they are unstable and are susceptible to degradation by proteases. Cytokeratin profile reflects both cell type and differentiation status in different types and different layers of epithelia. For example, the suprabasal layers of masticatory mucosa express CK1 and 10, whereas CK4 and 13 are expressed by suprabasal cells of lining mucosa. Among the nonkeratinized epithelium,

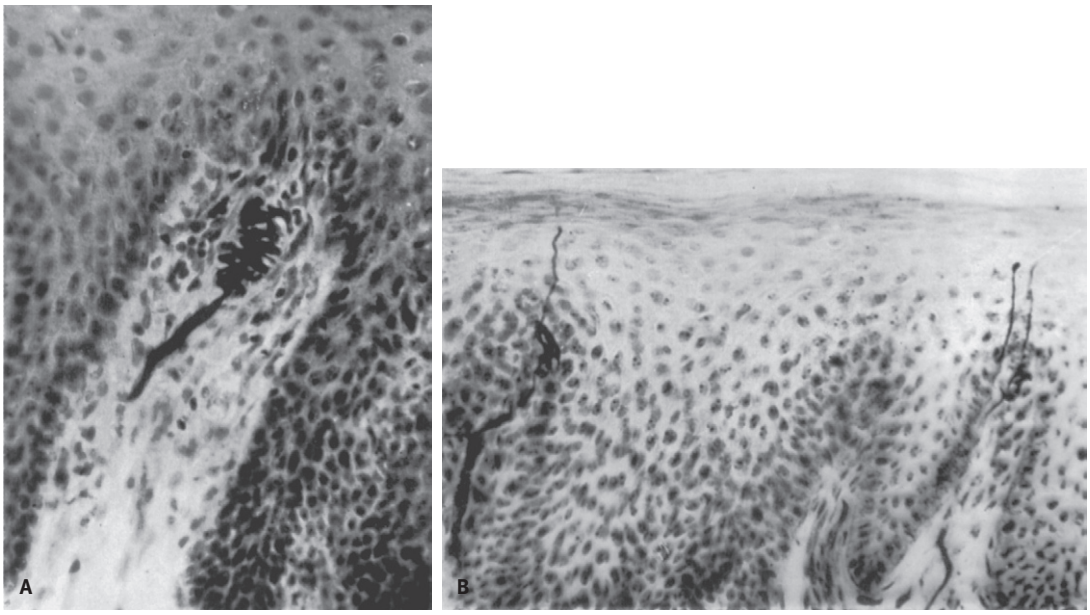


Figure 10.5 **(A)** Meissner tactile corpuscle in human gingiva (silver impregnation after Bielschowsky-Gros). **(B)** Intraepithelial 'ultraterminal' extensions and nerve endings in human gingiva (silver impregnation after Bielschowsky-Gros) (From Gairns FW and Aitchison JA: Dent Rec 70:180, 1950).

Table 10.3 Major Contents of Submucosa

1. Salivary glands
2. Blood vessels
3. Nerves
4. Adipose tissue

regional variations in cytokeratin expression have been observed; ventral surface of tongue express CK5, 6 and 14 but soft palate express CK7, 8 and 18.

Keratinized Epithelium

Keratinizing oral epithelium has the keratinocytes arranged in four cell layers: basal, spinous, granular, and

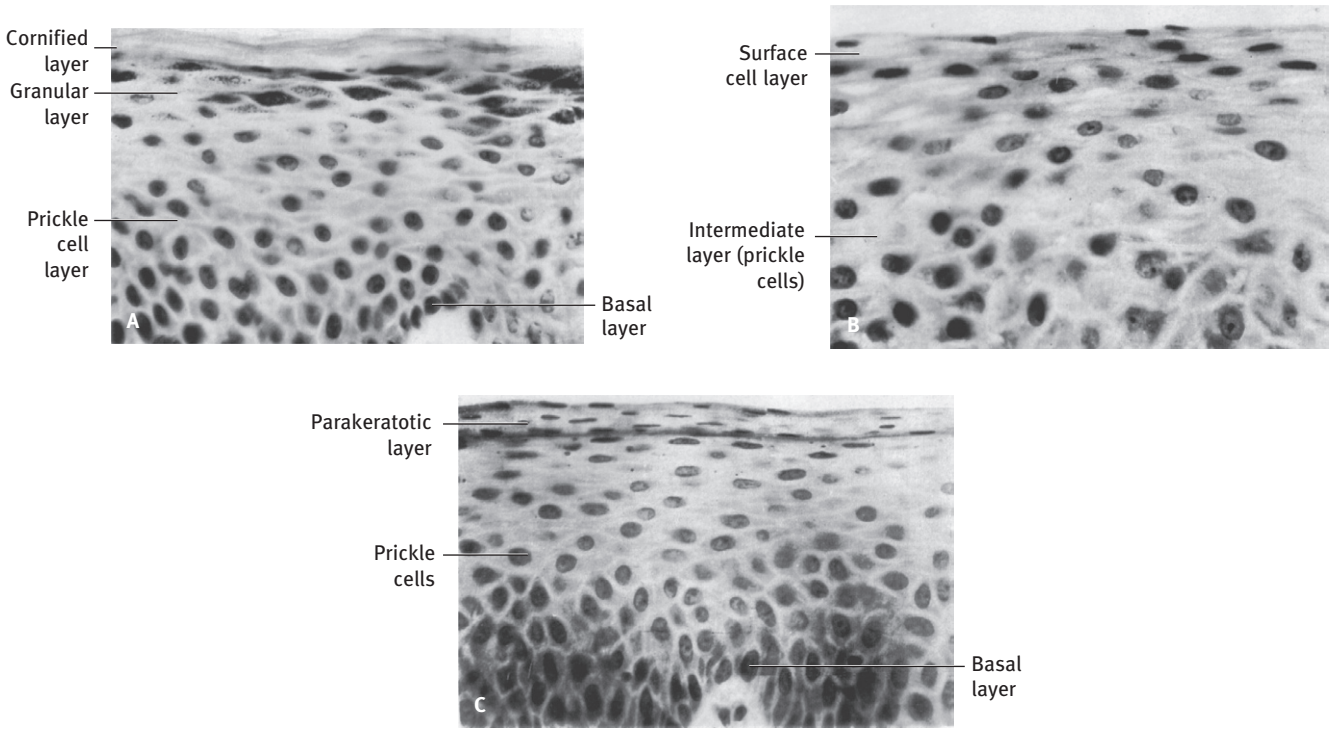


Figure 10.6 Variations of gingival epithelium. **(A)** Keratinized. **(B)** Nonkeratinized. **(C)** Parakeratinized.

cornified. These are also referred to in Latin as *stratum basale, stratum spinosum, stratum granulosum,* and *stratum corneum* (Fig. 10.7). These layer stake their names from their morphologic appearance. A single cell is, at different times, a part of each layer. After mitosis, it may remain in the basal layer and divide again or it may become determined, during which time it migrates and is pushed upward. During its migration as a keratinocyte it becomes committed to biochemical and morphologic changes. This is termed as *differentiation.* Differentiation ends with the formation of a keratinized squama, a dead cell filled with densely packed protein contained within a toughened cell membrane. After reaching the surface it is shed or cast off. This process of shedding of surface epithelial cells is called *desquamation.* The process of cell migration from the basal layer to the surface is called *maturation.* The time taken for a cell to divide and pass through the entire epithelium is termed *turnover time* or *turnover rate.* The turnover time in oral mucosa is faster than skin but slower than intestinal mucosa. Regional differences in the turnover time do exist within oral cavity. Turnover time of nonkeratinized oral epithelium is faster than keratinized oral epithelium.

Maturing cells produce growth inhibitors that restrict it from further cell division. There is evidence for EGF and TGF alpha in promoting cell proliferation and differentiation.

During migration cells increase in size and shape, more so in nonkeratinized epithelium than in keratinized epithelium. Increase in the synthesis of tonofilament occurs much more in keratinized epithelium. The tonofilaments are not only fewer in number but also not aggregated into bundles in the nonkeratinized epithelium.

This whole process from the onset of determination is called *keratinization.* A determined keratinocyte can no longer divide. It can only differentiate. For the tissue to remain in a steady state, undifferentiated cells must remain in the basal layer and form one differentiated cell for each cell that desquamates.

Stratum basale

The basal layer is made up of a single layer of cuboidal cells. The basal layer is made up of cells that synthesize DNA and undergo mitosis, thus providing new cells (Fig. 10.8). New cells are generated in the basal layer. However, some mitotic figures may be seen in spinous cells just beyond the basal layer. These cells have become determined as they leave the basal layer. The basal cells and the parabasal spinous cells are referred to as the *stratum germinativum* but only the basal cells can divide.

Basal cells show ribosomes and elements of rough-surfaced endoplasmic reticulum, indicative of protein-synthesizing activity. Basal cells synthesize some of the proteins of the basal lamina. They also synthesize proteins, which form the intermediate filaments of the basal cells.

It has been proposed that the basal cells are made up of two populations. One population is serrated and heavily packed with tonofilaments, which are adaptations for attachment, and the other is nonserrated and is composed of slowly cycling stem cells. The stem cells give rise to slowly dividing cells which serve to protect the genetic information to the tissue and a large number of amplifying cells which increase the number of cells for maturation.

The serrated basal cells are a single layer of cuboid or high cuboid cells that have protoplasmic processes (pedicles) projecting, from their basal surfaces toward the connective tissue (Fig. 10.4). Specialized structures called hemidesmosomes, which abut on the basal lamina

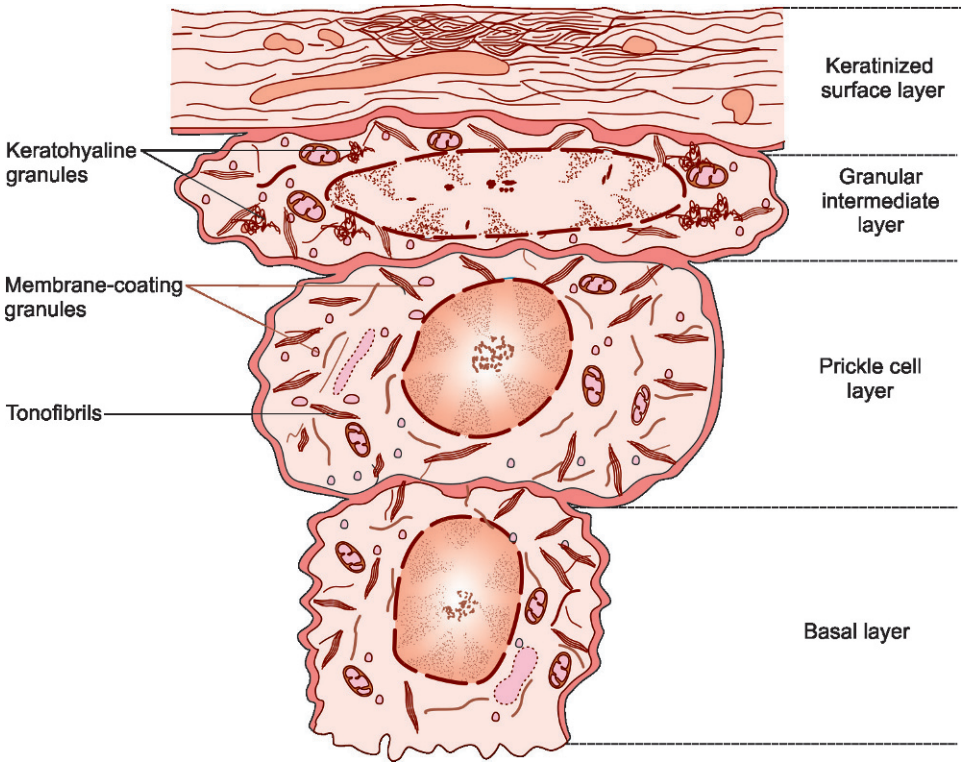


Figure 10.7 Diagram showing details of the different cell layers of the orthokeratinized epithelium.

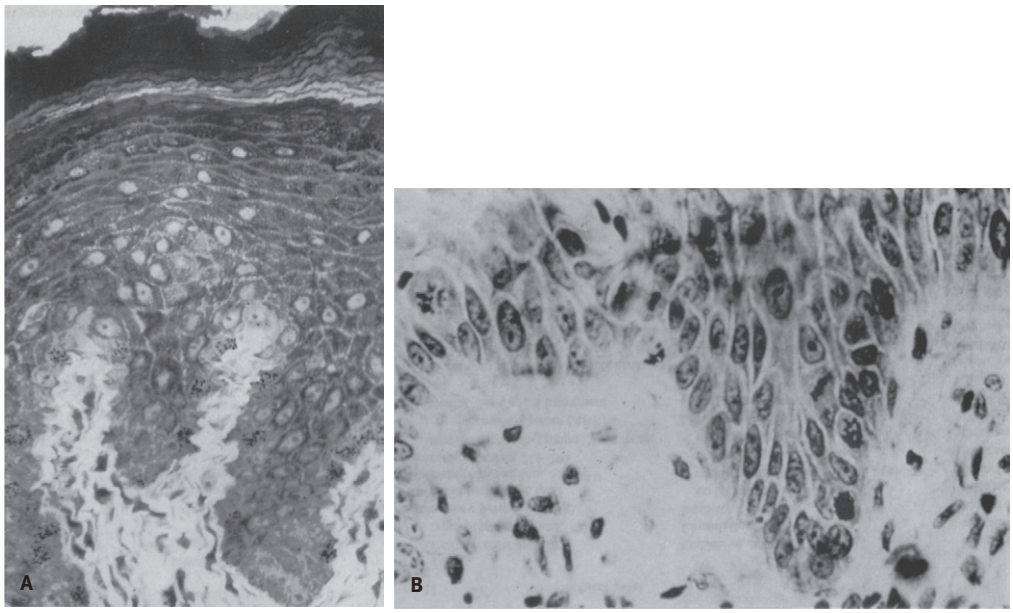


Figure 10.8 **(A)** Arrangement of labeling in oral epithelium 30 minutes after administration of tritiated thymidine. Grains are localized over nuclei in stratum basale. **(B)** Oral epithelium showing many mitotic figures (**A** from Anderson GS and Stern IB: Periodontics 4:115, 1966).

(Fig. 10.3), are found on the basal surface. They consist of a single attachment plaque, the adjacent plasma membrane, and an associated extracellular structure that appears to attach the epithelium to the connective tissue.

The lateral borders of adjacent basal cells are closely apposed and connected by desmosomes (Fig. 10.9B). These are specializations of the cell surface, consisting of adjacent cell membranes and a pair of denser regions (attachment plaques) as well as intervening extracellular structures (Fig. 10.10).

The basal cells contain tonofilaments, which course toward, and in some way are attached to the attachment plaques. Desmosomes consist of two principal types of proteins—the transmembranous proteins and proteins within the cell and related to the attachment plaque. The transmembrane proteins, the desmogleins and desmocollins, are members of the cadherin family. The desmosomal cadherins are linked to the keratin cytoskeleton via several cytoplasmic attachment plaque proteins, including desmoplakin, plakoglobin (gamma-catenin), plakophilins, envoplakin and periplakin. Desmosomal junctions (and hemidesmosomal junctions) which provide mechanical linkages are frequently seen amongst oral epithelial cells. Gap junctions are low resistance junctions and it allows electrical and chemical communication; are occasionally seen. Tight junctions are not observed amongst oral epithelial cells.

Stratum spinosum

The spinous cells which make up this layer are irregularly polyhedral and larger than the basal cells. On the basis of light microscope, it appears that the cells are joined by 'intercellular bridges' (Fig. 10.9A). Tonofibrils seem to course from cell to cell across these bridges. Electron microscopic studies have shown that the 'intercellular bridges' are desmosomes and the tonofibrils are bundles of tonofilaments (Fig. 10.11). The tonofilament-sturn or loop adjacent to the attachment plaques do not cross over into adjacent cells. It is suspected that an agglutinating material joins them to the attachment plaques. The desmosome attachment plaques contain the polypeptides desmoplakin and plakoglobin. Monoclonal antibodies to these polypeptides can be used to detect carcinomas (an epithelial tumor) by immunofluorescent microscope. The intercellular spaces contain glycoprotein, glycosaminoglycans, and fibronectin.

The tonofilament network and the desmosomes appear to make up a tensile supporting system for the epithelium. The percentage of cell membrane occupied by desmosomes is higher in gingiva and palate than in alveolar mucosa, buccal mucosa, and tongue. The intercellular spaces of the spinous cells in keratinizing epithelia are large or distended; thus the desmosomes are made more prominent, and these cells are given a prickly appearance. The spiny appearance of the spinous layer is due to the shrinkage of cells during tissue preparation causing them to separate at points where desmosomes do not anchor them together.

The spinous (prickle) cells resemble a cocklebur or sticker that has each spine ending at a desmosome. of the four layers, the spinous cells are the most active in protein synthesis. These cells synthesize additional proteins that differ from those made in the basal cells. This change indicates their biochemical commitment to keratinization. In terms of number and length the desmosomes of the spinous layer occupy more of the membrane in the tongue, gingiva, and palate than in either alveolar or buccal mucosa.

Stratum granulosum

This layer contains flatter and wider cells. These cells are larger than the spinous cells. This layer is named for the basophilic keratohyalin granules (blue staining with hematoxylin and eosin) (Figs 10.12A to C) that it contains. The nuclei show signs of degeneration and pyknosis. This layer still synthesizes protein, but reports of synthesis rates

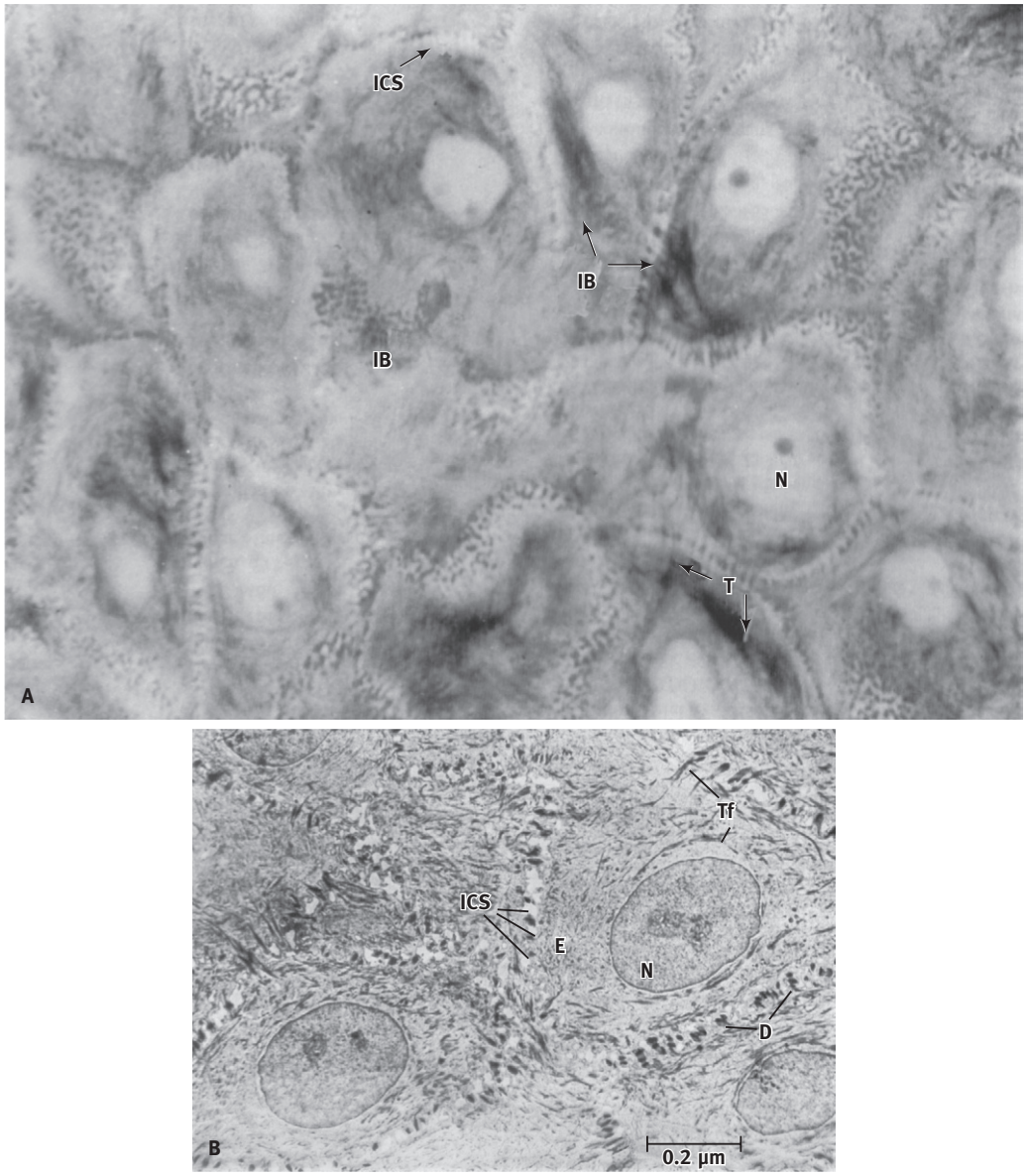


Figure 10.9 **(A)** High magnification light micrograph showing epithelial cells with nuclei, N; intercellular spaces, ICS; tonofibrils, T; and intercellular bridges, IB. Speckled areas are intercellular bridges (desmosomes) cut tangentially or 'en face.' **(B)** Electron micrograph of prickle cells of human gingiva. Portions of epithelial cells, E, are evident, separated by intercellular space, ICS. Several nuclei, N, are evident. Tonofilaments, Tf, are present in cytoplasm and extend toward desmosomes, D, located at periphery of cells (**B** from Grant DA, Stern IB, and Listgarten MA: Periodontics in the tradition of Orban and Gottlieb, ed 6, St Louis, 1988, The CV Mosby Co).

at this level differ. However, as the cell approaches the stratum corneum, the rate diminishes. Tonofilaments are more dense in quantity and are often seen associated with keratohyalin granules (Figs 10.12D to F). Sometimes dense networks of tonofilaments and keratohyalin granules are evident. Epidermal and oral keratinocytes express additional differentiation markers, including filaggrin and trichohyalin, that associate with the keratin cytoskeleton during terminal differentiation. Calcium and retinoids influence epithelial differentiation by altering the transcription of target genes and by regulating activity of enzymes critical in epithelial differentiation, such as transglutaminases, proteinases, and protein kinases.

In the stratum granulosum the cell surfaces become more regular and more closely applied to adjacent cell surfaces. At the same time the lamellar granule, a small organelle (also known as *keratinosome, Odland body* or membrane-coating granule) forms in the upper spinous and granular cell layers. The membrane coating granules are glycolipids. It has an internal lamellated structure (Figs 10.13A and B). Lamellar granules discharge their contents into the intercellular space forming an intercellular lamellar material, which contributes to the permeability barrier (Fig. 10.13C). This barrier forms at the junction of granular and cornified cell layers. The intercellular space of this region has a lamellar structure similar to that of the lamellar granule (Fig. 10.13C) and contains glycolipid. At approximately the same time during differentiation, the inner unit of the cell membrane thickens, forming the 'cornified cell envelope.'

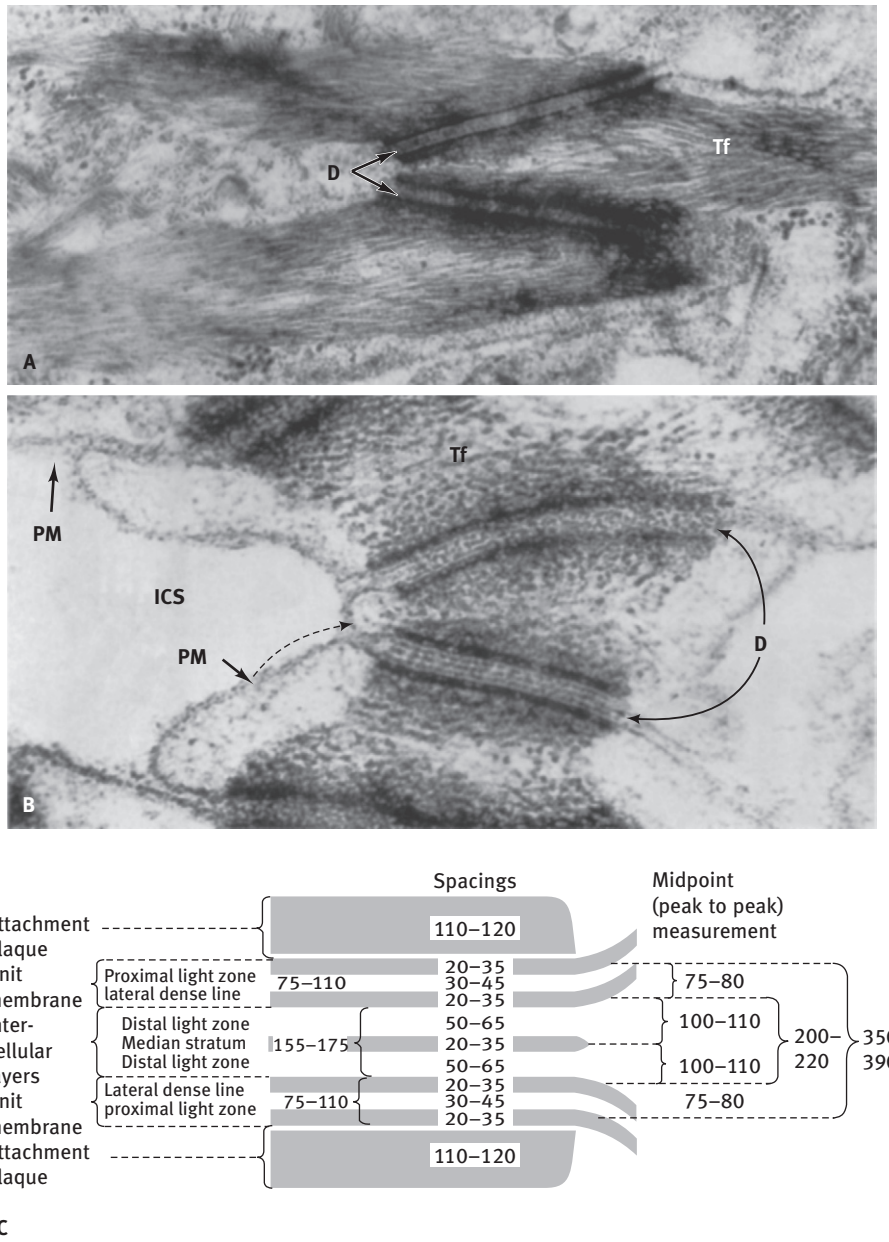


Figure 10.10 **(A)** Tonofilaments, Tf, extending to series of desmosomes, D. Tonofilaments are sectioned in long axis (human gingiva). **(B)** Higher magnification of two desmosomes, D, showing substructure. Tonofilaments are cross-sectioned. Intercellular space, ICS, is bounded by adjacent cell membranes, PM, whose unit membrane is clearly evident (dashed arrow). Unit membranes form part of substructure of desmosome. **(C)** Diagrammatic cross-sectioned representation of desmosome and dimensions (in Ångström units) of various components (From Stern IB: Periodontics 3:224, 1965).

Several proteins contribute to this structure, of which involucrin is important. Involucrin and loricrin become crosslinked by enzyme transglutaminase to form a thin (10 nm) highly resistant electron dense cornified envelope just beneath the plasma membrane. The orderly crosslinking of tonofilaments starts first with the crosslinking of involucrin, periplakin and envoplakin which form a scaffold on which other proteins like loricrin and small proline rich protein (SPRR) are added. Influx of calcium and cell death are said to be the causes for this formation. Thereafter the thickened membrane contains sulfur-rich proteins stabilized by covalent crosslinks. It forms a highly resistant structure. All the genes involved in the expression of the proteins of the cornified envelope are located in the chromosome Iq21 region and are known as epidermal differential complex.

In nonkeratinizing oral epithelium a small organelle similar to the lamellar granule forms. The granules differ in appearance from keratinized and nonkeratinized epithelium; in being elongated and lamellar in keratinized and circular and amorphous in nonkeratinized epithelium.

Stratum corneum

The stratum corneum is made up of keratinized squamae, which are larger and flatter than the granular cells. Thickness of stratum corneum varies at different sites in the oral cavity and is thicker than most areas of the skin. Here all of the

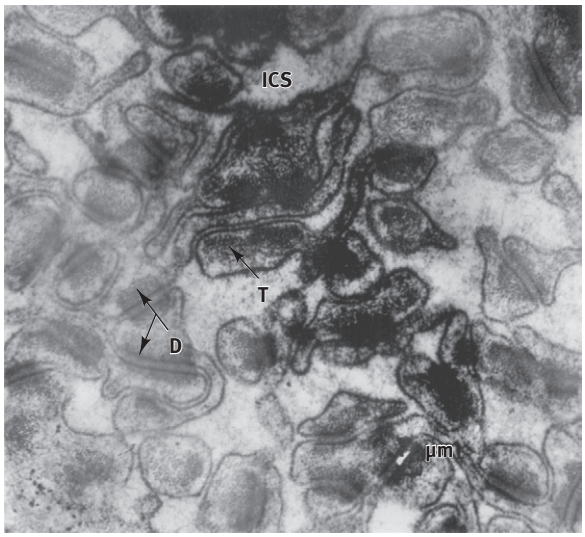


Figure 10.11 Electron micrograph of prickle cell layer of human gingival epithelium showing intercellular bridges and tonofibrils. Here desmosomes are cut tangentially or 'en face' as shown in light micrograph (Figure 10.9, A). Note relatively close adaptation of cell processes ending in desmosomes, D. These processes contain tonofilaments, T, cut on end, which appear as fine dots. Relatively large intercellular space, ICS, contains cell-coating material.

nuclei and other organelles such as ribosomes and mitochondria have disappeared (Fig. 10.12D). The layer is acidophilic (red staining with hematoxylin and eosin) and is histologically amorphous. The keratohyalin granules have disappeared. Ultrastructurally the cells of the cornified layer are composed of densely packed filaments developed from the tonofilaments, altered, and coated by the basic protein of the keratohyalin granule, filaggrin.

The cells of the stratum corneum are densely packed with filaments in this nonfibrous interfilamentous matrix protein, filaggrin (named for its function in filament aggregation). Crosslinking of tonofilaments by disulfide bonds facilitates close packing of the filaments and gives mechanical and chemical resistance to this layer. When the purified solubilized matrix protein obtained from the epithelium is combined with solubilized keratin filaments in vitro, aggregates of matrix and highly oriented filaments form instantaneously. Their ultrastructural appearance is similar to that of the contents of the stratum corneum. The active matrix protein, filaggrin, is derived from a precursor in the keratohyalin granules. Studies of the interaction of matrix and filaments have been performed with filaggrin and keratin filaments obtained from epidermis; however, the same proteins can also be demonstrated in keratinizing oral epithelium. The keratinized cell becomes compact and dehydrated and covers a greater surface area than does the basal cell from which it developed. It does not synthesize protein. It is closely applied to adjacent squamae. The cell surface and desmosomes are altered, and the plasma membrane is denser and thicker than in the cells of deeper layers.

In orthokeratinization, keratinized squamae form as has been described. In parakeratinization, the cells retain pyknotic and condensed nuclei and other partially lysed cell organelles until they desquamate.

Keratinocytes and Nonkeratinocytes

Keratinocytes

These are epidermal/epithelial cell that synthesizes keratin and its characteristic intermediate filament protein is cytokeratin. The above descriptions of cells are those of keratinocytes. As described earlier, they show cell division, undergo, maturation and finally desquamate. Keratinocytes increase in volume in each successive layer from basal to superficial. The cells of each successive layer cover a larger area than do the cells of the layers immediately below.

Nonkeratinocytes

The epithelium contains a smaller population of cells that do not possess cytokeratin filaments; hence they do not have the ability to keratinize. These groups of cells are termed nonkeratinocytes. Unlike keratinocytes, nonkeratinocytes do not show mitotic activity, undergo maturative changes or desquamate. They are not arranged in layers and do not form desmosomal attachments with adjacent keratinocytes. They are usually dendritic and appear unstained or clear in the routine hematoxylin and eosin stains. They are identified by special stains or by immunocytochemical methods. These cells migrate to the oral epithelium from neural crest or from bone marrow. Melanocytes, Langerhans cells and Merkel cells are the nonkeratinocytes found in the oral epithelium. Inflammatory cells often seen especially in certain regions, like lymphocytes, are also considered as nonkeratinocytes.

Melanocytes

Melanin is elaborated by specific cells, melanocytes, residing in the basal layer. The melanocytes are derived from the embryologic neural crest and migrate into the epithelium (Fig. 10.14). Each melanocyte establishes contact with about 30–40 keratinocytes through their dendritic processes. Melanin produced by the melanocytes are transferred through their dendritic process to the adjacent basal cell keratinocytes which store the pigment in the form of melanosomes. Oral pigmentation can be studied by use of either the dopa reaction or silver-staining techniques. In the dopa reaction the cells containing tyrosinase enzyme appear dark. Therefore the melanin-producing cells, which contain tyrosinase (dopa oxidase), are demonstrated. Silver stains also dye the melanin pigment. They are also stained by Mason- Fontana stain. Melanin pigment dispersed in the connective tissue will be phagocytosed by the macrophages. These macrophages termed melanophages, also stain positively for dopa. Melanocytes appear as clear cells in hematoxylin sections. Silver stains reveal a spider like (dendritic) appearance. Thus melanocytes are referred to as clear cells or dendritic cells.

Keratinocytes release mediators essential for normal melanocyte function. Melanocytes vary in number in different regions but variation in the degree of pigmentation (gingivae is highly pigmented), is not related to the numbers but is due to their activity, i.e. number, size and dispersal of melanosomes, the quantity of melanin within the melanosomes and the rate of degradation of the pigment.

Langerhans cell

The Langerhans cell is another clear cell or dendritic cell found in the upper layers of the skin and the mucosal

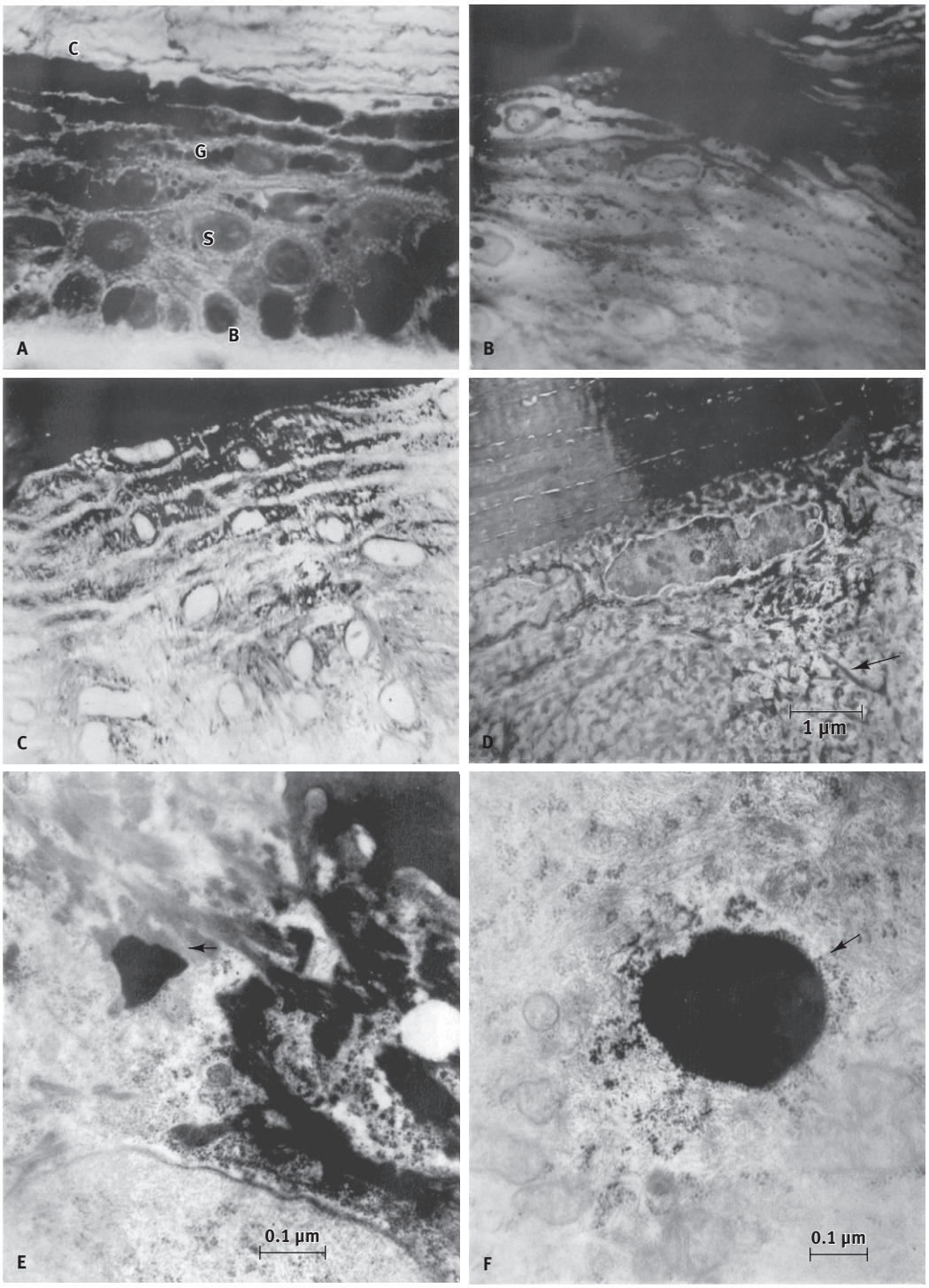


Figure 10.12 **(A)** Light micrograph of newborn rat skin showing basal cells, B, spinous cells, S, granular cells with numerous dense granules, G, and C, cornified (keratinized) components. **(B)** and **(C)** Keratohyalin is formed as discrete spherical granules in some tissues or is formed as angular amorphous material in other tissues. **(D)** and **(E)** Angular form is associated with tonofilaments primarily (arrow); **(F)** whereas spherical form is surrounded by ribosomes (arrow) and may contain more than one material (small arrows).

epithelium. There is a correlation in the occurrence of stratum granulosum and Langerhans cells. The cell has a convoluted nucleus and characteristic rod-like granules in the cytoplasm, termed Birbeck granules. This cell is free of melanin and does not give a dopa reaction. It stains with gold chloride, ATPase, and immunofluorescent markers. The Langerhans cell is a cell of hematopoietic origin. Langerhans cells penetrate the epithelium from lamina propria. It has vimentin-type intermediate filaments. Langerhans cells are involved in the immune response. In the presence of antigenic challenge by bacterial plaque Langerhans cells migrate into the gingiva. They also migrate into the epithelium in response to chemotactic factors released by the keratinocytes to the surface receptors of Langerhans cells. They contain la antigens, which they present to primed T cells (thymocytes). They may function, as do macrophages,

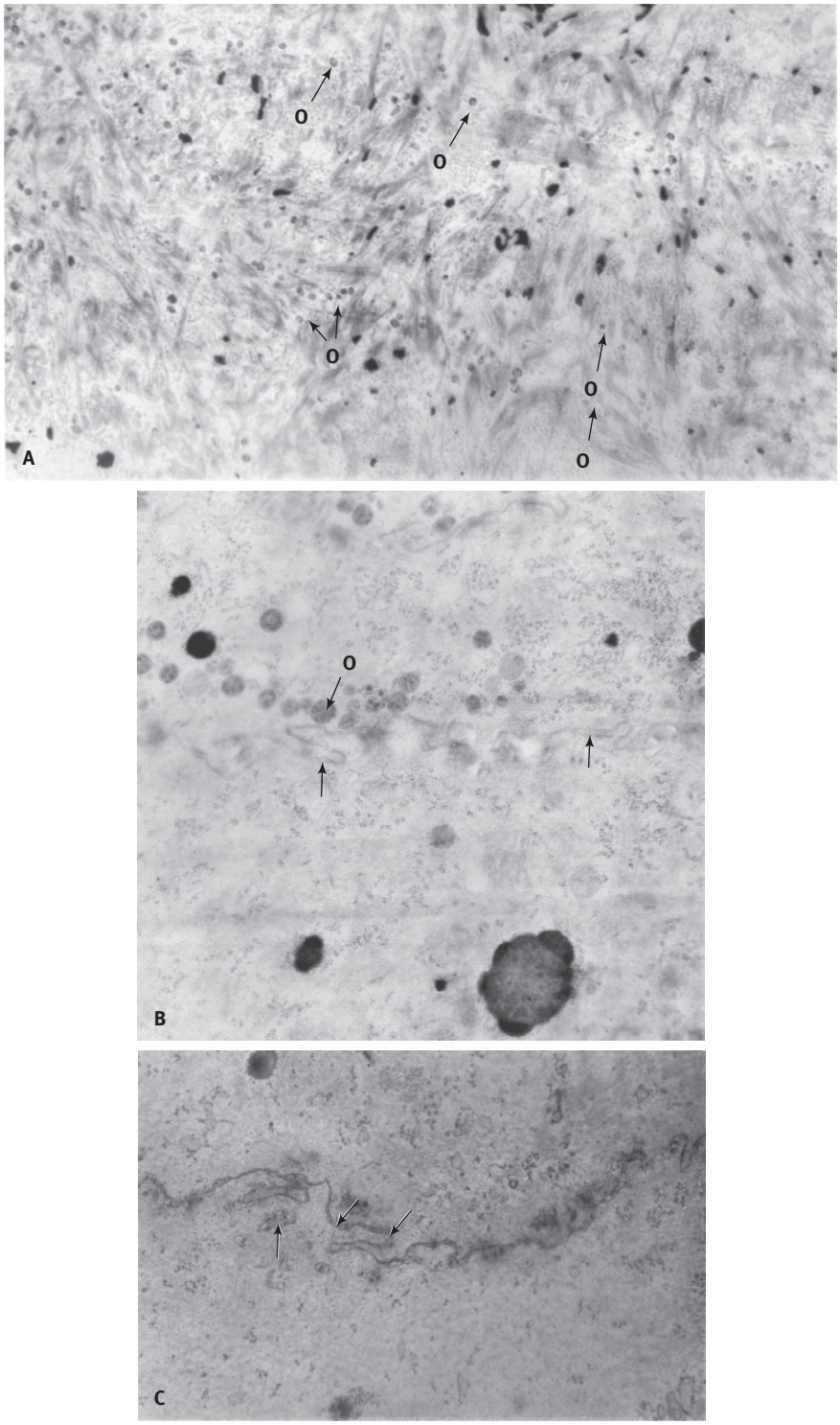


Figure 10.13 **(A)** Lamellar granules, O, are found close to cell membrane (arrows) and desmosomes in granular cells. **(B)** Lamellar granules, O, lying close to plasma membrane (arrows) and in cells containing ribosome-associated keratohyalin granules. Note that some of keratohyalin granules have two densities and perhaps two components. Lamellar granules contain an internal lamellar structure. **(C)** Lamellar structure in intercellular space (arrows). It is presumed that these lamellae are derived from lamellar granules that are no longer present.

by picking up antigen and presenting it to lymphocytes, either locally or at lymph nodes. Langerhans cells with HLA-DR and T6 antigens also interact with lymphocytes but do so differently from keratinocytes. Langerhans cells present the antigen to specific helper T cells. The interleukin-1 secreted by the keratinocytes induces the T cells to produce interleukin-2, which binds to responsive T cells, causing them to proliferate. Another factor with common biochemical and biophysical properties to interleukin-1 is epidermal cell-derived thyrocyte-activating factor (ETAF), which apparently is produced by a subset of keratinocytes. It plays important role in contact hypersensitivity, in anti-tumor immunity and in graft rejection. They shuttle between epithelium and regional lymph nodes.

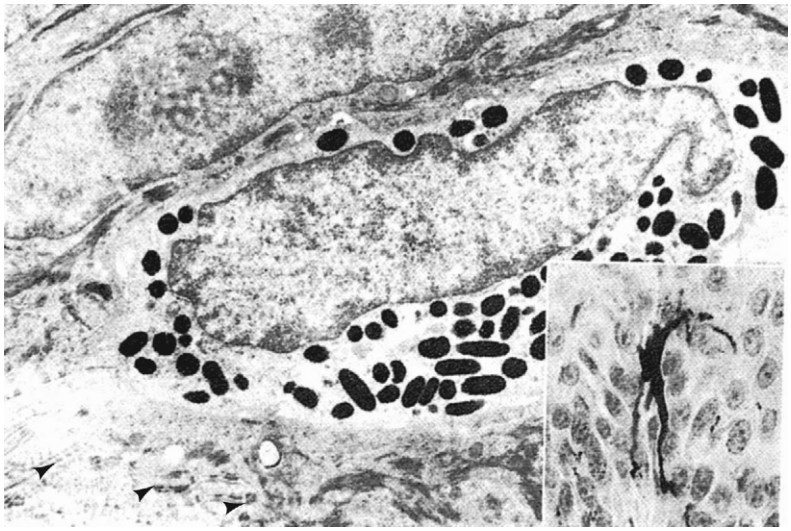


Figure 10.14 Ultrastructure of melanocyte. Note. numerous melanosomes. Arrowheads indicate basal lamina. Inset: Dendritic appearance of melanocyte in light microscope.

The importance of these observations is that the skin and presumably the oral mucosa have an epithelial immunologic function, and through this function the epithelium of the skin and oral mucosa interacts with the entire lymphoid system in concert with the Langerhans cells to help mount an immune response.

Merkel cells

Merkel cells are found among the basal cells. It has nerve tissue immediately subjacent and is presumed to be a specialized neural pressure-sensitive receptor cell. It responds to touch sensation. They are commonly seen in masticatory mucosa, but are usually absent in lining mucosa. Merkel cells differ from other nonkeratinocyte in that they are not dendritic. Ultrastructurally, the nucleus shows a deep invagination and characteristic rodlet. They contain numerous characteristic electron-dense granules that are located almost exclusively at the side of cytoplasm in contact with axon terminals. Intermediate-type junctions are noted between axon terminals and Merkel cells. The function of these granules are not known. Merkel cells migrate from the neural crest. They are stained by PAS stain.

Other cells, such as lymphocytes and polymorphonuclear leukocytes, are also found at various levels of the epithelium. These cells are transient and can pass through the epithelium to the surface.

Keratinocytes and lymphocytes interact. Keratinocytes can activate lymphocytes through the production of interleukin-1, but they may also inhibit lymphocyte proliferation. Stimulated lymphocytes produce gamma-interferon, which can stimulate keratinocytes to express HLA-DR antigen (Table 10.4).

Table 10.4 Nonkeratinocytes and its Functions

Cells	Location	Function
1. Melanocytes	Basal layer	Melanin synthesis
2. Langerhans cells	Upper layer	Involved in immune response
3. Merkel cells	Basal cells	Specialized neural Receptor sensitive cells
4. Inflammatory cells like lymphocytes & neutrophils	Transits and passes through the epithelium to the surface	Defense

Nonkeratinized Epithelium

Nonkeratinizing epithelia differ from keratinizing epithelia primarily because they do not produce a cornified surface layer, but there are other differences as well. The layers in nonkeratinizing epithelium are referred to as basal, intermediate, and superficial (stratum basale, stratum intermedium, stratum superficiale) (Figs 10.15, 10.6B). Thus there are only three layers in the nonkeratinized epithelium. The basal cells of both types are similar. The cells of the stratum intermedium are larger than cells of the stratum spinosum. The intercellular space is not obvious or distended and hence the cells do not have a prickly appearance. These cells do contain some intermediate keratin filaments, but they differ biochemically from those in keratinizing epithelia and are sparsely distributed within the cells. The cells of the stratum intermedium are attached by desmosomes and other junctions, and their cell surfaces are more closely applied than are spinous cells. There is no stratum granulosum (although incomplete or vestigial granules may form), nor is there a stratum corneum. Stratum superficiale contains nucleated cells. They contain less number of tonofilaments and lack keratohyaline granules (Fig. 10.16). These cells ultimately desquamate, as do the cornified squamae. In general, nonkeratinizing oral mucosa have higher rates of mitoses than do the keratinizing oral mucosa.

Tissues that are not keratinized at one stage of development may keratinize at another (Fig. 10.13). Similarly, tissues may be modulated from keratinized-parakeratinized and nonkeratinized variants in pathologic states. Although the terms 'keratinized' and 'parakeratinized' may be used interchangeably with the terms 'parakeratosis' and 'keratosis,' the former terms refer to physiologic and the latter terms refer to pathologic stages. When keratinization occurs in a normally nonkeratinized tissue, it is referred to as keratosis. When normally keratinizing tissue such as the epidermis becomes parakeratinized, it is referred to as parakeratosis (Table 10.5).

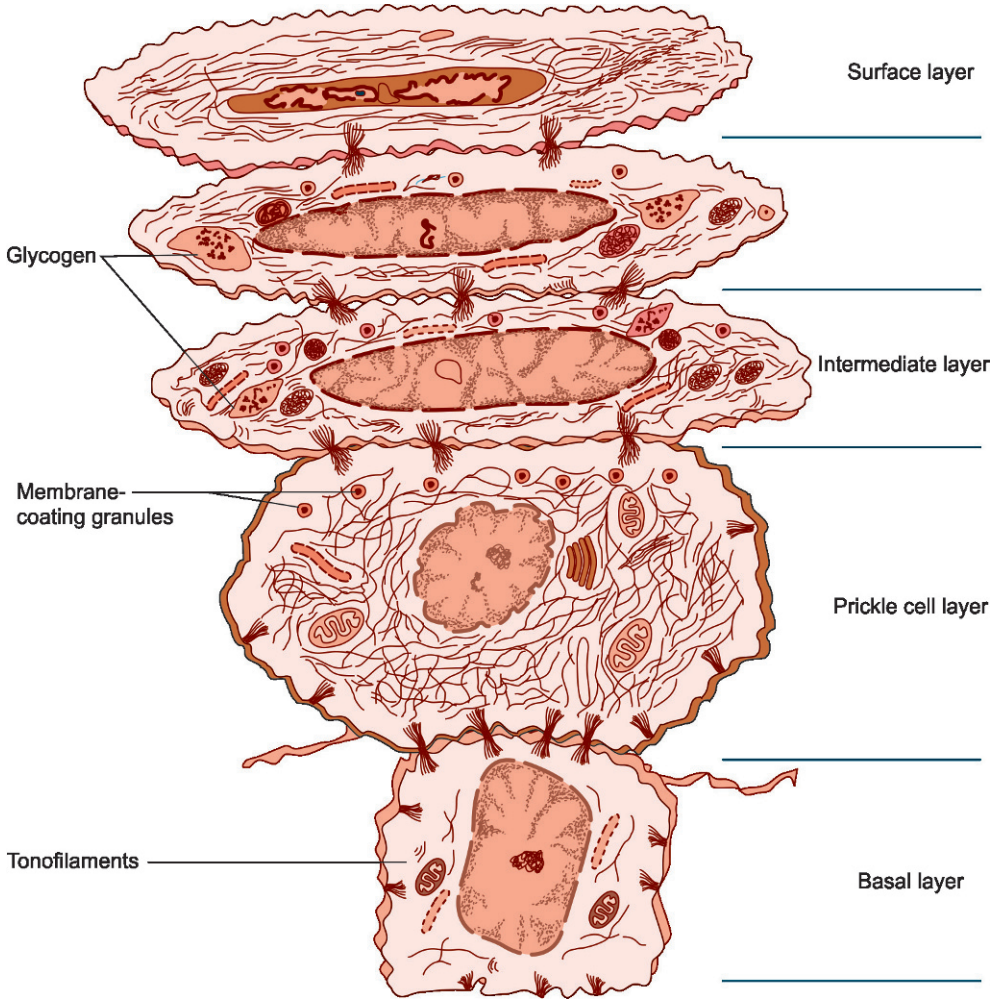


Figure 10.15 Diagram showing details of the different cell layers of the nonkeratinized epithelium.

Table 10.5 Keratinized and Nonkeratinized Mucosal Regions

Areas with Keratinized Mucosa
Masticatory mucosa
1. Gingiva
2. Hard palate
Specialized mucosa
3. Dorsum of tongue
Vermilion zone
Areas with Nonkeratinized Mucosa
Lining mucosa
1. Labial and buccal mucosa
2. Soft palate
3. Alveolar mucosa and vestibular fornix
4. Mucosa of ventral aspect of the tongue
5. Mucosa of the floor of the mouth

SUBDIVISIONS OF ORAL MUCOSA

For descriptive purposes the oral mucosa may be divided into the following areas:

Keratinized areas
  Masticatory mucosa
  Vermilion border of lip
Nonkeratinized areas
  Lining mucosa
Specialized mucosa

Keratinized Areas

Masticatory mucosa (gingiva and hard palate)

The masticatory mucosa is keratinized and is made up of the gingiva and the hard palate. They have similarities in thickness and keratinization of epithelium; in thickness, density, and firmness of lamina propria; and in being immovably attached. However, there are differences in their submucosa.

Hard palate

The mucous membrane of the hard palate is tightly fixed to the underlying periosteum and therefore immovable. Like the gingiva it is pink. The epithelium is uniform in form with a rather well-keratinized surface. The cells of the stratum corneum exhibit stacking, and in the rat there are complementary grooves and ridges between the apposing surfaces of the cells. The pedicles, the increase in number and length of desmosomes, the density of the tonofilaments, and the complementary grooves and ridges all appear to be adaptations of keratinizing epithelium to resist forces and to bind the epithelium to the connective tissue. The lamina propria, a layer of

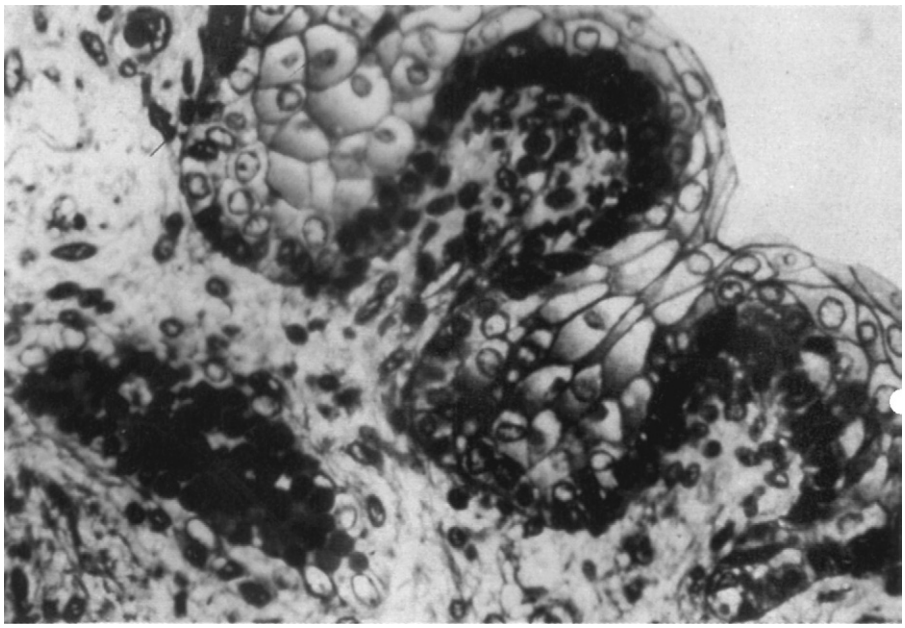


Figure 10.16 Section of human fetal tongue showing three cell strata of nonkeratinized epithelium.

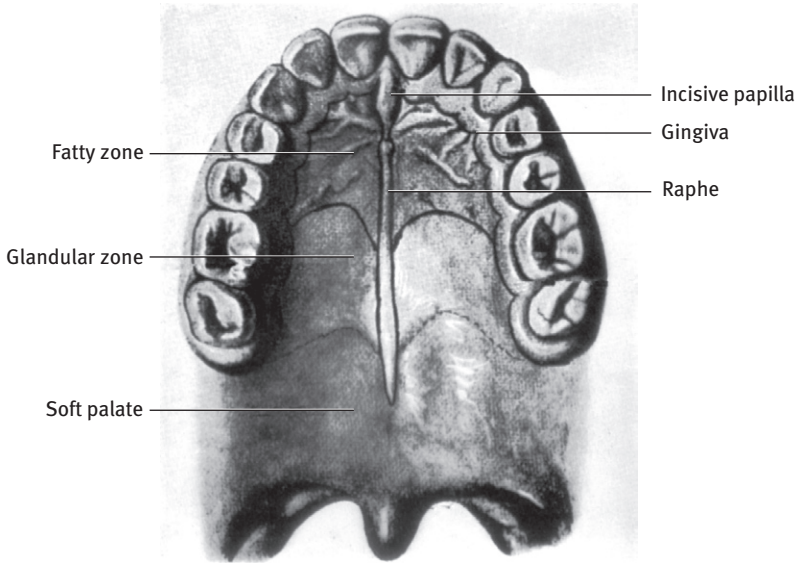


Figure 10.17 Surface view of hard and soft palates. The different zones of palatine mucosa are outlined.

dense connective tissue, is thicker in the anterior than in the posterior parts of the palate and has numerous long papillae. Various regions in the hard palate differ because of the varying structure of the submucous layer. The following zones can be distinguished (Fig. 10.17):

1. Gingival region, adjacent to the teeth
2. Palatine raphe, also known as the median area, extending from the incisive or palatine papilla posteriorly
3. Anterolateral area or fatty zone between the raphe and gingiva
4. Posterolateral area or glandular zone between the raphe and gingiva.

Except for narrow and specific zones, the palate has a distinct submucous layer. The zones that do not have a submucous layer occur peripherally where the palatine tissue is identical with the gingiva and along the midline for the entire length of the hard palate—the palatine raphe (Fig. 10.17). The marginal area shows the same structure as the other regions of the gingiva. Only the lamina propria and periosteum are present below the epithelium (Fig. 10.18). Similarly, a submucosa is not found below the palatine raphe or median area (Fig. 10.19). The lamina propria blends with the periosteum.

The submucous layer occurs in wide regions extending between the palatine gingiva and palatine raphe. Despite this extensive submucosa, the mucous membrane is immovably attached to the periosteum of the maxillary and palatine bones. This attachment is formed by dense bands and trabeculae of fibrous connective tissue that join the lamina propria of the mucous membrane to the periosteum. The submucous space is thus subdivided into

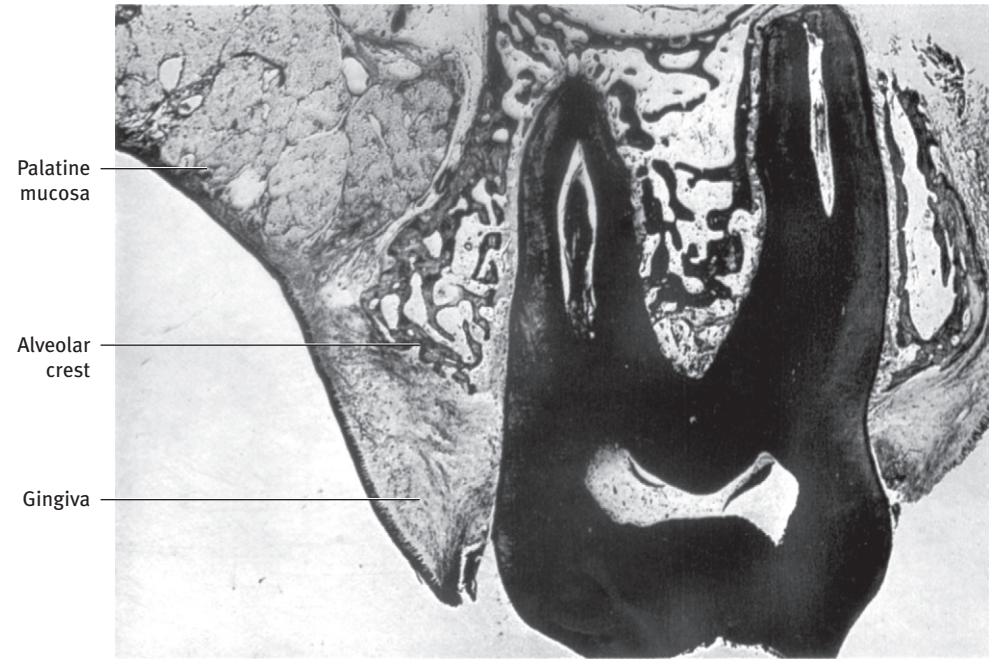


Figure 10.18 Structural differences between gingiva and palatine mucosa. Region of first molar.

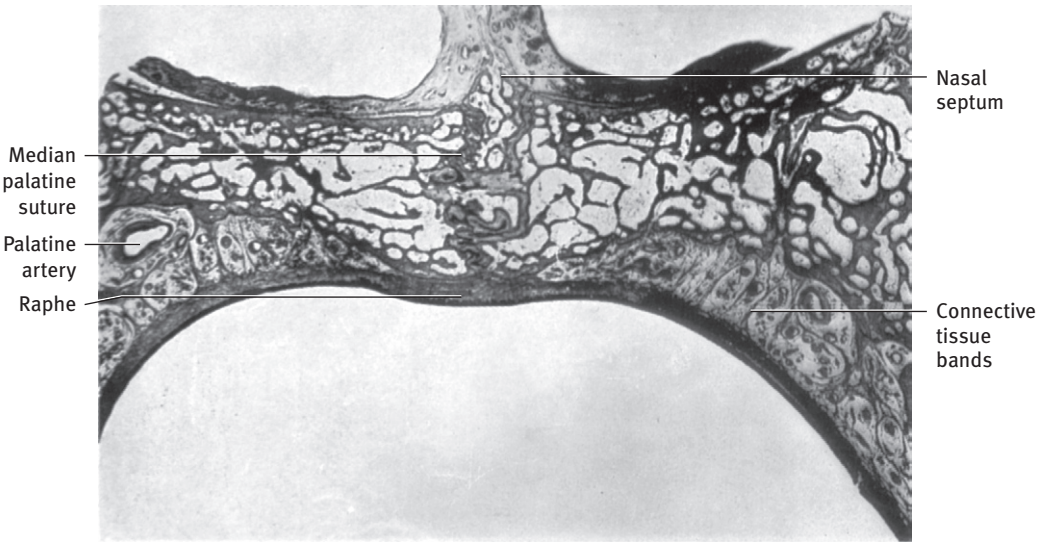


Figure 10.19 Transverse section through hard palate. Palatine raphe. Fibrous bands connecting mucosa and periosteum in lateral areas. Palatine vessels (From Pendleton EC: J Am Dent Assoc 21:488, 1934).

irregular intercommunicating compartments of various sizes. These are filled with adipose tissue in the anterior part and with glands in the posterior part of the hard palate. The presence of fat or glands in the submucous layer acting as a cushion is comparable to the subcutaneous tissue of the palm of the hand and the sole of the foot.

When the submucosa of hard palate and that of gingiva are compared, there are pronounced differences. The dense connective tissue that makes up the lamina propria of gingiva is bound to the periosteum of the alveolar process or to the cervical region of the tooth. A submucous layer, as such, cannot generally be recognized. In the lateral areas of the hard palate (Fig. 10.19), in both fatty and glandular zones, the lamina propria is fixed to the periosteum by bands of dense fibrous connective tissue. These bands are arranged at right angles to the surface and divide the submucous layer into irregularly shaped spaces. The distance between lamina propria and periosteum is smaller in the anterior than in the posterior parts. In the anterior zone the connective tissue contains fat (Fig. 10.20), whereas in the posterior part it contains mucous glands (Fig. 10.18). The glandular layers of the hard palate and of the soft palate are continuous.

At the junction of the alveolar process and the horizontal plate of the hard palate the anterior palatine vessels and nerves course, surrounded by loose connective tissue. This wedge-shaped area (Fig. 10.21) is large in the posterior part of the palate and smaller in the anterior part. It is important for oral surgeons and periodontists to know the distribution of these vessels.

Incisive papilla. The oral incisive (palatine) papilla is formed of dense connective tissue. It contains the oral parts of the vestigial nasopalatine ducts. They are blind ducts of varying length lined by simple or pseudostratified

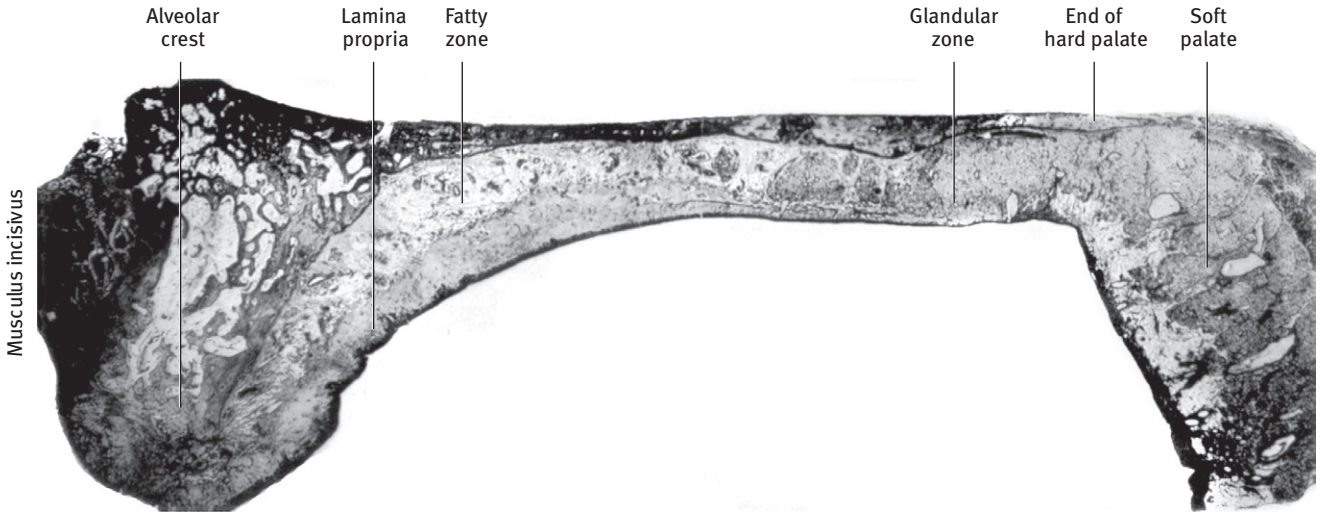


Figure 10.20 Longitudinal section through hard and soft palates lateral to midline. Fatty and glandular zones of hard palate.

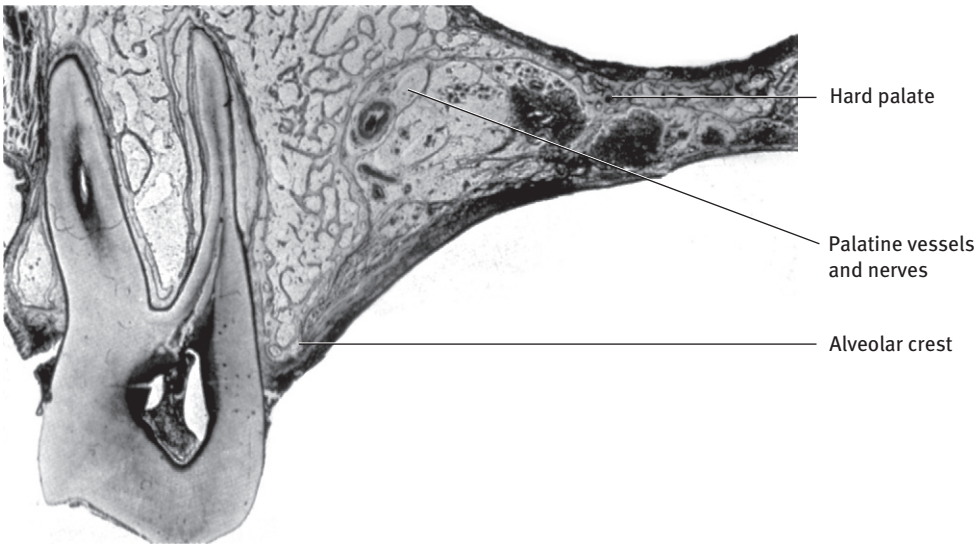


Figure 10.21 Transverse section through posterior part of hard palate, region of second molar. Loose connective tissue in groove between alveolar process and hard palate around palatine vessels and nerves.

columnar epithelium, rich in goblet cells. Small mucous glands open into the lumen of the ducts. These ducts sometimes become cystic in humans. Frequently the ducts are surrounded by small, irregular islands of hyaline cartilage, which are the vestigial extensions of the paraseptal cartilages. In most mammals the nasopalatine ducts are patent and, together with Jacobson's organ, are considered as auxiliary olfactory sense organs. Jacobson's organ (the vomeronasal organ) is a small ellipsoid (cigar-shaped) structure lined with olfactory epithelium that extends from the nose to the oral cavity. In humans, Jacobson's organ is apparent in the twelfth to fifteenth fetal week, after which it undergoes involution. In humans, cartilage is sometimes found in the anterior parts of the papilla. In this location it bears no relation to the nasopalatine ducts (Fig. 10.22).

Palatine rugae (transverse palatine ridges). The palatine rugae, irregular and often asymmetric in humans, are ridges of mucous membrane extending laterally from the incisive papilla and the anterior part of the raphe. Their core is made of a dense connective tissue layer with fine interwoven fibers.

Epithelial pearls. In the midline, especially in the region of the incisive papilla, epithelial pearls may be found in the lamina propria. They consist of concentrically arranged epithelial cells that are frequently keratinized. They are remnants of the epithelium formed in the line of fusion between the palatine processes (see Chapter 2).

Gingiva

The gingiva extends from the dentogingival junction to the alveolar mucosa. It is subject to the friction and pressure of mastication. The morphology of both epithelium and connective tissues indicates the adaptation to these forces. The stratified squamous epithelium may be keratinized or nonkeratinized but most often is parakeratinized. The underlying lamina propria is dense. The collagen fibers of the lamina propria may either insert into the alveolar bone and the cementum or blend with the periosteum.

The gingiva is limited on the outer surface of both jaws by the mucogingival junction, which separates it from the alveolar mucosa (Fig. 10.23). The alveolar mucosa is red and contains numerous small vessels coursing close

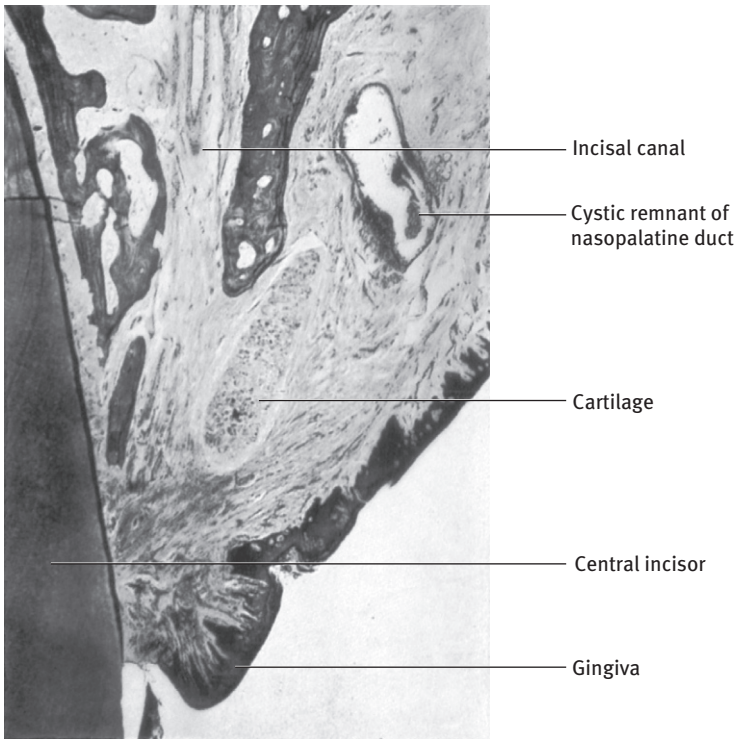


Figure 10.22 Sagittal section through palatine papilla and anterior palatine canal. Note cartilage in papilla.

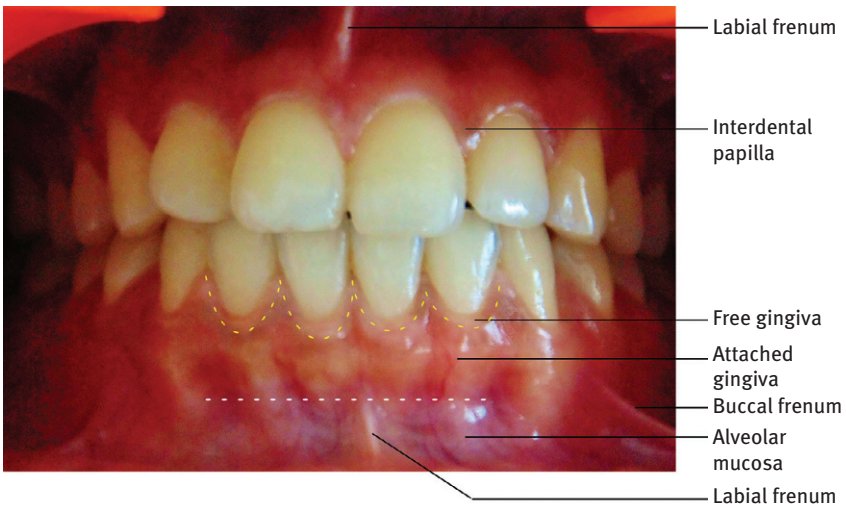


Figure 10.23 Vestibular surface of gingiva of young adult.

to the surface. Clinically the mucogingival junction is identified as the junction between bright pink alveolar mucosa and pale pink gingiva. On the inner surface of the lower jaw, a line of demarcation is found between the gingiva and the mucosa on the floor of the mouth. On the palate, the distinction between the gingiva and the peripheral palatal mucosa is not so sharp.

The gingiva is normally pink but may sometimes have a grayish tint. The color depends in part on the surface (keratinized or not) and thickness and in part on pigmentation. The surface may be translucent or transparent, permitting the color of the underlying tissues to be seen. The reddish or pinkish tint is attributable to the color given to the underlying tissue by the blood vessels and the circulating blood.

The gingiva can be divided into the free gingiva, the 'attached' gingiva[†] (Fig. 10.24), and the interdental papilla. The dividing line between the free gingiva and the (attached) gingiva is the *free gingival groove*, which runs parallel to the margin of the gingiva at a distance of 0.5 to 1.5 mm. The free gingival groove, not always visible microscopically, appears in histologic sections

[†]At the International Conference on Research in the Biology of Periodontal Disease, Chicago, June 12–15, 1977, it was voted to drop the use of 'attached' and simply refer to gingiva.

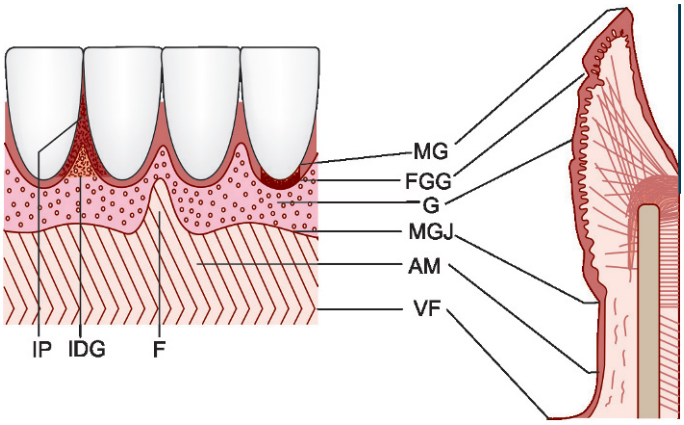


Figure 10.24 Diagrammatic illustration of surface characteristics of the clinically normal gingiva. IP, Interdental papilla; IDG, interdental groove; F, frenum; MG, marginal gingiva; FGG, free gingival groove; G, gingiva; MGJ, mucogingival junction; AM, alveolar mucosa; VF, vestibular fornix (From Grant DA, Stem IB, and Listgarten MA: Periodontics in the tradition of Orban and Gottlieb, ed 6, St Louis, 1988, The CV Mosby Co).

(Fig. 10.25A) as a shallow V-shaped notch at a heavy epithelial ridge. The free gingival groove develops at the level of, or somewhat apical to, the bottom of the gingival sulcus. In some cases the free gingival groove is not so well defined as in others, and then the division between the free gingiva and the gingiva is not clear. The free gingival groove and the epithelial ridge are probably caused by functional impacts on the free gingiva. In the absence of a sulcus there is no free gingiva.

The attached gingiva is about 4–6 mm. The mucogingival junction is 3–5 mm below the level of the crest of the alveolar bone.

The gingiva is characterized by a surface that appears stippled (Fig. 10.25B). Portions at the epithelium appear to be elevated, and between the elevations there are shallow depressions, the net result of which is stippling. The depressions correspond to the center of heavier epithelial ridges. There may be protuberances of the epithelium as well as stippling. They probably are functional adaptations to mechanical impacts. The disappearance of stippling is an indication of edema, an expression of an involvement of the gingiva in a progressing gingivitis.

Although the degree of stippling (Fig. 10.23) and the texture of the collagenous fibers vary with different individuals, there are also differences according to age and sex. In younger females the connective tissue is more finely textured than in the male. However, with increasing age the collagenous fiber bundles become more coarse in both sexes. Males tend to have more heavily stippled gingivae than do females. Like the human epidermis, the cells of the oral epithelium show another sex difference. In females the majority of the nuclei contain a large chromatin particle adjacent to the nuclear membrane.

The gingiva appears slightly depressed between adjacent teeth, corresponding to the depression on the

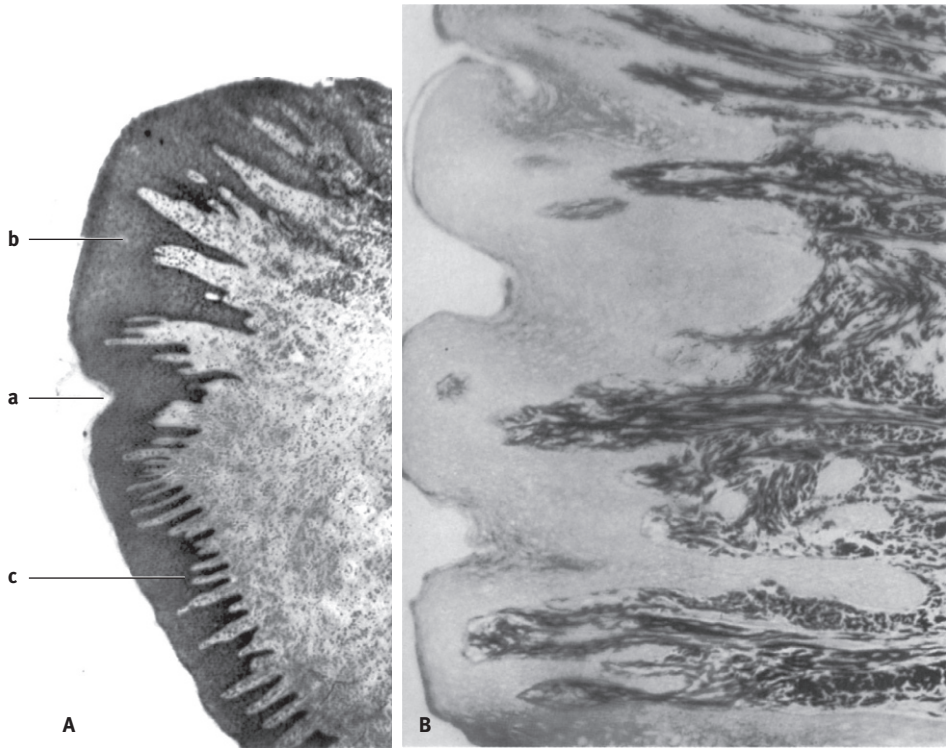


Figure 10.25 **(A)** Biopsy specimen of gingiva showing free gingival groove, a, and corresponding heavy epithelial ridge; b, free gingiva; c, gingiva. **(B)** Gingival specimen showing stippling. Note relation of connective tissue fiber bundles to stippled surface (Mallory stain) (From Grant DA, Stern IB, and Listgarten MA: Periodontics in the tradition of Orban and Gottlieb, ed 6, St Louis, 1988, The CV Mosby Co).

alveolar process between eminences of the sockets. In these depressions the gingiva sometimes forms slight vertical folds called interdental grooves.

The interdental papilla is that part of the gingiva that fills the space between two adjacent teeth. When viewed from the oral or vestibular aspect, the surface of the interdental papilla is triangular. In a three-dimensional view, the interdental papilla of the posterior teeth is tent shaped, whereas it is pyramidal between the anterior teeth. When the interdental papilla is tent shaped, the oral and the vestibular corners are high, whereas the central part is like a valley. The central concave area fits below the contact point, and this depressed part of the interdental papilla is called the *col*. The col is covered by thin nonkeratinized epithelium, and it has been suggested that the col (the nonkeratinized epithelium) is more vulnerable to periodontal disease.

The gingiva is parakeratinized in 75%, keratinized in 15%, and nonkeratinized in 10% of the population. It has been suggested that inflammation, which is seen in almost all gingival specimens, interferes with keratinization. The more highly keratinized the tissue, the whiter and less translucent is the tissue.

The presence of melanin pigment in the epithelium may give it a brown to black coloration. Pigmentation is most abundant at the base of the interdental papilla. It can be increased considerably in a number of pathologic states.

The lamina propria of the gingiva consists of a dense connective tissue that does not contain large vessels. Small numbers of lymphocytes, plasma cells, and macrophages are present in the connective tissue of normal gingiva subjacent to the sulcus and are involved in defense and repair. The papillae of the connective tissue are characteristically long, slender, and numerous. The presence of these high papillae makes for ease in the histologic differentiation of gingiva and alveolar mucosa, in which the papillae are quite low (Fig. 10.26). The tissue of the lamina propria contains only few elastic fibers, and for the most part they are confined to the walls of the blood vessels. Other elastic fibers known as oxytalan fibers (because of special staining qualities) are also present. On the other hand, the alveolar mucosa and the submucosa contain numerous elastic fibers. These fibers are thickest in the submucosa.

The gingival fibers of the periodontal ligament enter into the lamina propria, attaching the gingiva firmly to the teeth (see Chapter 8). The gingiva is also immovably and firmly attached to the periosteum of the alveolar bone. Because of this arrangement it is often referred to as mucoperiosteum. Here a dense connective tissue, consisting of coarse collagen bundles (Fig. 10.27A), extends from the bone to the lamina propria. In contrast, the submucosa underlying the alveolar mucous membrane is loosely textured (Fig. 10.27B). The fiber bundles of the lamina propria of the alveolar mucosa are thin and regularly interwoven.

The collagen fibers in the lamina propria of the gingiva are arranged in various groups, sometimes referred to as the gingival ligament. They serve to support the free gingiva, bind attached gingiva to the alveolar bone and tooth, link one teeth with the other. They are divided into the following groups:

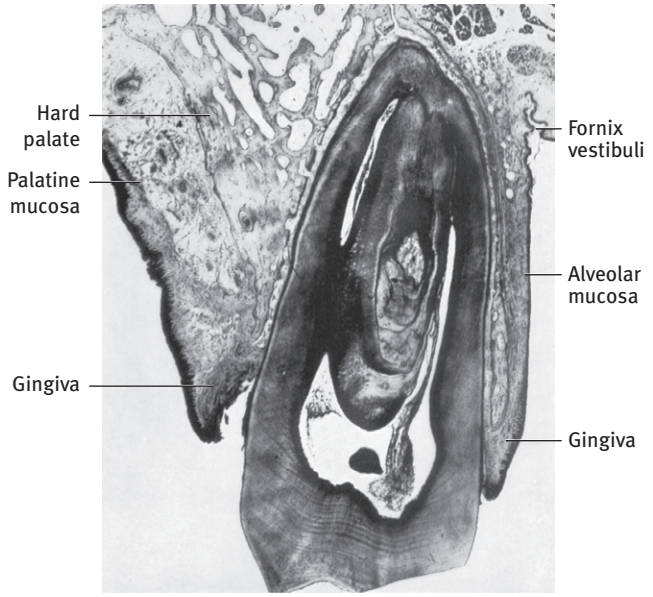


Figure 10.26 Structural differences between gingiva and alveolar mucosa. Upper premolar.

1. Dentogingival. Extends from the cervical cementum into the lamina propria of the gingiva. The fibers of the gingival ligament constitute the most numerous group of gingival fibers.
2. Alveologingival. The fibers arise from the alveolar crest and extend into the lamina propria.
3. Circular. A small group of fibers that circle the tooth and interlase with the other fibers.
4. Dentoperiosteal. These fibers can be followed from the cementum into the periosteum of the alveolar crest and of the vestibular and oral surfaces of the alveolar bone.

There are also accessory fibers that extend interproximally between adjacent teeth and are also referred to as transseptal fibers. These fibers make up the interdental ligament.

Apart from dentogingival, dentoperiosteal, alveologingival and circular fibers, interdental, semicircular, longitudinal fibers, vertical and transgingival fiber groups are described (Figs 10.28A–D). The interdental fibers connect the buccal and lingual papillae and the vertical fibers run coronally from alveolar mucosa or attached gingiva to the marginal gingiva or interdental papillae. The semicircular fibers connect the cementum on one side of the tooth to the opposite side after coursing through the free gingiva. The transgingival fibers pass from cementum of one tooth to the marginal gingiva of the adjacent tooth merging with circular and semicircular fibers.

The lamina propria of gingiva differs from other regions not only in the arrangement of collagen fiber but also in the composition and response of its matrix to certain stimuli and in the nature of the fibroblast. The fibroblast has less contractile protein and also lacks alkaline phosphatase. The matrix contains less of type III collagen, more of hyaluron and has a lower turnover

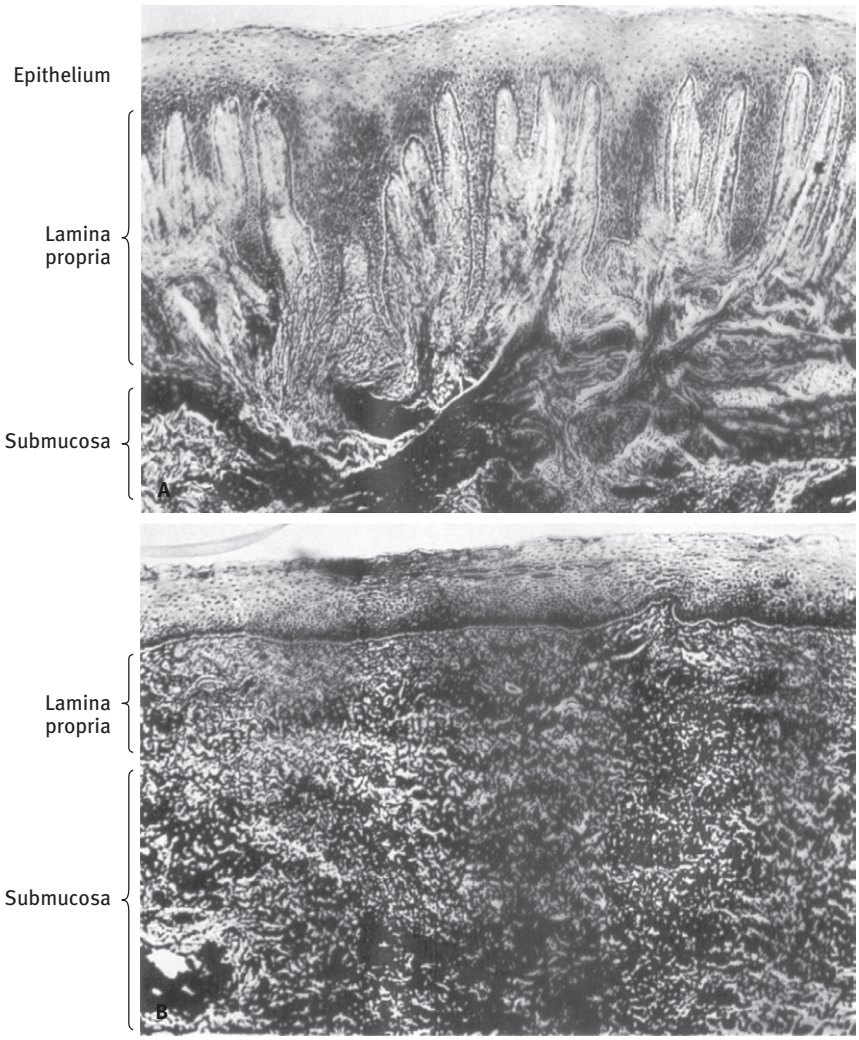


Figure 10.27 Differences between **(A)** gingiva, and **(B)** alveolar mucosa. Silver impregnation of collagenous fibers. Note coarse bundles of fibers in gingiva and finer fibers in alveolar mucosa.

rate. They release more prostaglandin in response to histamine (Table 10.6).

Blood and nerve supply

The blood supply of the gingiva is derived chiefly from the branches of the alveolar arteries that pass upward through the interdental septa. The interdental alveolar arteries perforate the alveolar crest in the interdental space and end in the interdental papilla, supplying it and the adjacent areas of the buccal and lingual gingiva. In the gingiva these branches anastomose with superficial branches of arteries that supply the oral and vestibular mucosa and marginal gingiva, for instance, with branches of the lingual, buccal, mental, and palatine arteries. The numerous lymph vessels of the gingiva lead to submental and submandibular lymph nodes.

The gingiva is well innervated. Different types of nerve endings can be observed, such as the Meissner or Krause corpuscles, end bulbs, loops, or fine fibers that enter the epithelium as 'ultraterminal' fibers (Fig. 10.5).

Vermilion zone

The transitional zone between the skin of the lip and the mucous membrane of the lip is the red zone, or the vermilion zone. The line that separates the skin from the vermilion zone is termed the vermilion border. It is found only in humans (Fig. 10.29). The skin on the outer surface of the lip is covered by a moderately thick, keratinized epithelium with a rather thick stratum corneum. The papillae of the connective tissue are few and short. Many sebaceous glands are found in connection with the hair follicles. Sweat glands occur between them.

The transitional region is characterized by a thicker but mildly keratinized epithelium and numerous, densely arranged, long papillae of the lamina propria, reaching deep into the epithelium and carrying large capillary loops close to the surface. Thus blood is visible through the thin parts of the translucent epithelium and gives the red color to the lips. The keratinization decreases towards the lip, but the thickness of the epithelium increases. The inner aspect of the lip is the thicker nonkeratinized labial mucosa. Like the skin, the transitional zone is exposed to the atmosphere, but unlike it there are no glands to keep it moist or prevent it from drying.

It is for this reason lips become dry easily and we lick our lips to moisten it.

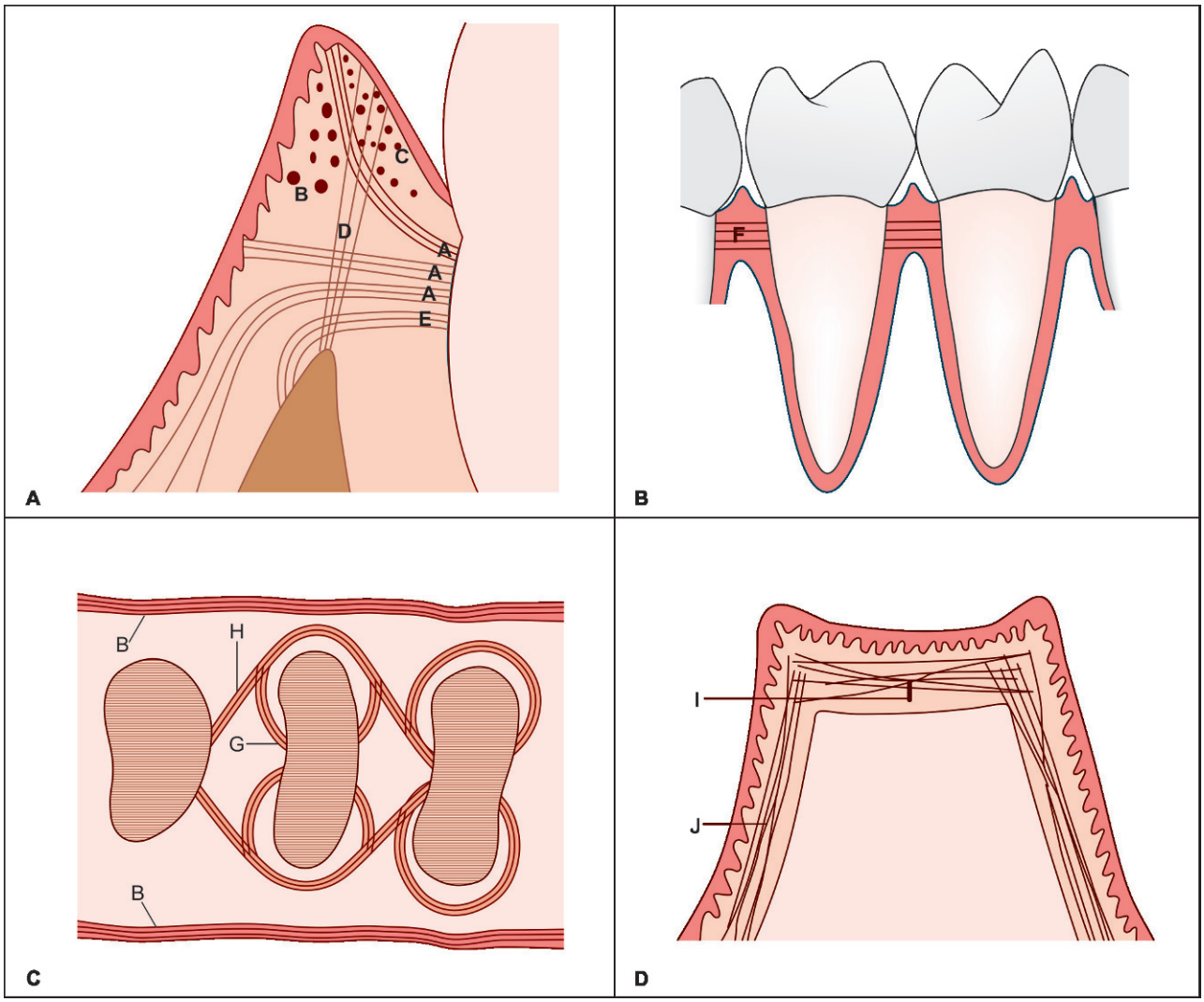


Figure 10.28 Diagrammatic representation of different fiber groups within gingiva. **(A)** buccolingual section, **(B)** mesiodistal section, **(C)** horizontal section, **(D)** buccolingual section along interdental col. A–dentogingival fibers, B–longitudinal fibers, C–circular fibers, D–alveologingival fibers, E–dentoperiosteal fibers, F–transseptal fibers, G–semicircular fibers, H–transgingival fibers, I–interdental fibers, J–vertical fibers.

Table 10.6 Various Groups of Fibers in Gingiva

1. Dentogingival
2. Alveologingival
3. Circular
4. Dentoperiosteal
5. Transseptal fibers (accessory fibers)

Nonkeratinized Areas

Lining mucosa

Lining mucosa is found on the lip, cheek, vestibular fornix, and alveolar mucosa. All the zones of the lining mucosa are characterized by a relatively thick nonkeratinized epithelium and a thin lamina propria. Different zones of lining mucosa vary from one another in the structure of their submucosa. Where the lining mucosa reflects from the movable lips, cheeks, and tongue to the alveolar bone, the submucosa is loosely textured. The reflectory mucosa found in the fornix vestibuli and in the sublingual sulcus at the floor of the oral cavity has a submucosa that is loose and of considerable volume. The mucous membrane is movably attached to the deep structures and does not restrict the movement of lips, cheeks and the tongue.

Where lining mucosa covers muscle, as on the lips, cheeks, and underside of the tongue, the mucosa is fixed to the epimysium or fascia. In these regions the mucosa is also highly elastic. These two characteristics permit the mucosa to maintain a relatively smooth surface during muscular movement. Thus heavy folding, which could lead to injury during chewing if such folds were caught between the teeth, does not occur.

The mucosa of the soft palate is intermediate between this type of lining mucosa and the reflecting mucosa: the mucosa is flexible but not very much mobile.

Lip and cheek

The epithelium of the mucosa of the lips (Fig. 10.29) and of the cheek (Fig. 10.30) is stratified squamous nonkeratinized

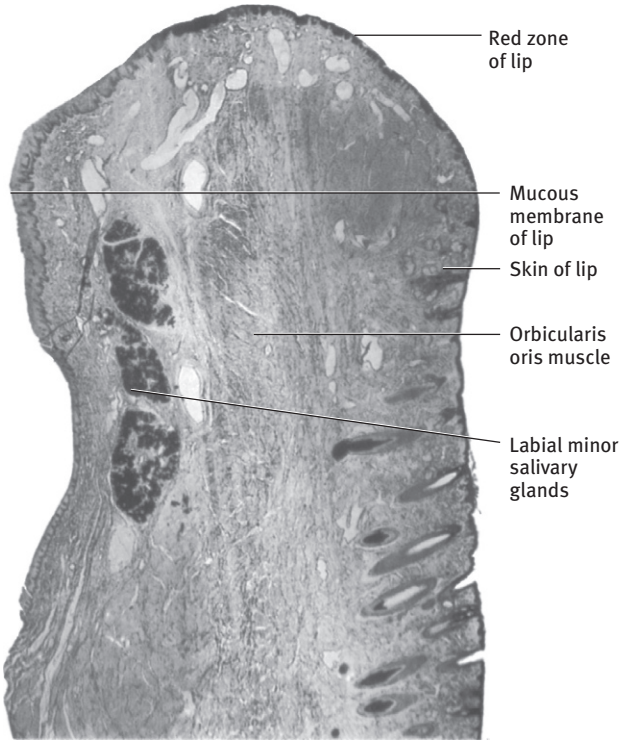


Figure 10.29 Section through the lip.

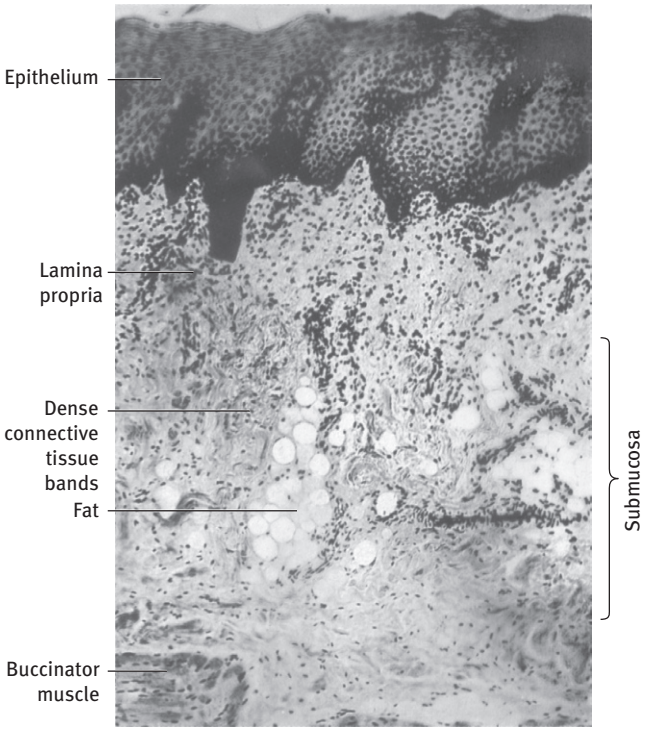


Figure 10.30 Section through mucous membrane of cheek. Note bands of dense connective tissue attaching lamina propria to fascia of buccinator muscle.

epithelium. The lamina propria of the labial and buccal mucosa consists of dense connective tissue and has short, irregular papillae.

The submucous layer connects the lamina propria to the thin fascia of the muscles and consists of strands of densely grouped collagen fibers. There is loose connective tissue containing fat and small mixed glands between these strands. The strands of dense connective tissue limit the mobility of the mucous membrane, holding it to the musculature and preventing its elevation into folds. This prevents the mucous membrane of the lips and cheeks from lodging between the biting surfaces of the teeth during mastication. The mixed minor salivary glands of the lips are situated in the submucosa, whereas in the cheek the glands are larger and are usually found between the bundles of the buccinator muscle and sometimes on its outer surface. The cheek, lateral to the corner of the mouth, may contain isolated sebaceous glands called Fordyce spots (Fig. 10.31). These may occur lateral to the corner of the mouth and are often seen opposite the molars.

A comparison of masticatory and buccal mucosa shows that in the keratinized tissue the epithelium is thinner. It has a granular layer, the basal cells are larger, but the average cell size is smaller, and the cells have an angular shape. Furthermore, it is characterized by having many tonofibrils, wider intercellular spaces, and 'prickles' that form 'intercellular bridges.' The cells of both tissues are joined by desmosomes. The appearance of the two differs by the heightened prominence of the 'prickles' in the keratinized tissues, brought about by the increased width of the intercellular space and the greater density of the tonofibrils. Even the lamina propria of the two differ. In masticatory mucosa the basement membrane contains more reticular fibers, and its papillae are high and more closely spaced.

Vestibular fornix and alveolar mucosa

The mucosa of the lips and cheeks reflects from the vestibular fornix to the alveolar mucosa covering the bone. The mucous membrane of the cheeks and lips is attached firmly to the buccinator muscle in the cheeks and orbicularis oris muscle in the lips. In the fornix the mucosa is loosely connected to the underlying structures, and so the necessary movements of the lips and cheeks are permitted. The mucous membrane covering the outer surface of the alveolar process (alveolar mucosa) is attached loosely to the periosteum. It is continuous with, but different from, the gingiva, which is firmly attached to the periosteum of the alveolar crest and to the teeth.

The median and lateral labial frenula are folds of the mucous membrane containing loose connective tissue. No muscle fibers are found in these folds.

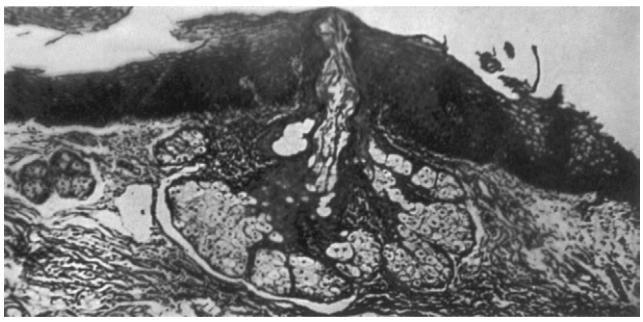


Figure 10.31 Sebaceous gland in cheek (Fordyce spot).

Gingiva and alveolar mucosa are separated by the *mucogingival junction*. The gingiva is stippled, firm, and thick, lacks a separate submucous layer, is immovably attached to bone and teeth by coarse collagen fibers, and has no glands. The gingival epithelium is thick and mostly parakeratinized or keratinized. The epithelial ridges and the papillae of the lamina propria are high.

The alveolar mucosa is thin and loosely attached to the periosteum by a well-defined submucous layer of loose connective tissue (Fig. 10.27B), and it may contain small mixed glands. The epithelium is thin and nonkeratinized, and the epithelial ridges and papillae are low and often entirely missing. These differences cause the variation in color between the pale pink gingiva and the red lining mucosa.

Inferior surface of tongue and floor of oral cavity

The mucous membrane on the floor of the oral cavity is thin and loosely attached to the underlying structures to allow for the free mobility of the tongue. The epithelium is nonkeratinized, and the papillae of the lamina propria are short (Fig. 10.32). The submucosa contains adipose tissue. The sublingual glands lie close to the covering mucosa in the sublingual fold. The sublingual mucosa and the lingual gingiva have a junction corresponding to the mucogingival junction on the vestibular surface. The sublingual mucosa reflects onto the lower surface of the tongue and continues as the ventrolingual mucosa.

The mucous membrane of the inferior surface of the tongue is smooth and relatively thin (Fig. 10.33). The epithelium is nonkeratinized. The papillae of the connective tissue are numerous but short. Here the submucosa cannot be identified as a separate layer. It binds the mucous membrane tightly to the connective tissue surrounding the bundles of the muscles of the tongue.

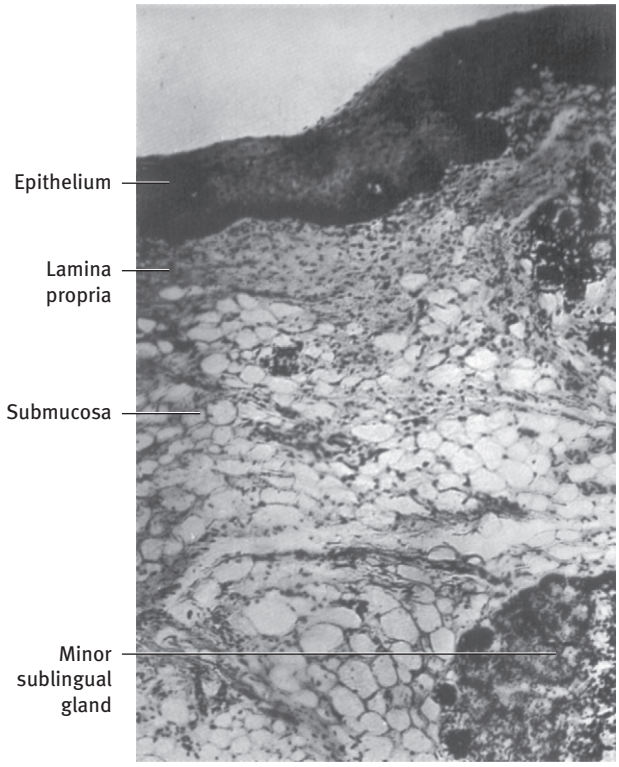


Figure 10.32 Mucous membrane from floor of mouth.

Soft palate

The mucous membrane on the oral surface of the soft palate is highly vascularized and reddish in color, noticeably differing from the pale color of the hard palate. The papillae of the connective tissue are few and short. The stratified squamous epithelium is nonkeratinized (Fig. 10.34). The lamina propria shows a distinct layer of elastic fibers separating it from the submucosa. The latter is relatively loose and contains an almost continuous layer of mucous glands. It also contains taste buds. Typical oral mucosa continues around the free border of the soft palate for a variable distance and is then replaced by nasal mucosa with its pseudostratified, ciliated columnar epithelium.

Specialized Mucosa

Dorsal lingual mucosa

The superior surface of the tongue is rough and irregular (Fig. 10.35). A V-shaped line divides it into an anterior part, or body, and a posterior part, or base.

The former comprises about two thirds of the length of the organ, and the latter forms the posterior one third. The fact that these two parts develop embryologically from different visceral arches (see Chapter 2) accounts for the different source of nerves of the general senses: the anterior two thirds are supplied by the trigeminal nerve through its lingual branch and the posterior one third by the glossopharyngeal nerve.

The body and the base of the tongue differ widely in the structure of the mucous membrane. The anterior part can be termed the 'papillary' and the posterior part the 'lymphatic' portion of the dorsolingual mucosa. On the anterior part are found numerous fine-pointed, cone-shaped papillae that give it velvet like appearance. These projections, the filiform (thread-shaped) papillae, are epithelial structures containing a core of connective tissue from which secondary papillae protrude toward the epithelium (Fig. 10.36A). The covering epithelium is keratinized and forms tufts at the apex of the dermal papilla. The filiform papillae do not contain taste buds.

Interspersed between the filiform papillae are the isolated fungiform (mushroom-shaped) papillae (Fig. 10.36B), which are round, reddish prominences. Their color is derived from a rich capillary network visible through the relatively thin epithelium. Fungiform papillae contain a few (one to three) taste buds found only on their dorsal surface.

In front of the dividing V-shaped terminal sulcus, between the body and the base of the tongue, are eight to ten vallate (walled) papillae or circumvallate papillae (Fig. 10.37). They do not protrude above the surface of the tongue but are bounded by a deep circular furrow so that their only connection to the substance of the tongue is at their narrow base. Their free surface shows numerous secondary papillae that are covered by a thin, smooth epithelium. On the lateral surface of the vallate papillae, the epithelium contains numerous taste buds. The ducts of small serous glands called von Ebner's glands open

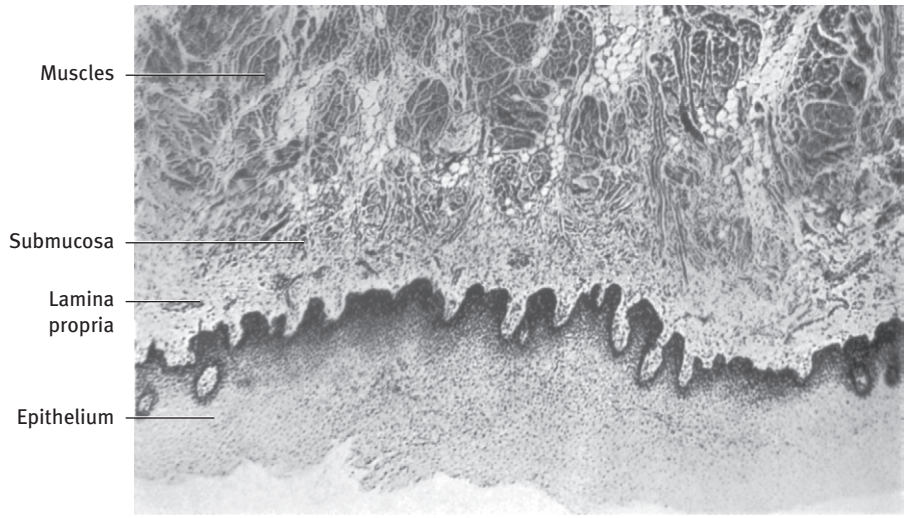


Figure 10.33 Mucous membrane on inferior surface of tongue.

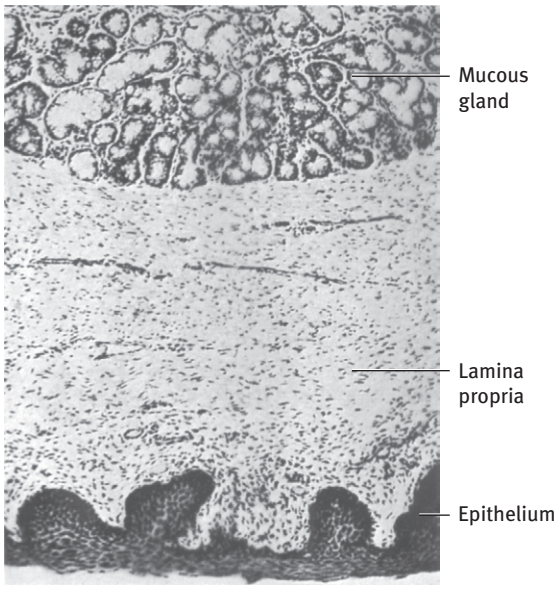


Figure 10.34 Mucous membrane from oral surface of soft palate.

into the trough. They may serve to wash out the soluble elements of food and are the main source of salivary lipase.

On the lateral border of the posterior parts of the tongue, sharp parallel clefts of varying length can often be observed. They bound narrow folds of the mucous membrane and are the vestige of the large foliate papillae found in many mammals. They contain taste buds.

Taste buds

Taste buds are small ovoid or barrel-shaped intraepithelial organs about 80 μm high and 40 μm thick (Fig. 10.38). They extend from the basal lamina to the surface of the epithelium. Their outer surface is almost covered by a few flat epithelial cells, which surround a small opening, the *taste pore* (a taste bud may have more than one taste pore). It leads into a narrow space lined by the supporting cells of the taste bud. The outer supporting cells are arranged like the staves of a barrel. The inner and shorter ones are spindle shaped. Between the latter are arranged 10 to 12 neuroepithelial cells, the receptors of taste stimuli. They are slender, dark-staining cells that carry finger like processes at their superficial end. The fingers like processes are visible at the ultrastructural level and resemble hairs at the light microscope level. The hairs reach into the space beneath the taste pore.

A rich plexus of nerves is found below the taste buds. Some fibers enter the epithelium and end in contact with the sensory cells of the taste bud.

Taste buds are numerous on the inner wall of the trough surrounding the vallate papillae, in the folds of the foliate papillae, on the posterior surface of the epiglottis, and on some of the fungiform papillae at the tip and the lateral borders of the tongue (Fig. 10.39).

The classic view maintains that the primary taste sensations, that is, sweet, salty, bitter, and sour, are perceived in different regions of the tongue and palate (sweet at the tip, salty at the lateral border of the tongue, bitter and sour on the palate and also in the posterior part of the tongue—bitter in the middle and sour in the lateral areas of the tongue). The classic view also diagrammatically and arbitrarily correlates the distribution of the receptors for primary taste qualities with the different types of papillae (vallate papillae with bitter, foliate papillae with sour, taste buds of the fungiform papillae at the tip of the tongue with sweet and at the borders with salty taste). Bitter and sour taste sensations are mediated by the glossopharyngeal nerve, and sweet and salty taste are mediated by the intermediofacial nerve by the chorda tympani.

On the other hand, many authorities believe that taste cannot be broken down into these four primaries, sweet, sour, salty, and bitter, but that it consists of a range of stimuli that form a spectrum of sensations making up all taste senses. Taste occurs when a chemical substance contacts a receptor cell in the taste bud. Each taste bud is innervated by many fibers. The reception of a chemical

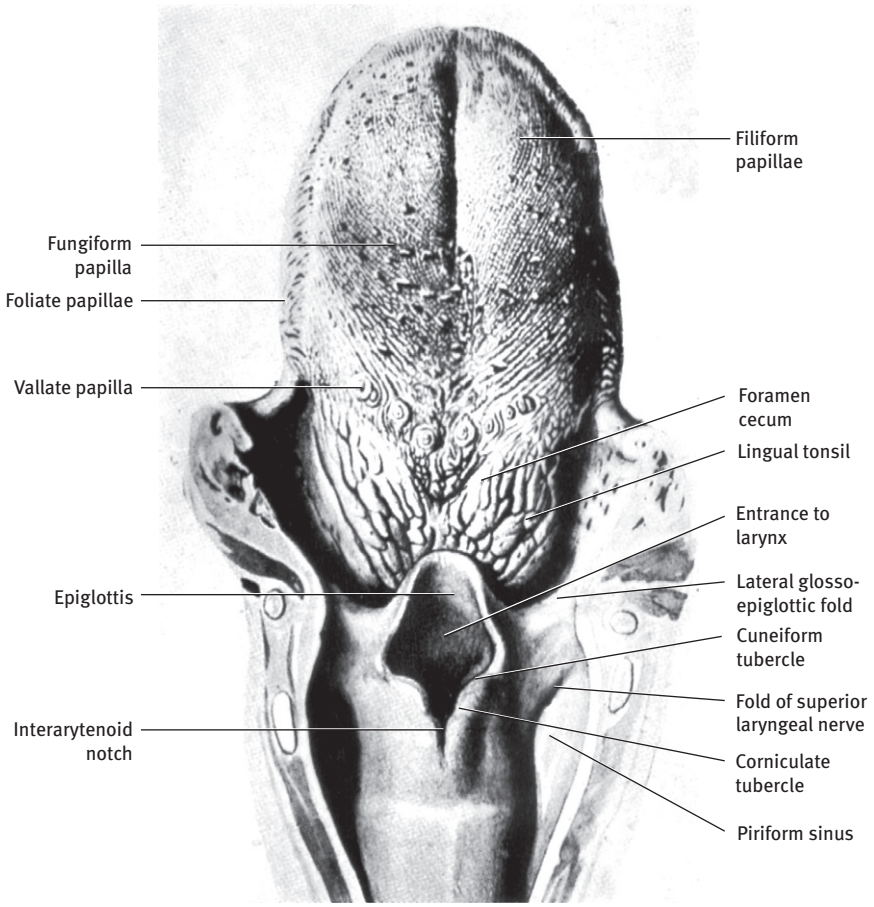


Figure 10.35 Surface view of human tongue (From Sicher H and Tandler J: Anatomie für Zahnärzte, Vienna. 1928. Julius Springer Verlag).

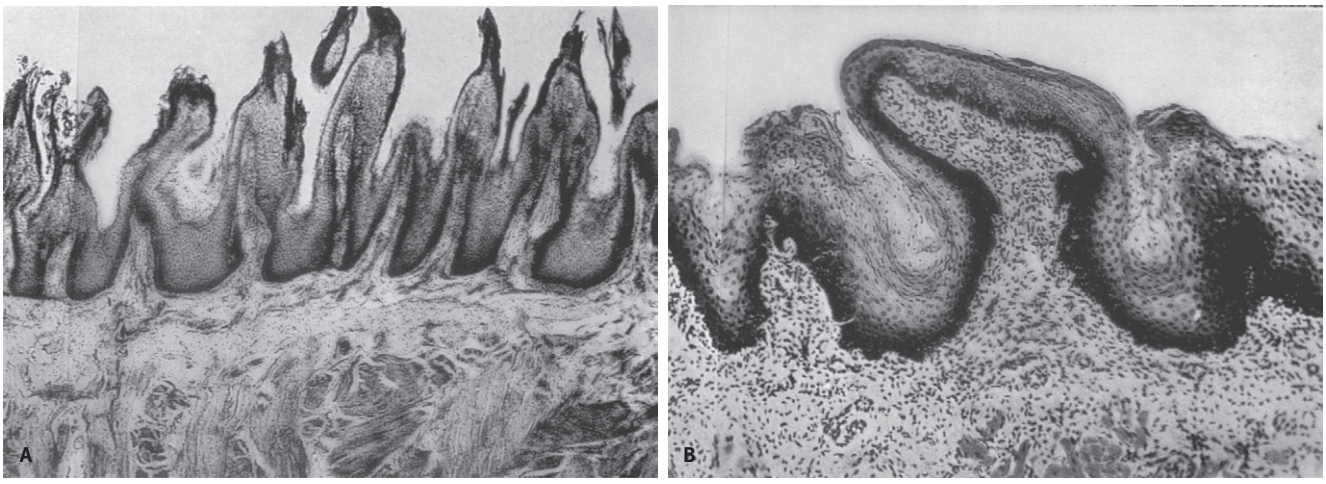


Figure 10.36 **(A)** Filiform, and **(B)** fungiform papillae.

substance fires the nerve fiber. Thus taste may be a continuum or a composite of the firing of many fibers.

At the angle of the V-shaped terminal groove on the tongue is located the foramen cecum, which represents the remnant of the thyroglossal duct (see Chapter 2). Posterior to the terminal sulcus, the surface of the tongue is irregularly studded with round or oval prominences, the *lingual follicles*. Each of these shows one or more lymph nodules, sometimes containing a germinal center (Fig. 10.40). Most of these prominences have a small pit at the center, the lingual crypt, which is lined with stratified squamous epithelium. Innumerable lymphocytes migrate into the crypts through the epithelium. Ducts of the small posterior lingual mucous glands open into the crypts. Together the lingual follicles form the *lingual tonsil* (Table 10.7).

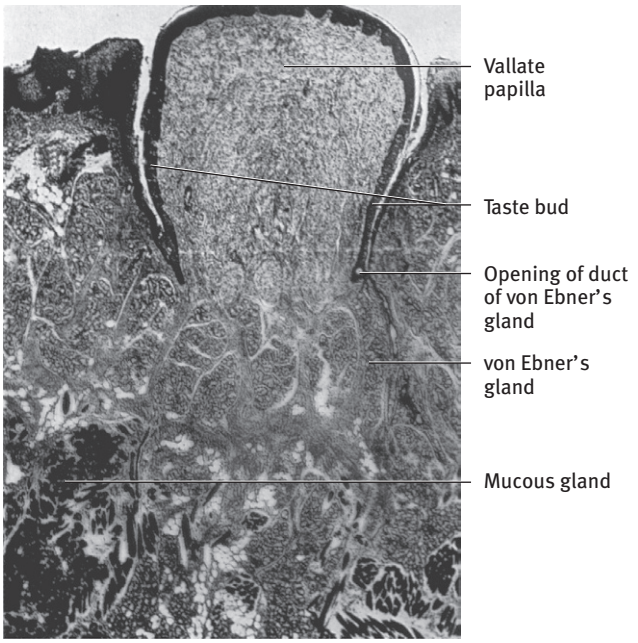


Figure 10.37 Vallate (or circumvallate) papilla.

GINGIVAL SULCUS AND DENTOGINGIVAL JUNCTION

Gingival Sulcus

The gingival sulcus or crevice is the space between the inner aspect of gingiva and the tooth. It is a continuous space present all around the tooth. The sulcus extends from the free gingival margin to the dentogingival junction. In healthy state its depth is at the approximate level of the free gingival groove on the outer surface of the gingiva. The sulcus may be responsible for the formation of the groove since it leaves the gingival margin without firm support. The groove is believed to be formed by the functional folding of the free gingival margin during mastication. The sulcular (crevicular) epithelium is nonkeratinized in humans. It lacks epithelial ridges and so forms a smooth interface with the lamina propria. It is thinner than the epithelium of the gingiva. The sulcular epithelium expresses CK4, which is typical of lining epithelium. The sulcular epithelium is continuous with the gingival epithelium and the attachment epithelium. These three epithelia have a continuous and co-extensive basal lamina. Under normal conditions the depth of the sulcus is variable; 45% of all measured sulci are below 0.5 mm. The average sulcus is 1.8 mm. The normal gingival sulcus depth is generally taken to be around 2 mm. Its depth increases due to periodontal diseases. Such a deepened gingival sulcus is termed periodontal pocket.

Dentogingival Junction

It is the junction between the gingiva and the tooth. The epithelium of the gingiva which gets attached to the tooth is called junctional or attachment epithelium. The union between this epithelium and tooth is referred to as epithelial attachment.

The junctional epithelium resembles reduced enamel epithelium in its structure in that they have a basal layer

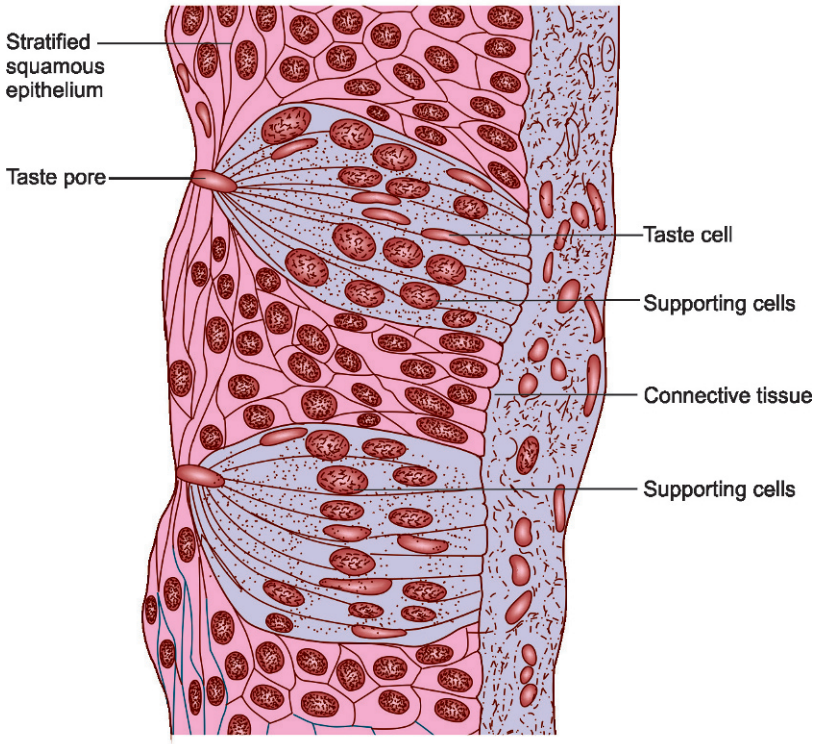


Figure 10.38 Taste buds from slope of vallate papilla (From Schaffer J: Lehrbuch der histologie und histogenese, ed 2, Leipzig, 1922, Wilhelm Engelmann).

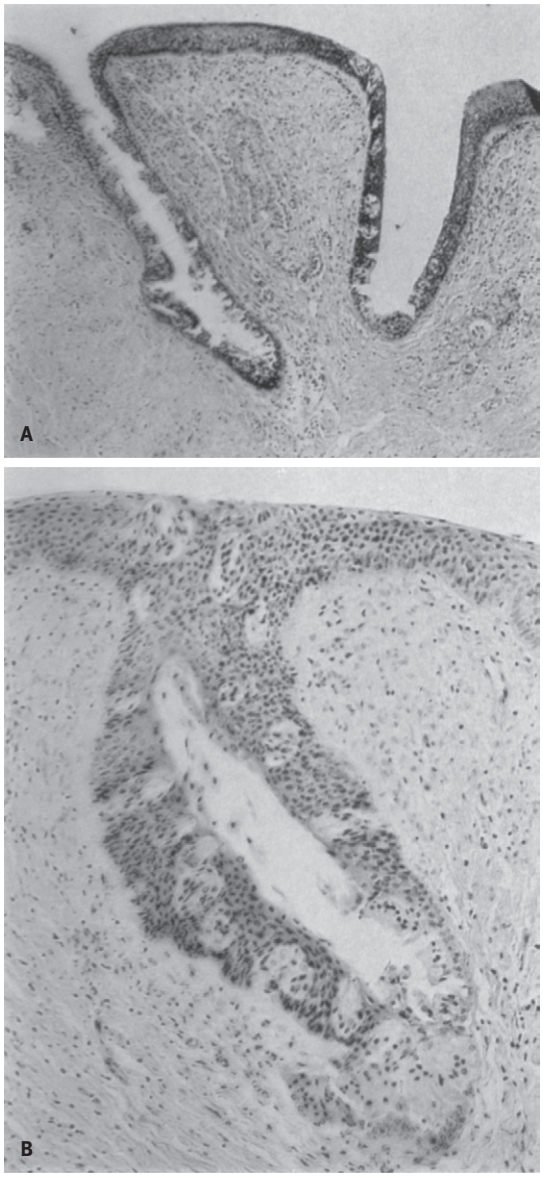


Figure 10.39 **(A)** Circumvallate papilla showing trough and numerous taste buds (light areas), **(B)** Higher magnification of trough and taste buds.

Table 10.7 Taste Buds

- Barrel shaped—intra epithelial organs
- Consisting of outer supporting cells arranged like staves of barrel and inner spindle shaped cells
- Between inner spindle shaped cells neuroepithelial cells are present
- Taste buds are seen in:
  - Vallate papillae
  - Folds of foliate papillae
  - Posterior surface of epiglottis
  - Occasionally in fungiform papillae

and few layers of flattened cells. The junctional epithelium is best regarded as a nondifferentiating, nonkeratinizing tissue lacking a gradient of change in cell types such as is present in the gingival epithelium. The intermediate filaments found in the junctional epithelium differ from those found in the keratinizing oral epithelia. They express CK5, 14 and 19, which are expressed in nondifferentiating tissues like reduced enamel epithelium. The junctional epithelium extends up to 2 mm on the surface of the tooth. It has the highest turnover rate of 5–6 days, therefore it regenerates readily. The junctional epithelium is highly permeable and it has large intercellular spaces, so that neutrophils have an easy passage in and out of the epithelium. It also permits the easy flow of crevicular (gingival) fluid. Both epithelium and connective tissue are attached to the tooth, and in health each contributes to the integrity of the dentogingival junction. Again the firmness of this junction is maintained by the gingival portion of the periodontal ligament.

Although the firmness and mechanical strength of the dentogingival junction is mainly attributable to the connective tissue attachment, the attachment of the epithelium to the enamel is by no means loose or weak. This can be demonstrated with ground histologic sections of frozen specimens where enamel and soft tissues are retained in their normal relation. When an attempt is made to detach the gingiva from the tooth in these preparations, the epithelium tears but does not peel off from the enamel surface (Fig. 10.41).

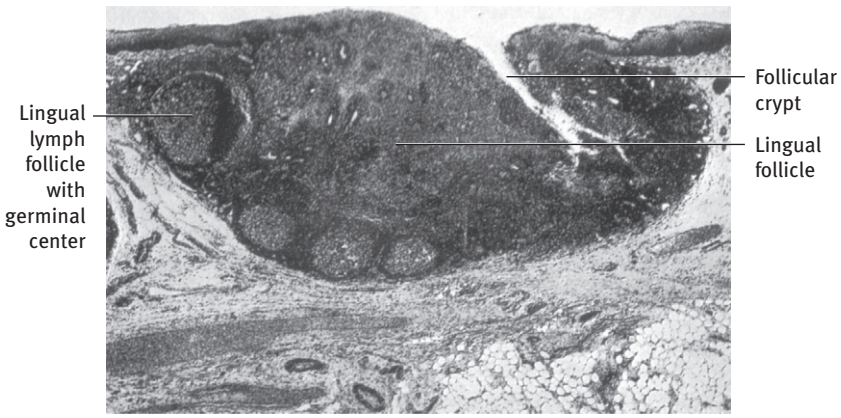


Figure 10.40 Lingual lymph follicle.

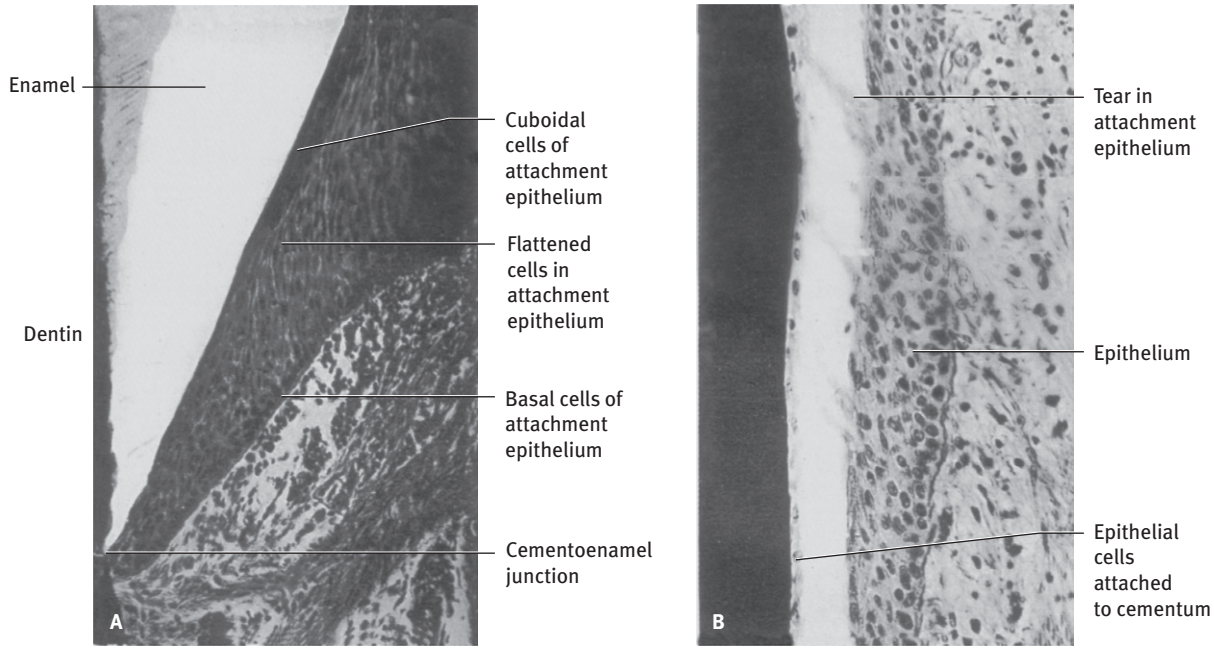


Figure 10.41 **(A)** Arrangement of cells in attachment epithelium indicates functional influences. **(B)** Artificial tear in attachment epithelium. Some cells remain attached to cementum, while others bridge tear (**A** from Orban B: Z Stomatol 22:353, 1924; **(B)** from Orban B and Mueller E: J Am Dent Assoc 16:1206, 1929).

The junction of the gingiva and the tooth is of great physiologic and clinical importance. This union is unique in many ways and may be a point of lessened resistance to mechanical forces and bacterial attack. The gingiva consists of two tissues maintaining the junction intact. Their biology differs. The dense, resilient lamina propria takes up impacts produced during mastication. In a similar sense so does the keratinized or parakeratinized surface of the gingiva. When the epithelium is injured, the injury is repaired by the turnover of cells and their ability to migrate. When the connective tissue is injured, ribosomes within the fibroblasts form molecules of the precursor protein of collagen (procollagen) and ground substances as well, contributing to repair.

Defense against bacterial injury is a function of the defense mechanism of the body. Lymphocytes and plasma cells are routinely seen in the connective tissue at the bottom of the gingival sulcus and below the attachment epithelium. Langerhans cells migrate to the sulcular and oral epithelium when infection or inflammation is present. The lysosomes of the junctional epithelium may have a phagocytic function. These defense reactions to the bacteria in the gingival sulcus constitute barriers against the invasion of bacteria and the penetration of toxins. The bacterial products may act directly or indirectly via the immune responses.

Development of dentogingival junction

When the ameloblasts finish formation of the enamel matrix, they leave a thin membrane on the surface of the enamel, the primary enamel cuticle. This cuticle may be connected with the interprismatic enamel substance and the ameloblasts. The ameloblasts shorten after the primary enamel cuticle has been formed, and the epithelial enamel organ is reduced to a few layers of flat cuboid cells, which are then called reduced enamel epithelium. Under normal conditions it covers the entire enamel surface, extending to the cementoenamel junction (Fig. 10.42), and remains attached to the primary enamel cuticle. During eruption, the tip of the tooth approaches the oral mucosa, and the reduced enamel epithelium and the oral epithelium meet and fuse (Fig. 10.43). The remnant of the primary enamel cuticle after eruption is referred to as Nasmyth's membrane.

The epithelium that covers the tip of the crown degenerates in its center, and the crown emerges through this perforation into the oral cavity (Fig. 10.44). The reduced enamel epithelium remains organically attached to the part of the enamel that has not yet erupted. Once the tip of the crown has emerged, the reduced enamel epithelium is termed the primary attachment epithelium.* Changes in keratin expression, as demonstrated by monoclonal antibody reactions to intermediate filaments, suggest that during the transition from ameloblast to junctional epithelium the changes in keratin expression occur as a form of all differentiations.

At the margin of the gingiva the attachment epithelium is continuous with the oral epithelium (Fig. 10.45). As the tooth erupts, the reduced enamel epithelium grows gradually shorter. A shallow groove, the gingival

*Some confusion may result if the student refers to the older literature in which the attachment epithelium is referred to as the epithelial attachment. It was first named the epithelial attachment (Epithelansatz) by Gottlieb, but after it was examined electron microscopically, it was renamed the junctional, or attachment epithelium by Stern. This epithelium synthesizes the material that attaches it to the tooth. This material, its morphology, mode, and mechanism of function, is what is now called the epithelial attachment. Thus the cellular structure is referred to as junctional or attachment epithelium, and its extracellular tooth attaching substance is referred to as the epithelial attachment.

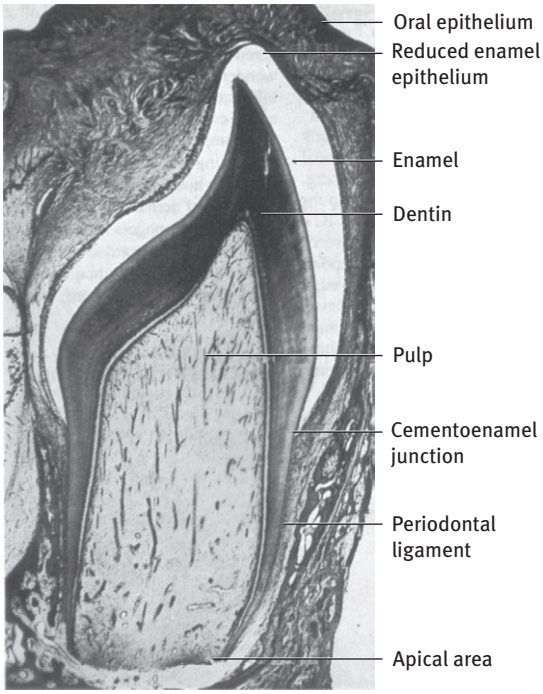


Figure 10.42 Human permanent incisor. Entire surface of enamel is covered by reduced enamel epithelium. Mature enamel is lost by decalcification (From Gottlieb B and Orban B: Biology of the investing structures of the teeth. In Gordon SM, editor: Dental science and dental art, Philadelphia, 1938, Lea and Febiger).

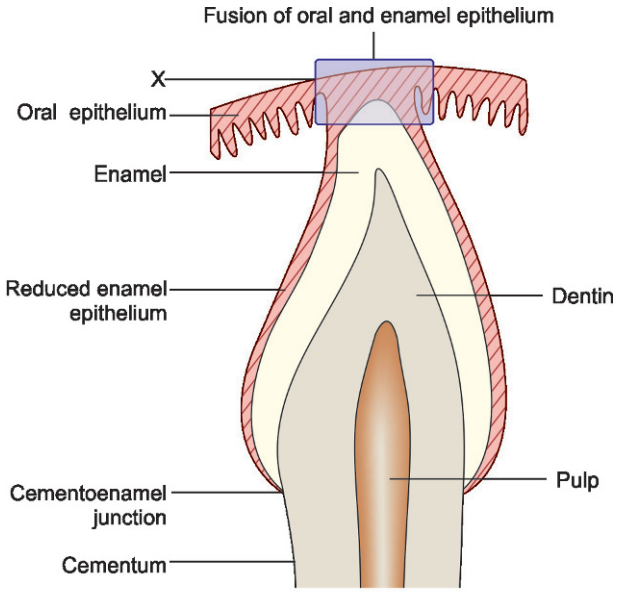


Figure 10.43 Reduced enamel epithelium fuses with oral epithelium. X in diagram indicates area from which photomicrograph was taken.

sulcus (Fig. 10.44), may develop between the gingiva and the surface of the tooth and extend around its circumference. It is bounded by the attachment epithelium at its base and by the gingival margin laterally. It deepens as a result of separation of the reduced dental epithelium from the actively erupting tooth. The gingiva encompassing the sulcus is the free or marginal gingiva.

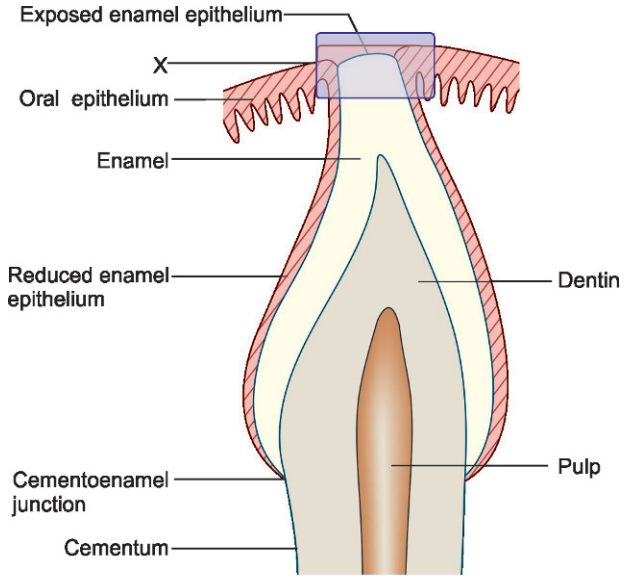


Figure 10.44 Tooth emerges through perforation in fused epithelia. X in diagram indicates area from which photomicrograph was taken.

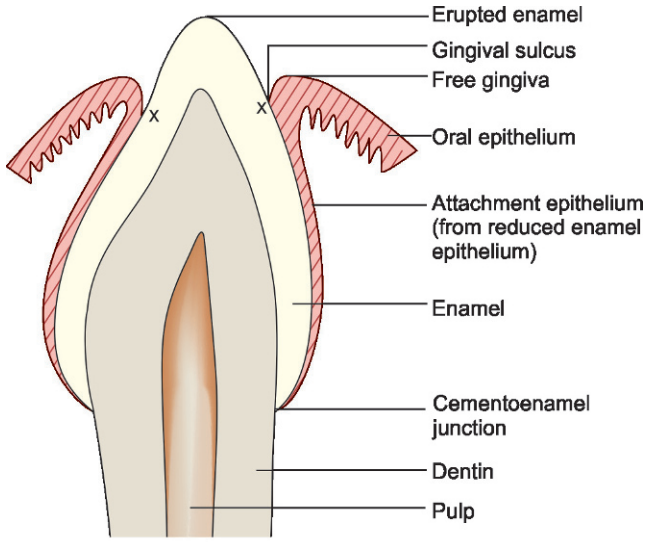


Figure 10.45 Diagram of attached epithelial cuff and gingival sulcus at an early stage of tooth eruption. Bottom of sulcus at X.

Shift of dentogingival junction

The position of the gingiva on the surface of the tooth changes with time. When the tip of the enamel first emerges through the mucous membrane of the oral cavity, the epithelium covers almost the entire enamel (Fig. 10.46). The tooth erupts until it reaches the plane of occlusion (see Chapter 13). The attachment epithelium separates from the enamel surface gradually while the crown emerges into the oral cavity. At first after the tip of the crown has appeared in the oral cavity, the epithelium separates rapidly from the surface of the tooth. Later, when the tooth comes to occlude with its antagonist, the separation of the attachment from the surface of the tooth slows down. When the tooth first reaches the plane of occlusion, one third to one fourth of the

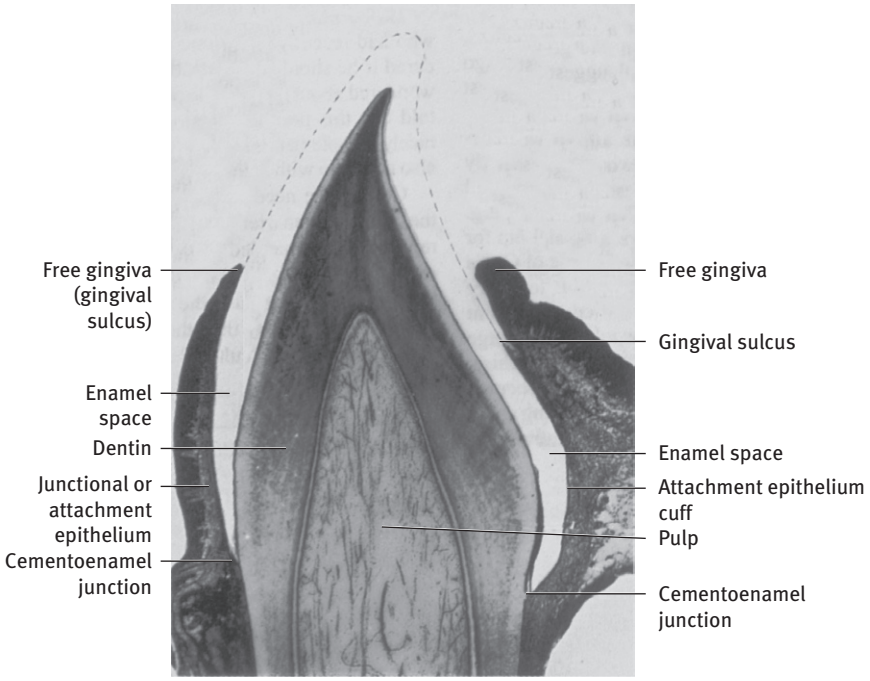


Figure 10.46 Attachment epithelium and gingival sulcus in erupting tooth. Dotted line, Erupted part of enamel. Enamel is lost in decalcification (From Kronfeld R: J Am Dent Assoc 18:382, 1936).

enamel still remains covered by the gingiva (Fig. 10.47). A gradual exposure of the crown follows. The actual movement of the teeth toward the occlusal plane is termed active eruption. This applies to the preclinical phase of eruption also. The separation of the primary attachment epithelium from the enamel is termed passive eruption. Further recession exposing the cementum

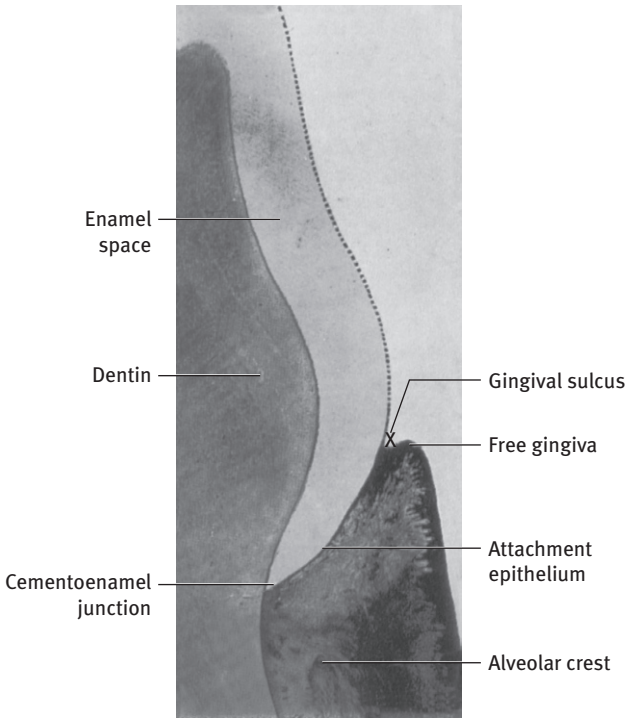


Figure 10.47 Tooth in occlusion. One fourth of enamel is still covered by attachment epithelium (From Kronfeld R: J Am Dent Assoc 18:382, 1936).

may ultimately occur. While the reduced ameloblasts are still present, the cells of the oral epithelium join them by forming desmosomes. Gradually the reduced enamel epithelium is lost, and the cells of the oral epithelium contact the tooth surface, forming hemidesmosomes and a lamina lucida, by means of which the cells attach themselves to the tooth. The replacement of primary attachment epithelium by cells derived from the gingival epithelium is called secondary attachment epithelium.

There is a conceptual construct, called passive eruption, that may be useful in describing the various levels of attachment that may occur as the gingiva recedes onto the cementum. Some persons believe passive eruption to be a normal occurrence with aging. The belief that this is a 'normal' occurrence is probably incorrect. Crown exposure involving passive eruption and further recession has been described in four stages. The first two may be physiologic. Many conceive of the last two as normal also, but they are probably pathologic.

First stage. The bottom of the gingival sulcus remains in the region of the enamel-covered crown for some time, and the apical end of the attachment epithelium (reduced enamel epithelium) stays at the cementoenamel junction (Fig. 10.48).

This relation persists in primary teeth almost up to 1 year of age before shedding and in permanent teeth, usually to the age of 20 or 30 years. However, this relation is subject to a wide range of variation (Fig. 10.49).

Second stage. The bottom of the gingival sulcus is still on the enamel, and the apical end of the attachment epithelium has shifted to the surface of the cementum (Fig. 10.50).

The downgrowth of the attachment epithelium along the cementum is but one facet of the shift of the dentogingival junction. This entails dissolution of fiber bundles that were anchored in the cervical parts of the cementum, now

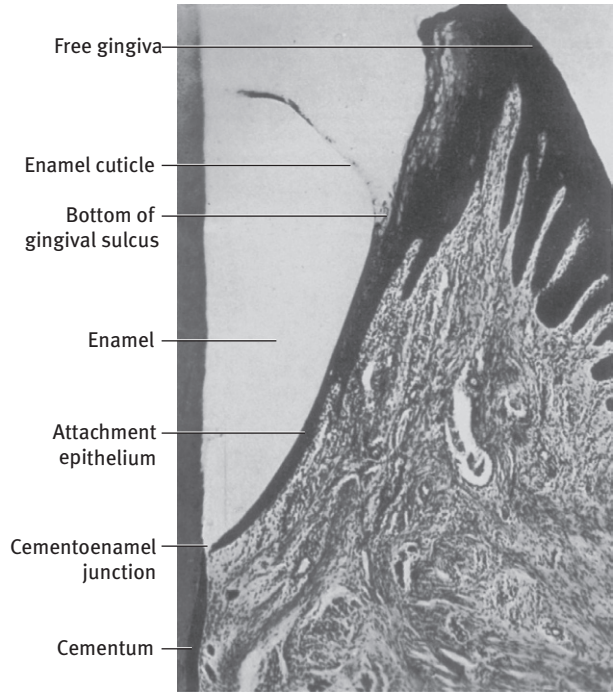


Figure 10.48 Attachment epithelium on enamel. First stage of crown exposure (From Gottlieb B and Orban B: Biology and pathology of the tooth [translated by M Diamond], New York, 1938, The Macmillan Co).

covered by the epithelium, and an apical shift of the gingival and transseptal fibers. The destruction of the fibers may be caused by enzymes formed by the epithelial cells, by plaque metabolites or enzymes, or by immunologic reactions as manifestations of periodontal disease. This stage of tooth exposure may persist to the age of 40 years or later.

Third stage. When the bottom of the gingival sulcus is at the cementoenamel junction, the epithelium attachment is entirely on the cementum, and the enamel-covered crown is fully exposed (Fig. 10.51). This stage in the exposure of a tooth no longer is a passive manifestation. The epithelium shifts along the surface of the tooth and does not remain at the cementoenamel junction. This discontinuous and slow process is regarded as the body's attempt to maintain an intact dentogingival junction in the face of factors that cause its deterioration.

Fourth stage. The fourth stage represents recession of the gingiva. When the entire attachment is on cementum, the gingiva may appear normal but is believed to have receded as a result of pathology (Figs 10.52, 10.53). It may occur without other clinical evidence of inflammatory periodontal disease.

The rates of crown exposure and recession vary in different persons. In some cases the fourth stage is observed in persons during their twenties. In others, even at 50 years of age or older, the teeth are still in the first or second stage. The rate varies also in different teeth of the same jaw and on different surfaces of the same tooth. One side may be in the first stage and the other in the second or even the fourth stage (Fig. 10.53).

Gradual exposure of the tooth makes it necessary to distinguish between the anatomic and the clinical crowns of the tooth (Fig. 10.54). That part of the tooth covered by enamel is the anatomic crown. The clinical crown is the part of the tooth exposed in the oral cavity. In the first and second stages the clinical crown is smaller than the anatomic crown. With recession (third stage) the entire enamel-covered part of the tooth is exposed, and the clinical crown is equal to the anatomic crown. Later the clinical crown is larger than the anatomic crown because parts of the root have been exposed (fourth stage). This type of crown exposure is to be differentiated from crown exposure that is produced by periodontal disease, loss of attachment (bone), and pocket formation.

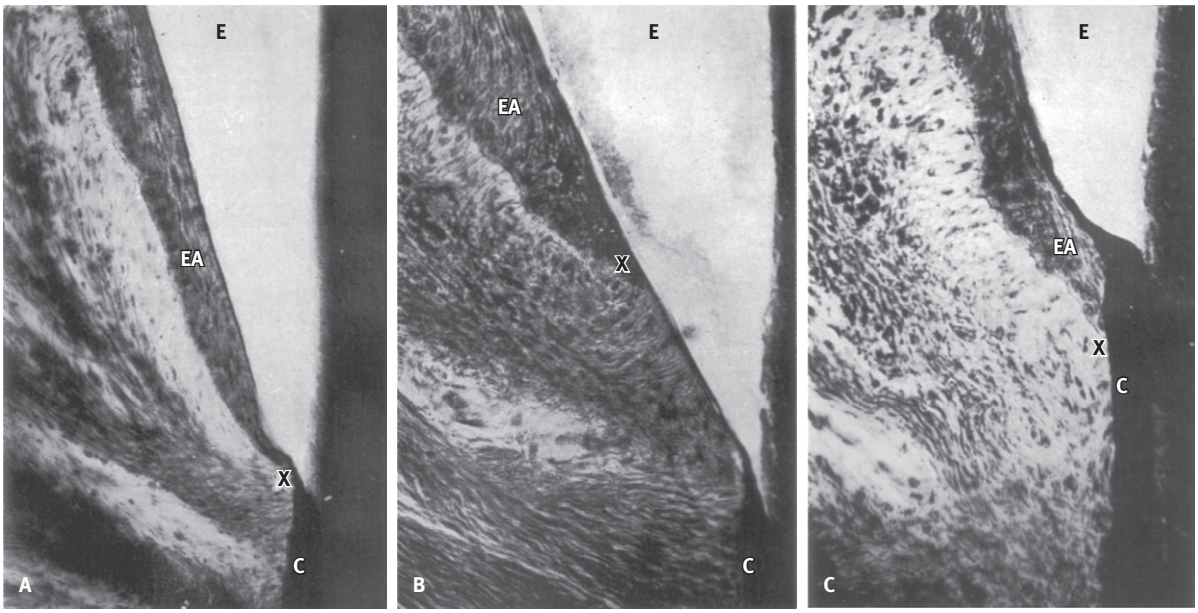


Figure 10.49 Three sections of same tooth showing different relations of tissues at cementoenamel junction. **(A)** Attachment epithelium reaching to cementoenamel junction. **(B)** Attachment epithelium ends coronally to cementoenamel junction. **(C)** Attachment epithelium covers part of cementum. Cementum overlaps edge of enamel. C, Cementum; E, enamel (lost in decalcification); EA, attachment epithelium; X, end of attachment epithelium (From Orban B: J Am Dent Assoc 17:1977, 1930).

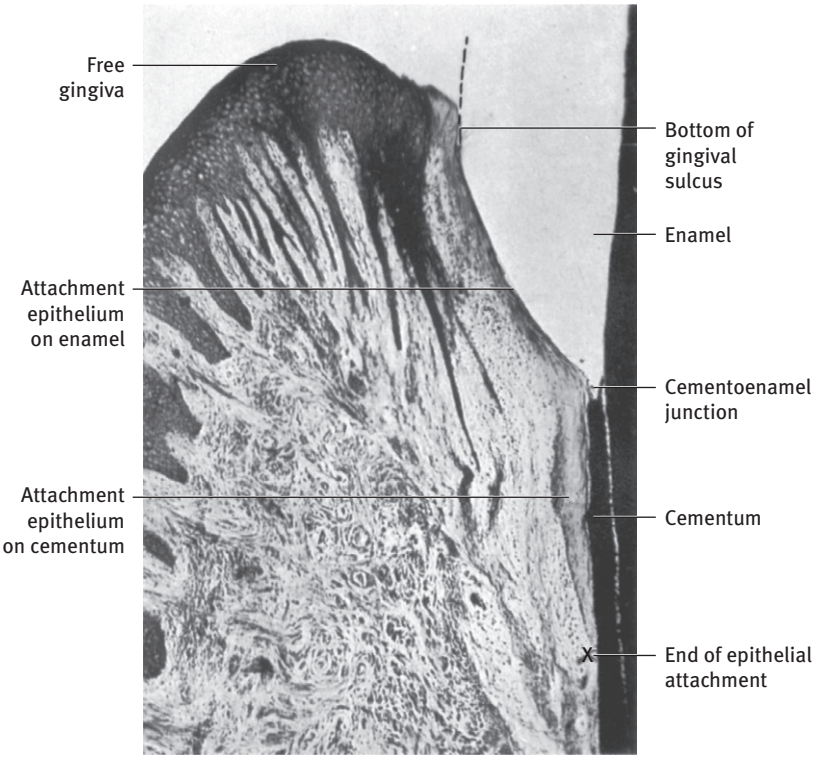


Figure 10.50 Attachment epithelium partly on enamel and partly on cementum. Second stage of passive tooth exposure (From Gottlieb B and Orban B: Biology and pathology of the tooth [translated by M Diamond], New York, 1938, The Macmillan Co).

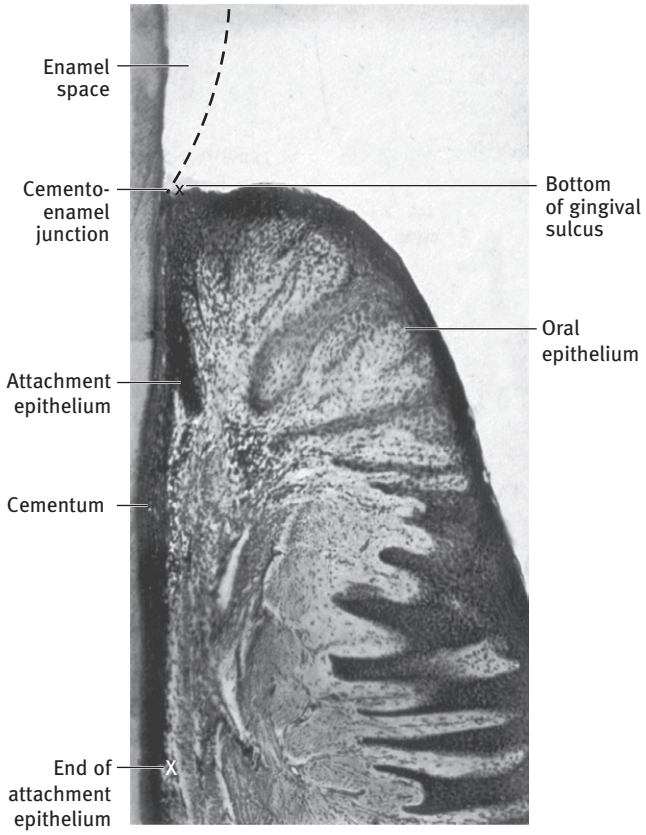


Figure 10.51 Recession is at bottom of gingival sulcus at cementoenamel junction, and attachment epithelium is on cementum (From Gottlieb B: J Am Dent Assoc 14:2178, 1927).

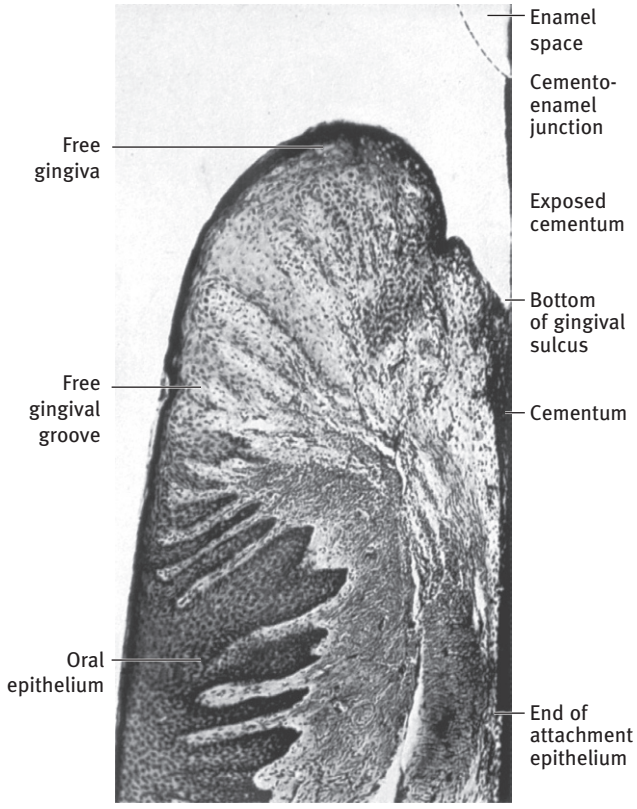


Figure 10.52 Recession. Bottom of gingival sulcus and attachment epithelium both on cementum. Continued recession may reduce the width to gingiva (From Gottlieb B: J Am Dent Assoc 14:2178, 1927).

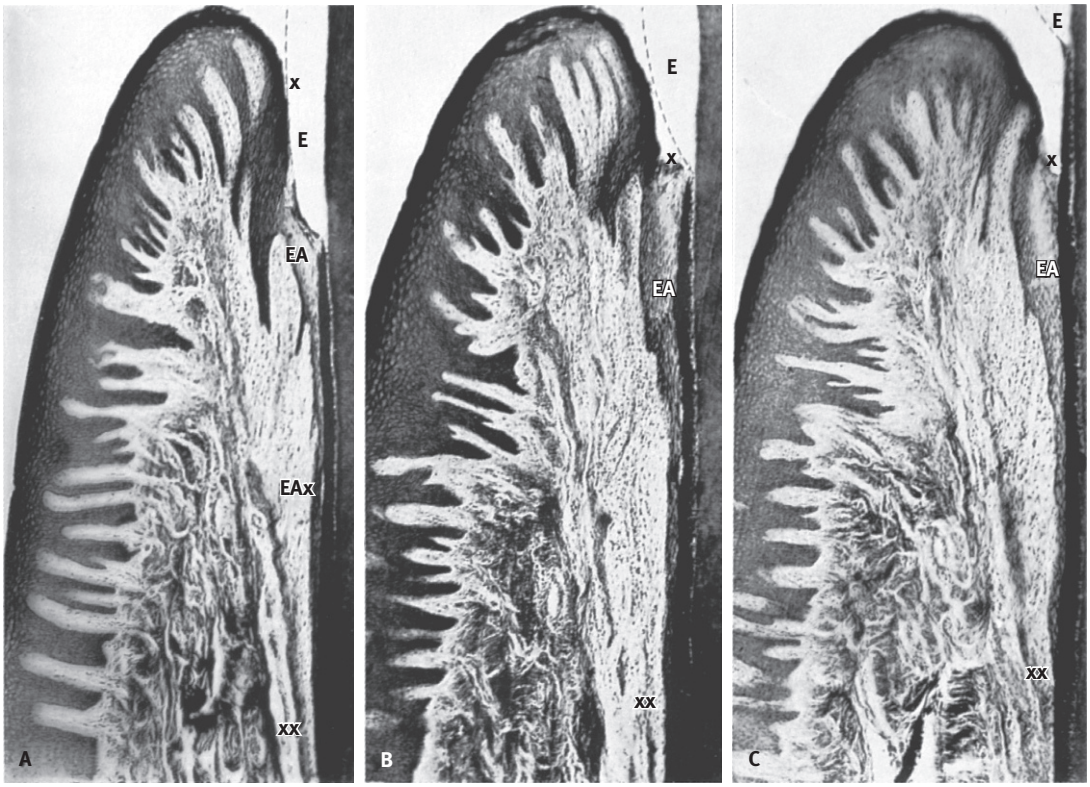


Figure 10.53 Three sections of same tooth showing different relation of soft to hard tissues. **(A)** Bottom of sulcus on enamel (second stage). **(B)** Bottom of sulcus at cementoenamel junction (third stage). **(C)** Bottom of sulcus on cementum (fourth stage). E, Enamel lost in decalcification (dotted line); EA, attachment epithelium; X, bottom of gingival sulcus; XX, end of attachment epithelium.

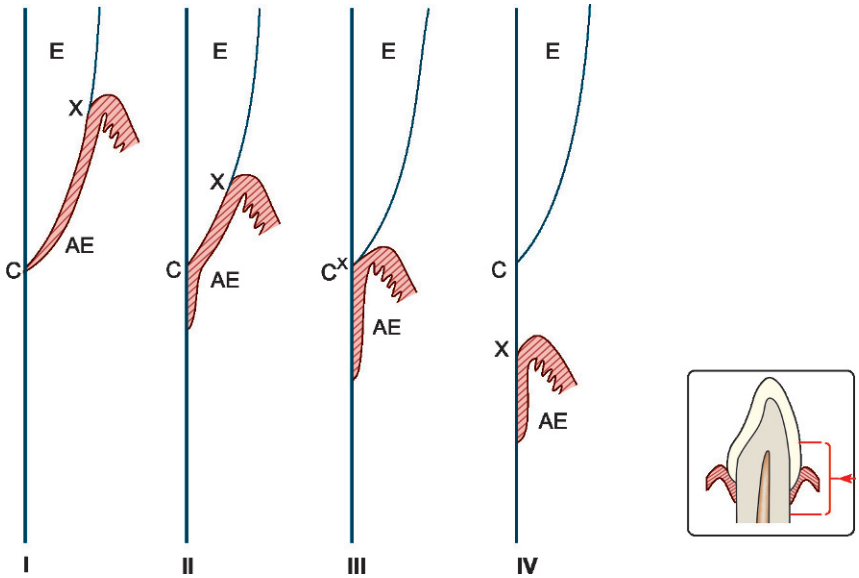


Figure 10.54 Diagram of four stages in eruption. In stages I and II (passive eruption), anatomic crown is larger than clinical crown. III and IV represent recession. In stage III, anatomic and clinical crowns are equal. In stage IV, clinical crown is larger than anatomic crown. Arrow in small diagram indicates area from which drawings were made. C, Cementoenamel junction; E, enamel; AE, attachment epithelium; X, bottom of gingival sulcus.

Sulcus and cuticles

When the ameloblasts are replaced by the oral epithelium, a secondary cuticle is formed. When the epithelium proliferates beyond the cementoenamel junction, the cuticle extends along the cementum (Figs 10.55, 10.56). Secondary enamel cuticle and the cemental cuticle are referred to as dental cuticle. These cuticles are microscopically evident as an amorphous material between the attachment epithelium and the tooth.

Epithelial attachment

The ultrastructural attachment of the ameloblasts (primary attachment epithelium) to the tooth was first shown

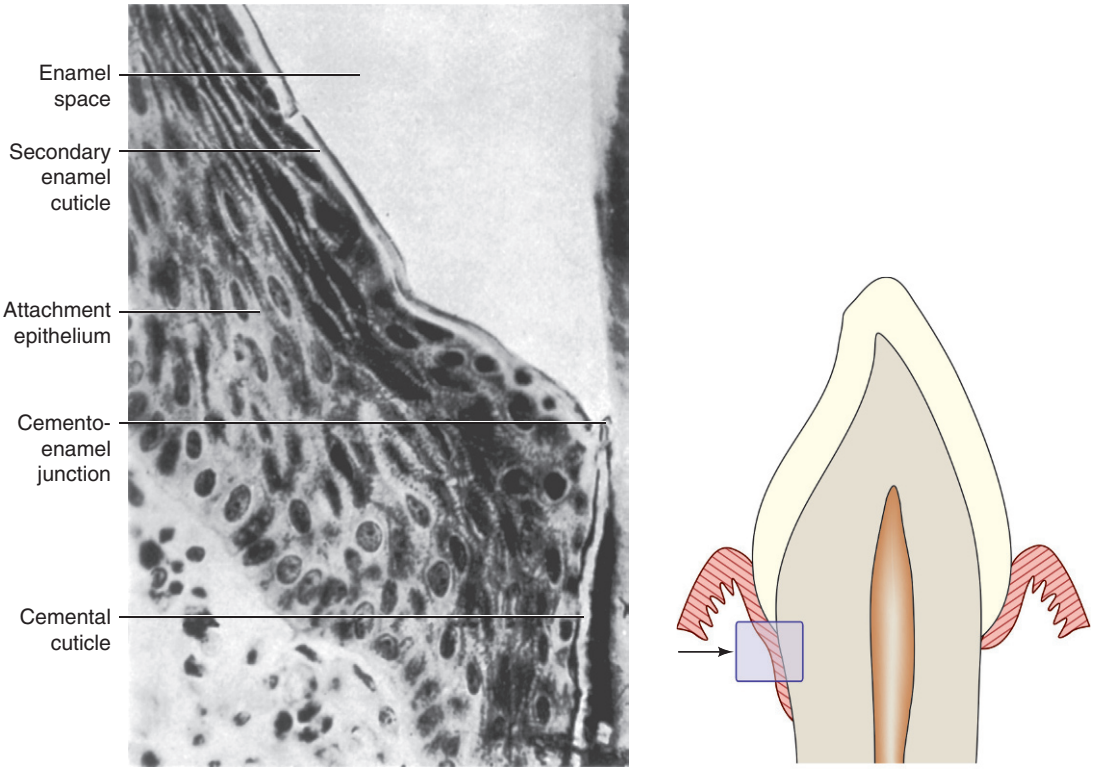


Figure 10.55 'Secondary enamel cuticle' follows attachment epithelium to cementum forming the dental cuticle. Arrow in diagram indicates area from which photomicrograph was taken.

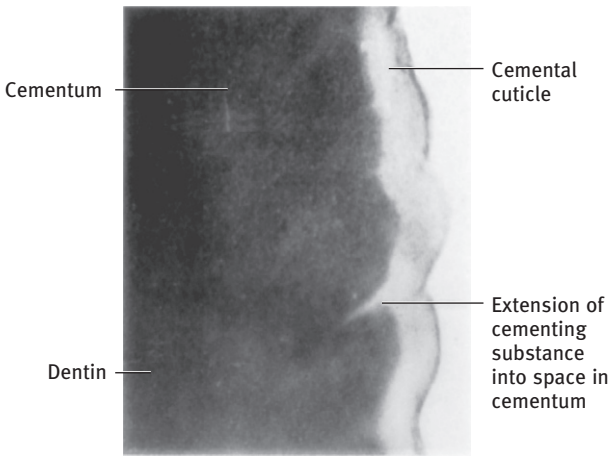


Figure 10.56 Cemental cuticle extending into cementum (From Gottlieb B and Orban B: Biology and pathology of the tooth [translated by M Diamond], New York, 1938, The Macmillan Co).

by Stern and confirmed by Listgarten and Schroeder, among others, to be basal lamina to which hemidesmosomes are attached. This mode of attachment is referred to as the epithelial attachment. It is submicroscopic, approximately 40 nm (400 Å) wide, and formed by the attachment epithelium. The adhesive forces in this zone are molecular in nature and act across a distance smaller than 40 nm (400 Å).

The secondary attachment epithelium composed of cells derived from the oral epithelium forms an epithelial attachment identical with that of the primary attachment epithelium, that is, a basal lamina and hemidesmosomes. Both reduced ameloblasts and gingival epithelial cells have been shown to form an electron microscopic basal lamina on enamel and cementum. Hemidesmosomes of these cells attach to the basal lamina in the same manner as all basal cells. This basal lamina is referred to as internal basal lamina so as to distinguish between the basal lamina present between the junctional epithelium and lamina propria which is referred to as external basal lamina. The internal basal lamina differs from the external basal lamina in that they lack laminin, anchoring fibrils and type IV collagen (Figs 10.57, 10.58).

Migration of attachment epithelium

Mitotic figures have been observed in cells adjacent to the tooth. When tritiated thymidine is administered to experimental animals, cells about to undergo DNA synthesis pick up radioactive thymidine. The radioactivity can be detected in histologic sections by the use of photographic emulsion. After the administration of the tritiated thymidine, labeled cells are found in the attachment epithelium (Fig. 10.59).

When cells leave the stratum germinativum, they become specialized. For instance, in oral epithelium cells specialize and undergo keratinization. In attachment epithelium the cells may remain relatively unspecialized and synthesize a basal lamina (the epithelial attachment). The lamina propria below the junctional epithelium is the deep connective tissue of the periodontal ligament (unlike other lamina propria) which keeps the epithelial cells of the junctional epithelium immature so that it can develop hemidesmosomes and attach to the tooth. They then migrate over it, with their attachment being maintained by the hemidesmosomes. It turns about the most

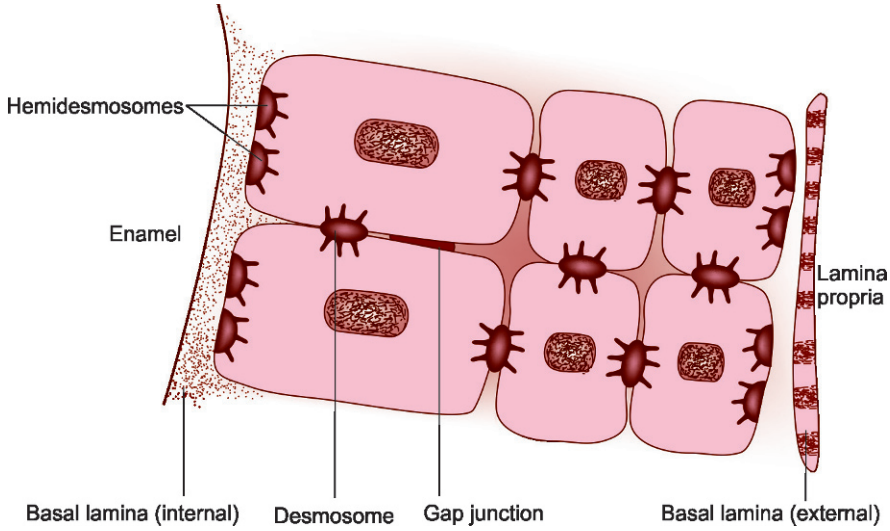


Figure 10.57 Diagrammatic representation of junctional epithelium.

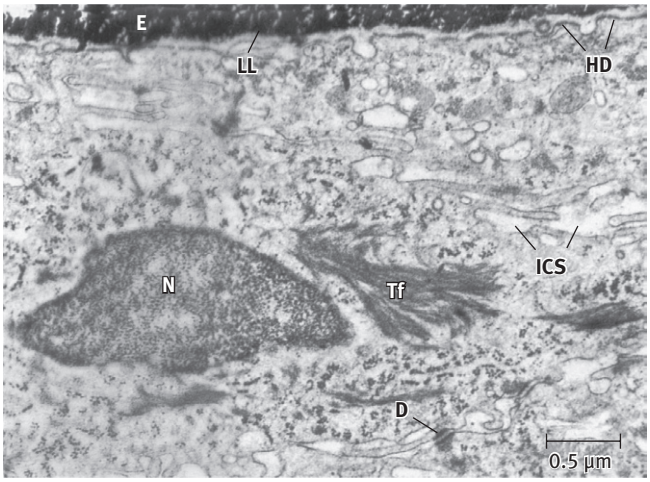


Figure 10.58 Electron micrograph of cells of attachment epithelium of rat incisor adjacent to enamel, E. Hemidesmosomes, HD, abut on and attach to lamina lucida, LL. Lamina densa is fully calcified and cannot be demonstrated in this calcified specimen. Lamina lucida is approximately 40 nm (400 Å) wide. Note that intercellular space, ICS, is wider than lamina lucida. Cells are attached to each other by desmosomes, D N, Nucleus; Tf, a bundle of tonofilaments (From Grant DA, Stern IB, and Listgarten MA; Periodontics in the tradition of Orban and Gottlieb, ed 6. St Louis, 1988, The CV Mosby Co).

apical cell and extends up along the tooth surface. The cells can then migrate along this basal lamina (Fig. 10.60). The hemidesmosomes hold the cells to this structure so that the strength of the attachment is not diminished despite the migration. The physical integrity of the attachment is maintained during the four stages of tooth exposure by this same biologic mechanism.

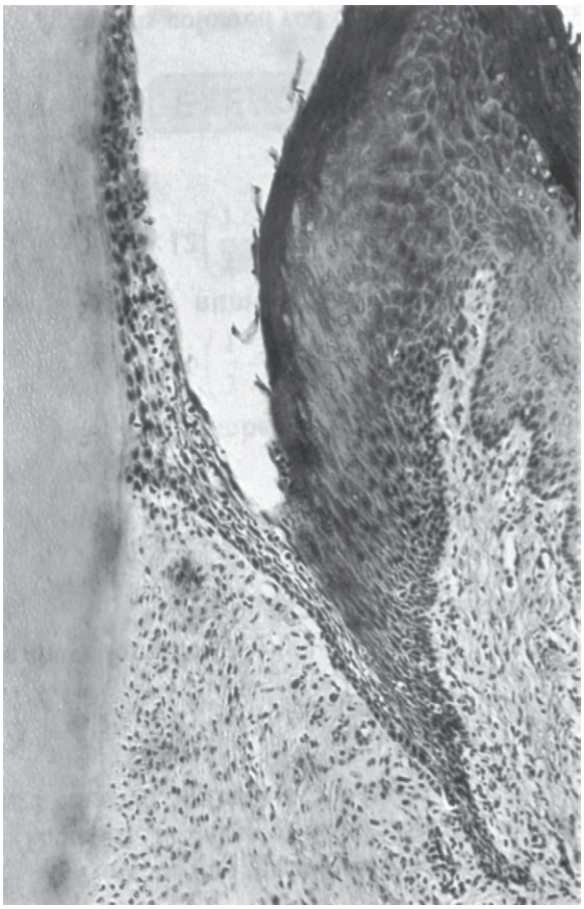
The apical migration of the sulcus is the result of a detachment of basal cells and a re-establishment of their epithelial attachment at a more apical level. It is not the result of degeneration and peeling off of the most coronal cells of the attachment epithelium. Perhaps toxic or inflammatory influences diminish the ability of the basal cells to synthesize DNA or otherwise interfere with the physiology of these cells. Perhaps collagenolysis destroys the subjacent collagen fibers, permitting the epithelium to migrate apically. Perhaps immunologically competent cells or antibody complexes produce tissue damage and permit the epithelium to migrate apically. In any event the junctional epithelium moves apically, replicates a new basal lamina, and re-establishes the epithelial attachment. If this results in a deepening of the sulcus, as gauged by a difference in the position of the top of the epithelial attachment relative to the marginal gingiva, a pocket will have formed.

The time it takes for labeled attachment epithelial cells to migrate and desquamate, i.e. the turnover time is about 72 to 120 hours for primates, and presumably much the same for humans (Fig. 10.59).

The reduced ameloblasts do not divide; however, on the other hand, basal cells adjacent to the tooth do divide and then migrate up and along the tooth, desquamating in 4 to 6 days. They seem to migrate from a mitotically DNA-synthetic active area, a locus of proliferation, in the basal layer at the junction of the oral and the attachment epithelia.

DEVELOPMENT OF ORAL MUCOSA

The epithelium of the oral cavity is derived from both the ectoderm and the endoderm. The anterior part of the oral cavity is lined by the epithelium derived from the ectoderm. The structures that develop from the branchial arches have their epithelium derived from the endoderm. These include the tongue, epiglottis and pharynx. The vestibular lamina separates from the primary epithelial band at about 6 weeks. Degeneration of the cells in the central part of this process leads to the formation of labial and buccal sulcus and the delineation of lips and cheeks from the alveolar mucosa. By 13–20 weeks differences between keratinized and nonkeratinized mucosa becomes apparent. Keratohyalin granules in the keratinized mu-



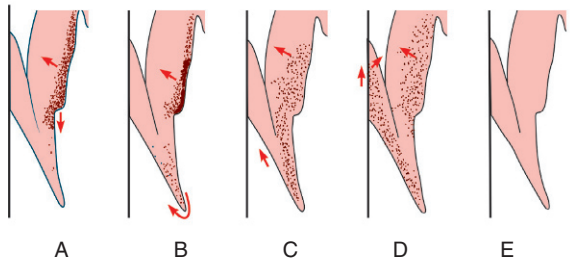


Figure 10.59 Composite of labeled cells and their positions: (A) ½ hour; (B) 6 hours; (C) 24 hours; (D) 72 hours; and (E) 144 hours after administration of tritiated thymidine to rats. Diagram of morphology of attachment epithelium and adjacent tissues is representative of gingiva on cemental (oral) surface of continuously growing rat incisor. Large arrows, Migration of attachment (junctional) epithelium toward and along tooth surface. Small arrows, Migration of cells toward sulcus (From Anderson GC and Stern IB: Periodontics 4:115, 1966).

cosa and region specific cytokeratins appear. The epithelium remains parakeratinized in masticatory mucosa, orthokeratinization is seen only after teeth erupt. Langerhans cells and melanocytes also appear during this period. Lingual papillae appear early at about 7th week; the circumvallate and foliate papillae appear earlier than filiform papillae, which can be recognized by 10–12 weeks.

In the lamina propria, reticular fibers are the first to appear (6–8 weeks) followed by the collagen fibers (8–12 weeks) and the elastic fibers appear much later by about 17–20 weeks.

AGE CHANGES IN ORAL MUCOSA

With age the oral mucosa becomes smooth and dry. These are due to epithelium becoming thin mainly due to the reduction in the thickness of epithelial ridges and decrease in the salivary secretion. The filiform papilla becomes reduced and the tongue appears smooth owing to the reduction in the thickness of the epithelium. Nutritional deficiencies may also be a contributing factor for this change. Varicose veins on the ventral aspect of tongue are often seen and these are termed as lingual varices. Langerhans cells become fewer with age, which may contribute to decline in cell mediated immunity. In the lamina propria cellularity decreases but collagen content increases. Minor salivary glands show considerable atrophy with fibrous replacement. Nerves and end organs in the oral mucosa may also be affected by age. The effects include a progressive loss of sensitivity to thermal, chemical and mechanical stimuli, and with decline in taste perception.

CLINICAL CONSIDERATIONS

It is essential to be thoroughly familiar with the structure and biologic interrelations of the various periodontal tissues in order to understand the pathogenesis of periodontal disease. Periodontal disturbances produce a deepened gingival sulcus, called periodontal pocket, as a response to plaque toxins and the subsequent immunologic response. Reduction in pocket depth is the primary objective of treatment. Treatment methods should be judged by their ability to reduce the depth of pockets and to prevent their recurrence.

Injury of the junctional epithelium may occur through accidental or intentional trauma, toothbrushing, flossing, or clinical probing. Since the junctional epithelium is located at a strategically important and delicate site, it may be expected that it should be very well adapted to cope with mechanical insults. The conversion of the junctional epithelium to pocket epithelium is regarded as a hallmark in the development of periodontitis.

The level of the gingival attachment to the tooth plays an important role in restorative dentistry. In young persons the clinical crown is smaller than the anatomic crown. It is therefore very difficult to prepare a tooth properly for an abutment or crown in young individuals. Moreover, when recession occurs at a later time, the restoration may require replacement.

When the root is exposed by recession and a restoration is to be placed, the preparation need not extend to the gingiva. The first requirement is that the restoration be adapted to mechanical needs. In extension of the gingival margin of any restoration the following rules should be observed. If the gingiva is still on the enamel and the gingival papilla fills the entire interdental space, the gingival margin of a cavity should be placed at the sulcus. Special care should be taken to avoid injury to the gingiva and the dentogingival junction, and to prevent premature recession of the gingiva. When periodontal disease is present, treatment should precede the placing of a restoration. If the gingiva has receded to the cementum and the gingival papilla does not fill the interdental space, the margin of a cavity need not necessarily be carried to the gingiva.

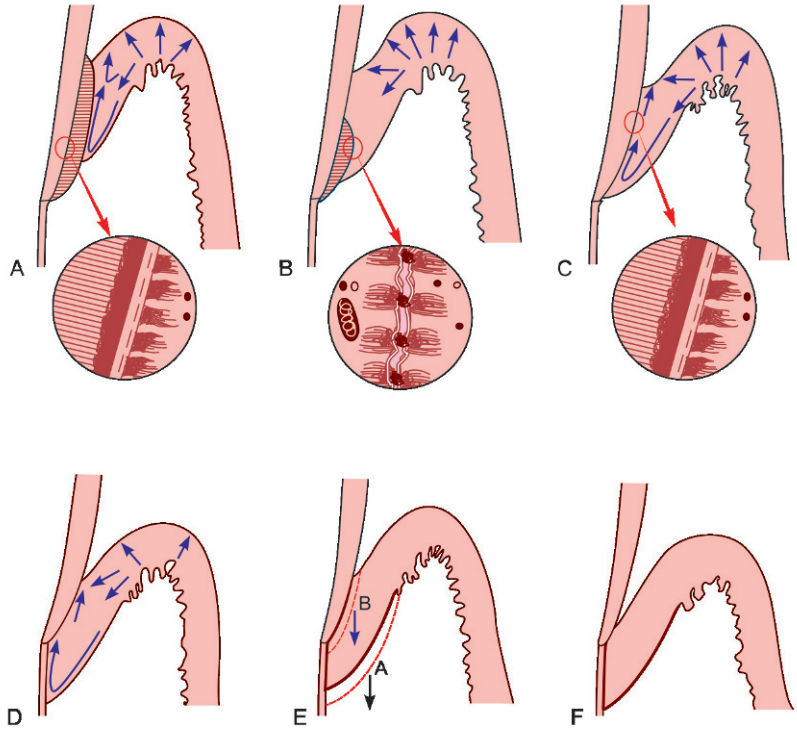


Figure 10.60 Dynamics of migration of tissues of dentoepithelial junction. **(A)** Dentoepithelial junction first consists of reduced ameloblasts attached by hemidesmosomes to lamina lucida. Oral epithelial cells migrate to gingival surface and keratinize (arrows). Some cells join reduced enamel epithelium, to which they attach. **(B)** Reduced ameloblasts are gradually displaced by junctional epithelium, cells of which are joined by desmosomes and by tight and gap junctions. When reduced enamel epithelium gives rise to junctional epithelium, mitotic activity is increased. Here cells of outer enamel epithelium, and possibly stratum intermedium, form a locus of proliferation. **(C)** With complete replacement of reduced enamel epithelium by junctional epithelium, attachment occurs by same mechanism as shown in A. **(D)** In time junctional epithelium may be found attaching to both enamel and cementum. (**E**) Junctional epithelium renews itself in a matter of days, as does gingival epithelium. Cells migrate in pathways denoted by arrows in D. Cells of junctional epithelium travel from basal lamina to epithelial attachment. In inflammation, basal cells at A migrate apically and laterally into areas of collagenolysis. They form new basal lamina. Arrow at B represents deepening of sulcus. (**F**) Even when junctional epithelium has completely migrated onto cementum, attachment is still mediated by basal lamina and by hemidesmosomes.

With gingival recession and exposure of the cervical part of the anatomic root, cemental caries or abrasion may occur. Improperly constructed clasps, overzealous scaling, and strongly abrasive dentifrices may result in pronounced abrasion. After loss of the cementum, the dentin may be extremely sensitive to thermal or chemical stimuli. Desensitizing drugs, judiciously applied, may be used to accelerate sclerosis of the tubules and reparative dentin formation.

The difference in the structure of the submucosa in various regions of the oral cavity is of great clinical importance. Whenever the submucosa consists of a layer of loose connective tissue, edema or hemorrhage can cause much swelling, and infection can spread speedily and extensively. Incision in lining mucosa creates gaping wounds, because it is loosely bound to the underlying structures and therefore suturing becomes mandatory. Incision in masticatory mucosa does not cause gaping wounds owing to the firmness in its attachment to the underlying bone. For a similar reason infiltration of local anesthesia into masticatory mucosa is difficult and causes pain but in the lining mucosa, infiltration is easy, the fluid gets dispersed easily and swelling or pain does not occur. Injections should be made into loose submucous connective tissue (i.e. the fornix and the alveolar mucosa). The only place in the palate where larger amounts of fluid can be injected without damaging the tissues is the loose connective tissue in the furrow between the palatal and the alveolar processes (Fig. 10.20).

Permeability of oral mucosa varies for different areas within oral cavity. Differences in permeability may be related to regional differences in the prevalence of certain mucosal diseases and can be utilized to advantage for local drug delivery, the floor of the mouth is an appropriate area for some medication for the rapid absorption.

In regeneration of oral epithelium, lamina propria plays a major part in determining the type of epithelium: keratinized or nonkeratinized. Healing in oral mucosa is faster than in skin owing to its profuse blood supply and due to its higher turnover rate of epithelium. Healing in oral mucosa invariably is not accompanied by a scar. This is explained in the gingiva as being probably due to the fetal like nature of its collagen.

Anticancer drugs, which affect the mitosis of rapidly proliferating cells affect the oral mucosa due to its high turnover rate, resulting in ulceration.

The bacteria colonized to the most superficial layer of epithelium are shed along with the cell. If the cells have a nucleus they have the ability to proliferate. The desquamated cells are present in the saliva usually settle on the

rough surface of the dorsum of tongue forming a white coating. The thickness of the coating increases in states, where mouth becomes dry as occurring in fever.

The gingiva is exposed to heavy mechanical stresses during mastication. Moreover, the epithelial attachment to the tooth is relatively weak, and injuries or infections can cause permanent damage. Keratinization of the gingiva may afford relative protection. Therefore steps taken to increase keratinization can be considered as preventive measures. One of the methods of increasing keratinization is by massage or brushing, which acts directly by stimulation, and by minimizing plaque accumulation.

Unfavorable mechanical irritation of the gingiva may ensue from sharp edges of carious cavities, overhanging fillings or crowns, and accumulation of plaque. These may cause chronic inflammation of the gingival tissue.

Many systemic diseases cause characteristic changes in the oral mucosa. For instance, metal poisoning (lead, bismuth) causes characteristic discoloration of the gingival margin. Leukemia, pernicious anemia, and other blood dyscrasias can be diagnosed by characteristic infiltrations of the oral mucosa. In the first stages of measles, small red spots with bluish white centers can be seen in the mucous membrane of the cheeks, even before the skin rash appears. They are known as Koplik's spots. Endocrine disturbances, including those of the sex hormones and of the pancreas, may be reflected in the oral mucosa.

Changes of the tongue are sometimes diagnostically significant. In scarlet fever the atrophy of the lingual mucosa causes the peculiar redness of the strawberry tongue. Systemic diseases such as pernicious anemia and vitamin deficiencies, especially Vitamin B-complex deficiency, lead to characteristic changes such as magenta tongue and beefy red tongue.

In denture construction it is important to observe the firmness or looseness of the mucous membrane. In denture-bearing areas the mucosa should be firm.

In a large percentage of individuals the sebaceous glands of the cheek are visible as spots, yellowish spots called Fordyce spots. They do not represent a pathologic change (Fig. 10.30).

Autoantibodies are produced against autoantigens in certain vesiculobullous dermatologic disorders like pemphigus (desmogleins), linear IgA disease (basement membrane zone antigens), which have similar oral manifestations. Monoclonal antibodies to these specific antigens are of diagnostic value.

Mutation of genes encoding for cytokeratin causes various epithelial diseases affecting skin and oral mucosa. For example, mutation of genes encoding for CK5 and CK14 are the causes of epidermolysis bullosa, a vesiculobullous lesion and CK4 and CK13 for white spongy nevus, a congenital keratin disorder.

Various genes like p53, p16, p21, bcl-2, etc. are involved in the regulation of epithelial cell proliferation. The altered expression of these genes occurs in oral cancer.

SUMMARY

Oral mucosa lines the oral cavity. It is continuous with skin of the lip through the vermilion border and with the mucosa of pharynx posteriorly.

Classification (Regions) of Oral Mucosa

Oral mucosa is classified into three distinct regions namely, the masticatory mucosa comprising of gingiva and hard palate, the lining mucosa comprising of lips, cheeks and all other regions except the dorsum of tongue. The mucosa of the anterior 2/3rd of the dorsum of tongue is known as specialized mucosa.

Functions of Oral Mucosa

The functions of oral mucosa are to protect the underlying structures, lubricate the mucosa for swallowing and speech function and to defend against the entry of bacteria and their toxins.

Parts of Oral Mucosa

Mucosa consists of a stratified squamous epithelium and the connective tissue called lamina propria. The mucosa is attached to the underlying structure, which is either bone or muscle, by a loose connective tissue called the submucosa.

Differences between Masticatory and Lining Mucosa

The masticatory mucosa is tightly bound to the bone while the lining mucosa is loosely attached to muscles to allow distention. The epithelium is keratinized, the lamina propria is dense and the submucosa is thin in masticatory mucosa while in lining mucosa the epithelium is nonkeratinized, lamina propria is not dense and submucosa is prominent.

Differences between Skin and Oral Mucosa

Oral mucosa differs from skin in that the epithelium of skin is always orthokeratinized and it contains appendages like hair follicles, sweat glands, and sebaceous glands.

Basement Membrane

The epithelium is separated from the lamina propria by the basement membrane. Ultrastructurally this interface is called basal lamina and it consists of a clear lamina lucida and a dense lamina densa. The lamina densa consists of collagen fibrils of which type VII forms loops (anchoring fibrils) through which pass types I and II. The lamina lucida is a glycoprotein layer containing collagen fibrils type IV, and is immediately subjacent to the epithelium.

Lamina Propria

The lamina propria consists of a papillary layer which occupies the spaces between the epithelial projections called epithelial ridges and a reticular layer below it. The papillary layer depends upon the length of the epithelial ridges, it can be even absent as in alveolar mucosa. Lamina propria contains type I and II collagen fibers and elastic fibers in lining mucosa which helps to restore tissue form after stretching. Sensory nerve endings of various types are found in the papillae.

Submucosa

The submucosa consists of connective tissue with larger blood vessels, adipose tissue, and minor salivary glands. The submucosa is not well developed in masticatory mucosa, therefore adipose tissue and minor salivary glands may be absent in these regions.

Cells of the Epithelium: Keratinocytes and Nonkeratinocytes

The epithelial cells belong to two groups–the keratinocyte (those having cytokeratin filaments) and the nonkeratinocyte (those without cytokeratin filaments).

The keratinocytes are arranged in different layers, undergo mitotic division, ascend or move towards the surface, also show changes in their morphology, structure and in their function. These changes are known as differentiation and their ascent or cell migration is called maturation. The time taken for a cell to divide and pass through the entire epithelium is called turnover time. Once a cell reaches the surface it gets shed. This process is called desquamation. The nonkeratinocytes, on the other hand, do not show any of these changes—no mitosis, maturation, differentiation, or desquamation. The cells are present in much fewer numbers and have varied functions. Melanocytes, Langerhans cells, and Merkel cells are the nonkeratinocytes. The nonkeratinocytes do not stain with routine H&E stains and therefore are referred to as clear cells. They are also known as dendritic cells because they show dendritic processes.

Nonkeratinocytes: Melanocytes, Langerhans Cells and Merkel Cells

Melanocytes are present amongst the basal cells. They produce melanin pigment and transfer it to the adjacent keratinocytes through their dendritic process. Melanocytes can be studied using Dopa reaction or silver stains. Though melanocytes vary in number it is the amount of melanin that makes the gingiva highly pigmented. Langerhans cells are present in the spinous layers of keratinized mucosa. The cells show characteristic Birbeck granules and stain with gold chloride and ATPase. They are of hemopoietic origin. Their function is to pick up the antigens and present it to the macrophages. Merkel cells are found among basal cells in the keratinized mucosa. They are nondendritic, stain with PAS, and they function like a touch receptor.

Arrangement of Cell Layers in the Epithelium

The keratinocytes are arranged in four layers in the masticatory mucosa or keratinized epithelium and in three layers in the lining mucosa or nonkeratinized epithelium. The layers in the keratinized epithelium are stratum basale, stratum spinosum, stratum granulosum, and the stratum corneum. The basal cells are a single layer of cuboidal cells which rests on the basement membrane, show mitotic activity and protein synthesis. Ultrastructurally they may show serrations and get attached to basement membrane by hemidesmosomes or be nonserrated so that they can undergo differentiation and migrate. Desmosomal junctions exist between adjacent basal cells which contain proteins of the cadherin family. The stratum spinosum shows larger polyhedral cells which show intercellular bridges. Towards surface the cells become flatter with fewer intercellular bridges. The cells synthesize proteins and contain bundles of tonofilaments. The stratum granulosum contains cells which are flatter and wider than the spinous cells. Protein synthesis becomes diminished and the nuclei start to show signs of degeneration. Tonofilaments increase in number and are associated with keratohyaline granules. The contents of the Odland bodies are discharged intercellularly to act as permeability barrier. Involucrin protein thickens the inner cell membrane and helps in crosslinking of tonofilaments. The stratum corneum contains flatter and wider cells with dense bundles of tonofilaments aggregated by filaggrin. In orthokeratinized epithelium, the cells show no organelles or nucleus but in parakeratinized epithelium the cells show pyknotic nuclei and partially lysed organelles. The nonkeratinized epithelium shows only three layers—the stratum basale, stratum intermedium, and the stratum superficiale. The basal cells are same but in the stratum intermedium the polygonal cells are larger, do not exhibit the prickly appearance and show very less amount of tonofilaments. The superficial layer contains wider and flatter cells having nucleus and organelles but with few tonofilaments which are not tightly packed.

Hard Palate

The hard palate mucosa is tightly bound to the bone and shows four zones: the gingival region adjacent to the gingiva, the median or palatine raphae area in the midline, the anterolateral (fatty zone), and the posterolateral (glandular zone) zones. Only the anterolateral and posterolateral zones contain the submucosa, which shows minor salivary glands in the posterolateral and adipose tissue in the anterolateral zones. Vestigial nasopalatine ducts and hyaline cartilage and embryonic epithelial remnants in the form of epithelial pearls may be seen in the incisive papillae area.

Gingiva

The gingiva is that part of oral mucosa which covers the alveolar processes. The mucogingival line separates the gingiva from the alveolar mucosa. The gingiva can be divided into the free gingiva which is coronal most portion of the gingiva, the apical attached gingiva and the interdental papilla in between the teeth. The attached gingiva (which is attached to the underlying bone/tooth) shows a stippled surface and is demarcated from the free gingiva by the free gingival groove. The interdental papilla of the buccal and lingual side is connected by col, which occupies the area below the contact point. The space between the inner aspect of the gingiva and the teeth which are present all around the tooth is called gingival sulcus. Its normal depth is about 1.8 mm. The depth is measured by a probe as the distance between the crest of the free gingiva and the dentogingival junction. The bottom of the gingival sulcus corresponds to the free gingival groove. The free gingiva is absent when there is no gingival sulcus. The epithelium of the gingiva is keratinized but the epithelium of the col and that lining the sulcus (sulcular epithelium) is nonkeratinized. The epithelial ridges of the free gingiva are characteristically slender in appearance. The lamina propria of the gingiva contains dense collagen fibers arranged in groups. These serve to support the free gingiva, bind attached gingiva to bone or teeth and connect one teeth with the other. The main groups of collagen fibers are the dentogingival (connect cervical cementum to gingiva), alveologingival (connect alveolar crest to gingiva) dentoperiosteal (connect cementum to periosteum of the alveolar crest), transseptal (connect adjacent cementum interproximally) and circular (encircle the teeth) fibers. Apart from these groups interdental, semicircular, vertical and transgingival fiber groups are present.

Dentogingival Junction

The dentogingival junction is a unique junction because it is an attachment of an epithelium with a hard tissue. The epithelium of the gingiva which attaches with the enamel or cementum of the tooth is referred to as attachment epithelium or

junctional epithelium. The junctional epithelium extends to about 2 mm on the surface of the tooth. The attachment of the epithelium to the tooth is firm and is further reinforced by the collagen fibers. The union between the tooth and the epithelium is called epithelial attachment. The epithelium resembles the reduced enamel epithelium in that it has a basal layer and few layers of flattened cells which do not show differentiation or keratinization like other parts of oral mucosa. It has a higher turnover rate and is highly permeable to neutrophils and to the passage of gingival fluid into the sulcus. The outward flow of gingival fluid, the lysosomal production and constant presence of defense cells ensure adequate defense against bacterial invasion. Ultrastructurally a basal lamina exists between the tooth and the epithelium with lamina densa towards tooth, and this basal lamina is referred to as internal basal lamina so as to distinguish from the external basal lamina between this epithelium and the lamina propria. The internal basal lamina lacks laminin, anchoring fibrils and type IV collagen.

The position of dentogingival junction changes with time. The epithelium detaches from the crown gradually exposing more amount of crown, and grows apically on to the tooth surface. With the result the bottom of gingival sulcus shifts apically. The physical integrity of the epithelial attachment is maintained at all times during the process. The shift of dentogingival junction has been described in stages; first and second are considered physiological and stages three and four as pathological. In the first and second stages, the bottom of gingival sulcus is on the enamel, in the third it is at CE junction and in the fourth stage it is on the cementum. The apical end of epithelial attachment is at the CE junction in the first stage, in the other stages it is on the cementum. When the tooth makes its appearance in the oral cavity the reduced enamel epithelium is attached to the tooth and this is referred to as primary attachment epithelium. Later the reduced enamel epithelium gets gradually replaced by the growth of gingival epithelium and this is known as secondary attachment epithelium.

Structural Variations in Lining Mucosa in Different Regions

The lining mucosa of different regions differ from one another in the thickness of their epithelium (the epithelium of the floor of the mouth is the thinnest and those of buccal mucosa thickest), the presence of elastic fibers in the lamina propria (soft palate) and in the thickness and content of submucosa (thin submucosa in inferior aspect of tongue, thickest with mixed glands and adipose tissue in labial and buccal mucosa, only mucous glands in soft palate, few mixed glands in alveolar mucosa).

Specialized Mucosa

The dorsolingual mucosa has numerous papillae in the anterior part, many of which contains taste buds which help in the perception of taste and therefore regarded as specialized mucosa. Papillae are epithelial projections with a core of connective tissue. The most numerous of the papillae–the filiform papillae are long thread- like structures with a highly keratinized epithelium, but do not contain taste buds. The large circumvallate papillae present in front of the junction between the anterior and the posterior part of the tongue do not protrude above the surface and has a circular furrow. Von Ebner's glands open into these furrows. The foliate papillae are sometimes present in the lateral border of the posterior part of the tongue. The taste buds are present in large numbers in the lateral wall of the circumvallate papillae, and to some extent in the mushroom- shaped fungiform papillae. Taste buds are barrel-shaped intraepithelial organs which have a taste pore at their free surface. The neuroepithelial cells which are receptors of taste stimuli are slender and show finger like process which extend into the taste pore. Supporting cells are flat on the outer aspect and spindle shaped on the inner aspect of the taste bud. Taste buds are richly supplied with nerves some of which end in contact with the sensory cells. The posterior part shows many lymphoid follicles. With age the oral mucosa becomes dry, smooth and thin. The dorsum of tongue becomes smooth due to loss of the papillae and varicose veins are seen in the floor of the mouth.

REVIEW QUESTIONS

1. Classify oral mucosa. What is the basis of this classification? What are the main differences between the different regions?
2. Describe the light and ultramicroscopic appearance of the various layers of oral epithelium. What are the differences between keratinized and nonkeratinized epithelium?
3. What are nonkeratinocytes? Describe them. How do they differ from keratinocytes?
4. Explain the terms differentiation, maturation and turnover rate in oral epithelium.
5. What are cytokeratins? What are their clinical significance?
6. What is gingiva? With aid of diagrams describe the appearance of different parts of gingiva.
7. Describe the histology of the gingiva.
8. What are the different zones of the hard palate? How do they differ from each other microscopically? What is the clinical significance of the structures found in the hard palate?
9. Classify papillae of tongue. Describe the macroscopic and microscopic appearance of dorsum of the tongue.
10. Describe the dentogingival junction. With aid of diagrams explain the shift of dentogingival junction. What is its clinical significance?
11. What are the age changes in oral mucosa?

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11 Salivary Glands

CHAPTER CONTENTS

The salivary glands are a group of compound exocrine glands secreting saliva. Saliva is a complex fluid produced by the salivary glands. The saliva forms a film of fluid coating the teeth and mucosa thereby creating and regulating a healthy environment in the oral cavity.

The parenchymal elements are derived from the oral epithelium and consist of terminal secretory units leading into ducts that eventually open into the oral cavity. The connective tissue forms a capsule around the gland and extends into it, dividing groups of secretory units and ducts into lobes and lobules. The blood and lymph vessels and nerves that supply the gland are contained within the connective tissue.

The salivary glands are compound glands as they have more than one tubule entering the main duct. A duct is a passage that allows the glandular secretion emptied directly into an anatomic location where the secretion is to be used. The salivary glands have numerous ducts associated with them hence they are exocrine glands. The architectural arrangement of the salivary glands is tubuloacinar, where acini are secretory units. These tubuloacinar units are merocrine as they release only the secretion of the cell from the secreting units.

STRUCTURE OF TERMINAL SECRETORY UNITS

The basic functional unit of a salivary gland is the terminal secretory unit called acini. The terminal secretory unit irrespective of size and location is made up of epithelial secretory cells, namely serous and mucous cells. The serous, mucous along with myoepithelial cells are arranged in an acinus or acini (multiple) with a roughly spherical or tubular shape and a central lumen (Fig. 11.1).

The cells in the acini rest on a basement membrane. They are arranged in a single layer. The intercellular spaces of the apical ends of the cells are separated from the lumen by junctional complexes which are tight (zonula occludens), intermediate junction (zonula adherens), and one or more desmosomes (maculae adherens). The junctional complexes hold the cells together in an acinus and regulate the permeability. Tight junctions seal the adjacent secretory cells, controlling paracellular ion influx. This helps in maintaining cell polarity and tissue homeostasis. The main tight junctional proteins are claudin, occludin and junctional adhesion molecules. The myoepithelial cells are located on the surface of the acini.

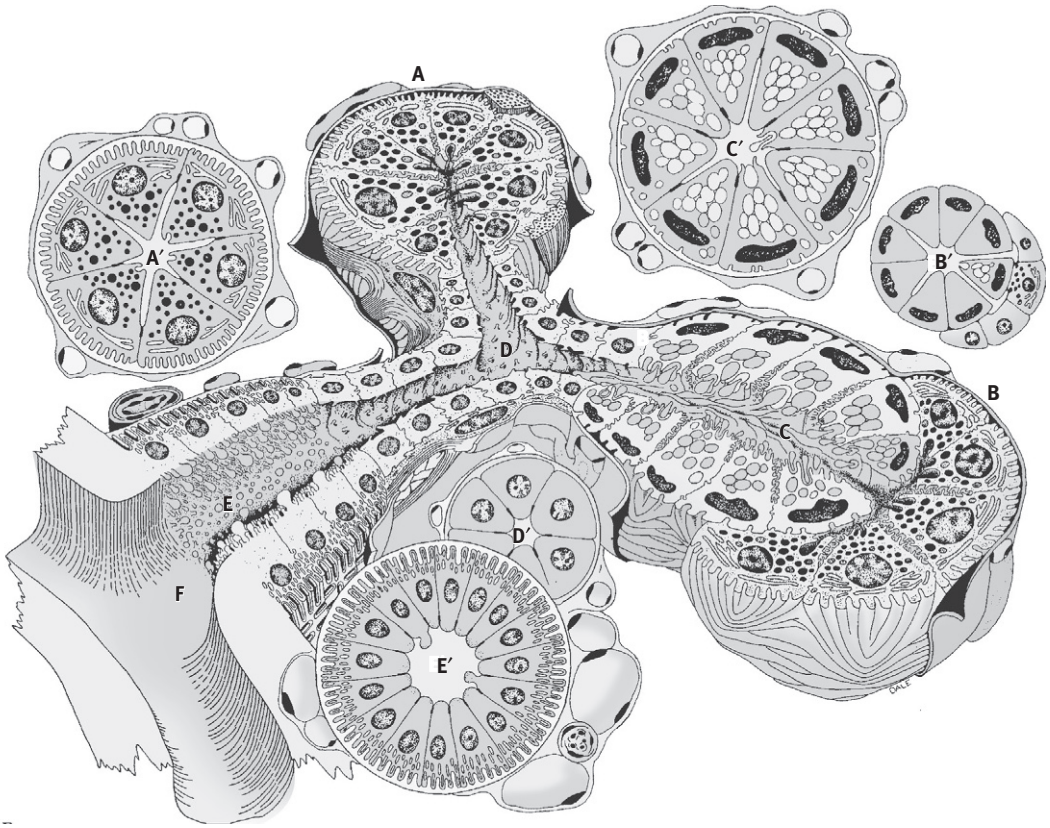

Figure 11.1 Schematic diagram of a typical salivary gland. (A) Serous acini, (A′) serous acini in cross-section. (B) Serous demilunes, (B′) serous demilunes in cross-section. (C) Mucous acini, (C′) mucous acini in cross-section. (D) Intercalated duct, (D′) intercalated duct in cross-section. (E) Striated duct, (E′) striated duct in cross-section. (F) Terminal excretory duct.

The central lumen of each acini may have a star-shaped morphology because of extension of lumen in between the cells called intercellular canaliculi. The central lumen of the acini continue via a fine series of ducts which constantly merge with each other and grow larger eventually to merge into the main excretory duct. These ducts comprise the ductal system.

The mucous acini have a larger lumen than serous acini (end piece).

The secretory terminal unit in serous acini is generally made of 8–12 serous acini surrounding a central lumen (Fig. 11.1).

Secretory end piece of mucous cells have a tubular configuration. The mucous cells are joined to each other by a variety of intracellular junctions but unlike the serous acini, they lack the presence of intercellular canaliculi. The intercellular canaliculi are said to be present only in acini with demilunes. Sometimes mucous acini have bonnet or crescent shaped covering which is made of serous cells. These are called demilunes (Fig. 11.1). The presence of demilunes is questioned. It has been shown that demilunes are as a result of artifact during tissue preparation. Recent methods like rapid freezing, freeze substitution and three-dimensional reconstruction techniques have shown that serous cells align with mucous cells to surround a common lumen.

Serous Cells

Serous secretory cells are pyramidal with a broad base on the basement membrane, the apex faces the lumen. The serous cells have a spherical nucleus placed at the basal region. The apical cytoplasm of these cells shows accumulation of secretory granules. The secretory granules are 1 μm in diameter with a distinct limiting membrane. In human beings the granules contain a dense core or a twisted skin like structure with a lighter matrix. They can be visualized in semi thin plastic embedded tissue section, stained with toluidine blue or specific cytochemical techniques. The granules are closely apposed to each other but retain their individuality (Fig. 11.2).

The granules are zymogen granules and are formed by glycolated proteins which are released into a vacuole. In electron microscope the immature granules appear paler in density as compared to electron dense granules which are maturing and moving towards the luminal plasma membrane. The numbers of the secretory granules also vary with different levels of activity in an unstimulated or resting cell. There are numerous granules in the luminal portion of the cell, whereas in a stimulated cell the granules are few as they are depleted in huge numbers into the lumen by exocytosis.

The serous cells show acid phosphates, esterases, glucuronidase, glucosidase and galactoside activity.

The ultrastructural feature of a serous cell is typical of a protein secreting cell. A typical serous cell spends most of its synthetic capacity for producing the secretory protein. The basal cytoplasm is packed with parallelly stacked, with ribosome studded RER (rough endoplasmic reticulum). The RER is placed basal and lateral to the cell nucleus. A closed system of cisternae or membranous sacs

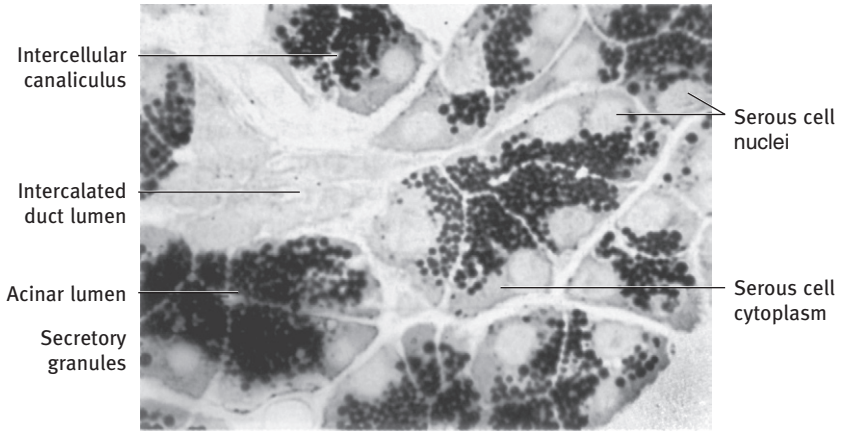

Figure 11.2 Light micrograph of rat parotid gland illustrating general arrangement and cytologic features of serous cells. Gland was incubated in cytochemical medium to demonstrate the secretory enzyme peroxidase, resulting in unstained nuclei, lightly stained cytoplasm, and heavily stained secretory granules. Cells of intercalated duct are unreactive (1μm; ×990).

constitutes RER (Fig. 11.3). The ribosomes consist of RNA and proteins.

The nucleus of a cell by the way of m-RNA sends an encoded message which is translated by ribosomes. An appropriate amino acid with a specific sequence is synthesized. These proteins, or the preproteins have a NH_2 terminal extension of 16–30 amino acids called the *signal sequence*. As signal sequence is ready and emerges it is attached to the membrane of RER. RER recognizes them with the help of certain proteins and crosses the RER membrane along with the growing polypeptide chain. A proteolytic enzyme, signal peptidase removes the signal sequence and the protein newly synthesized reaches the cisternal space of RER. From here the protein is sent to the Golgi apparatus. The Golgi apparatus is a membranous cisternae of several stacks of 4 to 6 smooth surfaced saccules located apically and laterally to the cell nucleus. The Golgi apparatus is functionally connected to RER

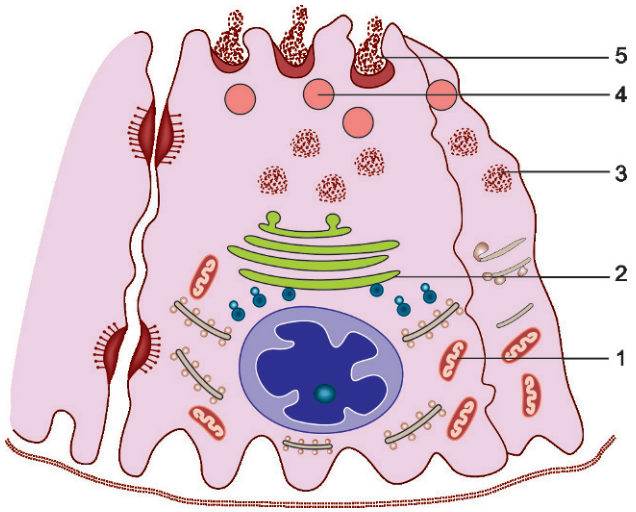

Figure 11.3 Diagrammatic representation of serous cell and pathway of synthesis, storage and exocytosis of secretory protein. 1–Rough endoplasmic reticulum synthesizing protein, 2–Golgi complex transfer protein to transface, 3–Immature granules, 4–Mature granules with concentrated protein, 5–Exocytosis.

through budding vesicles at the end of RER. Each of the Golgi apparatus has a cis or convex face and a trans or concave face. The budding vesicles of RER enter the Golgi bodies from the cis face where the vesicles fuse with the Golgi saccules emptying its contents. The proteins migrate from the cis to trans face in the Golgi saccules where they are packed into vacuoles of variable density and size. These vacuoles are the forming secretory granules known by the name of condensing vacuoles, presecretory granules or immature granules. The immature granules are connected to the smooth membrane of the trans face. The limiting membrane of immature granules has irregularities which allow fusion of small vesicles. The immature granules which are pale increase in size and density to mature. This happens in a process of concentration gradient which continues during the transportation and packing of granules.

Following their synthesis, many secretory proteins undergo one or more covalent structural modifications prior to their secretion. The most common modification of salivary proteins is glycosylation (i.e. the addition of carbohydrate side chains to the amino acids asparagine, serine, and threonine in the protein). The carbohydrates of secretory glycoproteins include galactose, mannose, fructose, glucosamine, galactosamine, and sialic acid. Glycosylation is a multistep process that begins in the RER and is completed in the Golgi apparatus.

The mature granule stored at the apex of the cell is emptied into the lumen by exocytosis. This process involves the membrane of the granule to fuse with the plasma membrane of the cell at the lumen. This process prevents the loss of cell cytoplasm. Sometimes during rapid secretion a chain of granules may be released in the form of a string of pearls. This is called compound exocytosis. The serous cells devote 80% of its capacity in the production of zymogen granules, but there are other activities also happening in the cell depicted by the other cell granules (Fig. 11.4).

True or unattached ribosomes are seen which synthesize nonsecretory cellular proteins. A good number of mitochondria are seen in relation with RER and Golgi apparatus. They show the presence of enzymes of oxidative phosphoregulation, citric acid cycle and electron

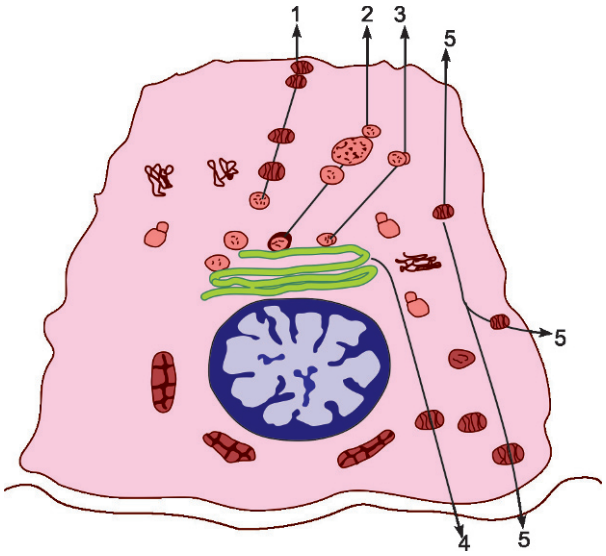

Figure 11.4 Diagrammatic representation of possible vesicular protein secretory pathways in parotid acinar cells. 1–Storage granule pathway (main pathway), 2–Constitutive like pathway, 3–Constitutive pathway to the apical membrane, 4–Constitutive pathway to the basolateral membrane, 5–Transcytosis from basolateral to the apical membrane.

transport. In general they are powerhouses for numerous synthetic and transportation process. Lysosomes are seen with hydrolytic enzymes which help to destroy foreign material and worn out cell organelles.

Mucous Cells

The mucous cell, like the serous cell, is specialized for the synthesis, storage, and secretion of a secretory product. However, its structure differs from that of the serous cell. In routine histologic preparations, the apex of the cell appears empty except for thin strands of cytoplasm forming a trabecular network. The nucleus and a thin rim of cytoplasm are compressed against the base of the cell (Figs 11.1, 11.5).

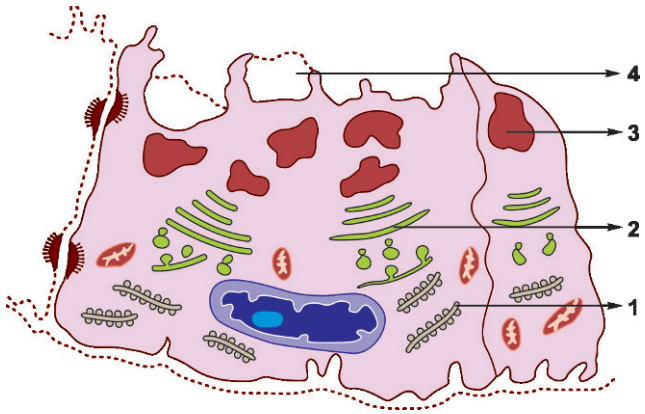

Figure 11.5 Mucous cell and pathway of synthesis and exocytosis of mucus. 1–Rough endoplasmic reticulum synthesizing mucous protein, 2–Golgi complex transfer protein to transface, 3–Formation of mucous pool, 4–Exocytosis of mucus

The mucous cell shows accumulations of large amounts of secretory product at the apical cytoplasm. The secretory product pushes the nucleus and endoplasmic reticulum against the basal cell membrane. The mucous secretion differs from secretion of serous in two important respects:

1. They have little or no enzymatic activity and probably serve mainly for lubrication and protection of the oral tissues.
2. The ratio of carbohydrate to protein is greater and larger amounts of sialic acid and occasionally sulfated sugars are present.

The differences in the carbohydrate content of a mucous cell and a serous cell can be demonstrated by histochemical staining techniques (Table 11.1).

Most of the times the mucous secretion in a cell appears unstained in routine histologic section. However, when special stains like PAS or alcian blue are used they are strongly stained (Figs 11.6, 11.7).

The nucleus of the mucous cell is oval or flattened in shape and located just above the basal plasma membrane (Fig. 11.7). The RER is limited to a narrow band of cytoplasm along the base and lateral borders of the cell and to an occasional patch of cytoplasm between the mucous droplets. The mitochondria and other organelles are also primarily limited to this band of basal and lateral cytoplasm. The Golgi apparatus is large, consisting of several stacks of 10 to 12 saccules sandwiched between the basal RER and mucous droplets forming from the *trans* face. The Golgi apparatus plays an important role in these cells because of the large amount of carbohydrate that it adds to the secretory products.

The secretion of mucous droplets occurs by a somewhat different mechanism than the exocytotic process seen in the serous cells. When a single droplet is discharged, its limiting membrane fuses with the apical plasma membrane, resulting in a single membrane separating the droplet from the lumen. This separating membrane may then fragment, being lost with the discharge of mucus, or the droplet may be discharged with the membrane intact, surrounding it. During rapid droplet discharge, the apical cytoplasm may not seal itself off, and the entire mass of mucus may be spilled into the lumen (Fig. 11.5).

Myoepithelial Cells

Myoepithelial (ME) cells are closely related to the secretory and intercalated duct cells.

They are stellate or spider-like, with a flattened nucleus, scanty perinuclear cytoplasm and long branching processes that embrace the secretory and duct cells (Fig. 11.8). In case of intercalated ducts the myoepithelial cells have a more fusiform shape and are elongated with a few short processes. The processes in the acini lie in the 'gutters', hence the outline of the acini appears smooth but in the intercalated duct the processes runs longitudinally on the surface creating a bulge. Their appearance is reminiscent of a basket cradling the secretory unit, hence the terms '*basket cell*'. ME cells are similar to smooth muscle cells but are derived from epithelium. These cells are located around the terminal secretory

Table 11.1 Differences between Serous and Mucous Acini

Light Microscopy	
Mucous Cell	**Serous Cell**
Pyramidal in shape	Pyramidal in shape with narrow apex near the lumen
Flattened nucleus at base	Spherical nucleus near basal one-third
Apical portion of cell appears empty	Apical portion contains zymogen granules
Apical portion stains weakly with H&E	Apical portion stains strongly with H&E
Apical portion stains strongly with carbohydrate stains like PAS, Alcian blue	Apical portion shows numerous eosinophilic secretory granules which stains with toluidine blue
Apical cytoplasm not sealed – Mucus spilled into lumen	Secretion of granules as string of pearls – No loss of cytoplasm
Mucous Acini	**Serous Acini**
Elliptical in shape	Spherical in shape
Larger in size	Smaller in size
Have larger lumen	Have smaller lumen
Lacks intercellular canaliculi	Intercellular canaliculi is present
Secretion – No enzymatic activity	Secretion – Enzymatic activity – Acid phosphatase, esterases, Glucuronidase etc.
Produce more carbohydrate components than proteins	Produce secretory proteins, carbohydrate content less
Secretory glycoproteins have **O**-linked oligosaccharide chains	Secretory glycoproteins have **N**-linked oligosaccharide chains
Electron Microscopy	
Prominent Golgi regions – located between nucleus and secretory droplets	Extensive RER – in parallel aggregates – lateral and basal to nucleus. Golgi located apical to nucleus
Secretory droplets are irregular and larger than serous	Secretory granules smaller, seen at apical area – covered by unit membrane

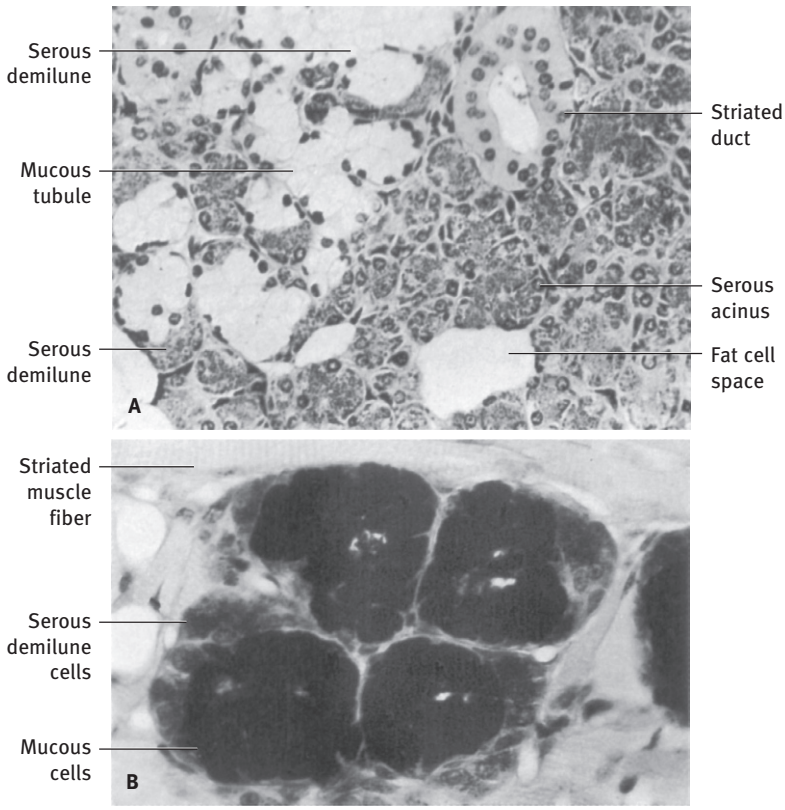

Figure 11.6 (**A**) Light micrograph of human submandibular gland illustrating different appearance of mucous and serous cells. Mucous tubules are capped by serous demilunes. Two striated ducts are cut in cross-section. (**B**) Light micrograph of posterior lingual mucous gland of rat, stained with alcian blue and periodic acid-Schiff (PAS). Mucous secretory glycoprotein stains with both alcian blue and PAS, indicating acidic carbohydrate residues. Granules of serous demilune cells stain only with PAS, indicating neutral glycoproteins (**A**, ×265; **B**, ×420).

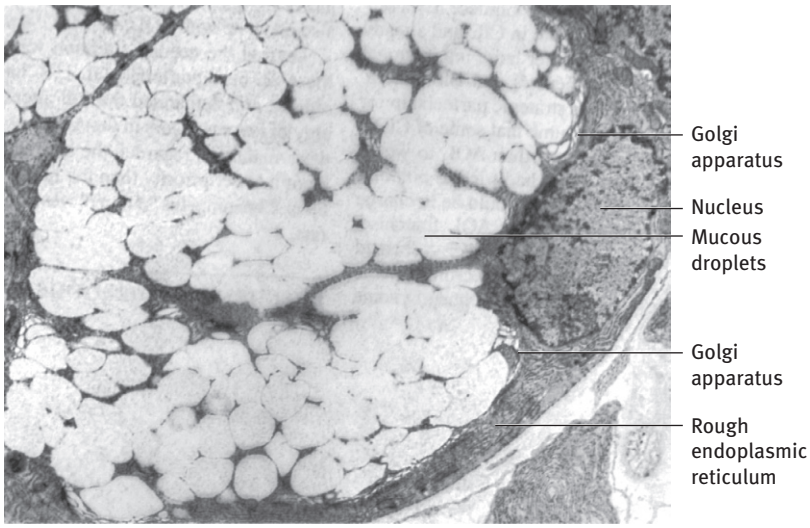

Figure 11.7 Electron micrograph of mucous cell. Pale mucous droplets have flocculent content and tend to coalesce into larger masses. Golgi apparatus is well developed; rough endoplasmic reticulum and nucleus are compressed against base of cell (×7000).

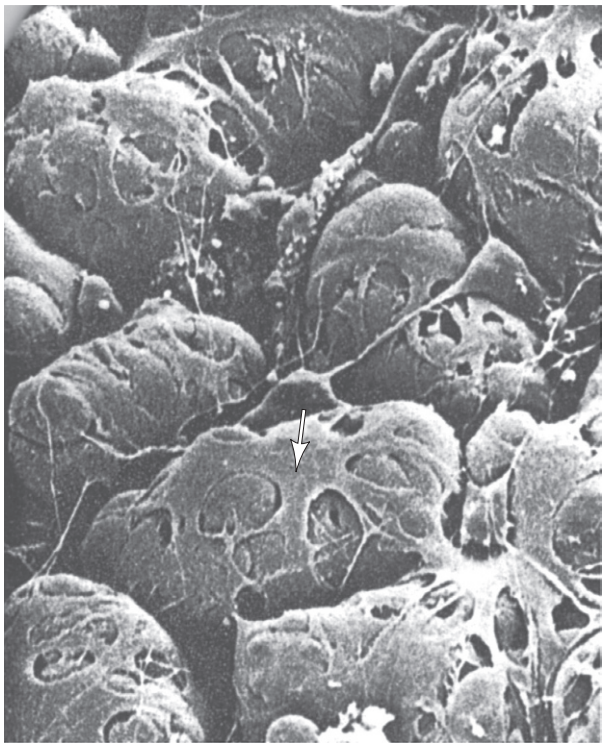

Figure 11.8 Scanning electron micrograph of myoepithelial cells (arrow) and their processes covering the basal surfaces of the acinar cell. The basal lamina has been digested to reveal the basal surface of the acinar cells (From Nagato T, Yoshida H, Yoshida A, et al: Cell Tissue Res 209:1,1980).

units and the first portion of the duct system. They lie between the basement membrane of the parenchyma cells and are attached to the cells by desmosomes.

Myoepithelial cells are difficult to identify in routine histologic preparations, but their typical stellate shape can be observed in sections stained by special histochemical or immunofluorescent techniques. ME cells contain cytokeratin intermediate filament and contractile actin filaments. These can be used to help identify them using immunocytochemistry. The presence of cytokeratin confirms the epithelial origin of myoepithelial cells.

The usual appearance of myoepithelial cells in electron micrographs is a section through one of their processes lying in a groove on the surface of a secretory or duct cell. The processes are filled with longitudinally oriented fine filaments about 6 nm (60 Å) thick. Small dense bodies are frequently present between the thin filaments; these are also present in smooth muscle cells, where they appear to form a cytoskeletal network in association with 10 nm (100 Å) diameter filaments. The usual cytoplasmic organelles are largely restricted to the perinuclear cytoplasm. The body of the cell, containing the nucleus, often lies in the space where the basal regions of two or three parenchymal cells come together. The plasma membrane of the myoepithelial cell closely parallels the basal membrane of the parenchymal cell, and the two are joined by occasional desmosomes. Numerous micropinocytotic vesicles, or caveolae are located on the plasma membranes of the myoepithelial cells. The myoepithelial cells are innervated through the parasympathetic motor nerve.

The precise functional role of ME cells in salivary secretion is not very clear; however the following structural details clearly indicate its contractile function.

1. Structure of ME cells is similar to that of smooth muscles.
2. Immunofluorescent studies indicate presence of myosin, actin and related proteins.
3. After appropriate stimulation, the measurement of ductal pressure of ME cell indicates a contractile process.
4. Cinemicrography of individual secretory unit stimulated to secrete in vivo reveal a regular pulsatile movement of the entire unit.

The functions related to ME cells indicate clearly that ME cells actively can:

1. Accelerate the initial outflow of saliva from the acini.
2. Reduce luminal volume. The intercalated ducts may shorten and widen the ducts helping to maintain their patency.

3. Contribute to secretory pressure in the acini or duct.
4. Support the underlying parenchyma and reduce the back permeation of fluid.
5. Help salivary flow to overcome increase in peripheral resistance of the ducts.

Recent studies show ME cells are involved in signaling the secretory cells and protecting the salivary gland tissue. The ME cells provide signals to the acinar secretory cells that are needed for maintaining cell polarity and the structural organization of the acinus. There is evidence that ME cells also produce a number of proteins that have tumor suppressor activity, such as proteinase inhibitors and anti-angiogenesis factors, which act as barriers against invasive epithelial neoplasms.

Ducts

The ductal system of salivary glands consists of hollow tubes connected initially with the acinus and then with other ducts as the ducts progressively grow larger from the inner to the outer portion of the gland. Each type of duct is lined by different type of epithelium, depending on its location in the gland. In comparison each major salivary gland displays differences in the length or types of duct present.

The ductal system is not just a pipeline or conduit for the passageway for the saliva; it also actively participates in the production and modification of saliva.

In a salivary gland the smallest ducts are the intercalated ducts connecting the terminal secretory units to the next larger duct, the striated ducts. In the interlobar tissue the ducts continue to join one another increasing in size until the main excretory duct is formed.

Salivary glands have varying number of lobules depending on their size and each lobule is surrounded by connective tissue. The ductal system is sometimes also named according to its location. Some within the lobule, meaning intralobular ducts and some are interlobular ducts, which lie within the connective tissue within the lobules of the gland.

There are two types of intralobular ducts—the *intercalated* and *striated ducts*. The excretory ducts are interlobular.

Intercalated ducts

The intercalated ducts (Figs 11.1, 11.9) are lined by a single layer of low cuboid cells with relatively empty-appearing cytoplasm. They are often difficult to identify in the light microscope because they are compressed between secretory units. In electron micrographs the intercalated duct cells share several characteristics of serous cells (Fig. 11.9B). A small amount of RER is located in the basal cytoplasm, and a Golgi apparatus of moderate size is found apically. In proximally located cells (near the secretory units) a few small secretory granules may be found. The lateral membranes of adjacent cells are joined apically by junctional complexes and several desmosomes. One or two areas of prominent interlocking folds of the lateral surface are located further basally (Fig. 11.10). At the periphery of the duct, processes and

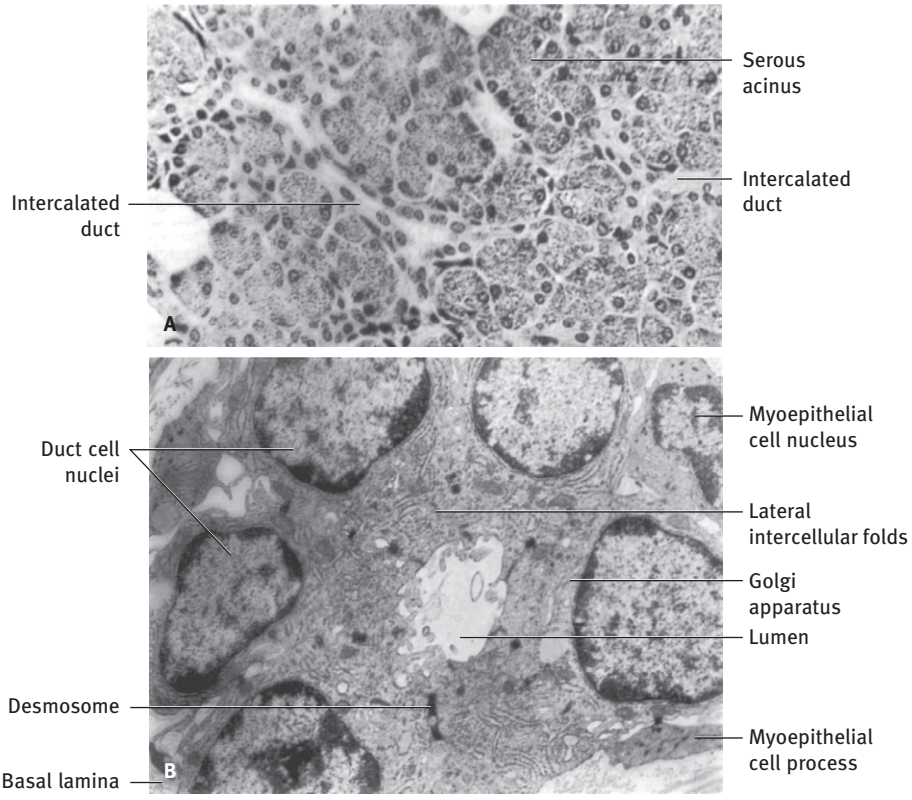

Figure 11.9 (**A**) Light micrograph of human parotid gland showing long branching intercalated ducts between serous acini. (**B**) Electron micrograph of intercalated duct cut in cross-section. Duct cells contain moderate amount of rough endoplasmic reticulum and a prominent Golgi apparatus but few or no secretory granules. Prominent desmosomes and interlocking folds are present between adjacent cells. Myoepithelial cell processes extend longitudinally along duct, inside basal lamina (Rat parotid gland **A**, ×265; **B**, ×9600).

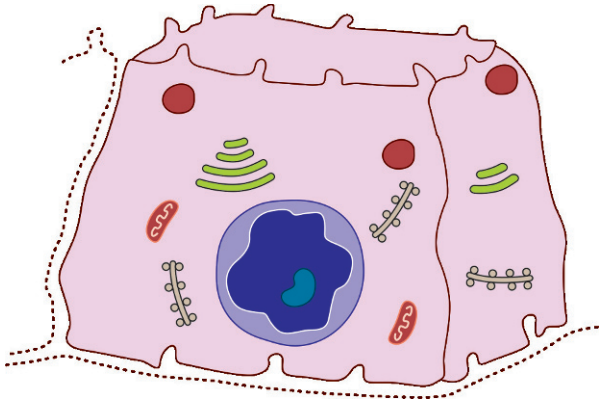

Figure 11.10 Diagrammatic representation of intercalated duct cells.

cell bodies of myoepithelial cells may be found, attached by desmosomes to the duct cells.

The intercalated ducts do not act as a simple conduit but modify the saliva through secretory and absorptive process. The intercalated ducts contribute to macromolecular components like lysozymes, lactoferrin and some unknown components to the saliva. These are stored in the secretory granules of the cells. It is believed that intercalated duct also house undifferentiated cells which can undergo differentiation to replace damaged or dying cells in the end piece or striated ducts.

Striated ducts

The striated ducts receive saliva from the intercalated ducts. They form the largest portion of the duct system constituting the intralobular component of the duct system.

The striated ducts are lined by a layer of tall columnar epithelial cells with large, spherical, centrally placed nuclei (Figs 11.2, 11.11). The cytoplasm is abundant and eosinophilic and shows prominent striations at the basal ends of the cells, perpendicular to the basal surface. An occasional basally located cell can be identified by the position of its nucleus, below the level of those of the other cells.

In electron micrographs the basal cytoplasm of the striated duct cells is partitioned by deep infoldings of the plasma membrane, producing numerous sheet-like folds that extend beyond the lateral boundaries of the cell and interdigitate with similar folds of adjacent cells (Fig. 11.12). Abundant large mitochondria, usually radially oriented, are located in portions of the cytoplasm between the membrane infoldings (Figs 11.13, 11.14). The combination of infoldings and mitochondria accounts for the striations seen in the light microscope.

The striations corresponding to multiple infolding of the plasma membrane of the cells packed with mitochondria occupying a large surface area with high levels of energy, clearly indicating that the cell is involved in active transport. The striated ducts are site of electrolyte reabsorption especially of sodium and chloride, and secretion of potassium and bicarbonate. This reabsorption is against a concentration gradient, hence requires a substantial amount of energy. The luminal content is converted from an isotonic or a slightly hypertonic fluid into a hypotonic

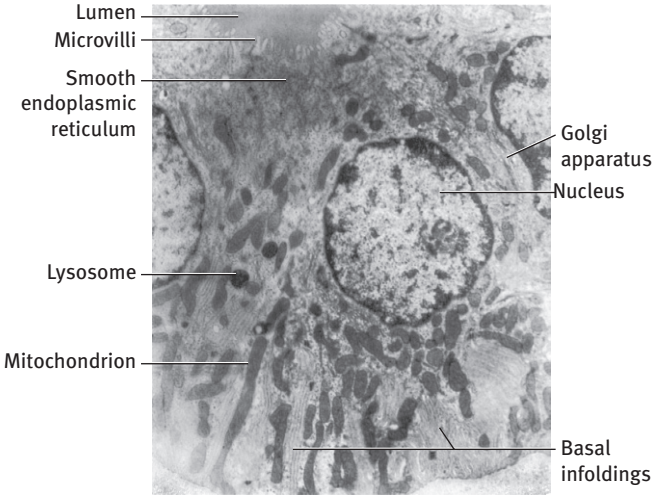

Figure 11.12 Electron micrograph of striated duct cells of rat parotid gland. Numerous mitochondria are located between infoldings of basal plasma membrane. A few lysosomes and the Golgi apparatus are located in perinuclear region, and smooth endoplasmic reticulum is found in cell apices. Short microvilli project into lumen (×7400).

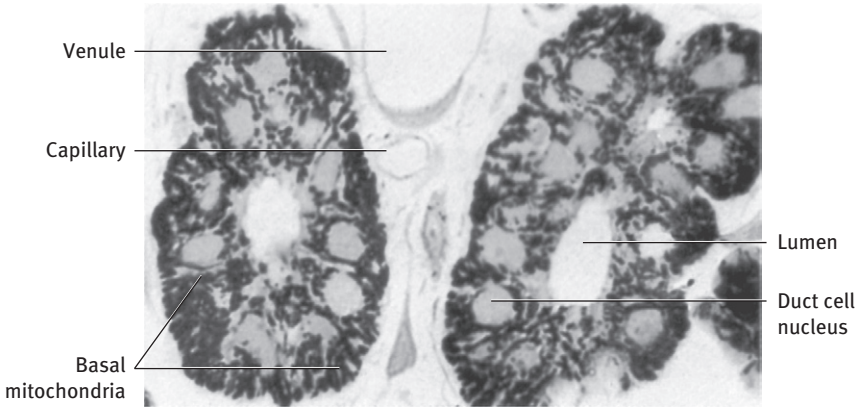

Figure 11.11 Light micrograph of two striated ducts cut in cross-section. Large, primarily radially oriented mitochondria, stained for cytochrome oxidase activity, fill basal regions of duct cells. Unstained nuclei are centrally located, and small mitochondria are found in apical cytoplasm (Rat parotid gland; ×990).

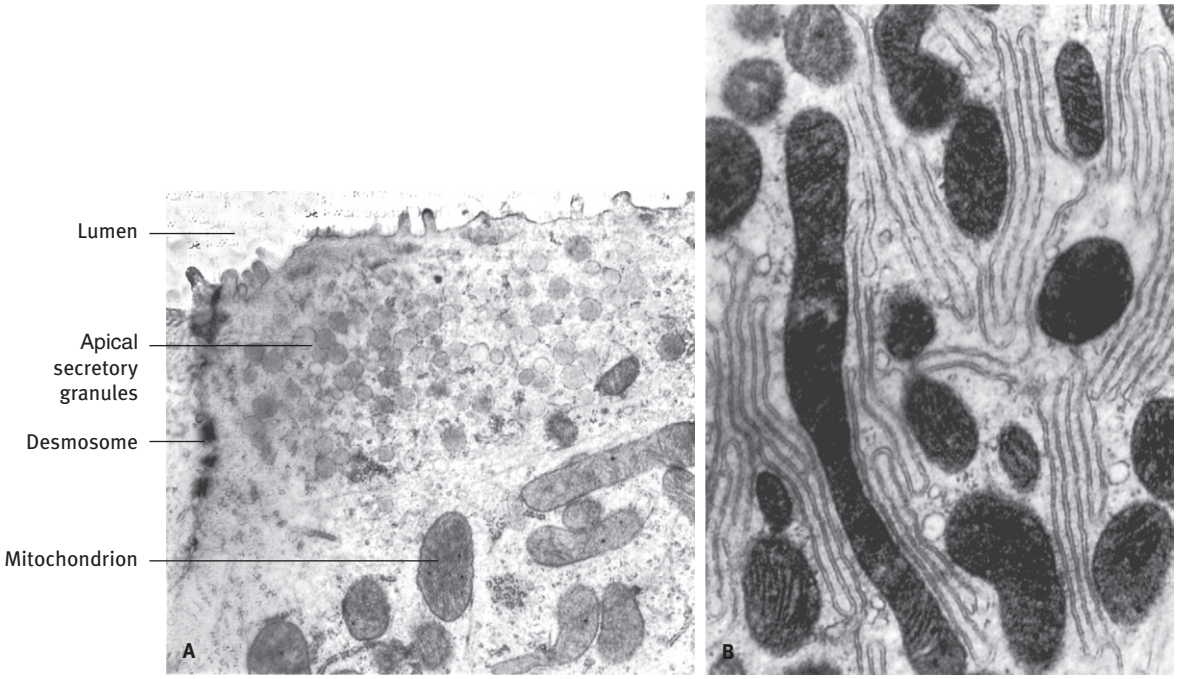

Figure 11.13 (**A**) Electron micrograph of apical cytoplasm of striated duct cell. Small secretory granules are located near lumen. Mitochondria, smooth and rough endoplasmic reticulum are also present. (**B**) Basal region of striated duct cell, showing mitochondria and infolded plasma membranes (Rat sublingual gland; **A**, ×22,200; **B**, ×23,900).

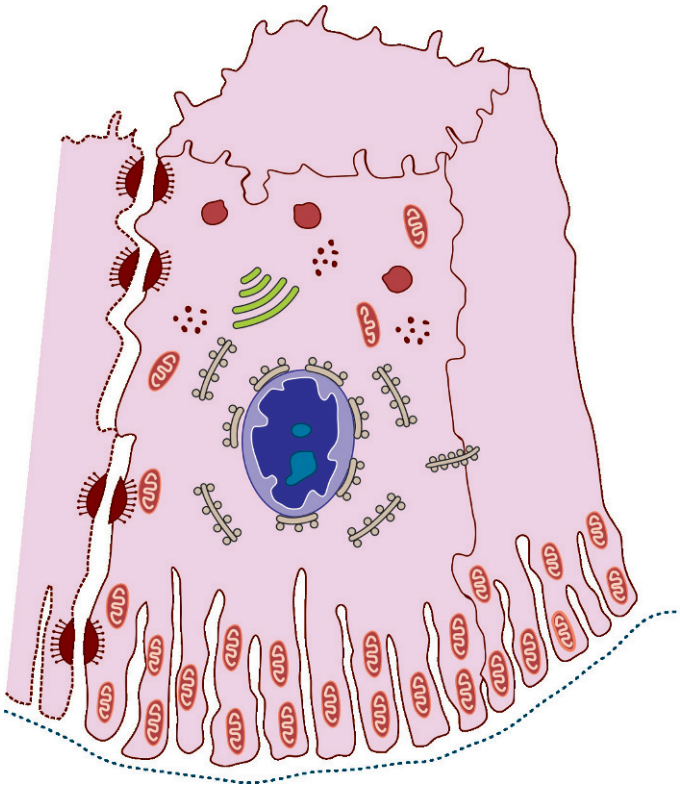

Figure 11.14 Diagrammatic representation of striated duct cell.

fluid. The striated ducts also modify the organic content of the primary saliva. The duct cells synthesize and secrete glycoproteins such as kallikrein and epidermal growth factor. The cells are also capable of reabsorbing proteins from the luminal surface by endocytic mechanisms this is evident by the presence of vesicles in the cytoplasm.

Essentially all of the water enters saliva at the level of the terminal secretory units; the striated and excretory ducts appear to be relatively impermeable to water. The ductal reabsorption of Na^+ and Cl^- exceeds the secretion of K^+ and HCO^-_3, leaving a hypotonic luminal fluid. Since active transport of water does not occur, the ducts cannot secrete water against the osmotic gradient to produce the final hypotonic saliva.

The single layered epithelium of striated ducts contains simple cytokeratin intermediate filaments 8 and 18.

Excretory ducts

The striated ducts join each other to form larger intralobular ducts. These ducts gradually increase in size and are surrounded by increasing layers of connective tissue. Progressively along the path, the duct becomes nonstriated and large, to become the excretory interlobular duct.

As the excretory duct enlarges it contains two layers: the mucosa and the outer connective tissue adventitia. The mucosal epithelium of the duct consists of pseudostratified columnar epithelium cells. In larger ducts occasional goblet cells and ciliated cells may be seen. The ductal epithelium slowly undergoes a transition to stratified epithelium, cuboidal epithelium and finally into stratified squamous epithelium when it merges with the epithelium of the oral cavity. When stratified, the duct epithelium contains keratin, the intermediate filament types are typical of stratified epithelium of oral cavity. The connective tissue on the external surface has collagen and elastic fibers which allow passive stretching of the duct to allow and accommodate varying volumes of saliva.

In the excretory ducts a small number of other types of cells are present. Tuft or brush cells with long stiff microvilli and apical vesicles are seen. They are thought to be receptor cells as they show nerve endings adjacent

to the basal portion of the cell. Sometimes cells with pale cytoplasm and dense nuclear chromatin are seen towards the base of the duct epithelium. They appear to be lymphocytes and macrophages.

Dendritic cells or antigen presenting cells are seen as cells with long branching processes that extend between the epithelial cells. These are involved in processing and presentation of foreign antigens to T-lymphocytes and in immune surveillance.

Differences among intercalated, striated and excretory ducts are depicted in Table 11.2.

Connective Tissue Elements

The cells found in the connective tissue of the salivary glands are the same as those in other connective tissues of the body and include fibroblasts, macrophages, mast cells, occasional leukocytes, fat cells, and plasma cells. The cells, along with collagen and reticular fibers, are embedded in a ground substance composed of proteoglycans and glycoproteins.

Connective tissue of the salivary gland consists of a surrounding capsule that demarcates the gland from the adjacent structures. The extension of the connective tissue as septa inward from the capsule divides the gland into lobes and lobules. The septa contain blood vessels and nerves that supply the parenchymal components (glandular components) and excretory ducts.

The plasma cells produce immunoglobulins that are secreted into the saliva by transcytosis.

The main immunoglobulin in the saliva is the IgA, which is synthesized as a dimer complexed with an additional protein called J chain. The receptor bound IgA clinging with a portion of the receptor called secretory component is released at the luminal surface of the cell. Small amounts of IgG and IgM are also secreted into the saliva.

In the lobules of the salivary gland, finer partitions of connective tissue extend in between the adjacent end pieces and ducts. They carry the arterioles, capillaries and venule of the microcirculation and branches of the autonomic nerves that innervate the secretory and ductal cells.

Blood Supply

The vascular supply to the gland is also embedded within the connective tissue, entering the glands along the excretory ducts and branching to follow them into the individual lobules.

Since the salivary secretion is predominantly 99% water it necessitates an extensive blood supply. The ducts, to the level of the intralobular striated ducts, are supplied with a dense capillary network: the capillary loops to the intercalated ducts and terminal secretory units are less extensive. Arteriovenous communications are seen around the larger interlobular ducts.

An extensive capillary plexus originates from separate arterioles that exist around the excretory ducts. The endothelium of the capillaries and postcapillary venules are fenestrated.

The venous return follows the arterial supply. In the arteriovenous anastomoses when the blood flow increases during increased secretion more blood is diverted through the anastomoses resulting in increased venous and capillary pressure, therefore increasing the

Table 11.2 Differences among Intercalated, Striated and Excretory Ducts

	Intercalated Ducts	Striated Ducts	Excretory Ducts
Location	Intralobular	Intralobular	Interlobular
Cell morphology	Single layer of low cuboidal cells	Single layer of tall columnar cells	Pseudostratified columnar epithelium to stratified squamous
Size	Smallest in diameter	Bigger than intercalated duct	Largest in diameter
Size comparison	Smaller than secretory end pieces	Larger than secretory end pieces	Larger than striated ducts
Lumen	Greater than secretory end pieces	Greater than secretory end pieces	Greater than secretory end pieces
Cell details	Centrally located nuclei surrounded by scant cytoplasm	Central nucleus with abundant cytoplasm	Different types of cells and nuclei
Basal plasma membrane	No indentation	Infoldings of basal plasma membrane partitioning basal cytoplasm	No indentation
Solute concentration	Isotonic / hypertonic	Hypotonic	Hypotonic
Electron microscopy	Small amount of RER located in basal cytoplasm and Golgi apparatus seen apically	Abundant large mitochondria between basal infoldings	Basal cells do not show such modifications
Function	Contribute to macromolecular components like lysozymes and lactoferrin	Site for electrolyte reabsorption – active transport Contribute kallikrein and EGF	No modification to saliva
Other cell types present	Undifferentiated cells that can undergo differentiation	None	Dendritic cells, APCs, lymphocytes and macrophages
Myoepithelial cells	At periphery of duct	Absent	Absent

fluid filtration rate across the capillary endothelium which takes care of the fluid necessary for maintaining the secretion.

Nerve Supply and Pattern of Innervation

The main branches of the nerves supplying the glands follow the course of the vessels, breaking up into terminal plexuses in the connective tissue adjacent to the terminal portions of the parenchyma.

Both postganglionic nerve fibers of the sympathetic and parasympathetic divisions of the autonomic nervous system innervate the glands. The preganglionic parasympathetic fibers originate in the superior or the inferior salivatory nuclei in the brainstem and travel via the facial and glossopharyngeal nerves to the submandibular and otic ganglia. In the ganglia they synapse with the postganglionic neurons. The postganglionic fibers reach the gland through the lingual and auriculotemporal nerve. Preganglionic sympathetic nerves originate in the thoracic spinal cord, synapse with postganglionic neurons in the superior cervical ganglion. The postganglionic fibers reach the glands traveling along with the arterial blood supply.

In the gland lobules branches of nerve follow blood vessels finally forming a plexus of unmyelinated fibers. The axons of the nerve are invested by the cytoplasmic processes of Schwann cells and are distributed to secretory cells, myoepithelial cells, the smooth muscles of the arterioles, and possibly intercalated nonstriated ducts.

The secretory cells receive their innervation by one of the two patterns. In the intraepithelial type (intraparenchymal), the axons split off from the nerve bundle and penetrate the basal lamina, lying adjacent to or between the secretory cells (Fig. 11.15). As the axons pass through the basal lamina, the Schwann cell covering is usually lost; occasionally it may be continued into the parenchyma and lie between the axons and the secretory cell. The site of innervation (neuroeffector site) is considered to be at varicosities of the axon, which contain small vesicles and mitochondria. The vesicles are believed to contain the chemical neurotransmitters norepinephrine and acetylcholine and presumably release them by an exocytosis like process. The membranes of the axon and secretory cell are separated by a space of only 10 to 20 nm (100 to 200 Å), but no specializations of the plasma membranes have been detected at these sites. A single axon may have several varicosities along its length, making contact with the same cell or with two or more cells.

The second type of innervation is subepithelial (extraparenchymal). Instead of penetrating the basal lamina, the axons remain associated with the nerve bundle in the connective tissue (Fig. 11.16). Where the nerve bundles approach the secretory cells, some of the axonal varicosities, which contain the small neurotransmitter vesicles, lose their covering of Schwann cell cytoplasm. Presumably, these bared axonal varicosities are the sites of transmitter release. The axons remain separated from the secretory cells by 100 to 200 nm (1000 to 2000 Å), and the transmitters must diffuse across this space, which includes the basal lamina of the secretory cells and the nerve bundle.

The intraparenchymal and extraparenchymal innervations vary not only among the glands but also among different cells within the same single gland. Intraparenchymal innervation is seen occurring in the human submandibular gland and in the minor glands of the lip, whereas extraparenchymal innervation occurs in the parotid gland. The different types of innervation have no functional difference.

In some glands both sympathetic (adrenergic) and parasympathetic (cholinergic) terminals (distinguished by special fixation and cytochemical techniques) have been observed in proximity to the secretory cells. Similarly, physiologic studies indicate that the cells of some glands respond to both sympathetic and parasympathetic stimulation by changes in their membrane potential. However, the extent of participation by each division varies between glands and the composition of the saliva secreted in response to stimulation of each division is distinctly different. In general, a copious flow of watery saliva is secreted in response to parasympathetic stimulation, whereas that produced by sympathetic stimulation is thicker, higher in organic content, and comparatively less in quantity.

The innervation of duct cells is not clear. Intraepithelial terminals in ducts have been observed only rarely, but histochemical studies suggest that cholinergic and adrenergic nerves are found in the connective tissue around the ducts. Physiologic studies indicate that the ductal system is responsive to autonomic

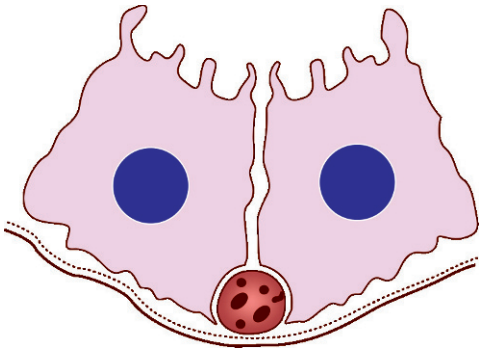

Figure 11.15 Diagrammatic representation of intraepithelial innervation.

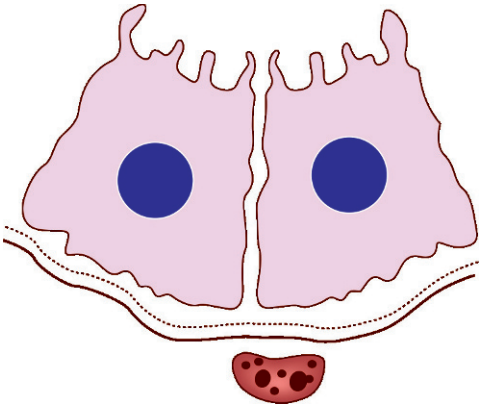

Figure 11.16 Diagrammatic representation of subepithelial innervation.

stimulation and membrane potential changes in the duct cells have been recorded, as well as changes in the transductal ion flux.

CLASSIFICATION AND STRUCTURE OF HUMAN SALIVARY GLANDS

The salivary glands have been classified in a variety of ways, the most commonly used groupings are based on:

1. Size and location; namely major and minor gland and based on location as labial and lingual, etc.
2. Histochemical nature of secretory product; namely serous and mucous.

Though it has been seen that all serous secretions are not alike they may differ considerably in the type and the amount of enzymes and proteins they produce. The carbohydrate component shows a similar variability. This is seen in mucous cells also. Hence, histochemical characterization of the secretory products of the different salivary glands may be useful for the comparison with the other glands.

On basis of weight of salivary glands producing saliva, the volume exceeds that of other digestive organs by as much as 40 times.

Major Salivary Glands

The largest of the glands are the three bilaterally paired major salivary glands. They are all located extraorally, and their secretions reach the mouth by variably long ducts.

Parotid gland

The parotid is the largest major salivary gland. Its superficial portion is located subcutaneously lying in front of the external ear and its deeper portion lies behind the ramus of the mandible, filling the retromandibular fossae.

The parotid is 5.8 cm craniocaudally and 3.4 cm ventrodorsally. It weighs between 14 and 28 grams.

The main excretory duct, Stensen's duct crosses the masseter muscle and turns medially at the anterior edge penetrating the buccinator muscle to open at a papilla at the buccal mucosa opposite the maxillary second molar. The duct measures 4–6 cm in length and 5 mm in diameter. A small portion of the parotid generally accompanies the duct forming an accessory gland, a few millimeter anterior to the superficial portion of the gland. The parotid gland receives its blood supply from the branches of the external carotid artery as they pass through the gland.The parasympathetic nerve supply is derived mainly from the ninth cranial nerve reaching the gland via the otic ganglion and the auriculotemporal nerve. The sympathetic innervation of all salivary glands is provided by the postganglionic fibers from the superior cervical ganglion and reaches the individual gland in association with their vascular supply.

The lymphatic drainage is via paraparotid and intraparotid lymph nodes into the superficial and deep cervical lymph nodes. The parotid gland is enclosed in a well defined connective tissue capsule which sends septa into the gland, separating it into lobes and lobules.

The parotid gland is a pure serous gland (Fig. 11.17A); all the acinar cells are similar in structure to the serous cells described earlier. In the infant, however, a few mucous secretory units may be found. Electron microscopic studies indicate that the serous granules may have a dense central core. The intercalated ducts of the parotid are long and branching (Fig. 11.9), and the pale-staining striated ducts are numerous and stand out conspicuously against the more densely stained acini. The connective tissue septa in the parotid contain numerous fat cells, which increase in number with age and leave an empty space in histologic sections.

Submandibular gland

The submandibular gland is the second largest salivary gland, also called the submaxillary salivary gland. It weighs half the weight of parotid gland.

The submandibular gland is on the medial aspect of the body of the mandible in the submandibular triangle. It is placed posterior and superficial to the mylohyoid muscle with an extension folded around the posterior border of the mylohyoid to be above the muscle. The main excretory duct, Wharton's duct, runs forward above the mylohyoid muscle lying just below the mucosa of the floor of the mouth in its terminal portion. It opens at the sublingual papillae also called the caruncula sublingualis, lateral to the lingual frenum.

The gland receives blood supply from the lingual and facial arteries. The parasympathetic innervation is derived primarily from the VII cranial nerve reaching the gland through the lingual nerve after synapsing in the submandibular ganglion.

The lymphatic drainage is to the deep cervical and jugular chain of nodes.

The submandibular gland is enveloped by a well defined capsule. It is a branched tubuloacinar gland of mixed type.

The submandibular gland is a mixed gland, with both serous and mucous secretory units (Fig. 11.17B). The serous units predominate, but the proportions may vary from one lobule to the next. The mucous terminal portions are capped by demilunes of serous cells. The existence of demilunes has been recently questioned. Although they appear similar by light microscope, notable differences between submandibular and parotid serous cells are observed in the electron microscope (Fig. 11.18). The basal and lateral plasma membranes are thrown into numerous folds, interdigitating with similar processes from adjacent cells. The serous granules exhibit a variable substructure, from a granular matrix with a dense core or crescent, to an irregular skein of dense material dispersed in the matrix. The intercalated ducts tend to be ducts that are somewhat shorter than those of the parotid, whereas the striated ducts are usually longer.

Sublingual gland

Sublingual gland is the smallest of the major salivary glands which is almond shaped. The sublingual gland lies between the floor of the mouth, below the mucosa and above the mylohyoid muscle (Fig. 11.1). It is composed of one main gland with several small glands. The main duct, Bartholin's duct opens with or near the

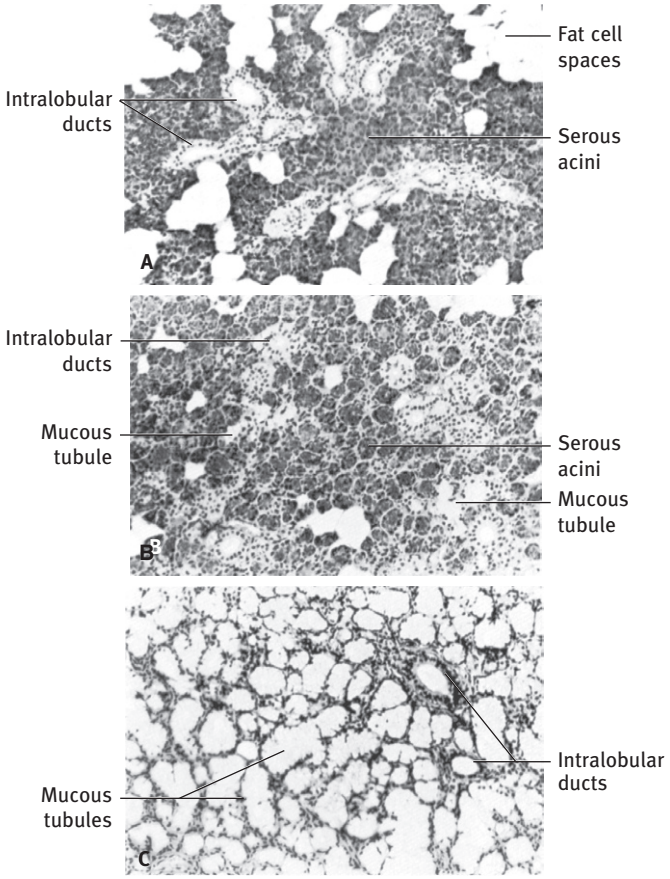

Figure 11.17 (**A**) Light micrograph of human parotid gland, showing serous acini, several intralobular striated ducts, and numerous fat cell spaces. (**B**) Light micrograph of human submandibular gland. Serous acini predominate, but a few mucous secretory units are present. Several intralobular striated ducts are cut in cross-section. (**C**) Light micrograph of human sublingual gland showing large mucous secretory units with typical tubular structure. Serous demilunes are difficult to distinguish at low magnification. Intralobular ducts are poorly developed (A to C, × 90).

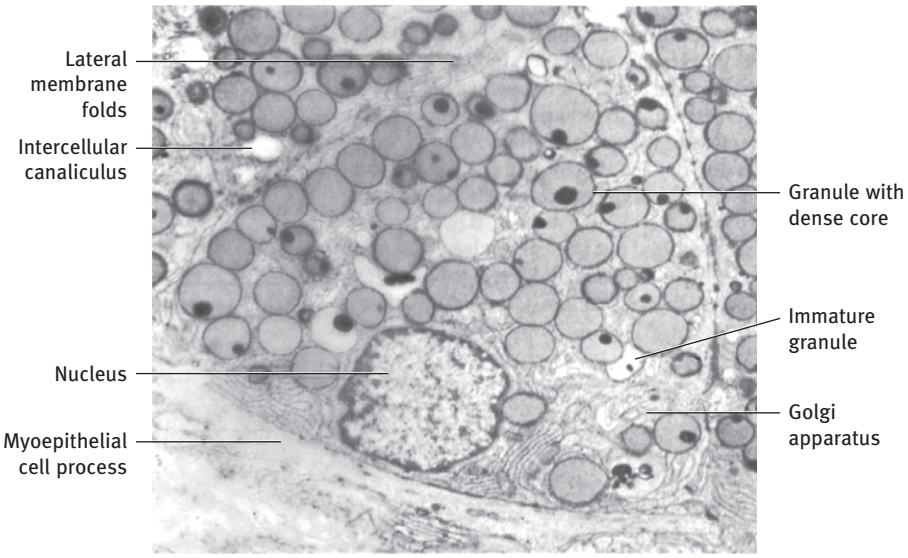

Figure 11.18 Electron micrograph of serous cells of human submandibular gland, showing secretory granules with dense core. Immature granules with similar cores are seen in Golgi regions. Several intercellular canaliculi are cut in cross-section, and extensive folding of lateral cell membranes occurs between adjacent cells. Myoepithelial cell process is present at base of cell (×6600) (From Tandler B and Erlandson RA: Am J Anat 135:419, 1972; reprinted by permission of the Wistar Institute Press).

submandibular duct. Several smaller ducts, duct of Rivinus, open independently along the sublingual fold.

The sublingual gland is also a mixed gland, but the mucous secretory units greatly outnumber the serous units (Figs 11.17C, 11.19). The mucous cells are usually arranged in a tubular pattern; serous demilunes may be present at the blind ends of the tubules. Pure serous acini are rare or absent. The intercalated and striated ducts are poorly developed; mucous tubules may open directly into ducts lined with cuboid or columnar cells without typical basal striations.

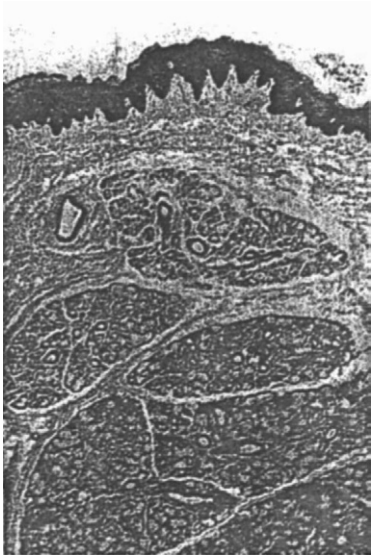

Figure 11.19 Sublingual salivary gland lying beneath oral mucosa shows predominantly pale staining mucous acini with serous demilunes.

The sublingual gland receives its blood supply from the sublingual and the submental arteries. The parasympathetic nerve supply is also derived from the VII cranial nerve. It reaches the gland via the lingual nerve after synapsing in the submandibular ganglion. The lymphatic drainage is to the submandibular lymph nodes.

Differences among parotid, submandibular and sublingual glands are depicted in Table 11.3.

Minor Salivary Glands

The minor salivary glands are located beneath the epithelium in almost all parts of the oral cavity. These glands usually consist of several small groups of secretory units opening via short ducts directly into the mouth. They lack a distinct capsule, instead mixing with the connective tissue of the submucosa or muscle fibers of the tongue and cheek.

There are 600 to 1000 minor salivary glands lying in the oral cavity and the oropharynx.

The minor salivary glands are classified according to their anatomic location, e.g. labial glands, buccal glands, lingual glands, palatine and glossopalatine glands, etc. They are not present in the gingiva, anterior raphe region of the hard palate or the anterior two thirds of the dorsum of the tongue.

Labial and buccal glands

The glands of the lips and cheeks classically have been described as mixed, consisting of mucous tubules with serous demilunes. However, ultrastructural studies of the labial glands have revealed the presence of mucous cells only. Intercellular canaliculi have also been observed between the mucous cells. The intercalated ducts are variable in length, and the intralobular ducts possess

Table 11.3 Differences among Parotid, Submandibular and Sublingual Glands

	Parotid	Submandibular	Sublingual
Development	4-6 weeks IUL	6th week IUL	8-12 weeks IUL
Secretion	Purely serous	Mixed (predominantly serous)	Mixed (predominantly mucous)
Flow rate	0.4ml/min (unstimulated) 1-2ml/min (stimulated)	0.1ml/min (Unstimulated) 0.8ml/min (Stimulated)	-
Secretion (viscosity)	Serous (Watery)	Seromucous	Mucous (thick and viscid)
Contribution to saliva	25%	70%	5%
Amylase activity	Maximum	Moderate	Minimum
Lysozyme activity	Absent	Predominant	Less
Intercalated duct	Long	Wide and long	Almost absent but rarely present
Striated duct	Long	Longer than parotid	Very short
Excretory ducts	Stenson's duct – Opens at buccal mucosa opposite to 2nd molar	Wharton's duct – Opens at sublingual papillae lateral to lingual frenum	Bartholin's (open near submandibular gland duct) and ducts of Rivinus (open independently in the floor of the mouth)
Capsule	Thick and well developed	Thin	Thin / absent
Nerve supply	Presynaptic – Glossopharyngeal Post synaptic -auriculotemporal nerve	Facial nerve – chorda tympani	Facial nerve – chorda tympani
Blood supply	External carotid artery	Lingual / facial artery	Sublingual / submental arteries
Lymphatic drainage	Paraparotid / Intraparotid nodes which drain into deep cervical nodes	Deep cervical, jugular nodes	Submandibular lymph nodes

only a few cells with basal striations. Although the buccal glands have not been examined by electron microscope, they are usually described as a continuation of the labial glands with a similar structure.

Glossopalatine glands

The glossopalatine glands are pure mucous glands. They are principally localized to the region of the isthmus in the glossopalatine fold, but may extend from the posterior extension of the sublingual gland to the glands of the soft palate.

Palatine glands

The palatine glands are also of the pure mucous variety. They consist of several hundred glandular aggregates in the lamina propria of the posterolateral region of the hard palate and in the submucosa of the soft palate and uvula. The excretory ducts may have an irregular contour with large distentions as they course through the lamina propria. The openings of the ducts on the palatal mucosa are often large and easily recognizable.

Lingual glands

The glands of the tongue can be divided into several groups. The anterior lingual glands (glands of Blandin and Nuhn) are located near the apex of the tongue. The anterior regions of the glands are chiefly mucous in character, whereas the posterior portions are mixed. The ducts open on the ventral surface of the tongue near the lingual frenum. The posterior lingual mucous glands (Fig. 11.20) are located lateral and posterior to the vallate papillae and in association with the lingual tonsil. They are purely mucous in character, and their ducts open onto the dorsal surface of the tongue. The posterior lingual serous glands (von Ebner's glands) are an extensive group of purely serous glands located between the muscle fibers of the tongue below the vallate papillae (Fig. 11.20). Their ducts open into the trough of the vallate papillae and at the rudimentary foliate papillae on the sides of the tongue.

Von Ebner's glands

Of all of the minor salivary glands, the posterior lingual serous glands (von Ebner's glands) are among the most interesting. Classically, their secretions have been described as serving to wash out the trough of the papillae and ready the taste receptors (located in the epithelium of the trough) for a new stimulus. Although this may be a part of their function, studies suggest that these glands have significant protective and digestive functions. Histochemical studies have localized the antibacterial enzymes peroxidase and lysozyme to these glands in humans. Biochemical studies of the lingual serous glands have demonstrated the presence of a secretory enzyme with lipolytic activity; similar lipolytic activity has been detected in aspirates from the esophagus and stomach. This lingual lipase has an acid pH optimum so that it is capable of hydrolyzing triglycerides in the stomach. The fatty acids, monoglycerides and diglycerides produced by lingual lipase, help to emulsify the remaining fat and increase the efficiency of pancreatic lipase in the intestine. In the newborn, when fat intake is high and levels of pancreatic lipase are low, lingual lipase probably plays a significant role in lipid digestion. Amylase activity has also been detected in the lingual serous glands of some species.

Table 11.4 depicts differences between major and minor salivary glands.

DEVELOPMENT AND GROWTH

Salivary glands are specialized secretory apparatus present from the amphibian upward. They show varying differentiation, structure and arrangement in different

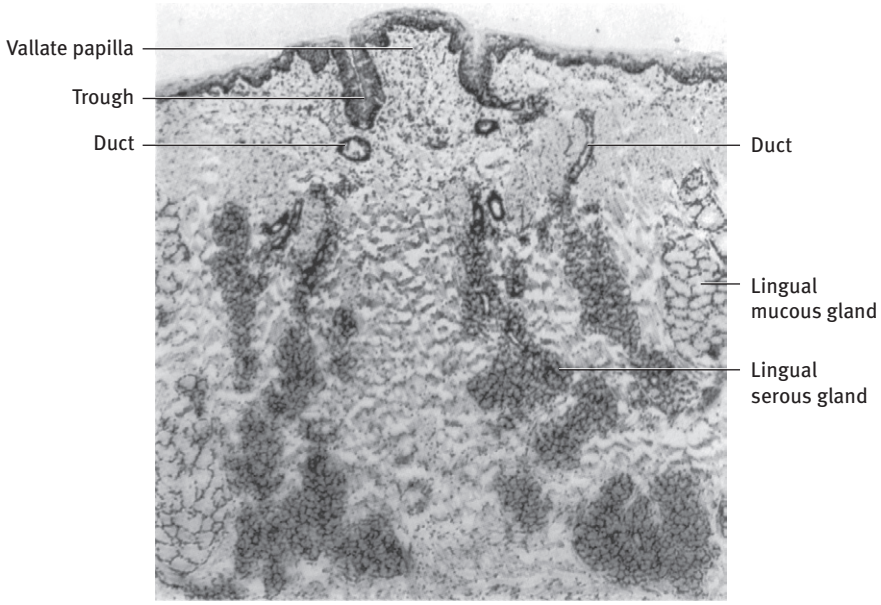

Figure 11.20 Light micrograph of minor salivary glands of rat tongue. Lingual serous (von Ebner's) gland is located between muscle fibers of tongue below vallate papilla. Its ducts empty into trough around papilla. Posterior lingual mucous glands are located lateral to serous glands; their ducts open onto surface of tongue (×36) (From Hand AR: J Cell Biol 44:340, 1970; reprinted by permission of the Rockefeller University Press).

Table 11.4 Differences between Major and Minor Salivary Glands

	Major Salivary Glands	Minor Salivary Glands
Size	Larger	Smaller
Number	Three in number, hence named individually	600-1000 in oral cavity and oropharynx, hence named as a group, according to their location Not named individually
Occurrence	Bilateral	Diffusely spread
Location	Extraoral with ducts opening intraorally	Intraoral
Contribution to whole saliva	90 to 95 %	5 to 10 %. More secretion during sleep
Capsule	Present	Absent
Excretory duct	Single main excretory duct	Multiple excretory ducts
Excretory duct size	Long excretory ducts	Short excretory ducts
Secretion	Mostly mixed except parotid, which is purely serous	Mucous or mixed except Von Ebner's gland which is serous
Secretion viscosity	Thin, watery	Thick, mucous
Secretion contents	Rich in enzymes, mucous and proteins	Proteins –important role in Innate immunity suggested

species, to cite an example, the parotid is present only in mammals.

In human beings all the salivary glands arise from the ectoderm of the oral cavity, and are comparable to other ectodermal derivatives such as sebaceous and mammary gland.

The minor salivary glands arise from the oral ectoderm and nasopharyngeal ectoderm. They develop after the major salivary glands.

During fetal life each salivary gland is formed at a specific location in the oral cavity through the growth of a bud of oral epithelium into the underlying mesenchyme. The primordia of the parotid and submandibular glands of humans appear during the 6th week, whereas the primordium of the sublingual gland appears after 7 to 8 weeks of fetal life. The minor salivary glands begin their development during the 3rd month. The epithelial bud grows into an extensively branched system of cords of cells that are first solid but gradually develop a lumen and become ducts. The secretory portions develop later than the duct system and form by repeated branching and budding of the finer cell cords and ducts.

The primordium of salivary glands is divided into pregland and preduct cells. The pregland cells take part in the formation of acini whereas the preduct cells are again divided into anterior and posterior domain. This gives rise to the common and the individual ducts respectively.

Three stages are seen in the development of the salivary gland. The 1st stage shows the formation of analogue and the development of the gland with dichromatic branched ducts. The 2nd stage shows further differentiation of the gland with early formation of lobules and canalization of the ducts. This stage lasts till the 7th embryonal month and encompasses the period of formation of functional units. The 3rd stage begins in the 8th embryonal month and leads to further structural maturation of the gland with acinar cells and intercalated duct differentiation. The development of the glandular tissue is characterized by reduction of the abundant interstitial connective tissue.

Studies of embryonic salivary glands in vitro have provided considerable information on the mechanism of glandular morphogenesis. The mesenchyme into which the glandular rudiment grows produces a factor or factors that stimulate the growth of the gland. If the mesenchyme and epithelium are separated and cultured on opposite sides of a filter, the growth of the epithelium proceeds normally; in the absence of the mesenchyme, the epithelium fails to grow. The process of branching morphogenesis, that is, the formation of hollow, tubular glands from an initially flat epithelial surface, appears to be related to the presence of microfilaments in the epithelial cells. Microfilaments about 5 to 7 nm (50 to 70 Å) thick form a network beneath the cell membrane of almost all cells; they consist of the contractile protein actin. In developing salivary epithelium they are particularly prominent at the apical and basal ends of the cells; differential contraction could cause a group of cells to pucker outward or clefts to form in a solid cord or sheet of cells, similar to the effect of pulling a purse string.

The presence of a functional innervation is also essential to proper growth and maintenance of salivary gland structure. Parasympathetic denervation of adult animals results in a 30% loss in glandular weight within 2 to 3 weeks. Sympathetic denervation causes variable responses, from atrophy of some glands to hypertrophy of others. Parasympathectomy of the developing rat parotid prevents attainment of adult gland size, cell number and size, and DNA and RNA content; sympathectomy has a moderate effect on cell and gland size only. Normal physiologic activity is also important for the proper growth of developing glands, as well as maintenance of adult structure and enzyme content. Feeding of a liquid diet to rats greatly diminishes the reflexly mediated secretory activity; the parotid rapidly decreases in weight and amylase content, and the normal diurnal pattern of synthesis and secretion is eliminated.

Conversely, chronically increased stimulation can cause an increase in glandular size. For example, increasing the bulk content of the food, which necessitates increased masticatory activity, results in hypertrophy of the rat parotid.

Treatment of mice and rat with isoproterenol, a α-adrenergic drug causes rapid and complete discharge of

the stored secretory products and stimulates protein synthesis; resulting in glandular enlargement up to 5 times to that of untreated animals.

These enlarged glands show enhanced production of certain proteins while others are reduced, additionally certain new proteins are also synthesized. The effect of these drugs on the growth of salivary gland has found wide application in experimental studies of cellular secretion protein and nucleic acid synthesis and regulation of gene expression.

The contractile myoepithelial cells which envelope each acinus as well as portions of the ductal system are derived from the neural crest cells and thus are ectodermal in origin. They become active between the 24th and 35th week of the prenatal development. It is important to note that most of the mesenchymal elements like the septa and the capsule are produced by the influence of the neural crest cells.

CONTROL OF SECRETION

The physiologic control of salivary gland secretion is mediated through the activity of the ANS; particularly parasympathetic nervous system. The control of secretion is also linked to changing taste and smell. Each of these is capable of modifying the amount and consistency of the salivary secretion though gustatory stimulus is more important than masticatory stimulus.

Postganglionic fibers of both the sympathetic (adrenergic) and parasympathetic (cholinergic) divisions innervate the secretory cells. Myoepithelial, arteriolar smooth muscle cells, intercalated and striated duct cells also receive direct innervation. Unmyelineated nerve invested by cytoplasmic processes of Schwann, forms a plexus in the connective tissue surrounding the terminal secretory units. Some instances show the pattern of innervation termed intraepithelial or hypolemmal. This type of innervation is seen in human submandibular gland and minor salivary glands of the lip whereas some areas show subepithelial or epilemmal innervation generally seen in human parotid gland. Both patterns of innervations show varicosities which are swellings or varicosities along their length which contain neurotransmitter vesicles. These sites are the sites of neurotransmitter release called nerve terminals. A single axon may have several varicosities and may effect innervation at several different cells.

The release of neurotransmitter from the vesicle in the nerve terminals adjacent to parenchymal cells stimulates them to discharge their secretory granules, secretes water and electrolytes and contraction of myoepithelial cells. The molecular events that occur during this process is called *stimulus secretion coupling*.

Norepinephrine, the sympathetic transmitter activates both α- and β-adrenergic receptors, while the parasympathetic transmitters activate cholinergic receptors. Protein secretion is mediated mainly through the α-adrenergic receptor; stimulation of the β-adrenergic and the cholinergic receptors also causes low levels of protein secretion, but these two receptors mainly appear to be involved in the secretion of water and electrolytes. Receptors for the peptide transmitter substance P are also present on salivary gland cells; substance P stimulates secretion similar to that caused by α-adrenergic and cholinergic agonists. Vasoactive intestinal polypeptide is present in the nerve endings in the salivary glands and has shown to induce secretion by some glands.

Receptor stimulation results in increases in the intracellular concentration of 'second messengers,' which trigger additional events leading to the cellular response. In the case of α-adrenergic, cholinergic, and substance P receptors, the membrane permeability to Ca^{++} is increased and a marked influx of Ca^{++} into the cell occurs. Recent experiments have linked the activation of these receptors to rapid changes in membrane phospholipid metabolism and release of Ca^{++} from intracellular stores such as the endoplasmic reticulum or the plasma membrane. The increased cytoplasmic Ca^{++} concentration causes K^+ efflux, water and electrolyte secretion, and a low level of exocytosis. Stimulation of the β-adrenergic receptor activates the plasma membrane enzyme adenylcyclase, which catalyzes the formation of 3′,5′- cyclic adenosine monophosphate (cyclic AMP) from adenosine triphosphate. The increased intracellular concentration of cyclic AMP activates cyclic AMP-dependent protein kinase, an enzyme that phosphorylates other proteins, which in turn may be involved in the process of exocytosis. Calcium activated K^+ channels function through voltage independent intermediate single channels and Max-k channels. Cyclic AMP may also stimulate release of Ca^{++} from intracellular stores, thereby increasing its cytoplasmic concentration. Thus Ca^{++} may be the common intracellular mediator for all of the receptors; the different cellular responses may reflect the different sources of Ca^{++} or differing local concentration, or both. Ca^{++} may have additional effects, including stimulation of guanylate cyclase activity and an increase in the concentration of 3′,5′-cyclic guanosine monophosphate (cyclic GMP). However, the role of cyclic GMP in the secretory process has not yet been determined.

Adjacent secretory cells are joined to one another by specialized intercellular junctions called *gap junctions*. These junctions are permeable to ions and small molecules; thus changes in the intracellular concentration of these substances in one cell are reflected by parallel changes in the adjacent cells. Therefore physiologic stimulation probably results in a response by secretory units (acini) rather than individual cells.

Similarly these junctions presumably allow for a coordinated contraction of several myoepithelial cells associated with each secretory unit. In some instances the gap junctions become electrically coupled or have decreased permeability, thus the physiologic significance of gap junction in relation to control of secretion still remains to be established.

Finally the blood vessels in the salivary gland are innervated by sympathetic vasoconstrictor and parasympathetic vasodilator fibers. The vascular response elicited by the autonomic stimulation ultimately determines the availability of water, electrolytes and metabolic substances during sustained secretory activity.

The secretion of saliva depends on many other factors which also to an extent alter the composition of saliva, namely age, duration and nature of stimulus, etc. Secretion of saliva is minimum at birth and does not

contain salivary amylase. The volume of saliva increases by 2–3 months and salivary amylase appears when the infant is given complex carbohydrates in diet. In old age the secretory reserve becomes decreased though the constituents appear to be stable.

The secretion of saliva can alter from 0.1 ml/min at rest to 4.0 ml/min at actively stimulated times.

It is believed that multiple methods of secretion coexist in the same acinar cells.

In the salivary glands, once the secretory cells are stimulated, the protein secretion in the salivary acini happens by two methods. In the first and main pathway, the cells store and secrete protein by a process of stored granule exocytosis. The time taken from the synthesis to exocytosis is about 3–5 hours. In the second pathway, the cells do not store protein but secrete it continuously by vesicular mechanism, i.e. vesicles traveling directly from the Golgi complex to the plasma membrane. This is called constitutive pathway. In this mechanism some proteins travel in the opposite direction; to the interstitial tissue. In addition, transcytosis is also seen which indicates passage of substances through the acinar cell like IgA, which passes from the interstitial tissue through the cell from the basolateral to the apical cell membrane (Fig. 11.4).

COMPOSITION OF SALIVA

Saliva consists primarily of water accounting for 99% or more of saliva (Box 11.1). Inorganic ions, secretory proteins, glycoproteins of serum constituents and other substances typically account for 1% or less. The main electrolytes of saliva are Na, K, Ca, Cl, HCO^3 and HPO^4. Other electrolytes present in smaller concentration are Mg, SO_4, F, SCN, and I. The main organic substances found in saliva are secretory proteins. They include enzymes such as amylase, ribonuclease, kallikrein, esterase, nystatin, cystatin, peroxidase, lysozymes, lactoferrin and acid phosphatase; mucin containing large amounts of bound carbohydrates which have a similar composition to their specific blood group substance and other proline rich proteins and glycoproteins. Other normal organic constituents of saliva include secretory immunoglobulins like IgG and IgM, blood clotting factors, amino acids, urea, uric acid, glucose, various lipid and hormones. The minor salivary glands secrete proteins which play an important role in innate immunity. They are bacterial pattern recognition receptors.

The proportions of these components are variable depending upon the source of saliva, the nature and the intensity of the secretory stimulus and the time of the day.

The saliva produced by major salivary gland differ from one another in composition. The parotid gland secretes a watery saliva rich in enzymes such as amylase, protein rich protein and glycoproteins. The submandibular gland contains higher proportion of glycosylated substances such as mucin in addition to the components already listed. The sublingual gland produces a viscous saliva rich in mucin.

As can be seen the composition collected will reflect on the cellular make up of a particular gland.

Box 11.1 Saliva: Composition

99%: water
1%: Inorganic ions, secretory proteins
Main electrolytes: Na, K, Ca, Cl, HCO_3, HPO_4
Organic substances: Amylase, ribonuclease, kallikrein, esterase, nystatin, cystatin, peroxidase, lysozymes, lactoferrin, acid phosphatase, mucin, proline rich proteins
Other organic substances: Clotting factors, amino acids, urea, glucose, lipids and hormones
Immunoglobulins: IgA, IgG and IgM
Mixed saliva (whole saliva): contains secreted saliva and desquamated oral epithelial cells, microorganisms and their products, leukocytes, food debris and gingival fluid

The stimulated saliva collected from the mouth is a complex mixture. It is also known by the name mixed saliva. The secretions of all the minor and major salivary glands contribute to whole saliva. In addition to the components derived by the gland, the whole saliva also contains desquamated oral epithelial cells, microorganisms and their products, leukocytes, serum constituents and the fluid from the gingival crevice, and food remnants. The total volume of saliva secreted by humans is approximately 750 to 1000 ml daily, of which submandibular accounts for 60%, the parotid about 30% and the sublingual about 5% or less, and about 1% of saliva is derived from the minor salivary glands.

Secretion elicited in response to sympathetic stimulation will differ in protein and electrolyte from that due to parasympathetic stimulation.

The resting flow rate of whole saliva is 0.2 to 0.4 ml/min, for the parotid is 0.4 ml/min, submandibular is 0.1 ml/min. On stimulation the rates increase to 0.2 to 0.4 ml/min for the whole saliva, 1.0 to 2.0 ml/min for parotid, and 0.8 ml/min for the submandibular salivary gland.

The concentration and proportion of different electrolytes depend in part on the flow rate of saliva.

The pH of whole saliva varies from 6.4 to 7.4, the parotid saliva varies over a greater range from pH of 6.0 to 7.8.

The time of the day also exerts an influence on the amount, source, and composition of the saliva. For instance during sleep, very little saliva is secreted by the major salivary gland and minor salivary gland becomes much more significant.

Other important factors affecting the composition of saliva are flow rate, differential gland contribution, circadian rhythm, duration of stimulus, nature of stimulus and diet.

The concentration of constituents in saliva depends only on the rate of flow and not on the nature of stimulus.

FUNCTIONS OF SALIVA

The most important function of the salivary gland is the production and the secretion of the saliva.

Protection of the oral cavity environment is the major function of the saliva, the other functions being assisting in digestion, speech, mastication, taste and tissue repair.

Protection of the oral cavity and oral environment

Saliva extends protection to the oral cavity and its tissues in many ways. The constant secretion of saliva prevents desiccation of the oral tissues. The absence of which can cause the oral mucosa to degenerate and atrophy. Its fluid-like nature provides a washing action to flush away debris and the nonadherent bacteria. The mucin and other glycoproteins provide lubrication for the movement of the oral tissues against each other allowing smooth and sliding movements.

Saliva protects the mucosa from chemical and thermal insults by reducing the concentration and lowering and buffering the temperature respectively.

The saliva causes dilution of detritus and oral acid neutralization. The saliva protects the oroesophageal mucosa. Saliva has high molecular weight glycoproteins responsible for oral, oropharyngeal and esophageal mucosal lubrication.

The primary buffering system of saliva is formed by bicarbonates (HCO_3). To some extent phosphate ion (HPO_4) and salivary proteins contribute to the buffering action. Additionally the metabolism of the salivary proteins and peptides provides urea and ammonia that help to increase the pH. This helps in maintaining a high pH, which is not conducive for cariogenic bacteria to survive, ferment carbohydrate and produce acid to cause tooth decay.

A large group of salivary proteins called proline rich proteins because of their high content of amino acid proline, and statherin, a small tyrosine rich protein, inhibit the precipitation of calcium phosphate from the saliva. Along with other salivary glycoproteins, statherin and certain of the proline rich proteins bind to the tooth surface, forming the acquired enamel pellicle. The resulting supersaturation of the calcium and phosphate reduces dissolution and promotes remineralization of the tooth enamel. On the surface of the tooth, a high concentration of calcium and phosphate causes posteruptive maturation of the enamel, increases surface hardness and resistance to demineralization. Remineralization of the initial carious lesions can be enhanced by fluoride ions in saliva.

Saliva has many antibacterial features. They are chiefly produced by the serous secretor cells of both major and minor salivary glands. Some high molecular weight salivary glycoproteins aggregate specific strains of oral microorganisms and/or prevent their adhesion to oral tissues, thus facilitating their oral clearance.

The acinar cells secrete peroxidase and ductal system secretes thiocyanate, both of which establish a bactericidal system in saliva. Salivary peroxidase in presence of hydrogen peroxidase and thiocyanate, catalyses the formation of hypothiocyanate ion (OSCN) which inhibits bacterial growth.

Another antibacterial protein in saliva is lysozyme, an enzyme that hydrolyzes the polysaccharide of bacterial cell walls, resulting in cell lysis.

The antioxidant defense mechanism is exerted by uric acid and ascorbic acid. A profound amount of these antioxidants is secreted by the parotid gland during meal times. This helps in reducing oxidant stress and maintains oral integrity.

An important group of defensive substances in saliva are the immunoglobulins. The predominant salivary immunoglobulin is IgA. Salivary or secretory IgA differs from serum IgA that is produced locally by plasma cells in the connective stroma of the glands and consist of a dimer of two IgA molecules and a protein called J chain. There is an additional glycoprotein produced by the parenchymal cells, called secretory component, that is also a part of the secretory IgA molecule. Secretory component acting as a receptor in the parenchymal cell membrane for dimeric IgA, facilitates the transfer of the IgA to the lumen, either by translation in the cell or by the endocytosis and secretion along with the secretory products of the parenchymal cells. Secretory component may also increase the resistance of the IgA molecules to denaturation or proteolysis in the oral cavity. Small amounts of IgG and IgM have also been detected in saliva, and occasional plasma cells in the glandular stroma can be stained by fluorescent antibodies specific for these immunoglobulins. Salivary immunoglobulins may also enter the saliva through the gingival crevice. Salivary immunoglobulins may act primarily through their ability to inhibit the adherence of the micro-organisms to the oral tissues. Another antibacterial substance found in the saliva is lactoferrin, an iron binding protein. In the presence of specific antibody, lactoferrin that is not saturated with iron enhances the inhibitory effect of the antibody on the microorganisms.

Saliva contains many antimicrobial substances. They include the antibacterial agents lysozymes, lactoferrins, calprotectin, lactoperoxidase, immunoglobulins, chromogranin A, cystatins, histatins, VEGP (von Ebner's gland protein), SLPI (secretory leukocyte proteinase inhibitor) and the antiviral agents; mainly cystatins, mucins, immunoglobulins, and SLPI. Antifungal action is exerted by histatins, chromogranin A and immunoglobulins.

Digestion

Saliva participates in digestion by providing a fluid environment for solubilization of food and taste substances, and through the action of the digestive enzymes, principally amylase have been identified in human beings; two of these, representing 25–30% of the total amylase protein, have small amounts of bound proteins. The action of amylase on ingested carbohydrates to produce glucose and maltose begins in the mouth and continues for up to 30 minutes in the stomach before the amylase is inactivated by the acid pH and proteolysis. Lingual lipase produced by lingual serous glands initiates the digestion of dietary lipids, hydrolyzing triglycerides to monoglycerides, diglycerides and fatty acids. Other hydrolytic enzymes have been detected in saliva, but their significance in food digestion has not been established.

Mastication and deglutition

Saliva moistens the food and helps its breakdown into smaller particles to initiate digestion. The moistening and lubricating properties of saliva allow the formation of bolus and facilitate deglutition.

Saliva not only moistens the dry food but also reduces the temperature of the hot foods.

Taste perception

The food taken into the oral cavity is emulsified in saliva and dissolved. This process is a prerequisite for the sense or perception of taste. This is due to the presence of

Table 11.5 Saliva: Functions

Functions	Components
1. Protection and Lubrication	Mucin, Proline rich proteins, Water
2. Antimicrobial action	Lysozyme, Lactoferrin, Lactoperoxidases, Mucins, Cystins, Histatins, IgM, PRP, IgA
3. Mucosal Integrity	Mucins, Electrolytes, Water
4. Dilution and Cleansing	Water
5. Buffer capacity and Remineralization	Bicarbonate, Phosphate, Calcium, Statherin, Proline rich anionic proteins, Fluoride
6. Deglutition	Water, Mucins
7. Digestion	Amylase, Lipase
8. Taste	Water, lipocalins
9. Phonation	Water, Mucin
10. Excretion	(through) Water

water and lipocalins in saliva. It also helps in maintaining taste receptors.

Speech

Saliva keeps the oral tissue moist and well lubricated which facilitates speech. It helps in vocalization and communication ability.

Tissue repair

A variety of growth factors and trefoil proteins are present in small quantities in saliva. Under experimental conditions these promote tissue growth, differentiation and wound healing.

Excretion

The salivary glands have an excretory function as do pancreas and gastric glands. Saliva is generally not lost to metabolism since it is reabsorbed in the GIT. Many substances from blood reach the saliva, thus saliva can be considered as a route of excretion.

The low molecular weight serum constituents can be demonstrated in saliva as for example, electrolytes and drug concentrations which can be assessed in the saliva. Infective agents from blood can reach the saliva. This is particularly true for hepatitis B virus.

The nitrates in the food reach the saliva and are reduced to nitrites by microorganisms, which are considered to be important in carcinogenesis.

The functions of different components of saliva are summarized in Table 11.5.

CLINICAL CONSIDERATIONS

An understanding of the anatomy, histology, and physiology of the salivary glands is essential for good clinical practice. In all aspects of clinical practice salivary glands and saliva play an important role.

Saliva regulates the oral environment and has widespread distribution of the salivary glands in the oral cavity. Hence there is a great impact of salivary gland pathology on clinical practice in dentistry.

With the exception of a portion of the anterior part of the hard palate, salivary glands are seen everywhere in the oral cavity. Because of this feature salivary gland lesion can occur everywhere in the oral cavity. In a differential diagnosis of oral lesions therefore a salivary gland origin must always be kept in mind.

The salivary glands are subject to a number of pathologic conditions. These include inflammatory infective diseases such as viral, bacterial, or allergic sialadenitis, a variety of benign and malignant tumors, autoimmune diseases such as Sjögren's syndrome, and genetic diseases such as cystic fibrosis. One of the most common surface lesions of the oral mucosa is a vesicular elevation called mucocele. This is produced from the severance of the duct of a minor salivary gland and pooling of the saliva in the tissues. A blockage of a salivary gland duct may occur after formation of a mucous or calcified plug within the duct. If this occurs in a minor salivary gland, it usually causes no symptoms, but in major glands such obstruction can be very painful and may require surgical intervention.

Another lesion associated with salivary gland is nicotinic stomatitis. In this the hard palate is whitened by hyperkeratinization around the duct opening of minor salivary gland caused due to heat from tobacco use. The heat causes inflammation of the duct openings which become dilated. Hence clinically these are seen as red macules scattered on white background of the palatal mucosa.

The salivary glands may also be affected by a variety of systemic and metabolic diseases. The major glands, especially the parotid, may become enlarged during starvation, protein deficiency, alcoholism, pregnancy, diabetes mellitus, and liver disease. The association of the major salivary glands with the cervical lymph nodes, brought about by a common area of development, necessitates the differentiation of pathologic conditions of these lymph nodes from salivary gland diseases.

Alteration of salivary gland function during disease states may have profound influences on the oral tissue. Loss of salivary function or reduction in volume of saliva secreted is called xerostomia. This leads to dryness of the mouth. The causes of xerostomia include disease states like Sjögren's syndrome, effects of chemotherapy or radiation therapy or as a result of variety of medications.

The common drugs causing dry mouth are anticholinergics, antidepressants, antipsychotics, antihypertensives, anoretics and drugs used in the treatment of parkinsonism.

Decreased salivary volume leads to difficulty in speech, mastication and taste perception, and swallowing becomes painful. The teeth become susceptible to caries. Oral tissues become susceptible to frequent oral infection; inflammation and ulceration of oral mucosa is commonly seen. In some instances excessive salivary secretion is seen because of certain physiologic states and rare pathologies. This is called ptyalism or sialorrhea.

As seen saliva is the principal protector of the hard and soft oral tissues. Any alteration in the saliva thereby affects the quality of life. Thus signs and symptoms of salivary dysfunction should be accurately diagnosed and treated.

Age changes in the salivary glands, particularly prominent in the parotid, consist of a gradual replacement of parenchyma with fatty tissue. Since the parotid is the major source of serous saliva; with advancing age, patients often complain of dryness and an increase in the viscosity

of saliva. Recent studies have shown that in the aged the flow of saliva is reduced during resting condition, but in composition and quantity stimulated saliva in healthy, aged individuals is similar to that of young adults.

Salivary gland and sialochemistry are often of value in the diagnosis of glandular and systemic diseases. It is valuable in diagnosing diseases like cystic fibrosis, Sjögren's syndrome and certain infectious diseases associated with *H. pylori* like peptic ulcer disease and chronic gastritis. Biopsy of the minor salivary gland of the lip is frequently used as an aid in the diagnosis of salivary gland disease. Sialochemistry is used to monitor plasma concentration of certain therapeutic drugs and toxic substances. Drug monitoring of a large variety of drugs is under trial. The therapeutic agents like antipyrine, carbamazepine, cyclosporin, digoxin, methadone, phenytoin, quinine and barbiturates are a few examples of drug monitoring. The blood type of individuals can be determined from salivary samples. The hormone levels can be monitored like in other body fluids, the efficacy of which needs to be established. Saliva can be used to monitor progesterone levels to find the ovulation time in females. The levels of testosterone, insulin and steroids can also be estimated in saliva. Hormonal levels of estrion and estradiol in saliva can give information about fetal growth. The determination of salivary electrolyte concentration and protein composition may aid in the diagnosis of salivary gland or systemic diseases, like alterations of salivary Na^+:K^+ ratio in patients with Addison's disease and Cushing's syndrome.

SUMMARY

Structure

Salivary glands are compound, exocrine glands secreting saliva. The secretory units are known as acini and the secretions are carried by tubules, which open into the oral cavity. Hence salivary glands are tubuloacinar in nature. The major salivary glands are large glands present outside the oral cavity whereas minor salivary glands are much smaller glands without a proper ductal system and present in the oral mucosa. The major glands are encapsulated but the minor glands do not have a capsule. The terminal secretory units or acini are made up of secretory cells, which are either serous or mucous.

The serous acini are smaller and spherical while the mucous acini are larger and tubular.

Serous Cells

The serous cells are pyramidal with the apex towards the lumen. They have a spherical nucleus near the base. The serous cells have all the features of a protein secreting cell. The secretory granules are called zymogen granules. The granules are discharged into the lumen by exocytosis.

Mucous Cells

The mucous cells are larger and their nucleus is flattened and is at the base of the cell. The cytoplasm of the mucous cells are in the form of a network enclosing mucinogen droplets. Unlike zymogen granules the mucous droplets are not membrane bound. The lumen of the serous acini has extensions between the cells and is known as intercellular canaliculi. The mucous secretion is discharged into the lumen along with a portion of the fused plasma membrane. The mucous secretion differs from the serous secretion in that the mucous secretion is more viscous having little or no enzymatic activity and has higher carbohydrate content.

Mixed Glands

In mixed glands having both serous and mucous acini, sometimes the serous acini are present as crescent-shaped caps over the mucous acini. These are called demilunes.

Myoepithelial Cells

The myoepithelial cells are stellate cells with a flattened nucleus and branching processes that embrace the acini or the striated ducts. The myoepithelial cells contain cytokeratin and contractile filaments. The cells contract to accelerate the flow of saliva.

Major Salivary Glands

The major salivary glands are larger encapsulated glands, fewer in number and located outside the oral cavity, but their well organized ducts open into the oral cavity. Parotid gland is a pure serous gland, the submandibular and the sublingual glands are mixed glands, but submandibular gland is predominantly serous and the sublingual gland is predominantly mucous gland. These major salivary glands together account for more than 90% of salivary secretion.

Minor Salivary Glands

The minor salivary glands are numerous (estimated to be between 600 and 1000) and are named by their location. They are present in all regions of the oral mucosa except gingiva, anterior raphe region of the hard palate and anterior two thirds of the dorsum of the tongue. The labial and buccal glands are mixed glands, the palatal glands and the glossopalatine glands are pure mucous glands, the von Ebner's glands (posterior lingual glands) are pure serous glands while anterior lingual glands are mixed glands. The secretions of von Ebner's glands serve to wash the trough of the circumvallate papillae and they also contain the lingual lipase enzyme.

Ducts

There are three order of ducts, namely intercalated ducts, striated ducts and excretory ducts. The intercalated duct lumen is continuous with the acini and is lined by a single layer of cuboidal cells. These ducts also secrete lysozymes, lactoferrin etc. The striated ducts are larger than intercalated ducts and are lined by a single layer of tall columnar cells. These cells show prominent striations. These striations are due to deep infoldings of the plasma membrane and contain numerous mitochondria. These ducts reabsorb sodium and chloride ions and secrete potassium and bicarbonate ions. This makes the saliva hypotonic. These ducts also secrete glycoproteins like kallikrein and epidermal growth factor. The next order of ducts is the excretory ducts, which are much larger. The ducts are lined by pseudostratified columnar epithelium which later on becomes stratified cuboidal and finally becomes stratified squamous epithelium. Ciliated cells, goblet cells and dendritic cells may be present.

Capsule

The connective which forms a capsule for the gland divides the gland into compartments of decreasing sizes such as

lobes and further divides the lobes into lobules. The connective contains the blood vessels and nerves that supply the epithelial cells of the acini and ducts. The intercalated ducts and the striated ducts are within the lobules (intralobular ducts), and the excretory ducts are between lobules (interlobular ducts).

Innervation

The innervations of secretory cells are by somatic sensory nerves and by autonomic nervous system. The axons of the nerves either penetrate the basal lamina (intraepithelial innervation) or end below the basement membrane (subepithelial innervation). Neurotransmitters like norepinephrine and acetylcholine are released in these sites.

Mechanism of Salivary Secretion

Norepinephrine, the sympathetic transmitter activates both α-adrenergic and β-adrenergic receptors resulting in salivary secretion, which is thicker, lesser in quantity but with a higher organic content than parasympathetic stimulated acetylcholine induced (cholinergic receptor) secretion which yields copious watery secretion. α-adrenergic receptor activation causes higher levels of protein secretion than other receptor's activation. Receptor stimulation leads to increased intracellular concentration of second messengers leading to increased permeability to calcium ions. The calcium influx causes efflux of potassium ions, water and electrolyte secretion and a low level of exocytosis. As adjacent secretory cells are joined by gap junctions, which are permeable to ions, the ionic changes in one cell are reflected in other cells as well. The amount of water available for secretion is controlled by parasympathetic vasodilator fibers and sympathetic vasoconstrictor fibers. In one mechanism of secretion called the main pathway, the protein secretion is stored before secretion while in the other mechanism called the constitutive pathway the secretion is continuous.

Functions of Saliva

Saliva has many functions. Saliva is necessary for all the functions of oral cavity like perception of taste, deglutition and speech. Saliva helps in digestion through its enzymes, amylase and lipase. It protects the oral cavity from drying and from bacterial invasion by flushing away the bacteria and also by the antibacterial substances like lysozymes, immunoglobulins, lactoferrins, etc. The buffering substances like bicarbonate ions neutralize the acids produced by cariogenic bacteria. Saliva has many growth factors for tissue repair and is also a route for excretion for many drugs.

Development of Salivary Glands

The salivary glands develop at about 7th to 8th week from the ectoderm by the growth of epithelial cells and its subsequent canalization into ducts and lumen of acini. Some cells like the myoepithelial cells are derived from the neural crest cells. Presence of functional innervation is essential for the proper growth and maintenance of salivary gland structure.

Clinical Considerations

Absence or decreased salivary secretion leads to a variety of pathology like caries, mucosal ulceration and oral infection. The salivary glands themselves are associated with many diseases which are inflammatory in nature like sialadenitis, viral infections like mumps, cysts, developmental disorders, metabolic diseases, autoimmune diseases like Sjögren's syndrome and salivary gland tumors. Saliva is of great significance in diagnostic pathology. Sialochemistry is valuable in diagnosis of glandular, systemic diseases and for monitoring level of therapeutic drugs.

REVIEW QUESTIONS

1. Describe the structure of a serous salivary gland (parotid gland).
2. Compare and contrast serous acini with mucous acini.
3. Write notes on:
   Myoepithelial cells (basket cells)
   Striated ducts
   Demilunes
   Von Ebner's glands.
4. Describe the modifications to saliva by salivary gland ducts.
5. Tabulate the differences between major salivary glands and minor salivary glands.
6. Describe the methods of salivary secretion. How are the secretions controlled?
7. Give a detailed account of the composition of saliva.
8. Describe the functions of saliva.

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12 Lymphoid Tissue and Lymphatics in Orofacial Region

CHAPTER CONTENTS

INTRODUCTION TO LYMPHATIC SYSTEM

The body is made of a variety of cells organized as tissue and organ system. The tissue is always bathed in tissue fluid, which is made of diffusible constituents of blood and the waste materials discarded by the cells. A good portion of this tissue fluid returns back to cardiac circulation via the venous end. The remainder, accounting for 1/10th of the tissue fluid is carried by the lymphatics. The tissue fluid diffuses through the permeable walls of the lymphatic capillaries to become lymph. The lymph is carried by the lymph vessels of increasing sizes. The lymph nodes intervene in the course of lymphatics, before the lymph is emptied into the venous circulation.

The constituents of the lymphatic system are the lymph, lymph vessels and capillaries, lymph nodes, lymphoid organs, diffuse lymphoid tissue and bone marrow.

TYPES OF LYMPHOID TISSUES

The lymphocytes play a vital and central role in all the lymphoid tissues and organs. The areas where the pre T and pre B lymphocytes mature into naive T and B cells are the *primary lymphoid organs*. The primary mammalian lymphoid organs are fetal liver, adult bone marrow and thymus. The naive T and B cells mature in the absence of foreign antigen and leave the primary lymphoid organ. The rearrangement of their genetic material generates a clone of cells which can recognize and respond to a diverse and large variety of foreign antigens. Further maturation of the normal lymphocytes in the primary lymphoid organ results in the expression of many chemokine receptors and adhesion molecules that lead them to secondary lymphoid organs.

The **secondary lymphoid organs** are the spleen, lymph nodes, tonsils and adenoids, NALT (nose associated lymphoid tissue) Payer's patches and MALT (mucosa associated lymphoid tissue). MALT is the collective term for all mucosa associated lymphoid tissues of gastrointestinal, respiratory, and genitourinary tracts. They are present at anatomically distinct sites and are static. In some instances, less anatomically restricted tissues in the form of lymphoid cell aggregation, which are well organized but lack discrete definition and delimitation by a capsule also come under this category, e.g. BALT (bronchus associated lymphoid tissue) and ILF (isolated lymphoid follicles) and cryptopatches. GALT (Gut associated lymphoid tissue) and analogous lymphoid system has been identified in glandular tissues such as salivary gland called DALT (duct associated lymphoid tissue). The lymphoid cells concentration is seen around the excretory ducts in salivary glands. In the secondary lymphoid organs, the naive antigen specific T and B lymphocytes encounter pathogens and antigens and generate an adaptive response. The naive cells circulate in the blood

stream after they exit the primary lymphoid the passage of time the naive cells migrate into the tissue and settle in the secondary lymphoid organs. The secondary lymphoid organs and MALT concentrate the antigens received from the local sites and also receive the antigen presenting cells in efficient numbers. MALT also plays a role in immune tolerance. The exposure of the naive cells to the above antigens leads to activation of antigen specific lymphocytes, thus initiating a specific adaptive immune response and generates a long-term protective immunity. The MALT (tonsils) provides a surveillance and reactive system designed to combat foreign antigens and pathogens before they gain access to the inner core of the body.

The **tertiary lymphoid organs** are the ectopic accumulation of lymphoid cells that arise in nonlymphoid organs. They are also called by the name of tertiary lymphoid tissues.

The tertiary lymphoid organ arises as a sequel to chronic inflammation resulting in the process of lymphoid neogenesis or lymphoid neo-organogenesis. The tertiary lymphoid organ is very similar to the secondary lymphoid organ in cellular composition, morphologic organization, chemokines and vasculature. The capacity to arise in almost any site or organ in the adult is a potential, the tertiary lymphoid organ possess.

DEVELOPMENT OF LYMPH NODES AND LYMPHATICS

The lymphatic system develops in close association with the cardiovascular system. The early signs of the lymphatic system are seen by the formation of a number of endothelial lined lymph sacs. It is not very clear if these sacs develop from the various systems or are independent formation from mesenchymal lymphangioblasts. In the fifth week of intrauterine life, six sacs are recognized to be formed. As early as in the first trimester, lymphatic plexuses form either by extension of the sacs or may form de novo and extend into various tissues. Once the plexuses are formed they are studded by small collection of lymphoblasts, which eventually lead to invasion of the sacs (all except one forming the cisterna chyli) by the lymphocytes and the connective tissues leading to the formation of groups of lymph nodes.

In the second trimester, differentiation into cortex and medulla begins, leading to the compartmentalized structures of the lymph node parenchyma. After which very few changes happen until the lymph node is exposed to an antigenic challenge. The blood vessels branch into the lymph nodes and later develop into high end venules. The secondary follicles appear at day 18 after birth indicating B cell migration into lymph node, though the first lymphocytes invading the lymph nodes are T lymphocytes during lymphoid organogenesis.

FUNCTIONS OF THE LYMPHATIC SYSTEM

The major functions of the lymphatic system are as follows.

1. Tissue drainage The cardiovascular system pumps around 21 liters of plasma fluid from the arterial end of the capillaries into the tissue. The plasma fluid has dissolved substances and some plasma proteins. Most of the tissue fluid returns via the capillaries at the venous end into main stream but approximately 3–4 liter of fluid still remains in the tissue which is drained passively by the lymph vessels. Failing which the fluid becomes tissue logged and results in fall of blood volume. Up to 50% of fluid and serum protein that escapes from capillary return to the circulation via the lymph nodes.

2. Immunity The lymphatic organs and nodes along with the bone marrow are responsible for the production and maturation of lymphocytes. The lymphocytes in the form of T and B cells play an important role in mounting immunity.

3. Fat absorption This function happens only in the lymph vessels of the villi in the intestine which take up fat and fat soluble materials which actually give lymph an offwhite or light yellow color.

LYMPH NODES

The human body is confronted constantly by numerous invading pathogens. To provide defense against these pathogens and foreign substances, a chain of well organized and compartmentalized lymph nodes are present clustered in small groups at all strategic locations.

In the head and neck area, the lymph nodes are an important and major component of the lymphatic system in human beings. They are the center for mechanical filtration of foreign substances in the lymph and act as site for antigen presentation, lymphocyte activation, differentiation and proliferation. Lymph nodes feature throughout the body but are more concentrated at areas draining the organs with environmental contact because in these areas antigenic contact is constant and more often than other areas of the body. A young adult will have around 450 lymph nodes. A good number of 60 to 70 are found in the head and neck region, predominantly in the neck. Some areas have lymphoid tissue or aggregates in the mucosal surfaces. MALT, GALT and BALT are all integral part of the lymphatic system. All these lymph nodes irrespective of the location converge in a single channel, the thoracic duct which empties the lymph into the venous system. The human brain is the only organ which does not depend on lymphatics but drains its extracellular fluid through the cerebrospinal fluid and the Virchow–Robin spaces.

Anatomy

Lymph nodes are yellowish oval or bean-shaped nodular, soft and encapsulated peripheral lymphoid organs. The lymph node generally shows a smooth surface on the outer side. The inner side shows an indentation or a dimpling called the hilum, through which vessels enter and exit. They are 2–20 mm in diameter. A cut section of

a lymph node is offwhite in color and homogeneous, frequently described as a fish meat appearance. It is composed of dense and compact accumulation of lymphoid tissue. Each lymph node is connected to the circulation by afferent and efferent lymphatics.

The characters and number of a lymph node vary with age, pathology and location in the body.

Microscopic Structure

A section of the lymph node studied under low power in light microscopy shows an encapsulated tissue with numerous lymphatic lobules, lymph filled sinuses. There are three areas other than the capsule that are well defined in any lymph node. They are: the cortex or cortical area where the lymphoid follicles are seen which are B cell centers, the paracortex or paracortical area surrounding the lymphoid follicles where prominent vessels are seen along with a dense population of T cells, and the medullary area or zone along with the sinuses.

The capsule of a lymph node is chiefly composed of collagen and elastin fibers with a few fibroblasts. The capsular dense connective tissue extends as trabeculae radiating into the deeper or interior areas of the lymph nodes. The fibers of the capsular and trabecular area continue with the fine reticular fibers in the deeper area.

All the lymph nodes irrespective of their size and location are made of *lymphoid lobule*. The lymphoid lobules are the structural and functional unit of lymph nodes. Variable number of these lobules make up the lymph node, sometimes varying from 1–2 lobules in the smallest lymph node and numerous in the larger lymph nodes.

The lymphoid lobules are arranged side by side radiating from the hilus to the capsular area in the form of cones. The lobules have a bulbous composition in the subcapsular area and taper into slender chords in the medullary and hilar areas. The bulbous area forms the nodal cortex and the slender chord areas form the nodal medulla. The nodal cortex is commonly divided into superficial cortex and deep cortex. The superficial cortex denotes the cortex area and the deep cortex, the paracortex area (Fig. 12.1 & 12.2).

The cortex contains spherical follicles in the form of primary and secondary follicles surrounded by diffuse cortex. The paracortical area is made up of deep cortical units (DCU). Each lobule has a single deep cortical unit which is further divided into central deep cortical unit and the surrounding peripheral deep cortical units. Sometimes the peripheral deep cortical units can coalesce from adjacent lobules to form a large multiunit complex.

The lobule in the lymph node is surrounded by an intricate and complex system of lymphatic sinuses. It is also compartmentalized into areas of T cells and B cells and their differentiated forms in different stages of maturity.

An individual is in constant contact with antigens which stimulate the lymph nodes. This increases the cell compartment of one type, generally at the cost of the other compartment. A constant antigenic challenge therefore results in a variety or varied appearance of a normal lymph node.

Cortical (follicle) area

This area of superficial cortex is made of lymphoid follicles with germinal centers. Therefore it is also called as follicle compartment. It plays a role in humoral immunity and is an area where B cells are seen. The cortical area shows primary, secondary or reactive lymphoid follicles (Fig. 12.3). The zonation and orientation of follicles depend on the stage of immune activity and on sectioning. The primary follicles are round nodular area composed of a homogeneous cell population of small darkly stained, inactive lymphocytes. The secondary follicles arise from

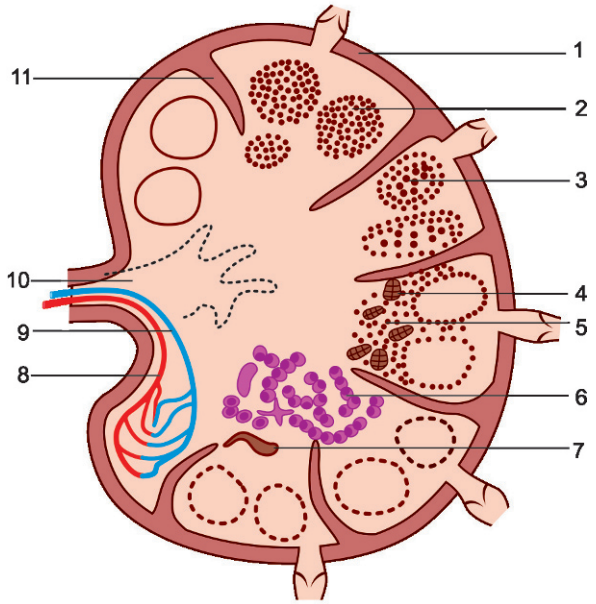


Figure 12.1 Diagrammatic representation of zones and structures in a lymph node 1. Capsule, 2. Primary follicle in cortical area (B cell areas), 3. Secondary follicle in the cortical area with reactive center (B cell areas), 4. High end venules (HEV), 5. Paracortical area (T cell areas), 6. Medullary cords, 7. Sinus, 8. Arteriole, 9. Venule, 10. Hilum, 11. Trabeculae.

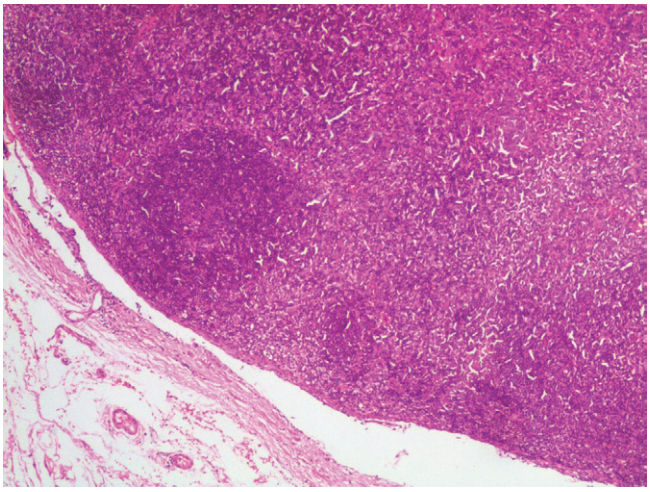


Figure 12.2 Capsule of a lymph node seen along with a primary follicle (H & E stain x40).

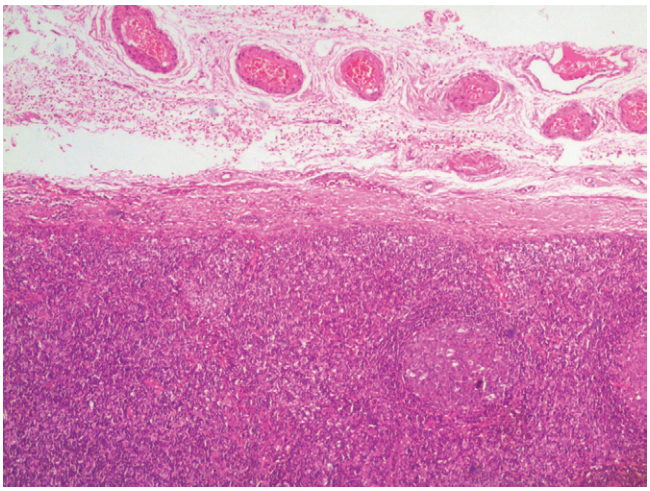


Figure 12.3 Capsule and trabeculae seen along with supracapsular arterioles. Lymph node shows reactive or secondary follicle. (H&E stain x40).

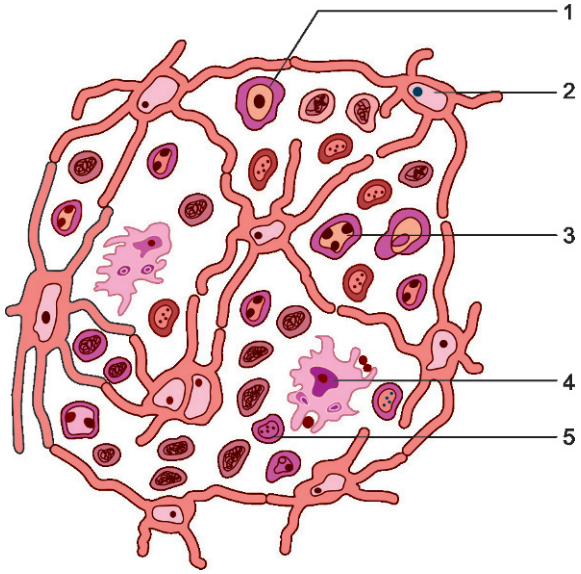


Figure 12.4 Diagrammatic representation of cells in the cortex or follicle area: 1. Immunoblast, 2. Follicular dendritic cells (FDC), 3. Centroblast, 4. Tingible body macrophages (TBM), 5. Naïve B cell.

the primary follicle due to antigenic stimuli. The peripheral area or mantle (mantle zone) in a secondary follicle is made of closely packed small lymphocytes and a central pale stained area (germinal center) having a heterogeneous population of cells (Table 12.1). In this group, centroblasts, centrocytes, macrophages, follicular dendritic cells (FDC) and lymphocytes in various phases of maturation are seen.

The stimulation of the follicle results in 'follicle center cell reaction'. This leads to differentiation of B cells which become direct precursor for antibody producing plasma cells and long-term memory cells.

The activity in the cortical area requires assistance from different types of cells. They are follicular dendritic cells, tingible body macrophages and the lymphoid cells (Fig. 12.4).

Table 12.1 Differences between Primary Follicle and Secondary Follicle

Primary Follicle	Secondary Follicle
• Gives rise to secondary follicle	• Arises from primary follicle due to antigenic stimulus
• Composed of homogeneous cell population	• Composed of different cell population
• Contains inactive lymphocytes	• Composed of different population of cells like centroblasts, centrocytes, macrophages, follicular dendritic cells and lymphocytes
• Shape: round, homogeneous and all cells are darkly stained	• Shape: round with central pale area and darkly stained peripheral area
• Does not have different zones	• Contains two two zones • Central pale area – geminal center • Peripheral area composed of closely packed small lymphocytes – mantle zone

Follicular dendritic cells (FDCs) The FDCs are inconspicuous cells with many long and slender dendritic cytoplasmic extensions. It has a large nucleus, a fine vesicular chromatin and a small nucleolus. The cytoplasmic extensions of the FDCs are linked with each other by the hemidesmosomes. These cells are said to originate from the mononuclear phagocyte system or perivascular mesenchyme or possibly from circulating mesenchymal stem cells. The follicular dendritic cells are antigen trapping cells which keep the antigen on their surface and present it to the B cells. They do not engulf the antigen or process it.

Tingible body macrophages (TBMs) Tingible body macrophages are histiocytes derived from circulating monocytes. These are large cells with an irregular morphology. An abundant pool of pale cytoplasm is seen containing apoptotic bodies and phagocytized debris. The cytoplasm is neither basophilic nor pyrinophilic. A large number of primary and secondary lysosomes, Golgi complex and mitochondria are present along with a few strands of endoplasmic reticulum. It has a large nucleus with finely dispersed chromatin. The TBM engulf the apoptotic bodies and nuclear debris formed by the selection process of the lymphocytes from the surrounding vicinity. Some amount of particulate and soluble antigen proteins are also engulfed, to be presented to lymphoid cells for antigen identification and further initiation of specific antibody production. The nuclear size of the TBM is used as a yard scale for estimating the size of lymphocytes especially in lymphomas (Fig. 12.5).

Lymphoid cells In a lymph node, a spectrum of lymphoid cells in various stages of differentiation and activation are seen. Depending on the site, varying

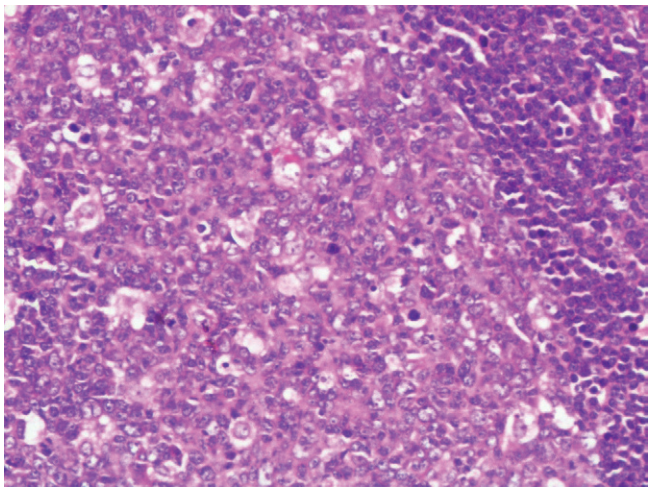


Figure 12.5 A reactive secondary follicle showing numerous tingible body macrophages, mitotic figures, apoptotic bodies and proliferating B cells (H&E stain x 400).

proportions of B cells, T cells, plasma cells with numerous subpopulation of transition cells are seen. The main cell population in the follicles is the B lymphocyte and its subpopulation. Mainly the small or naive B cells and the medium size B cells populate the primary follicle whereas in the secondary follicle, B cells in various stages of reaction and differentiation, the centroblasts, the centrocytes and the mature plasma cells are seen (Fig. 12.6).

Naive B cells small lymphocytes, mature or circulating lymphocytes These are round cells with an average cell diameter of 6 mm and a nuclear diameter of 5 mm showing a high nuclear cytoplasmic ratio. In light microscope, it is seen as a dense basophilic cell mainly depicting a large nucleus with a thin rim of perinuclear cytoplasm.

Centroblasts These are large round cells with a basophilic rim of cytoplasm and a large vesicular nucleus with distinct nucleoli. The small nucleoli are arranged peripherally on the nuclear membrane. This cell population shows increased mitotic figures and undergoes the process of selection through apoptosis. Centroblasts are produced when mature B lymphocytes encounter antigenic challenge, as a first step towards production of antibodies. Centroblasts gives rise to centrocytes.

Centrocytes These cells are smaller than the centroblasts. They have a folded irregular shaped nuclei, often referred to as cleaved nuclei, with a chromatin pattern, which is coarser than the centroblast. They migrate towards the paler zone of germinal center to undergo further division into immunoblasts.

Lymphoblasts These are round cells, which are medium to large in size, with a scanty basophilic cytoplasm, a round nucleus with finely dispersed chromatin and inconspicuous nucleoli. These cells represent a small portion of the lymphoid cells.

Immunoblasts These large cells, with a large vesicular nucleus and a conspicuous single centrally placed nucleus, are seen occasionally.

Plasma cells and T lymphocytes. These cells are occasionally seen in the follicular area.

Paracortex (paracortical area)

Paracortical area is also known as paracortex or the deep cortex. It is a densely cellular area extending beneath the cortex and in between the lymphoid follicles forming cellular strands from the capsule to the corticomedullary junction. This area is a thymus dependent area with predominantly lymphoid cells, (mainly T lymphocytes) epithelioid venules (postcapillary venules) and interdigitating dendritic cells—IDC (Fig. 12.7). Sometimes the follicular reticular cells (FRC) are often found at the periphery of paracortex.

T lymphocytes These are small or large round cells with small, irregular nuclei, coarse chromatin with little cytoplasm.

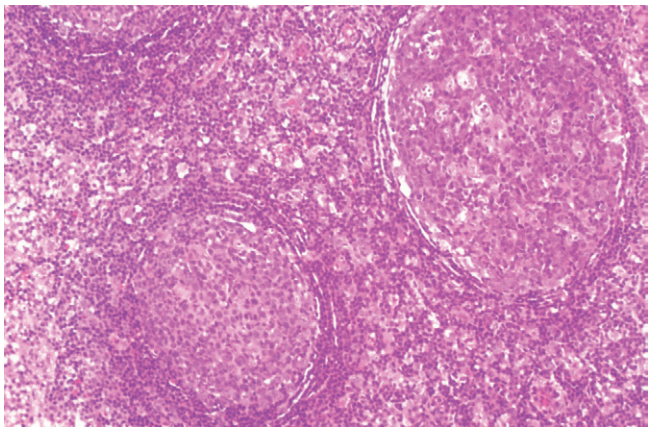


Figure 12.6 Secondary follicles with different levels of maturity along with the paracortex area (H&E stain x 200).

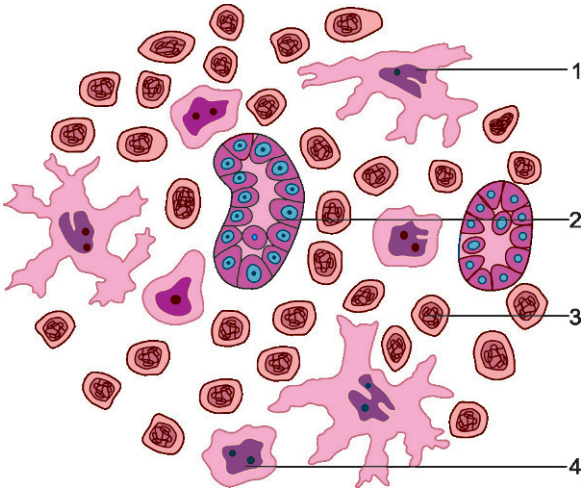


Figure 12.7 Diagrammatic representation of cells in the paracortex: 1. Interdigitating dendritic cells (IDC), 2. High endothelial venules (HEV), 3. T lymphocytes, 4. Immunoblast.

Interdigitating dendritic cells (IDCs) The IDCs are present in good numbers in the paracortex and can cause mottling of paracortex. These cells are large and irregular in shape. An abundant pale cytoplasm with ill-defined borders extend into blunt processes. They have large bizarre nuclei having deep clefts and folds with delicate chromatin pattern and inconspicuous nucleoli. IDCs represent bone marrow derived cells, closely linked to the Langerhans cell of skin sharing both functional and morphological features. They are antigen presenting cells to the T lymphocytes and play an important role to initiate and/or maintain the immune responses.

Epithelioid venules (postcapillary venules, high endothelial venules) The high endothelial venules (HEVs) are distinct vessels found in the paracortex conspicuous by the presence of plump cuboidal or cylindrical endothelial cells with large oval nuclei, with vesicular chromatin and indistinct nucleoli (Fig. 12.8). These vessels are vital for recirculation, distribution and homing of the lymphocytes in different lymphoid organs. Lymphocytes leave the blood stream by passing between the lining cells of the HEVs and enter the lymph node parenchyma.

Follicular reticular cells (FRCs) The FRC present at the edge of the paracortex is responsible for the transport of cytokines and/or antigens though the parenchyma of the lymph node.

Medullary area

In the lymph node, the medulla is an active site of plasma cell proliferation, differentiation and production of antibodies. The cells of the medulla form solid chords, which are intervened by medullary sinuses (Fig. 12.9). The cells in the chords are mature plasma cells, lymphocytes, immunoblasts, plasmacytoid lymphocytes and plasmablasts in different proportions (Fig. 12.10).

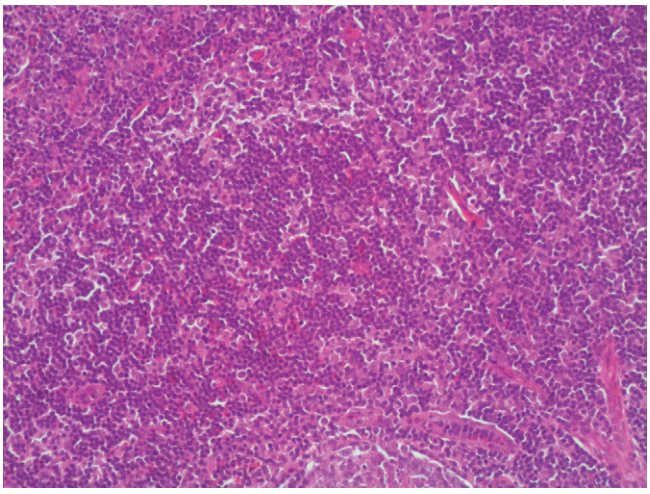


Figure 12.8 Paracortical T cell rich area seen with numerous high endothelial vessels (H& E stain x 200).

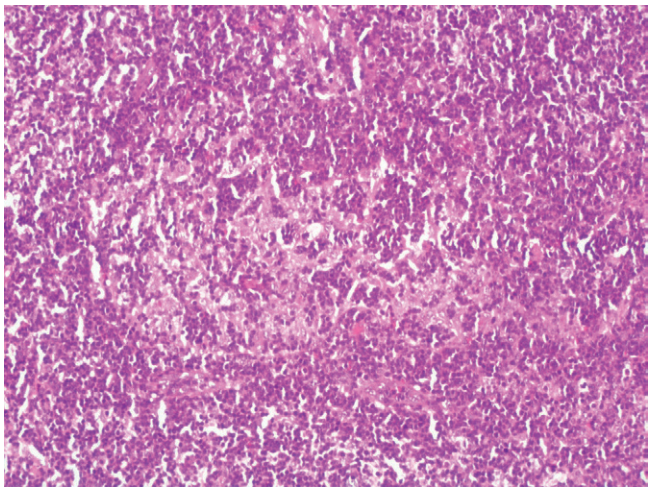


Figure 12.9 Medullary area showing numerous medullary cords made of plasmacytoid cells (H& E stain x 200).

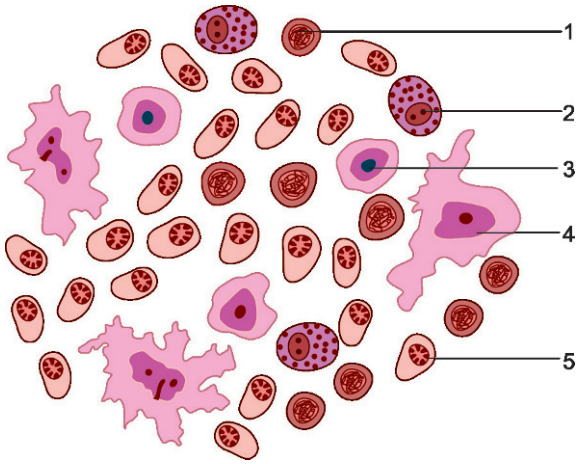


Figure 12.10 Diagrammatic representation of cells in the medullary area: 1. Small lymphocytes, 2. Mast cells, 3. Immunoblast, 4. Macrophage, 5. Plasma cells.

Small lymphocytes These cells appear as small cells with a round nuclei and scanty cytoplasm. They are present in large numbers.

Lymphoplasmacytoid cells or lymphoplasmacytic cells These are cells with clumped chromatin placed peripherally in the nucleus. Good amount of cytoplasm with perinuclear halo is seen in plasmacytoid differentiation (Fig. 12.11).

Plasma cells These are cells with a distinct 'clock face' nucleus which is eccentrically located in the cytoplasm with a perinuclear halo or zone of cytoplasmic clearing. These cells are found in good numbers in the medulla.

Macrophages They appear as large cells with abundant cytoplasm and with a large irregular nucleus. They actively participate in antigen handling and presentation than phagocytic activity. Only few macrophages are present in the medulla.

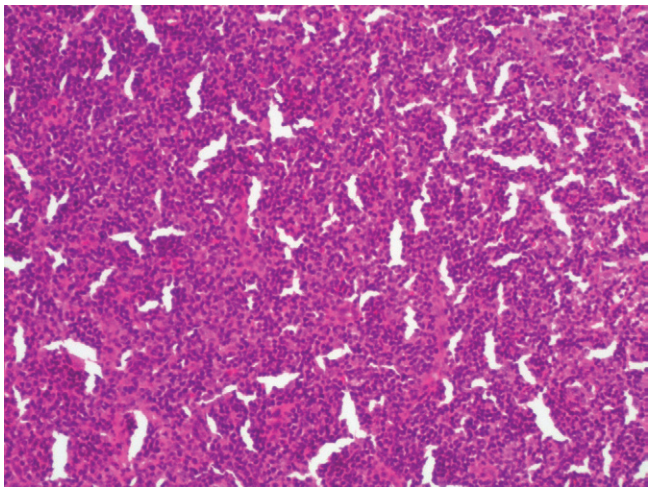


Figure 12.11 Sinuses lined by plasmacytoid lymphocytes (H & E stain x 200).

Mast cells These cells are seen occasionally, and are prominent because of the granular cytoplasm which can be demonstrated by metachromatic dyes like toluidine blue.

Immunohistochemistry

A variety of immunohistochemical staining is done to identify different cell types in the different areas or zones of the lymph nodes. They are proven to be of immense importance for identification of lymphoma cell types.

Cortical area

In the lymphoid follicles, the main cell types are the B cell lymphocyte cell staining positive for CD19, CD20, CD22 and CD79a. Centroblasts express CD10. The tingible body macrophages stain with monoclonal antibodies for CD11b, CD35 and CD68. The dendritic reticular cells (DRCs) stain with CD21, CD35, C3b and C3d.

Paracortical area

This area has a predominance of T cells which stain with pan T cell markers CD2, CD3, CD5, CD7, CD43 and HLA-DR. The interdigitating dendritic cells stain with CD24, HLA-DR and S-100 protein.

Medullary area

The plasma cells in this zone form the major population staining positive with CD138.

Lymphoid sinuses The sinus are inhabited by the macrophages which are positive for CD68 and S-100 protein.

Blood vessels The endothelial cells of the HEV and blood vessels stain with CD31 and CD34.

Lymph Sinuses

The lymph enters the lymph node through the afferent vessels. Each of the lobule has a single afferent lymphatic channel bringing a stream of lymph to the lymph node. Every lobule is connected by a cascading series of lymphatic sinuses. They are divided into subcapsular, trabecular, transverse and medullary sinus.

The lymph brought by the afferent vessels flows through the subcapsular sinus over the lobule, and down the trabecular sinus into the deeper areas of the lobule. Later the lymph travels through the transverse sinuses through the lobules and drains into the medullary sinuses. Lymph from the various lobules drains into a single efferent lymphatic vessel that leaves the lymph node through the hilar area along with the vessels.

Reticular Network

The lymph node has a dense collection of lymphocytes in the follicular and parafollicular areas which are supported by an intricate reticular meshwork. This reticular delicate meshwork is composed of spindle and stellate shaped elongated FRCs and the reticular fibers which are secreted by the fibroblastic reticular cells (Fig. 12.12). The fibroblastic reticular cells cover almost 90 of the reticular fiber surface by mapping along the reticular fibers.

The reticular network in the lobular area is made of stellate fibroblastic reticular cells with elongated cell process. They divide the lobule into a number of interstices and locules which are occupied by lymphocytes, macrophages and antigen presenting cells. The lymphocytes move in these interstitial areas by adhering to fibroblastic reticular cells and crawling along their surface. The boundaries of the lobules show the fibroblastic reticular cells modify their morphology and become flattened, so as to form a layer which defines and segregates a lobule from the surrounding cells and sinuses.

The sinus in the medullary area also shows the presence of the branched sinusal fibroblastic reticular cells.

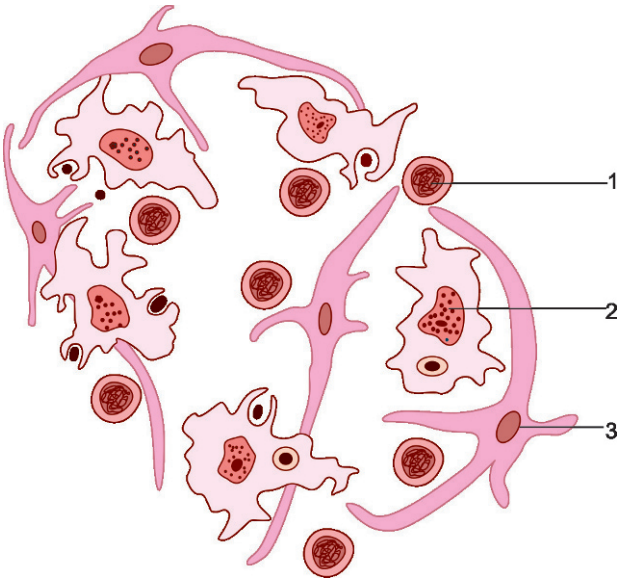


Figure 12.12 Diagrammatic representation of cells seen in the sinus: 1. Lymphocytes, 2. Sinus histiocytes, 3. Fibroblastic reticular cells (FRCs).

They form a loose reticular meshwork in the sinus where the lymphocytes flow along with the lymph in the sinuses, the sinus histiocytes cling to the fibroblastic reticular cells. This helps the sinus histiocytes to remove cells, cell debris, particulate antigen from the flowing lymph. The sinus network is thinner, more delicately branched and forms large interstices as compared to the lobular reticular meshwork. The reticular fibers keep the subcapsular, trabecular, medullary and other cortical sinuses patent.

The reticular fibers are densely laid in the area of medullary cords, periphery of cortical units and interfollicular cortex but are scanty in the central area of the cortical units and follicles. The fibers are always intimately connected to the fibroblastic reticular cells. The reticular fibers are delicate fibers of type III collagen with a dimension of 20 nm in diameter.

LYMPHATIC VESSELS AND CAPILLARIES

The lymph from the tissue is absorbed by the lymphatic capillaries, which in turn converge to form progressively larger diameter lymphatic vessels.

The afferent lymphatics are numerous and enter the lymph node though the capsule, branch extensively to form plexus and drain into the subcapsular sinus. From the numerous radical cortical sinus, the trabecular sinuses are seen which allow the cascading of the lymph secretions through the cortex into the medulla. The sinuses coalesce as they approach the hilum area in the medulla into larger, visible medullary sinuses. The medullary sinuses drain into the larger vessel that drains the lymph, called as efferent vessel. The efferent is a single large vessel that exits the lymph node along with the artery and vein in the hilum.

The larger sinuses are lined by endothelial cells, which might be lost along with the branching of the sinus. The sinuses are home to the large number of macrophages, so that their phagocytic activity can occur when exposed to antigens. The macrophages sit and migrate along the reticulin fibers, where they are also explored to the B and T lymphocyte.

The movement of lymph in the lymph vascular system is quite similar to the movement of blood in the venous system. The lymphatic vessels are found in every tissue in the body except in the CNS, cartilage, bone, bone marrow, thymus, placenta, cornea, and in the hard tissues of the teeth.

The lymph vessels differ from the venous system in their network because of the numerous aggregates of lymphoid tissue in the form of lymph nodes, which they traverse along their course. The lymph is sampled in these nodes for antigens and active cells of the immune system and antibodies enter the lymph stream.

The lymphatic vessels look similar to vein but can be distinguished by the presence of small number of lymphocytes in their lumen and by the absence of erythrocytes. The lymph vessels are generally seen in the vicinity of neurovascular bundles. The vessel wall of the lymph vessel is the same as in the case of small veins. It has an outer fibrous covering, a middle layer of smooth muscle and elastic tissue and an inner lining of endothelial cells. The endothelial cells in the lymph vessels are more compressed as compared to the endothelial cells in the blood vessels. The lymphatic capillaries have a greater permeability. They show very thin endothelial cells, the underlying basement membrane is rudimentary or absent and the pericytes are also absent. The capillary wall and endothelium is linked to the surrounding connective tissue by the fine collagenous filaments called the anchoring filaments which prevent the collapse of the lymphatic lumens. The lymphatic capillaries and vessels show the presence of numerous valves all along their course. The valves are better formed and more in number than in veins to prevent the backflow of the lymph.

The lymphatic vessels differ from blood vessels in that the lymphatic vessels have blind ends and the system is not circulatory. The flow of lymph is unidirectional. The lymphatic vessels or lymphatic capillaries absorb and remove interstitial fluid and macromolecules from extracellular spaces and transport the lymph. The accumulated cellular, macromolecular structures and lymph is transported to the thoracic duct from where it enters back into the blood circulation.

The lymph collected from the right head and neck area is drained into the right subclavian vein. The drained lymph from the left side accumulates into the thoracic duct and returns to the main blood circulation via the left subclavian vein.

BLOOD VESSELS OF LYMPH NODES

The arterioles enter the lymph nodes through the hilum. They branch in large numbers to form a plexus in the parenchyma of the lymph node, the arterioles reach the cortical and paracortical area and branch profusely and loop into arteriovenous communications (AVCs). These AVCs are modified into the high endothelial venules (HEVs). High endothelial venules are specialized vessels having a high cuboidal endothelium. The high endothelial venules receive many branches lined with high endothelial cells (Fig. 12.8). The luminar size of high endothelial venules progressively goes on increasing from the cortex to the medullary area till it finally merges into the vein at the large vein at the hilum area.

The lymphocytes which are circulating in the blood stream enter the lymph node through the arterioles. Once they reach the HEVs they exit the blood stream and migrate into the parenchyma of the lymph node. They return back to the circulation through the efferent lymphatic vessel, and finally through the thoracic duct enter the blood circulation systemically. The high endothelial venules and the lymphatic vessels play a vital role in the movement of the lymphocytes in the lymph node and its homeostasis.

CLINICAL SIGNIFICANCE OF LYMPH NODES

Lymph node enlargement is one of the most common finding as pathological mass in the submandibular and cervical area in the neck. Most of the lymph node

enlargement is a result of acute or chronic response to an infectious organism, deposition of malignant cells as metastasis (from a primary tumor) and lastly as manifestations of primary tumors of the lymph nodes (lymphomas).

Lymphadenitis is the inflammation and infection of a lymph node generally caused by infection in an area drained by the particular lymph node. The infection can be acute or chronic, solitary or multiple, and specific and non specific in nature.

Lymphadenopathy refers to nontender and nonpainful enlargement of the lymph nodes. Lymphadenopathies which are persistent are generally signs of viral illness and chronic bacterial infections. Infectious mononucleosis, HIV infection, tuberculosis and syphilis are a few diseases commonly associated with cervical lymphadenopathy.

The lymph nodes of the head and neck are involved more frequently by metastatic carcinomas than primary tumor of the lymph nodes. Primary tumor of the oropharyngeal area is the cause of metastatic lymph nodes. Rarely neoplastic proliferation within the reticuloendothelial system causes primary tumors in the lymph nodes, i.e. lymphomas. In children, leukemias are more frequent causes of lymph node enlargement with anemias.

While discussing infectious and nodal metastasis associated with the lymph nodes, the terms primary, secondary and tertiary nodes are often used. The primary nodes refer to the first group of lymph nodes affected by an infection associated with the area drained by the specific group of lymph node. One node or a group of nodes can be primarily involved by an infection. If the infection is not treated or contained it spreads into the next group of lymph nodes—the secondary group, and so on to the tertiary group. A similar process occurs in the metastatic spread of tumors. In relation with the metastatic spread of tumors, the primary lymph nodes are also called sentinel lymph nodes.

Each group of nodes act as resistance barrier against the spread of infection and malignancy. Hence an understanding of this concept is vital to understand the spread of infection or malignancy.

LYMPH

The lymph is a watery fluid, offwhite in color. It has a composition similar to plasma and almost identical to interstitial fluid or tissue fluid. The lymph also carries particulate matter in the form of bacteria, cell debris. The plasma protein forms a constituent along with a few lymphocytes. In certain areas, it is enriched by absorbed fat, as in case of lymph from the lacteals of the small intestine.

Lymph is derived from interstitial fluid (tissue fluid) that flows into the lymphatics. Therefore, lymph as it first enters the terminal lymphatics has almost the same composition as the interstitial fluid. The protein concentration in the interstitial fluid of most tissues averages about 2 g/dl, and the protein concentration of lymph flowing from these tissues are near this value. The mixture of lymph from all areas of the body usually has a protein concentration of 3–5 g/dl.

The lymphatic system is also one of the major routes for absorption of nutrients from the gastrointestinal tract, especially for absorption of virtually all fats in food. Large particles, such as bacteria, can push their way between the endothelial cells of the lymphatic capillaries, and in this way enter the lymph. As the lymph passes through the lymph nodes, these particles are almost entirely removed and destroyed.

Rate of Lymph Flow

In a resting human 100 ml per hour of lymph flows through the thoracic duct and approximately another 20 ml flows into the circulation each hour through other channels; making a total estimated lymph flow through the channels to about 120 ml/h or 2–3 liters per day.

Interstitial fluid pressure has a major effect on the normal lymph flow. An increase in pressure of interstitial tissue around 0 mmHg increases the flow by more than 20 folds. Around 0–2 mmHg results in a 'maximum flow rate'; for lymph flow that is 4 mmHg or a negative value of –6 mmHg will decrease or completely stop the lymphatic flow.

Factors that increase the formation and flow of lymph are:

1. Increased capillary pressure
2. Decreased plasma colloid osmotic pressure
3. Increased interstitial fluid colloid osmotic pressure
4. Increased permeability of the capillaries
5. Compression of lymph vessel.

Lymph flows due to the intrinsic intermittent contraction of the lymph vessel walls. Any external factor that compresses the lymph vessel can also cause its movement. Such factors include, skeletal muscles contraction in the surrounding area, movement of the parts of the body, pulsations of arteries adjacent to lymphatics and compression of the tissues by objects outside the body.

During exercise and movement, the lymphatic pumping becomes very active, often increasing the lymph flow by 10 to 30 folds. Similarly during rest, the lymph flow can be very sluggish or completely static. Thus, the rate of lymph flow is determined by the product of interstitial fluid pressure and the activity of the lymphatic pump.

TONSILS

The oropharynx is the entrance of the alimentary and respiratory tracts. Numerous natural and foreign substances come into this area. Thus to protect the oropharynx the tonsils form a lymphatic tissue in a ring called *Waldeyer's ring*.

The tonsillar tissue in the oropharynx is made up of a single pharyngeal tonsil or adenoid in the midline of the oropharynx, superiorly; a bilateral palatine tonsil laterally, adjacent to the posterior molars and bilateral lingual tonsils which are set inferiorly in the posterior one third of the tongue in the floor of the mouth. The tonsils are a part of the lymphatic system which includes lymph nodes, thymus, spleen and diffuse lymphatic tissue.

The basic structure of the tonsil is more or less similar. Each tonsil is composed of lymphatic tissues or nodules. Each lymphatic nodule has germinal centers, which are active areas of lymphocyte formation. The tonsils generally have a free surface covered by epithelium. The epithelium is continuous with the clefts or grooves of the tonsils.

The tonsils unlike the lymph nodes have no afferent lymphatic vessels leading to the tonsils but like lymph nodes they do has efferent lymphatic vessels draining them. Each tonsil is bound externally by a connective tissue capsule and has underlying mucous or seromucous associated glands.

The oropharyngeal tonsils have active lymphatic nodules with germinal centers commonly seen in lingual and palatine tonsils, whereas the pharyngeal tonsil shows simple aggregation of lymphocytes. The epithelium covering the pharyngeal tonsil is respiratory as the tonsil is located in the nasopharynx. The orally located lingual and palatine tonsils show stratified squamous epithelium.

Lingual Tonsils

The lingual tonsils are a paired tonsils located bilaterally on the posterior one third of the tongue. It extends from the circumvallate papillae to the base of the epiglottis posteriorly. This tonsillar mass is made of several dozens of nonbranching crypts. The lingual tonsil is made of wide mouthed crypts. They form rows of lymphatic nodules supported by connective tissue septa present in each lobule of the gland. These tonsils have a connective tissue capsule investing them. The capsule is covered by nonkeratinized stratified squamous epithelium. Mucous glands are seen underlying the tonsils. These glands with their ducts opening into the crypts help to flush and cleanse the area. This is an adjunct to the washing action of saliva. Collective effect helps to maintain the lingual tonsillar mass free of inflammation. In between these mucous glands, are skeletal muscles and adipose tissue. The deep superior cervical nodes drain the lingual tonsils.

Palatine Tonsils

The palatine tonsils are the largest tonsils in the Waldeyer's ring. It is situated between the palatoglossus muscle (anterior pillar) and the palatopharyngeus muscle (posterior pillar). The palatine tonsil is divided into lobules by the crypts. Each lobule contains numerous lymphatic nodules, which contain germinal centers. Connective tissue forms septa and supports the nodules of lymphatic tissue and inserts the gland in a capsule. The palatine tonsils have deep branching crypts, which house oral bacteria. They may become plugged with lymphocyte discharge and desquamated epithelial cells. The tonsils have seromucous glands beneath them but unlike the lingual tonsils they do not open into the crypts but on the surface of the glands. Thus, these glands lack the complete flushing action thereby leading to frequent tissue inflammation. The deep cervical lymph nodes drain the palatine tonsils.

Pharyngeal Tonsils

The pharyngeal tonsils also called adenoids are located in the midline in the posterior wall of the superior portion of the nasopharynx. Sometimes it will extend laterally around the opening of the auditory tube in the area of torus tubarius. Then the tonsillar tissue is called tubal tonsil.

This tonsil varies in the structural arrangement. Pharyngeal tonsil is a solo tonsillar tissue with no crypts associated with it, but it has many folds in the mucosa. These tonsils have no well defined lymphoid nodules or germinal centers. The lymphoid tissue is diffusely arranged. The epithelium covering the tonsil is respiratory or pseudostratified columnar epithelium though occasional patches of squamous epithelium are seen. The ciliated epithelial cells are accompanied by numerous goblet cells. In the lamina propria there are mixed glands that drain on the surface of the epithelium overlying the gland. Deeper plane shows the muscles of pharynx and the periosteum, which are attached to the bone of the sphenoid. The pharyngeal tonsils drain to the retropharyngeal nodes.

Differences between tonsil and lymph node are depicted in Table 12.2.

Development of Tonsils

The Waldeyer's ring of the oropharyngeal tonsillar ring forms the anterior limit of the embryonic foregut. Thus the epithelium that takes in the formation of tonsils is endodermal. The basal cells of the endodermal epithelium start to proliferate diffusely and start entering the underlying connective tissue or the mesenchyme. Along with the proliferation of epithelial cells a concomitant subepithelial condensation of the mesenchyme also occurs. The nodular epithelial projections extend into the lamina propria and become the foci of lymphocytic infiltration. The projecting epithelium shows sloughing in the central core area leading to the formation of crypts. The nodules grow into lymphoid tissue by mitosis of the existing lymphocytes and by the accompanied differentiation

Table 12.2 Diferrences between Tonsil and Lymph node

Tonsil	Lymph node
• Seen at the junction of oropharynx and nasopharynx.	• These are present in chain of well organized small clusters at all strategic location
• Tonsils have free surface covered by nonkeratinized stratified squamous epithelium • The epithelium is continuous with the clefts or grooves of the tonsil	• Lymph node does not have epithelium covering
• Tonsil is bound externally by a connective tissue capsule and has underlying mucous or seromucous associated glands	• Lymph node is covered by capsule which is chiefly composed of collagen and elastic fibers. No glands are seen
• Tonsils lack afferent lymphatic vessels but have efferent lymphatic vessels draining them	• Inner side shows indentation or a dimpling called the hilum through which vessels enter and exit

of the mesenchymal cells. Later on additional mass of lymphoid tissue aggregates around the crypt, which completes the formation of the nodules. The connective tissue condenses and forms a capsule along the base of the glands and sends intervening septa into the folds of lymphatic tissue. Growth of tonsils is rapid at birth, till the age of 6–7 years, and again during puberty after which tonsillar atrophy begins.

Functions

The tonsils are located at the beginning of the respiratory and alimentary tract. A number of allergens and variety of natural and foreign substance come into contact with the oropharynx during breathing and eating. Hence the foremost and most important function of the tonsils is to activate lymphocytes, which protect the body from the invasion by microorganisms.

The allergens are sensed by the lymphocytes, which start the complex process of coding for antibody production. They have the capacity to retain this information. Hence the lymphocytes are also called memory cells.

Some of the lymphocytes transform into 'T cells' and engulf bacteria or discharge substances to destroy them. The other lymphocytes become 'B cells' which differentiate into plasma cells. These plasma cells secrete antibodies that destroy antigens. Plasma cells join salivary gland cells in secreting secretory IgA. The plasma cells are found in other areas of oral cavity during chronic infections like periodontal and periapical diseases. Some foreign substances are absorbed from the crypts of the glands into the gland proper and are then destroyed. So, in more than one way tonsils help in neutralizing microorganisms and can form antibody to any air and food borne antigens.

Clinical Significance of Tonsils

The tonsillar tissue is involved by a number of infectious diseases, developmental disorders and tumors.

The most common pathology associated with the Waldeyer's rings is tonsillitis caused by microorganisms (bacterial more frequently). Tonsillitis generally involves the palatine tonsils. They are the largest in children and protrude into the oropharynx, when infected, making swallowing difficult. They appear red with streaks of white purulent material on their surface. The reason for palatine tonsils to be more commonly involved might be due to deep branching crypts which do not have the benefit of flushing by the secretion of seromucous glands as they open on the surface of the epithelium and not on the crypts.

The pharyngeal tonsils also become a subject to infection in childhood. The lingual tonsils generally become inflamed and swollen, making swallowing difficult when there are allergens in the food.

LYMPHATIC DRAINAGE OF HEAD AND NECK

In the oropharyngeal area and in head and neck, numerous lymph nodes are seen. Many of them are grouped together in small clusters connected by lymphatic channels. Every group is responsible for the lymphatic drainage of a particular area. In the head and neck area, the lymph nodes are classified into superficial and deep group. The superficial group lies superior to the fascia; they are divided into two groups, anterior cervical nodes and superficial cervical nodes (Fig. 12.13). The deep nodes are more in number than superficial nodes. They are arranged as vertical chain and circular chain of lymph nodes. The deep cervical lymph nodes are divided into the superior and inferior deep cervical nodes. The deep cervical nodes commonly referred to are the jugulodigastric node and the jugulo-omohyoid node. The circular chain of lymph nodes consist of the submental node, submandibular node, buccal or facial node, parotid nodes, the postauricular node and the occipital nodes (Fig. 12.14).

The deep cervical group of lymph nodes are also called the terminal group of lymph nodes. They are called terminal group as they directly or indirectly via the regional groups receive the lymph from all the vessels of the head and neck. All the lymphatics from the head and neck chain drain into the deep cervical nodes. These nodes drain into jugular trunk which ends in the thoracic duct.

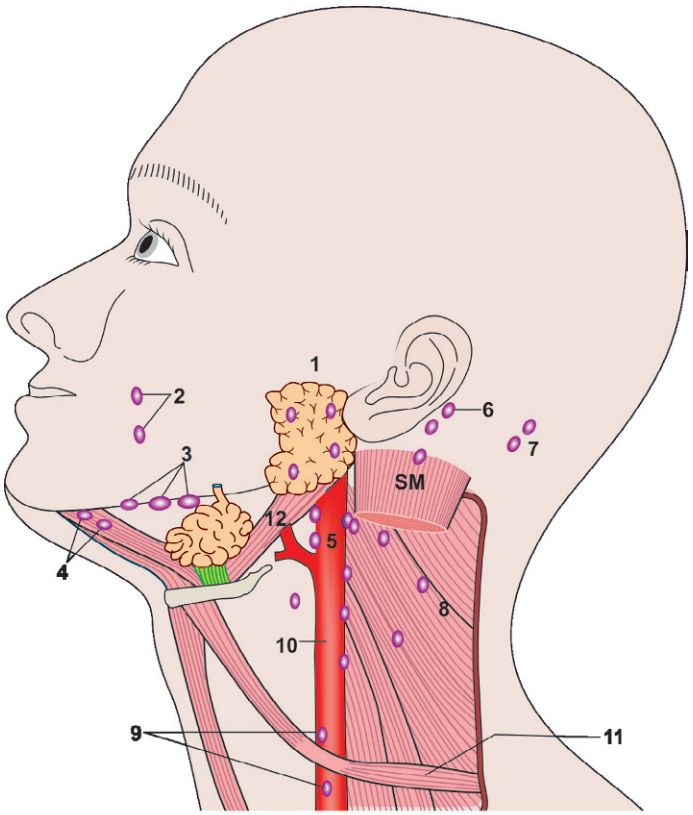


Figure 12.13 Diagram showing the location of lymph nodes of head and neck region: 1. Parotid nodes, 2. Buccal nodes, 3. Submandibular nodes, 4. Submental nodes, 5. Superior deep cervical nodes, 6. Posterior auricular nodes, 7. Occipital nodes, 8. Superficial cervical nodes, 9. Inferior deep cervical nodes, 10. Internal jugular vein, 11. Omohyoid muscle, 12. Posterior belly of digastric, SM—cut end of sternocleidomastoid muscle.

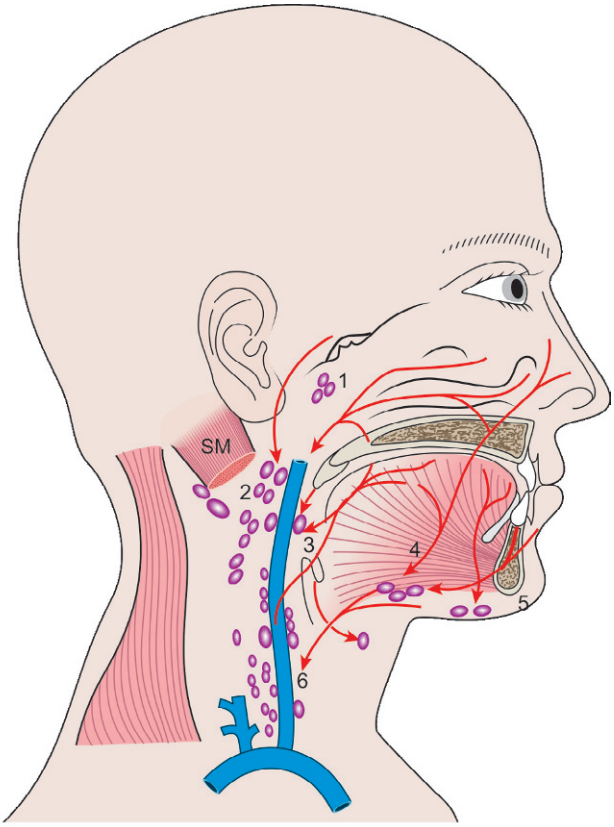
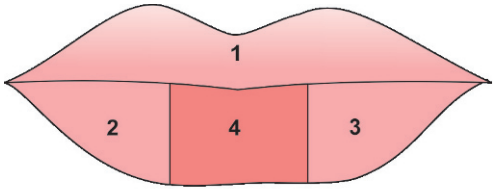


Figure 12.14 Diagram of head and neck lymph nodes, lateral view: 1. Parotid nodes, 2. Superior deep cervical nodes, 3. Middle deep cervical nodes, 4. Submandibular nodes, 5. Submental nodes, 6. Inferior deep cervical nodes, SM—cut end of sternocleidomastoid muscle.

Table 12. 3 Lymphatic Drainage of Specific Sites in Head and Neck Region

Skin of the head and neck	Superficial cervical nodes
Eyelids	Parotid and deep nodes
External nose and cheeks	Buccal nodes and submandibular nodes
Lateral part of the cheek	Parotid nodes
Buccal floor and lingual apex	Submental node
Deeper tissue of head and neck	Deep cervical nodes
Anterior part of the floor of the mouth	Upper deep cervical nodes via submental nodes
Lateral part of the floor of the mouth	Submandibular and superior deep cervical nodes
Tip of the tongue	Submental lymph nodes
Anterior 2/3rd of the tongue	Submental and submandibular nodes and then to lower deep cervical nodes
Posterior 1/3rd of the tongue	Upper deep cervical nodes
Hard palate	Retropharyngeal and superior deep cervical nodes
Soft palate	Retropharyngeal and superior deep cervical nodes
Tonsils	Superior deep cervical nodes and then to jugulodigastric nodes
Rich submucous lymphatic plexus of mouth and pharynx	Deep cervical lymph nodes
Teeth	Submandibular and deep cervical nodes
Gingiva	Submandibular nodes

The lymphatic drainage of the structures in the oral cavity are depicted in Table 12.3. Lymphatic vessels from the median area of the lower lip drain into the submental node, but from other areas of lip the drainage is to the submandibular node (Fig. 12.15). The lymph vessels from the buccal gingiva of maxillary and mandibular area drain into the submandibular lymph nodes. From the lingual region of mandibular gingiva, the lymph vessels from the anterior region drain into the submandibular nodes while from the posterior region and from the palatal gingiva drain into the deep cervical nodes. The lymph from all the teeth are drained into the submandibular nodes except those of mandibular incisors. The submental nodes receive lymph from mandibular incisors (Fig. 12.16).



1, 2, 3, Submandibular nodes
4, Submental nodes

Figure 12.15 Diagram showing lymphatic drainage of upper and lower lip.

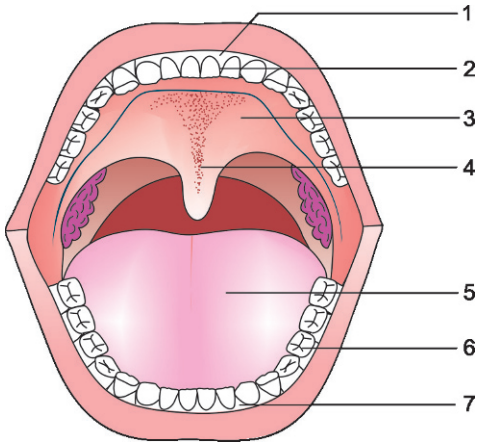


Figure 12.16 Diagram showing lymphatic drainage of different sites from the oral cavity. The draining areas are numbered and the draining lymph nodes are given in parenthesis: 1. Maxillary gingiva (buccal—submandibular lymph nodes) and palatal – (superior deep cervical lymph nodes); 2. Maxillary teeth (submandibular nodes); 3. Hard palate (superior deep cervical nodes and retropharyngeal nodes); 4. Soft palate (superior deep cervical nodes and retropharyngeal nodes); 5. Tongue—tip of the tongue (submental nodes), anterior 2/3rd (submandibular nodes and then to lower deep cervical nodes), posterior 1/3rd (upper deep cervical nodes); 6. Mandibular teeth—incisors (submental nodes), canines and posterior teeth (submandibular nodes); 7. Mandibular gingiva—buccal (submandibular) lingual (anterior—submandibular nodes), posterior (deep cervical nodes).

SUMMARY

The lymphatic system in the human body has a subtle but very vital function to play. The system performs the role of tissue drainage along with protection by activation of the defense cells of the body. The former role helps in maintaining the blood volume and the latter helps in defense and building up of immunity.

Types of Lymphoid Tissues

This system is made of primary, secondary and tertiary lymphoid organs and tissues along with lymph vessels and lymph. The primary lymphoid organs are the fetal liver before birth and thymus and bone marrow after birth. The secondary lymphoid organs are the spleen, lymph nodes and mucosa associated lymph nodes (MALT). MALT is a collective term for lymphoid tissues found associated with gastrointestinal mucosa like Payer's patches, respiratory mucosa and genitourinary mucosa. These structures lack a capsule.

Lymph Nodes

Lymph nodes are important secondary lymphoid organs densely populated in the head and neck region. They filter the foreign substances and are reservoirs of defense cells mainly in the form of lymphocytes. The lymphocytes mainly are activated in the lymph node following which they mature and differentiate for specific functions of defense.

The lymph node is small bean-shaped capsulated organ and shows three well developed zones on microscopic examination. The zones are the cortex, paracortex and the medulla. Afferent and efferent blood vessels enter and leave through the hilum.

In the head and neck area along with lymph nodes the MALT also plays a vital role in antigen concentration and initiation of specific adaptive immune response. The lymphoid follicles associated with the oropharynx is called Waldeyer's ring. They constitute the tonsillar tissue with bilateral palatine tonsil, bilateral lingual tonsil and pharyngeal tonsil. The tonsils perform the function of antigen presentation, neutralizing microorganisms and lymphocyte cell maturation very similar to what is seen in the lymph node. The tonsils have a mucosal covering over the capsule and seromucous glands open on their surface.

Microscopic Structure of Lymph Nodes

Lymph nodes have a capsule. It consists of three well defined zones: an outer cortex, a paracortical area and an innermost medullary zone. In the cortical zone and in the paracortical area lymphoid follicles are seen and in the medulla, cords of cells and sinuses are seen.

The microscopic appearance of lymph nodes varies due to state of antigenic challenge in the cortex. The lymphoid tissue is arranged in the form of follicles. The follicles have a lighter staining germinal centers where the cells are actively dividing.

Cortical Zone

The cortical area mainly consists of B lymphocytes. The other types of cells seen in this zone are follicular dendritic cells and tingible body macrophages. The follicular dendritic cells trap antigen in their dendritic process while the macrophages phagocytose antigen, process them before presenting to the lymphocytes. Apart from these cells centroblast, centrocytes, lymphoblast and immunoblast are seen.

Paracortical Zone

The paracortical zone shows T lymphocytes and interdigitating dendritic cells. These cells present antigen to the T lymphocytes. High epithelioid venules lined by plump cuboidal cells allow lymphocytes to exit the blood stream and enter the lymph node parenchyma.

Medullary Zone

The medulla shows cords of cells lined by lymphoplasmacytoid or plasma cells. The plasma cells produce the antibodies. The macrophages line the medullary sinus. These cells filter the antigen from the lymph that flows slowly through it. The afferent lymph vessels branch into spaces called sinuses below the capsule, and continues through the cortex into the medulla. Lymph from the various medullary sinuses joins together to form the efferent lymph vessel which exits from the hilum. The lymph vessels differ from the blood capillaries in that the lymph vessels have a more compressed endothelial cell, their walls are more permeable, and the basement membrane is rudimentary. Valves present in the lymph vessels ensure unidirectional flow of lymph.

The collagen fibers of the capsule invade the cortex forming trabeculae. It supports the parenchyma. The reticular tissue produced by the follicular reticular cells forms a network all over the parenchyma. The macrophages crawl over the reticular fibers.

Lymph Nodes of Head and Neck

The lymph nodes of neck are arranged in superficial and deep groups. The deep groups are arranged in horizontal chains like the submental, submandibular and parotid nodes. They are also arranged in vertical chains along the digastric and omohyoid muscles and close to internal jugular vein. They drain from all head and neck structures. Lymphatics from teeth and gingiva usually drain into the submandibular nodes.

Clinical Significance of Lymph Node Enlargement

The lymph nodes are involved in many diseases commonly from spread of infection and rarely to the formation of primary tumor and in metastasis (local spread) of tumors. As infection in the oral cavity are common, spread of infection to cervical nodes are common and cause painful enlargement of lymph nodes or lymphadenitis. Tonsillitis (inflammation of palatine tonsils) is common in children. Persistent painless enlargements of lymph nodes are associated with viral and chronic bacterial infection and sometimes with tumors.

REVIEW QUESTIONS

1. What are lymph nodes? Describe their anatomy.
2. Describe the zones in a lymph node.
3. What are primary and secondary follicles?
4. Describe the cells in the lymph node.
5. Explain the terms:
   MALT, GALT, BALT, DALT and NALT.
6. What are the factors affecting the lymphatic flow?
7. Write short notes on:
   Functions of lymphatic system
   Lymph
   Antigen presenting cells
   Importance of high endothelial venules (HEVs)
   Waldeyer's ring.
8. List out the differences between lymph vessels and blood vessels.
9. What are tonsils? Where are they located? What are their functions? Add a note on their clinical significance.
10. Describe the location of cervical lymph nodes. What are their drainage areas? Add a note on their clinical significance.
11. Describe the lymphatic drainage from different sites of the oral cavity.

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13 Tooth Eruption

CHAPTER CONTENTS

The term 'eruption', (derived from the Latin word *erumpere* = to break out) refers to the axial or occlusal movement of the tooth from its developmental position within the jaw to its functional position in the occlusal plane.

In common usage, eruption signifies the cutting of the tooth through the gum. However, the eruption of even a single tooth has to be understood in the broad context of various changes that occur in the jaws in preparation to, simultaneous with, and subsequent to the eruption of that tooth. These overlapping phases of physiologic tooth movements are studied as:

1. **Preeruptive tooth movements** made by a tooth germ within the tissues of the jaw prior to eruption. These movements are seen in the **preeruptive phase**.
2. **Eruptive tooth movements** made by a tooth from its original position within the jaw to its functional position in occlusion. These movements are seen in **prefunctional eruptive phase or eruptive phase.**
3. **Posteruptive tooth movements** made by a tooth that maintain it in functional occlusion. These movements are seen in **functional eruptive phase or posteruptive phase**.

The eruption of the deciduous dentition initially occurs followed by their phased replacement by the functionally larger and numerically greater permanent dentition.

For ease of understanding of this complex process, the individual phases of tooth eruption, although overlapping, will be separately considered. Each phase merits discussion of the pattern of movement of the tooth germ/tooth along with the associated histological changes.

PATTERN OF TOOTH MOVEMENT

Preeruptive Tooth Movement

When deciduous tooth germs first differentiate, they are very small and a good deal of space is between them. This space is soon used because of the rapid growth of the tooth germs, and crowding results, especially in the incisor and canine region. This crowding is then relieved by growth of the jaws in length, which permits drifting of the tooth germs. Bony remodeling of crypt wall occurs to facilitate movements of growing tooth germ and its movement. In bodily movement in a mesial direction, bone resorbs on the mesial side and forms on the distal side of the crypt.

Permanent teeth with deciduous predecessors also move before they reach the position from which they will erupt, but analysis and description of such movements are complicated by the fact that change in position of the tooth germ is the result of a number of factors involving body movement of the tooth germ, its growth, or a relative change in position of associated deciduous and permanent tooth germs. For example, in Figure 13.1 there appears to be a considerable change in position between the permanent incisor tooth germ and its deciduous predecessor in the first 2 years of life. But whether there has been much body movement of the tooth germ in its crypt is doubtful because the changes in the relative positions can be ascribed to growth of the permanent tooth and eruptive movement of the deciduous tooth. The same also holds good for the permanent molars (Fig. 13.2).

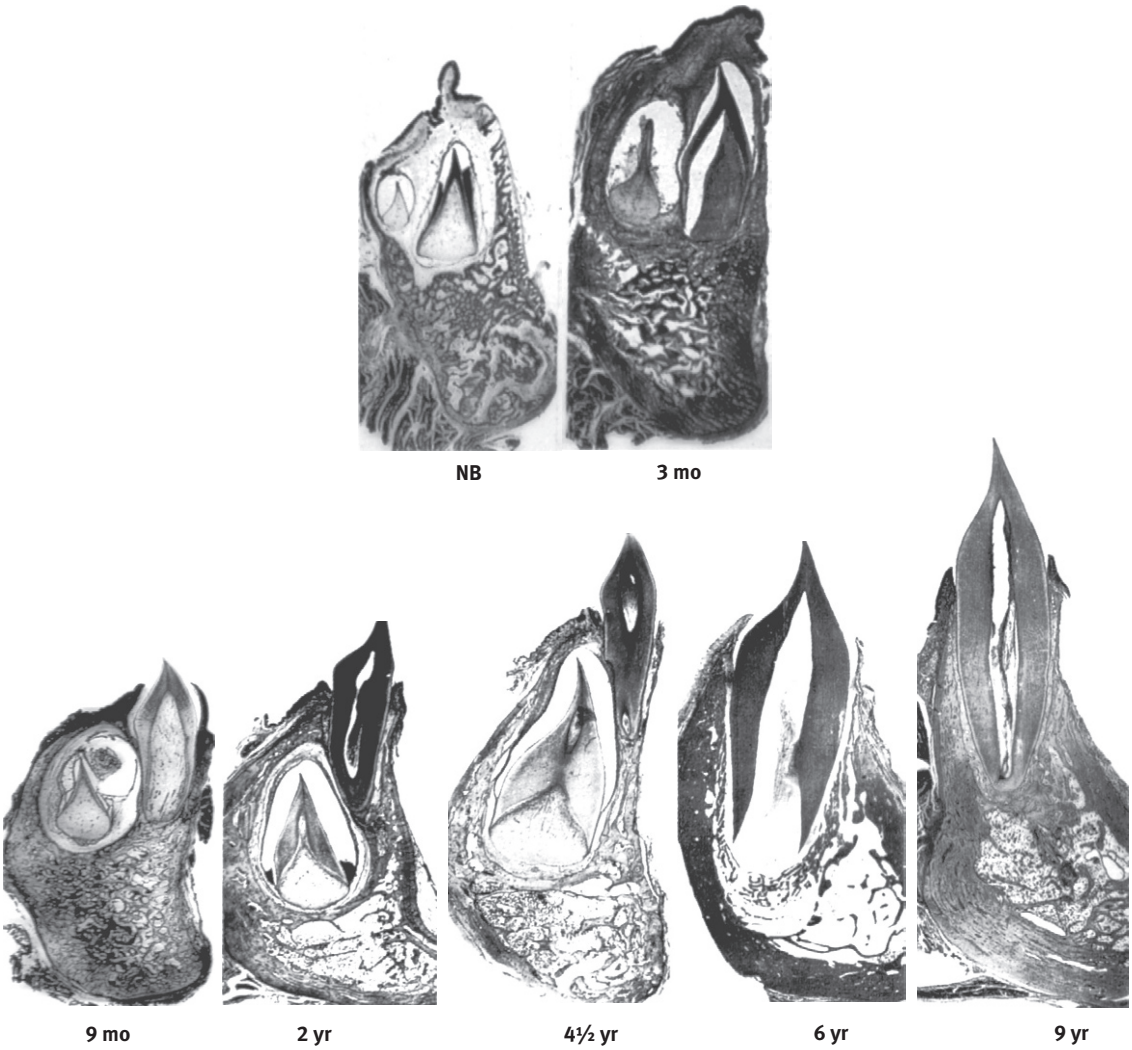

Figure 13.1 Buccolingual sections through central incisor region of mandible at representative stages of development from birth to 9 years of age. At birth both deciduous and permanent tooth germs occupy same bony crypt. Notice how, by eccentric growth and eruption of deciduous tooth, permanent tooth germ comes to occupy its own bony crypt apical to erupted incisor. At 4½ years, resorption of deciduous incisor has begun. At 6 years, deciduous incisor has been shed and its successor is erupting. Notice active deposition of new bone at base of socket at this time.

The permanent molars, which have no deciduous predecessors, also exhibit movement. For example, the upper permanent molars, which develop in the tuberosity of the maxilla, initially have their occlusal surfaces facing distally (Fig. 13.3) and swing around only when the maxilla has grown sufficiently to provide the necessary space. Similarly, mandibular molars develop with their occlusal surfaces inclined mesially and only become upright as room becomes available.

All these movements occur in association with growth of the jaws, which makes analysis of individual tooth movement even more difficult. For the beginning student, preeruptive tooth movement should be considered as movement positioning the tooth and its crypt within the growing jaws preparatory to tooth eruption.

Eruptive Tooth Movement

During the phase of eruptive tooth movement the tooth moves from its position within the bone of the jaw to its functional position in occlusion, and the principal direction of movement is occlusal or axial. However, as in the case of preeruptive tooth movement, jaw growth is still occurring while most teeth are erupting so that movement in planes other than axial movement is superimposed on eruptive movement. The term 'prefunctional' eruptive tooth movement is used to describe the movement of the tooth after its appearance in the oral cavity till it attains the functional position.

Posteruptive Tooth Movement

Posteruptive tooth movements are those that (1) maintain the position of the erupted tooth while the jaw continues to grow and (2) compensate for occlusal and proximal wear. The former movement, like eruptive movement, occurs principally in an axial direction to keep pace with the increase in height of the jaws. It involves both the tooth and its socket and ceases when jaw

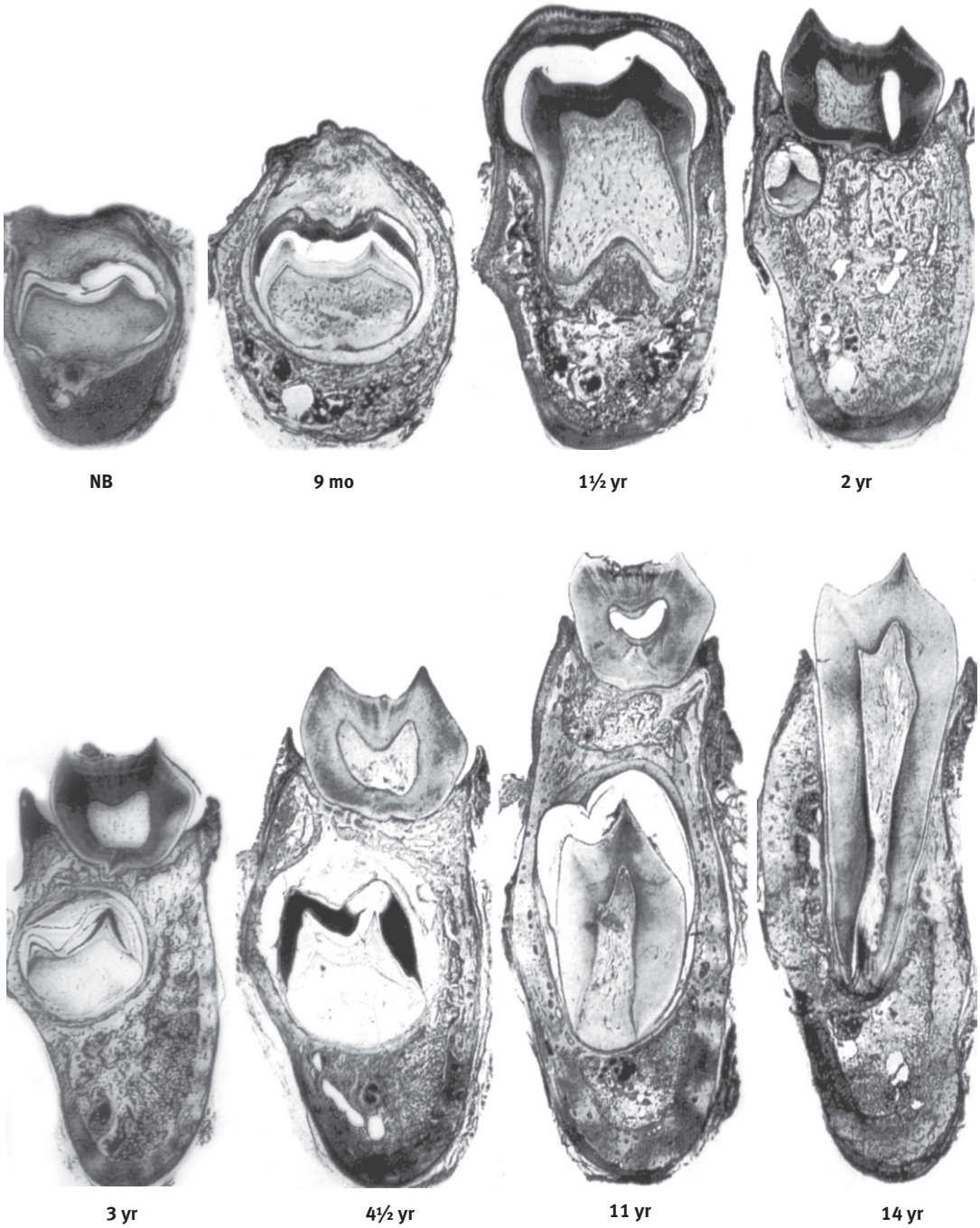

Figure 13.2 Buccolingual sections through deciduous first molar and first permanent premolar of mandible at representative stages of development from birth to 14 years. Notice how permanent tooth germ shifts its position. In section of 4½-year mandible, gubernacular canal is clearly visible. Lack of roots in the 2-, 3-, 4½-, and 11-year sections is not the result of resorption but of the sections being cut in midline of tooth with widely divergent roots.

growth is completed. The movements compensating for occlusal and proximal wear continue throughout life and consist of axial and mesial migration, respectively. Proximal wear, which can decrease the arch length by as much as 7 mm, is compensated by the mesial drift. The mesial drift involves the contraction of transseptal ligament and an anteriorly directed force that is the summation of mesially directed occlusal forces along cuspal planes.

Animal Experimental Studies in Eruption

The experimental studies in eruption are mainly carried out in experimental animals having teeth, which are either continuously growing as seen in rodents or continuously extruding as seen in sheep and cattle. The rapid and continuous growth of teeth and ease of handling of the rodents makes them useful for eruption studies. All the results from these studies cannot be

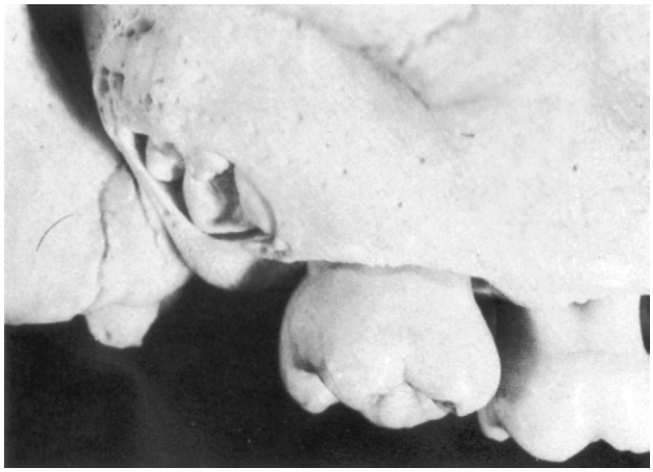

Figure 13.3 Region of maxillary tuberosity of dried skull of 4-year-old child. At this stage of development, first permanent molar is still within its bony crypt. Notice how occlusal surface faces backward. With further growth of maxilla, molar swings down so that it eventually erupts into occlusal plane.

applied to humans because there is no distinction between crown and root and that there is lack of evidence to show that the histological mechanisms of eruption are similar to humans. In the continuous extruding type of tooth eruption, there is distinction between crown and root but the supporting structures do not follow the extruding tooth. The teeth of the humans differ from these animals in that they show continuous eruption of teeth and that the supporting structures of the tooth follow the movement of the tooth. The differences in the various types of eruption are illustrated in Figure 13.4. Even molecules actively involved in tooth eruption like epidermal growth factor (EGF) and colony-stimulating factor-1 (CSF-1) have different effects on different modes of teeth eruption.

HISTOLOGY OF TOOTH MOVEMENT

Preeruptive Phase

Preeruptive tooth movement, whether it involves drifting or growth of the tooth germ, demands remodeling of the bony wall of the crypt. This is achieved by the selective deposition and removal of bone by osteoblastic and osteoclastic activity, but whether such bony remodeling is the cause of preeruptive tooth movements or reflects a response to forces produced by other factors is not known. It is of interest that there are instances that indicate normal skeletal morphogenesis might be involved in determining tooth position. Thus marrow spaces of consistent configuration develop in bones, and, as will be described later, eruptive pathways through bone form even in the absence of associated teeth. The intraosseous phase of tooth eruption can be considered as a process of bone modeling.

Eruptive Phase

During the eruptive phase of physiologic tooth movement, significant developmental events occur that are associated with eruptive tooth movement. They include the formation of the roots, the periodontal ligament, and the dentogingival junction.

Root formation is initiated by growth of Hertwig's epithelial root sheath, which initiates the differentiation of odontoblasts from the dental papilla. The odontoblasts then form root dentin, bringing about an overall increase in length of the tooth that is largely accommodated by eruptive tooth movement, which begins at approximately the same time as root formation is initiated. Shortly after the onset of root formation cementum, periodontal ligament, and the bone lining the crypt wall are formed (see Chapter 8). In addition, a number of structural changes are seen within the periodontal ligament, which could be responsible for tooth movement. Fibroblasts of the periodontal ligament possess as part of their cytoskeleton intermediate filaments that consist of contractile proteins. They also exhibit frequent cell-to-cell contacts of the adherence type and a further specialization involving the cell membrane, the fibronexus. This describes a morphologic relationship between the intracellular filaments of the fibroblast, transmembrane proteins, which produce an increased density of fibroblast cell membrane, extracellular filaments, and fibronectin. Fibronectin is a sticky glycoprotein that can stick to a number of extracellular components, including collagen. Ultrasructurally, fibronexus consists of intracellular actin filaments and extracellular fibronectin filaments associated with subplasmalemmal plaque material that contains proteins such as vinculin, talin, alpha-actin and integrin. Finally, the ligament fibroblast has the ability to ingest and degrade extracellular collagen while forming new collagen fibrils (Fig. 13.5). How these morphologic features might be related to tooth movement is discussed later in the section dealing with mechanisms.

Significant histologic changes also occur in the tissues overlying the erupting tooth. Bone removal is necessary for permanent teeth to erupt. In the case of those teeth with deciduous predecessors there is an additional anatomic feature, the *gubernacular canal* and its contents, the *gubernacular cord*, which may have an influence on eruptive tooth movement. When the successional tooth germ first develops within the same crypt as its deciduous predecessor, bone surrounds both tooth germs but does not completely close over them. As the deciduous tooth erupts, the permanent tooth germ becomes situated apically and is entirely enclosed by bone (Figs 13.1, 13.2) except for a small canal that is filled with connective tissue and often contains epithelial remnants of the dental lamina. This connective tissue mass is termed the 'gubernacular cord' (Figs 13.6-13.8), and it may have a function in guiding the permanent tooth as it erupts. After removal of any overlying bone there is loss of the intervening soft connective tissue between the reduced enamel epithelium covering the crown of the tooth and the overlying oral epithelium.

How this loss is achieved is not established. A simple explanation is that pressure from the erupting tooth causes local ischemia and therefore local necrosis, but other evidence indicates that this may be too facile an explanation. What is certain is that the changes taking

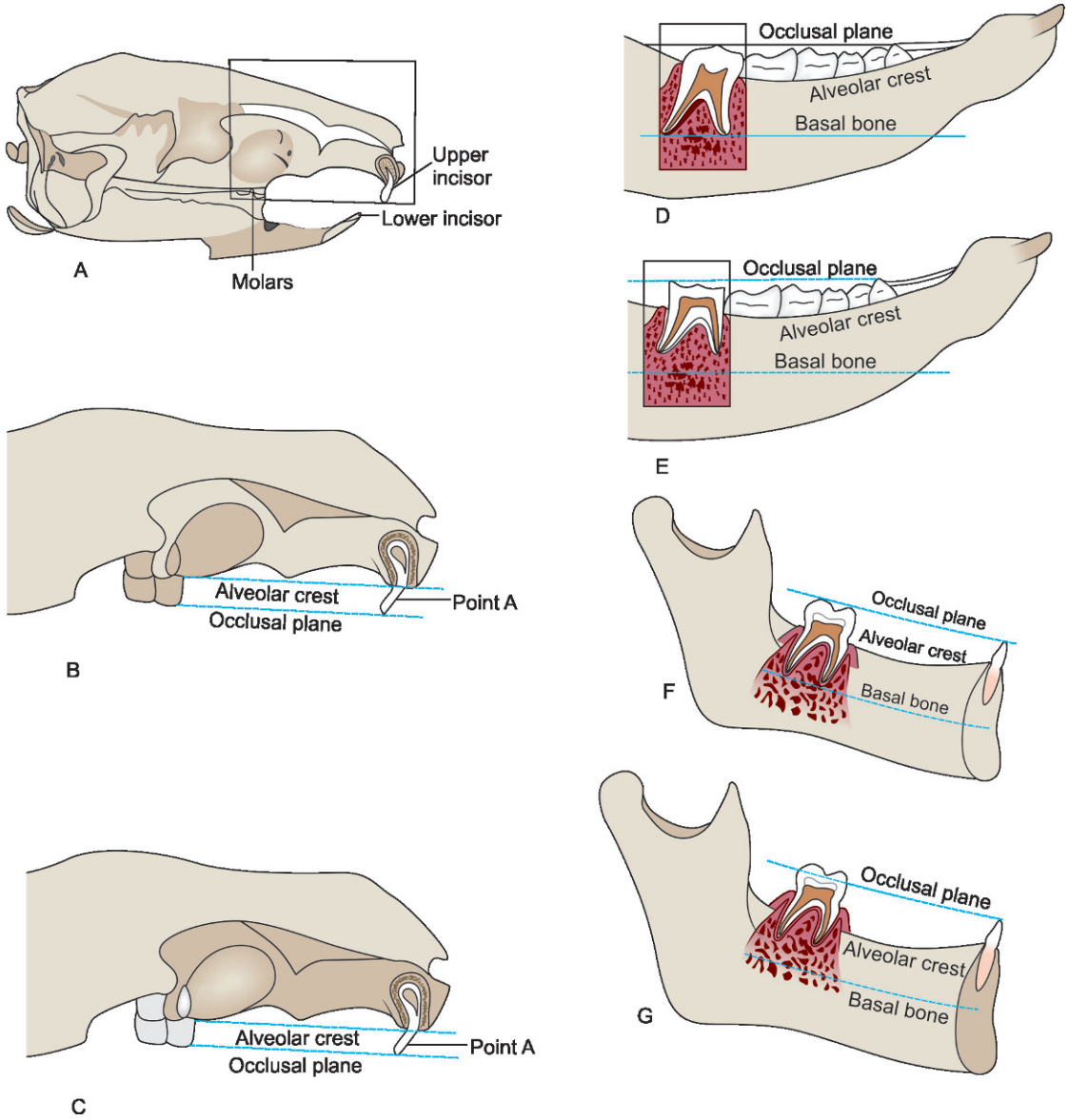

Figure 13.4 (**A–G**) Show the three different types of tooth eruption in rodents, cattle and humans. (**A**) Skull of the rodent, (**B**) Magnified portion of upper jaw from figure. A showing point A in the upper incisor, (**C**) Note the occlusal movement of point A in the continuously growing teeth of rodents. (**D** and **E**) Show the continuously extruding molar teeth in the lower jaw of the cattle. **(F** and **G**) Show the continuously erupting molar teeth in the lower jaw of the humans.

place in this connective tissue affect the epithelia it sustains and both the reduced dental epithelium and the overlying oral epithelium begin to proliferate and migrate into the disorganized connective tissue. The reduced enamel epithelium and the overlying epithelium come into contact with each other and eventually fuse (Figs 13.9, 13.10). The central cells of this epithelial mass degenerate and form an epithelium-lined canal through which the tooth erupts without any hemorrhage. This epithelial cell mass is also involved in the formation of the dentogingival junction (see Chapter 10).

Once the tooth has broken through the oral mucosa, it continues to erupt at the same rate until it reaches the occlusal plane and meets its antagonist. Rapid eruptive movement then ceases.

A longitudinal study of eruption times of permanent teeth of a large number of boys and girls showed a high degree of correlation of eruption time between teeth of same types (incisors, canines etc.) than between different types of teeth. The study also revealed association of eruption times to the pattern of innervation of jaws. Teeth eruption occurs earlier in females than in males of the same age and race.

Posteruptive Phase

In the posteruptive phase the tooth makes movements primarily to accommodate the growth of the jaws. The

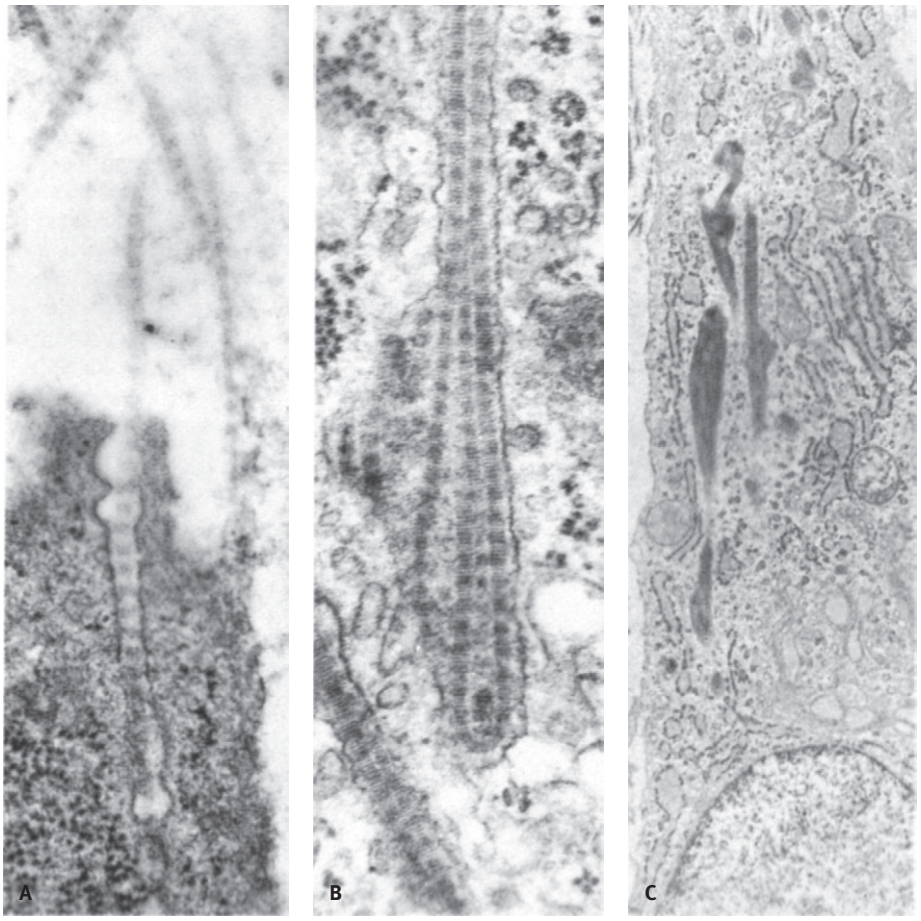

Figure 13.5 Three electron micrographs illustrating role of fibroblast in periodontal ligament remodeling and turnover. **(A)** Phagocytosis (ingestion) of collagen fibril. **(B)** Once within fibroblast, lysosomes containing catabolic enzymes fuse with vesicle containing collagen. **(C)** Degradation continues in phagolysosomes (From Ten Cate AR: Anat Rec 182:1, 1975).

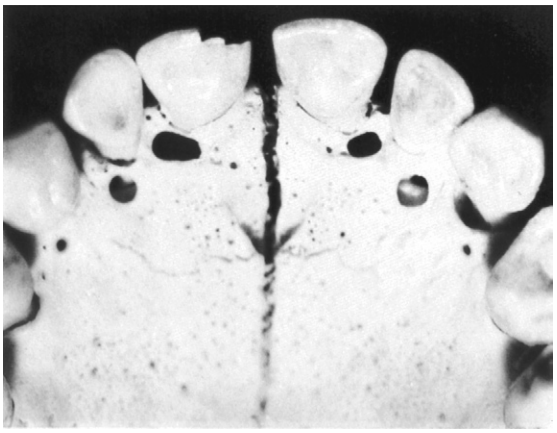

Figure 13.6 Incisor region of dried maxilla of 4-year-old child. Notice foramina lingual to deciduous teeth. These are gubernacular canals.

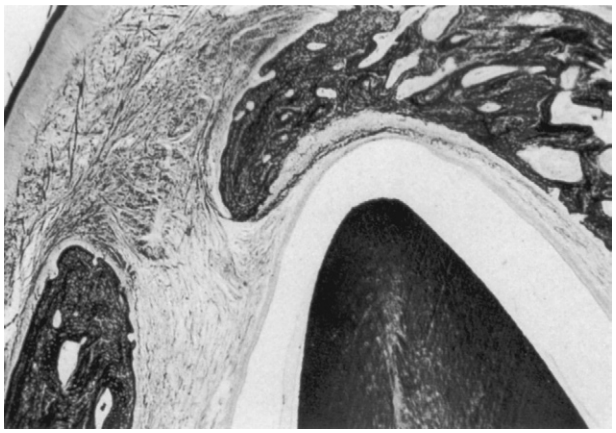

Figure 13.7 Gubernacular cord consists mainly of connective tissue and often contains a central strand of epithelium surrounded by connective tissue.

principal movement is in an axial direction. It occurs most actively between the ages of 14 and 18 and is associated with condylar growth, which separates the jaws and teeth. Although bone deposition occurs at the alveolar crest and on the socket floor (Fig. 13. 11), this is not responsible for tooth movement. The same forces responsible for eruptive tooth movement achieve axial posteruptive movement, with bone deposition occurring later. The bone formation at the fundus stabilizes the tooth during eruption.

Movements are also made to compensate for occlusal and proximal wear of the tooth. It is generally assumed

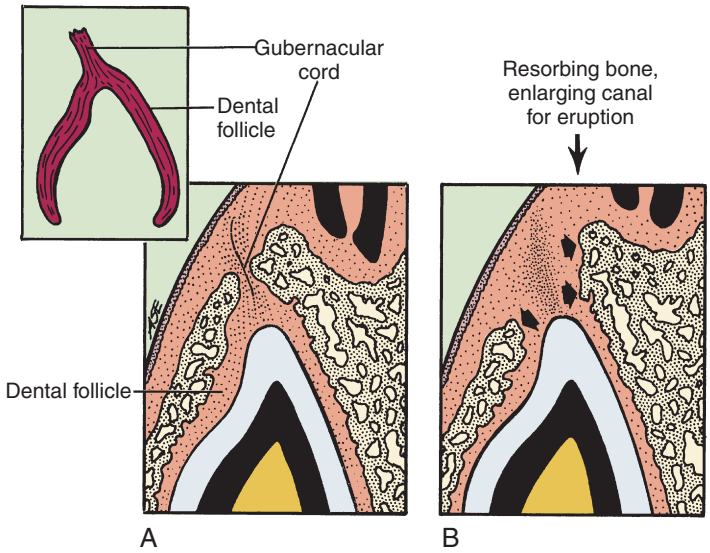

Figure 13.8 Diagram of a developing eruption pathway. **A,** Early developing eruption pathway **B,** Resorption of bone in eruption pathway (From James K. Avery and Daniel J. Chiego: Essentials of Oral Histology and Embryology, 3rd Edition, Mosby).

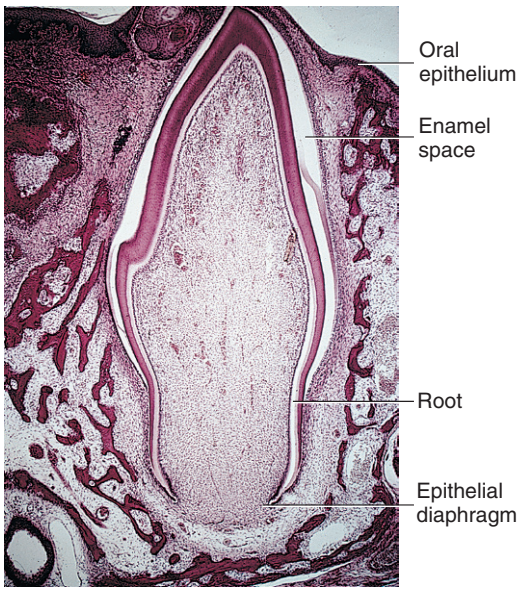

Figure 13.9 Histology of an erupting cuspid tooth. The crown tip is in contact with oral epithelium (From James K. Avery and Daniel J. Chiego: Essentials of Oral Histology and Embryology, 3rd Edition, Mosby).

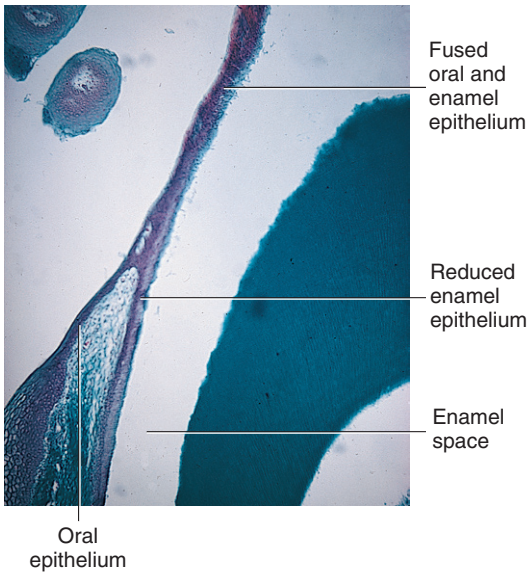

Figure 13.10 Fused reduced enamel epithelium and oral epithelium overlie the enamel of crown (Enamel space occurs as enamel is dissolved in preparation of slide) (From James K. Avery and Daniel J. Chiego: Essentials of Oral Histology and Embryology, 3rd Edition, Mosby).

that the continuous deposition of cement around the apices of the roots of teeth is sufficient to compensate for occlusal wear. However, there is no evidence that this deposition of cement actually moves the tooth. It is more likely that the forces causing tooth eruption are still available to bring about sufficient axial movement of the tooth to compensate for occlusal wear. The cement deposition that occurs is probably an infilling phenomenon. Bone formation in a ladder-like pattern is seen at the fundus (Fig.13.12).

Wear also takes place at the contact points between teeth, and to maintain tooth contact mesial or proximal drift takes place. Histologically this drift is seen as a selective deposition and resorption of bone on the socket walls by osteoblasts and osteoclasts respectively, and with the electron microscope collagen remodeling in both the periodontal and transseptal ligaments is seen.

The different stages in tooth eruption are diagrammatically represented in Figure 13.13.

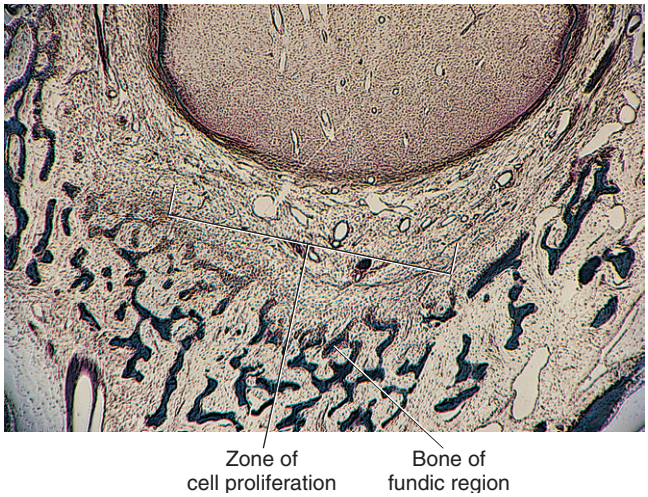

Figure 13.11 Histology of changes in fundic region during tooth eruption. Fine trabeculae of new bone appear near tooth apices that will aid in stabilizing the tooth during eruption (From James K. Avery and Daniel J. Chiego: Essentials of Oral Histology and Embryology, 3rd Edition, Mosby).

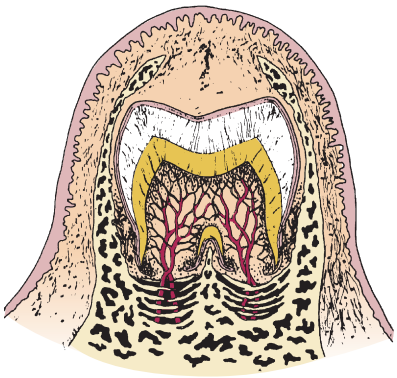

Figure 13.12 Diagram of a later stage of tooth eruption. The fundic region further develops a bony ladder (From James K. Avery and Daniel J. Chiego: Essentials of Oral Histology and Embryology, 3rd Edition, Mosby).

MECHANISM OF TOOTH MOVEMENT (THEORIES OF TOOTH ERUPTION)

The mechanism that brings about tooth movement is still debatable and is likely to be a combination of a number of factors. Although many possible causes (theories) have been proposed, only four theories merit serious consideration: (1) bone remodeling theory, (2) root formation theory, (3) vascular pressure theory, and (4) periodontal ligament traction theory. The role of dental follicle in tooth eruption is being increasingly recognized from data available from the recent studies of the molecular factors regulating eruption. Briefly stated, the bone remodeling theory supposes that selective deposition and resorption of bone brings about eruption. The root growth theory supposes that the proliferating root impinges on a fixed case, thus converting an apically directed force into occlusal movement. The vascular pressure theory supposes that a local increase in tissue fluid pressure in the periapical region is sufficient to move the tooth. The ligament traction theory proposes that the cells and fibers of the ligament pull the tooth into occlusion.

Bone Remodeling Theory

Bone remodeling clearly is important to permit tooth movement; for instance, in animals that exhibit a genetic deficiency of osteoclasts tooth eruption is prevented. Whether the bony remodeling that occurs around teeth causes or is the effect of tooth movement is not known, and both circumstances may apply. If the tooth germ is removed experimentally and the dental follicle left intact, an eruptive pathway forms in the overlying bone. Further, if a silicone replica is substituted for the tooth germ, it also erupts. On the other hand, if the dental follicle is removed, no eruptive pathway forms. These experiments establish the absolute requirement for a dental follicle to achieve bony remodeling and tooth

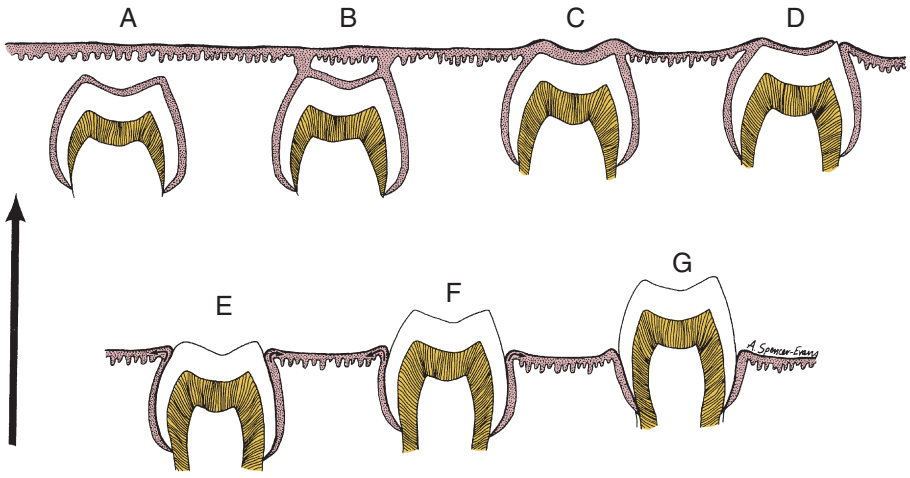

Figure 13.13 Stages of tooth eruption. **A,** Tooth crown approaching oral epithelium in preeruptive stage. **B,** Contact of reduced enamel epithelium including the developmental cuticle fusing with oral epithelium. **C,** Fusion of reduced enamel epithelium including the developmental cuticle and oral epithelia. **D,** Thinning of fused epithelia. **E,** Rupture of oral epithelium, formation of the attached gingiva and emergence. **F,** Clinical crown appearance into the oral cavity (prefunctional stage). **G,** Tooth erupting into functional occlusion (From James K. Avery and Daniel J. Chiego: Essentials of Oral Histology and Embryology, 3rd Edition, Mosby).

eruption, for it is the follicle that provides the source for new bone-forming cells and the conduit for osteoclasts derived from monocytes through its vascular supply. Other studies on bone remodeling have indicated that control may reside with the bone-lining cells, the osteoblasts. It is proposed that these cells, under hormonal influence, secrete collagenase and other proteolytic enzymes to remove the osteoid layer. In so doing these cells round up and expose the newly denuded mineralized bone surface, providing the stimulus to attract osteoclasts to the site.

Root Formation Theory

At first glance it would seem that root formation is the obvious cause of eruptive tooth movement. Root formation follows crown formation and involves cellular proliferation and formation of new tissue that must be accommodated by either movement of the crown of the tooth or resorption of bone at the base of its socket. It is the former that actually occurs, but if occlusal movement is prevented, resorption of bone occurs at the base of the socket. This is an important point, for it illustrates that if root formation is to result in an eruptive force, the apical growth of the root needs to be translated into occlusal movement and requires the presence of a fixed base. No such fixed base exists. The bone at the base of the socket cannot act as a fixed base because pressure on bone results in its resorption. Advocates of the root growth theory of tooth eruption postulated the existence of a ligament, the cushion-hammock ligament, straddling the base of the socket from one bony wall to the other like a sling. Its function was to provide a fixed base for the growing root to react against. But the structure described as the cushion-hammock ligament is the pulp-delineating membrane that runs across the apex of the tooth and has no bony insertion. It cannot act as a fixed base. Clinical observations also indicate that root formation cannot be responsible for eruptive tooth movement. For instance, some teeth move a distance greater than the length of their roots, and eruptive movement can occur after completion of root formation. Finally, experimental resection preventing further root formation does not stop eruptive tooth movement. Yet, as is discussed later, root formation may be a necessary prerequisite for eruption.

Vascular Pressure Theory

It is known that teeth move in synchrony with the arterial pulse, so local volume changes can produce limited tooth movement. Ground substance can swell by up to 50% with the addition of water, and a differential pressure sufficient to cause tooth movement between the tissues below and above an erupting tooth has been reported in the dog. Experimentally, increase of hydrostatic pressure induced by hypotensive drugs, increases the rate of eruption while stimulation of sympathetic nerves, which cause vasoconstriction and decrease of the hydrostatic pressure, decreases the rate of eruption. It has been observed that the number of fenestrated capillaries, increases with the eruption rate and their distribution varies; more numbers of fenestrated capillaries are seen near the base of the crypt than at the alveolar crest, thus accounting for the difference in the hydrostatic pressures.

Injection of 2% lignocaine with adrenaline 1:100,000 above the roots of erupting premolars (prefunctional phase of eruption), causes a burst in the increase of eruption of teeth receiving the injection with and without the vasoconstrictor. However the teeth receiving vasoconstrictor thereafter showed decrease in eruption rate, suggesting that vascular changes affect prefunctional eruption. Though vascular pressure can play an important role by generating an eruptive force, opinions differ to whether these pressures are primarily responsible for eruption.

Periodontal Ligament Traction Theory and Role of Dental Follicle in Tooth Eruption

There is a good deal of evidence that the eruptive force resides in the dental follicle–periodontal ligament complex. Experiments delineating the role of the follicle, from which incidentally the periodontal ligament forms, have already been presented in the section dealing with bony remodeling. Experiments in which roots were transected and metallic barrier was interposed showed the distal fragment of the root erupted. The results suggest that the fragment erupted because of the attachment of the dental follicle to the fragment and that vascular pressure, root growth or bone growth has apparently no role in eruption.

Experiments on the continuously erupting rodent incisor, designed to eliminate the effects of root growth and vascular supply, also show that, so long as periodontal tissue is available, tooth movement occurs. Abnormalities of dental follicle as seen in certain diseases like multiple calcifying hyperplastic dental follicle in which the dental follicles show calcifications and mucopolysaccharidoses VI, a genetic disorder of carbohydrate metabolism, in which there is excessive accumulation of dermatan sulfate are associated with delayed permanent teeth eruption.

Drugs that interrupt the proper formation of collagen in the ligament also interfere with eruption. All these experiments show that as long as dental follicle or developing periodontal ligament exists tooth eruption can occur.

Tissue culture experiments have shown that ligament fibroblasts are able to contract a collagen gel, which in turn brings about movement of a disk of root tissue attached to that gel. Thus there is no doubt that periodontal ligament fibroblasts have the ability to contract and transmit a contractile force to the extracellular environment and in particular to the collagen fiber bundles *in vitro*. All the morphologic features exist *in vivo* to permit similar movement. Thus the fibroblasts possess contractile filaments, are in contact with one another to permit summation of contractile forces, and exhibit fibronexuses by which such forces can be transmitted to the collagen fiber bundles. These not only remodel but are also inclined at the correct angle to bring about eruptive

movement. This angulation of the ligament fiber bundles is a prerequisite for tooth movement, and the orientation is believed to be established by the developing root, creating flow lines in the gel-like dental follicle. A simple analogy of the above is the sailor (fibroblast) pulling on a rope (collagen) attached to a sail (tooth). To move the sail the sailor must remain stationary and pull on the rope (contraction) and coil it on the deck (collagen remodeling).

Thus, according to this theory, eruption of teeth could be brought about by a combination of events involving a force initiated by the fibroblast. This force is transmitted to the extracellular compartment via fibronexuses and to collagen fiber bundles, which, aligned in an appropriate inclination brought about by root formation, bring about tooth movement. These fiber bundles must have the ability to remodel for eruption to continue, and interference with this ability affects the process. The removal of bone to create the eruptive pathway is also dictated by the tissues surrounding the tooth.

Opponents to the periodontal ligament traction theory have by their experiments disputed the observations with regard to the myofibroblastic nature of the fibroblast and the existence of fibronexus. In vitro experiments show that periodontal ligament fibroblast motility is affected by colchicines and generate tension by contractility like myofibroblast, but *in vivo* findings do not support, the migratory nature or features of myofibroblast. The existence of fibronexus between fibroblast and fibers is questioned. These junctions appear to be desmosomes and having no microfilament bundles. Therefore these cells would not be able to transmit a tractional force required to pull the tooth in eruption. Though studies of tritium labeled with thymidine show that the periodontal ligament fibroblast move occlusally at a rate equal to the rate of eruption, doubts have been expressed whether the cells are actively moving or they are passively transported.

The findings that support the ligament traction theory come mainly from the experiments conducted in continuously erupting rodent teeth. In humans, having limited eruption, fibers of dental follicle are not attached to the alveolar bone and are not oriented to move the teeth during the intraosseous phase. But, once the tooth has appeared in the oral cavity and before it attains its final position (prefunctional phase), the periodontal ligament fibers exert the tractional force due to its attachment to the alveolar bone and to its orientation.

Dental follicle plays a very important role in eruption. Dental follicle cells secrete colony stimulating factor-1 (CSF-1) and monocyte chemotactic protein-1 (MCP-1).These increase the rate of eruption by increasing the recruitment of monocytes and by promoting the formation of osteoclast. Transcriptor genes like NFκ (kappa) are necessary for osteoclast formation. The osteoclast formation is prevented by osteoprotegerin (OPG). Thus osteoclast formation and bone resorption is regulated. Moreover, dental follicle cells are shown to produce bone resorbing factors in the coronal half and bone forming factors in the basal half to facilitate eruption of teeth. These findings are supported by ultrastructural studies of the bony crypt. A detailed discussion of the molecular events in eruption is available in the online version of the book (see online resource Appendix).

In summary, eruptive movement is multifactorial, like vascular pressure at the apex along with contractile force generated by the dental follicle playing an important part and bone formation and resorption facilitating the process.

Posteruptive Tooth Movement

In posteruptive tooth movement the mechanisms for moving the tooth axially during eruption are most likely also used to compensate for occlusal wear. Mesial or proximal drift involves a combination of two separate forces resulting from occlusal contact of teeth and contraction of the transseptal ligaments between teeth. When the jaws are clenched, bringing teeth into contact, force is generated in a mesial direction because of the summation of cuspal planes and because many teeth have a mesial inclination. This can be demonstrated in a number of ways. When opposing teeth are removed, the rate of mesial drift is slowed but not eliminated. Selective grinding of cuspal slopes can either enhance or counter the effect of occlusal force, and when this is done, the rate of mesial drift is respectively enhanced or decreased but again not eliminated. These observations indicate that although an anterior component of occlusal force is responsible for mesial drift, it is not solely responsible.

Running between teeth across the alveolar process is the transseptal ligament, and there is evidence that this ligament has a key role in maintaining tooth position. For example, if a tooth is bisected, the two halves move away from each other, but if the transseptal ligament is previously removed, this separation does not occur. A simple but elegant experiment demonstrates that mesial drift is indeed multifactorial. By disking away the approximal contacts, room is made to permit mesial drift, and the teeth begin to move to re-establish contact. If teeth are ground out of occlusal contact, however, the rate of drift is slowed. The conclusion must be that mesial drift is achieved by contraction of transseptal fibers and enhanced by occlusal forces.

When the tooth is within the bony crypt, rate of eruption is about 1μ per day, when the tooth comes out of the bony socket, the eruption increases to 7.5μ and after its appearance in the oral cavity, the eruption rate accelerates to 1 mm per day. The final position of the teeth in the oral cavity is determined by the pressures exerted by the tongue, cheeks, lips and by the teeth which have come in contact. In addition abnormal habits like thumb sucking and lip biting, exert force on the erupting teeth, thus influencing its final position.

The force guiding the tooth in eruption should be sustained and overcome the resistance offered by the overlying tissues and it occurs alongside remodeling of periodontal ligament.

Table 13.1 Chronology of Human Dentition

Tooth	Formation of Enamel Matrix and Dentin Begins	Amount of Enamel Matrix Formed at Birth	Enamel Completed	Emergence Into Oral Cavity	Root Completed
Primary Dentition					
Maxillary					
Central incisor	14 wk in utero	Five sixths	1½ mo	7½ mo	1½ yr
Lateral incisor	16 wk in utero	Two thirds	2½ mo	9 mo	2 yr
Canine	17 wk in utero	One third	9 mo	18 mo	3¼ yr
First molar	12–15 wk in utero	Cusps united	6 mo	14 mo	2½ yr
Second molar	12–19 wk in utero	Cusp tips still isolater	11 mo	24 mo	3 yr
Mandibular					
Central incisor	18 wk in utero	Three fifths	2½ mo	6 mo	1½ yr
Lateral incisor	18 wk in utero	Three fifths	3 mo	7 mo	1½ yr
Canine	20 wk in utero	One third	9 mo	16 mo	3¼ yr
First molar	12–15 wk in utero	Cusps united	5½ mo	12 mo	2¼ yr
Second molar	12–18 wk in utero	Cusp tips still isolated	10 mo	20 mo	3 yr
Permanent Dentition					
Maxillary					
Central incisor	3–4 mo			7–8 yr	10 yr
Lateral incisor	10–12 mo			8–9 yr	11 yr
Canine	4–5 mo			11–12 yr	13–15 yr
First premolar	1½–1¾ yr			10–12 yr	12–13 yr
Second premolar	2–2¼ yr			10–12 yr	12–14 yr
First molar	At birth	Sometimes a trace		6–7 yr	9–10 yr
Second molar	2½–3 yr			12–13 yr	14–16 yr
Third molar	7–9 yr			17–21 yr	18–25 yr
Mandibular					
Central incisor	3–4 mo		4–5 yr	6–7 yr	9 yr
Lateral incisor	3–4 mo		4–5 yr	7–8 yr	10 yr
Canine	4–5 mo		6–7 yr	9–10 yr	12–14 yr
First premolar	1¾–2 yr		5–6 yr	10–12 yr	12–13 yr
Second premolar	2¼–2½ yr		6–7 yr	11–12 yr	13–14 yr
First molar	At birth	Sometimes a trace	2½–3 yr	6–7 yr	9–10 yr
Second molar	2½–3 yr		7–8 yr	11–13 yr	14–15 yr
Third molar	8–10 yr		12–16 yr	17–21 yr	18–25 yr

From Logan WHG and Kronfeld R: J Am Dent Assoc 20:379, 1933, slightly modified by McCall and Schour.

CLINICAL CONSIDERATIONS

Variation in scheduled time of eruption Generally, there is a remarkable consistency in the eruption schedule of the human dentition that reflects a programmed eruption process. An example will be the term '6-year molars' used synonymously with the permanent first molars. Different classes of teeth erupt in a set timeframe in different populations and races (Table 13.1). Tooth eruption occurs earlier in females compared to males of the same age and race. Teeth that emerge significantly outside of the normal ranges should be considered as abnormal or indicative of a fault in eruptive movement. Clinically the presence of both deciduous and permanent teeth helps in assessment of the age of the child. More accurate assessment of age can be made by studying the radiographs of the jaws, which show an age-related variability in the extent of crown and root formation.

Delayed or retarded eruption is the most common aberration relating to tooth eruption. It may be due to local or systemic factors. Systemic factors include nutritional, genetic and endocrine deficiencies. Local factors include such situations such as early loss of a deciduous tooth with consequent drifting of adjacent teeth to block the eruptive pathway. Increased density of fibrous tissue overlying the erupting tooth or the development of a cyst from remnants of the dental lamina may also be local factors that delay eruption. Severe trauma may eliminate the dental follicle, preventing formation of the periodontal ligament, resulting in a condition called ankylosis where the bone of the jaw fuses with the tooth. An ankylosed tooth is effectively unable to erupt.

Premature eruption occurs infrequently and may occasionally be seen with a permanent tooth that has prematurely lost its deciduous predecessor.

Natal teeth refer to those teeth, most commonly, the mandibular central incisors that are already present in the oral cavity of infants at the time of birth. Neonatal teeth are those teeth that erupt into the oral cavity within the first 30 days of life. However, it is now considered appropriate to designate all of them as natal teeth. Earlier, it was thought that natal teeth were predeciduous supernumerary teeth or that they represented gross aberrations in development and hence the removal of natal teeth was advocated to facilitate suckling. Also, natal and neonatal teeth showed abnormalities on microscopic examination such as absence of prism structure in cervical region, atubular dentin and absence of cementum. Recent evidence indicates that most of these are prematurely erupted teeth of the deciduous dentition (not supernumerary teeth) and they should not be extracted unless they are mobile and pose a risk for aspiration.

Orthodontic tooth movement The principal supporting tissues of the tooth, the periodontal ligament and the bone of the jaw, possess a remarkable 'plasticity' that enables the tooth to react favorably or unfavorably to its immediate environment. This plasticity and responsiveness of the supporting periodontal tissues is harnessed by the orthodontist to achieve correction of misaligned teeth by judicious application of forces to the tooth.

The tooth eruption is similar to orthodontic tooth movement except that the orthodontic tooth movement is brought about by biomechanical forces (mechanotransduction). At the molecular level, proinflammatory cytokines play a major role in orthodontic tooth movement in place of CSF-1 and VEGF. Therefore, many of these molecules like IL-1, 2, 6 and 8, TNFα have all been assayed in the gingival fluid for monitoring orthodontic tooth movement. Experimentally drugs which block prostaglandin pathway like aspirin, ibuprofen have shown to limit root resorption or reduce tooth movement

Impaction of teeth The white populations have shown an evolutionary trend towards decreased jaw sizes with a concomitant increase in the incidence of dental crowding within these relatively diminutive jaws. The third molars, and to a certain extent, the canines are often impacted as they erupt later than the rest of the dentition and most of the available space would already have been occupied by teeth that erupted before them.

Primary failure of eruption is a condition wherein permanent teeth, especially molars fail to erupt. The failure of eruption has no identifiable systemic or local causes and shows no difference in incidence between maxillary or mandibular teeth or between sexes. Family history of eruption problems and hypodontia (reduction in number of teeth) were identified in many cases. It is suggested that defects in genes like CSF-1, NFαB, c-fos may be responsible for this condition. Orthodontic treatment to force eruption is generally unsuccessful.

Hypereruption is a term used to describe a condition wherein the loss of an opposing tooth has caused the tooth/teeth to erupt to a greater than normal distance into the space provided

Teething The 'breaking out' of a tooth through the oral epithelium is often associated with an acute inflammatory response in the connective tissue adjacent to the erupting tooth. The child will manifest symptoms of inflammation such as pain, mild fever and general malaise that are popularly called *teething*.

SUMMARY

Eruption of teeth generally refers to emergence or appearance of teeth in the oral cavity. In a wider context it refers to movement of teeth in the oral cavity from its developmental position within the jaws to its functional position in the oral cavity and maintenance of this position throughout life. Therefore, three stages of eruption are described: a preeruptive phase, an eruptive phase and a posteruptive phase.

Preeruptive Phase

In the *preeruptive phase* the tooth germ and the developing teeth undergo bodily movement before they get into the eruptive position. The bone surrounding the tooth germ undergoes remodeling to accommodate the growing tooth germ. The deciduous teeth germs are first crowded together but get spaced when jaws grow in length and bodily movement of tooth germs occur. The permanent molars undergo tilting movements to correct their inclination–the maxillary molars tilt mesially and mandibular molars tilt distally along with the growth of the jaws.

Eruptive Phase

In the *eruptive phase,* the teeth with developing roots move in an axial direction, i.e. towards occlusal, to appear in the oral cavity and to contact its opposing tooth. When the permanent incisors erupt they are seen lingually to the deciduous incisors and come into proper position after the deciduous teeth are shed. The premolars erupt directly into their designated position. These permanent teeth (permanent successors) are guided in their eruption by a connective tissue structure called gubernacular cord.

Posteruptive Phase

In the *posteruptive phase,* the teeth have to maintain its functional position as the jaws grow and also to compensate for occlusal and proximal wearing of teeth. The movement of the teeth is in a mesio-occlusal direction. Therefore this movement is also called physiological mesial drift.

Animal Studies in Eruption

The understanding of the process of eruption in humans has come from experimental studies in animals, especially

rodents. All the results from animal studies cannot be applied to humans as their teeth structure and mode of teeth movement are different.

Mechanism of Eruption (Theories of Tooth Eruption)

The mechanisms involved in teeth eruption are not understood fully so, four main theories are put forth to explain the mechanism.

Bone Growth Theory

The *bone growth theory* states selective bone formation and bone resorption which occurs, is the cause for tooth eruption. Experiments in which tooth germ is removed and/or replaced with an inert material show the formation of eruptive pathway in bone.

Root Growth Theory

The *root growth theory* states that the growth of root impinges upon a sling of connective tissue called cushion-hammock ligament (which straddles across the bony socket) to produce the necessary thrust for eruption. This theory is challenged because cushion-hammock ligament is not inserted into the bone to have a fixed base to produce the necessary force for eruption. Also, the eruptive distances are greater than root length in many teeth and rootless teeth can also erupt.

Vascular Pressure Theory

The *vascular pressure theory* states that the increased vascular pressure present in the apex of a developing root produces the force for eruption. This theory though supported by experiments conducted in dogs is challenged on the grounds that the hydrostatic pressure may not be sufficient to sustain tooth eruption for long periods.

Ligament Traction Theory and Role of Dental Follicle in Tooth Eruption

The *ligament traction theory* states that the fibroblasts of the dental follicle by their contraction can generate a force, which can pull the teeth into occlusion. The fibroblasts have their processes attached to the collagen fibers by a sticky protein called fibronectin and as their processes are in contact with each other it produces a summative force for eruption. Support for this theory come from the experimental findings to decrease collagen formation or those in which eruption occurred in spite of metal barriers inserted in the developing root (to negate effects of root growth or vascular pressure) and in dental follicles replaced by silicone but with intact collagen fibers. However, the validity of this theory is doubted in that the fibroblast does not have contractile elements, their ability to migrate and that the collagen fibers were not inserted into the bone in early stages of eruption. In conclusion, the force of tooth eruption are due to several factors namely, selective bone remodeling, the dental follicle, the contractile force of fibroblast and vascular pressure at the apex.

The dental follicle plays an important part in eruption. It produces factors for promoting osteoclastic bone resorption in the coronal part and by promoting bone formation in the apical part. Dental follicle cells secrete MCP-1 (monocyte chemotactic proteins-1) and CSF-1 (colony stimulating factor-1), which promote osteoclast formation. OPG (osteoprotegerin) interferes with binding of RANKL to RANK receptor on mononuclear cells thereby decreasing osteoclast formation. In clinical situations teeth which are directly attached to the bone (tooth ankylosis) do not undergo posteruptive movement and lie below the occlusal level. They are called submerged teeth. Local and systemic factors influence the rate of eruption. Impaction of third molars and delayed eruption are commonly seen in dental practice while premature eruption and natal teeth are rarely seen.

Clinical Considerations

Eruption of teeth follows a strict pattern and time of eruption. The presence of deciduous and permanent teeth at the same time helps in assessment of age clinically and radiographic assessment of extent of crown and root formation helps in more accurate assessment of age. Eruption of teeth are influenced by systemic conditions, like hormonal disorders of thyroid. Increase in number of teeth called supernumerary teeth or decrease in number of teeth, termed hypodontia or teeth may be present at birth (natal teeth). The more common disorder is delayed eruption of permanent teeth in which local factors play a very important role.

REVIEW QUESTIONS

1. Describe the types of tooth movement seen in different phases of eruption.
2. Why one cannot apply all results of animal experimental studies of eruption to humans?
3. Enumerate the theories of tooth eruption. Discuss the ligament traction theory of eruption.
4. Write notes on:
   Gubernacular cord
   Fibronexus
   Root growth theory of eruption
   Vascular pressure theory of eruption
   Physiological mesial drift
5. Describe the cellular and molecular events of eruption.

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14 Shedding of Deciduous Teeth

CHAPTER CONTENTS

DEFINITION

The human dentition, like those of most mammals, consists of two generations. The first generation is known as the deciduous (primary) dentition and the second as the permanent (secondary) dentition. The necessity for two dentitions exists because infant jaws are small and the size and number of teeth they can support is limited. Since teeth, once formed, cannot increase in size, a second dentition, consisting of larger and more teeth, is required for the larger jaws of the adult. The physiologic process resulting in the elimination of the deciduous dentition is called *shedding* or *exfoliation*.

PATTERN OF SHEDDING

The shedding of deciduous teeth is the result of progressive resorption of the roots of teeth and their supporting tissue, the periodontal ligament. Most attention has been paid to the removal of the dental hard tissues, which is accomplished by easily identified multinuclear cells in every way similar to osteoclasts (Fig. 14.1). In general, the pressure generated by the growing and erupting permanent tooth dictates the pattern of deciduous tooth resorption. At first this pressure is directed against the root surface of the deciduous tooth itself (Fig. 14.2). Because of the developmental position of the permanent incisor and canine tooth germs and their subsequent physiologic movement in an occlusal and vestibular direction, resorption of the roots of the deciduous incisors and canines begins on their lingual surfaces (Fig. 14.3). Later, these developing tooth germs occupy a position directly apical to the deciduous tooth, which permits them to erupt in the position formerly occupied by the deciduous tooth (Fig.14.4). Frequently, however, and especially in the case of the permanent mandibular incisors, this apical positioning of the tooth germs does not occur, and the permanent tooth erupts lingual to the still functioning deciduous tooth (Fig. 14.5).

Resorption of the roots of deciduous molars often first begins on their inner surfaces because the early developing bicuspids are found between them (Fig. 14.6). This resorption occurs long before the deciduous molars are shed and reflects the expansion of their growing permanent successors. However, as a result of the continued growth of the jaws and occlusal movement of the deciduous molars, the successional tooth germs come to lie apical to the deciduous molars (Fig. 14.7). This change in position provides the growing bicuspids with adequate space for their continued development and also relieves the pressure on the roots of the overlying deciduous molars. The areas of early resorption are repaired by the deposition of a cementum-like tissue. When the bicuspids begin to erupt, resorption of the deciduous molars is again initiated, and this time continues until the roots are completely lost and the tooth is shed (Fig. 14.8). The bicuspids thus erupt in the position of deciduous molars.

HISTOLOGY OF SHEDDING

The cells responsible for the removal of dental hard tissue are identical to osteoclasts, and are called *odontoclasts*.

Odontoclasts are readily identifiable in the light microscope as large, multinucleated cells occupying resorption bays on the surface of a dental hard tissue. They are smaller than osteoclast, contain fewer nuclei, and produce smaller resorption lacunae. Their cytoplasm is vacuolated, and the surface of the cell adjacent to the resorbing hard tissue forms a 'ruffled' border (Fig. 14.1). The ruffled border is resolved with the electron microscope (Fig. 14.9) as an extensive folding of the cell membrane into a series of invaginations 2 to 3 µm deep, with mineral crystallites within the depths of

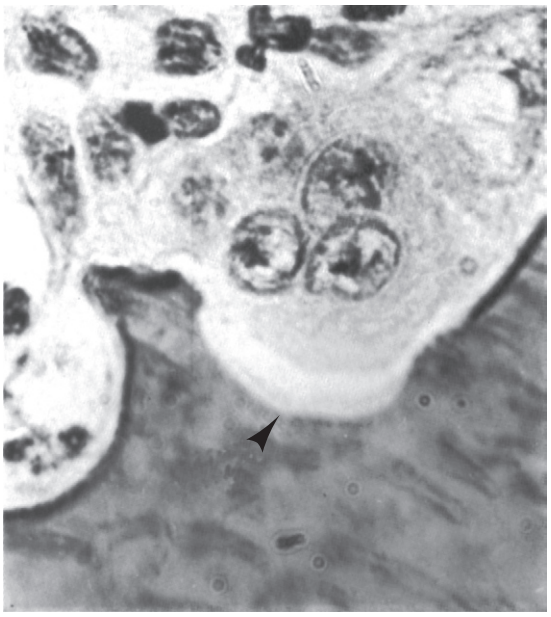

Figure 14.1 Photomicrograph of odontoclast resorbing dentin. Note ruffled border (arrow) where odontoclast is in contact with dentin (From Furseth R: Arch Oral Biol 13:417, 1968).

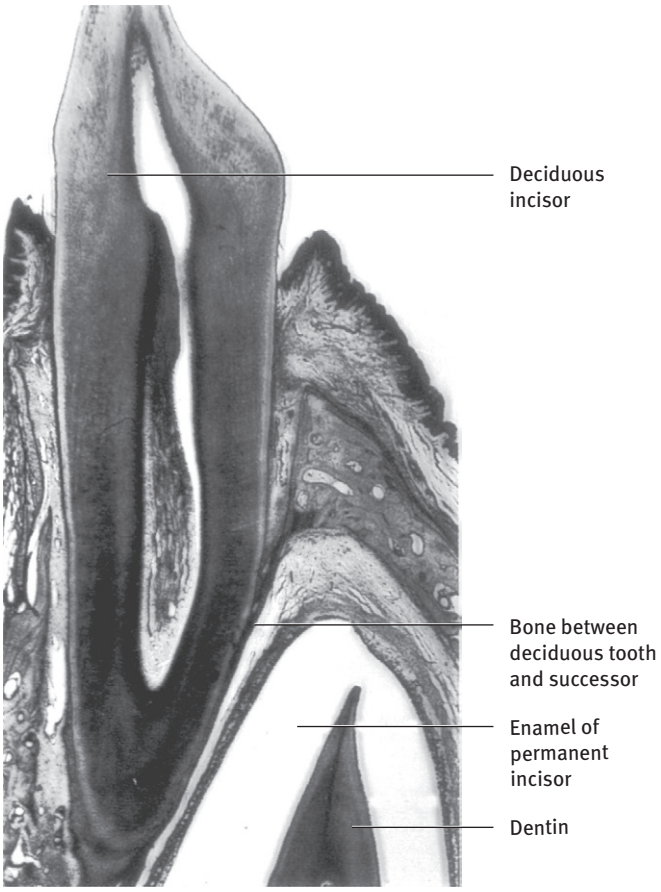

Figure 14.2 Thin lamella of bone separates permanent tooth germ from its predecessor lingual to the still functioning deciduous tooth.

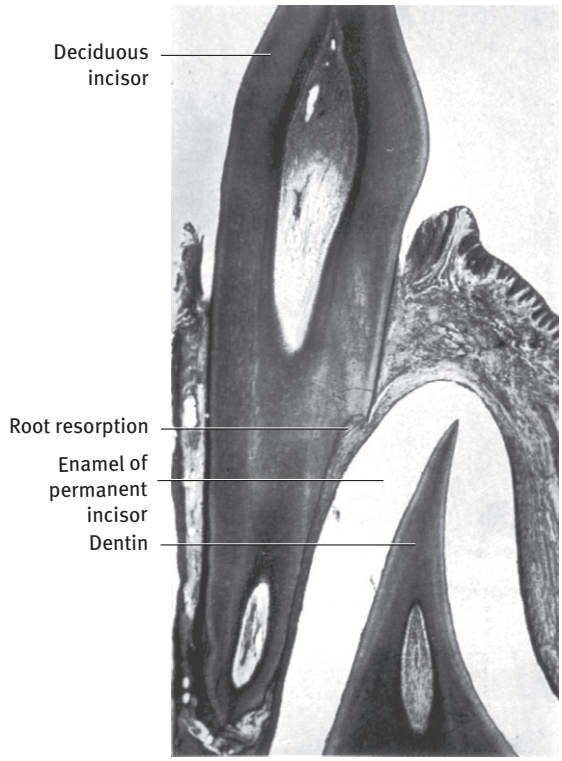

Figure 14.3 Resorption of lingual aspect of root of deciduous incisor caused by pressure of erupting successor.

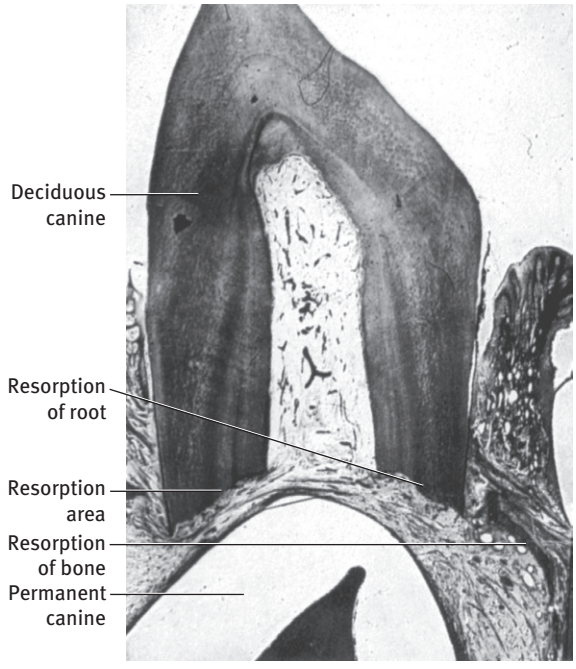

Figure 14.4 Resorption of root of deciduous canine. Note apical position of permanent successor (From Kronfeld R: Dent Cosmos 74:103, 1932).

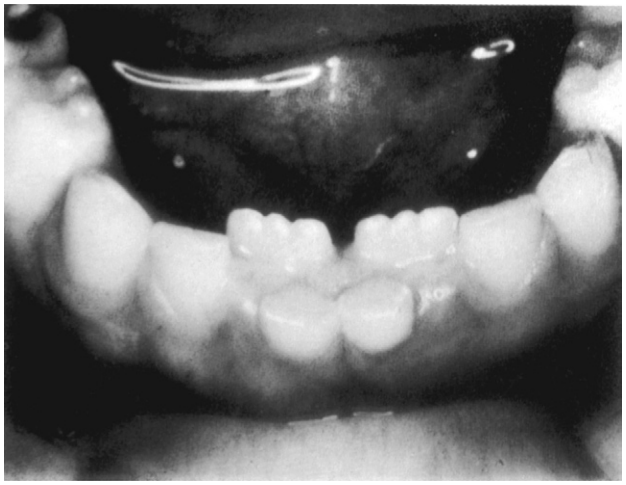

Figure 14.5 Dentition of 6-year-old child showing how permanent incisors frequently erupt lingually to deciduous incisors before latter teeth are shed.

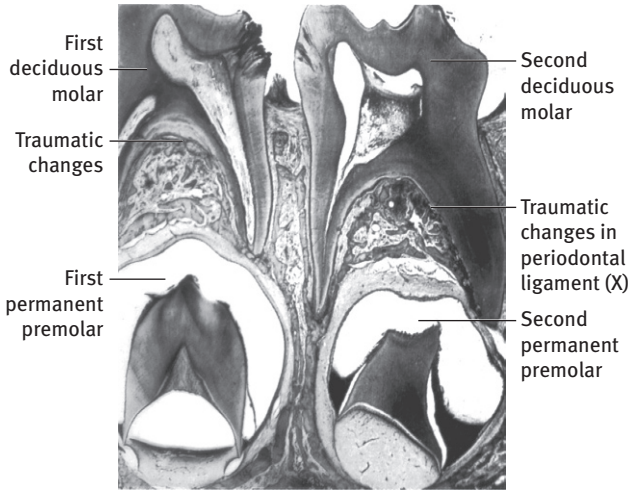

Figure 14.7 Germs of permanent premolars below roots of deciduous molars.

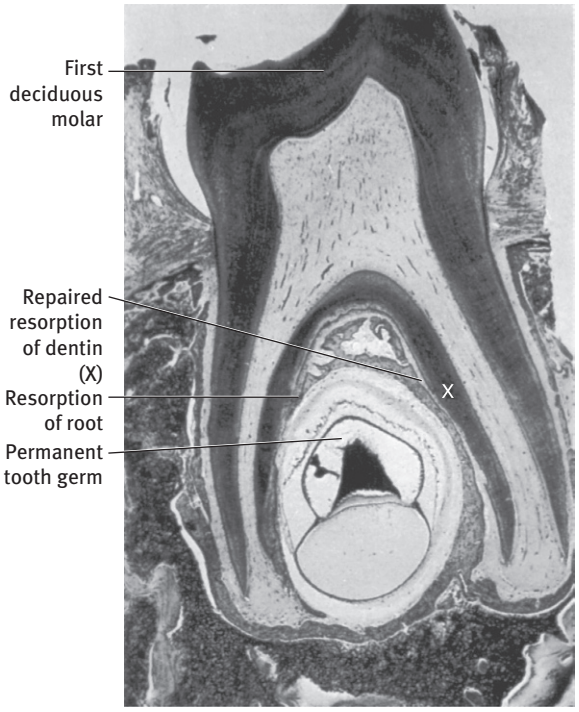

Figure 14.6 Germ of lower first permanent premolar between roots of first deciduous molar. Repair of previously resorbed dentin has occurred at X (See also Figs 14.16, 14.17).

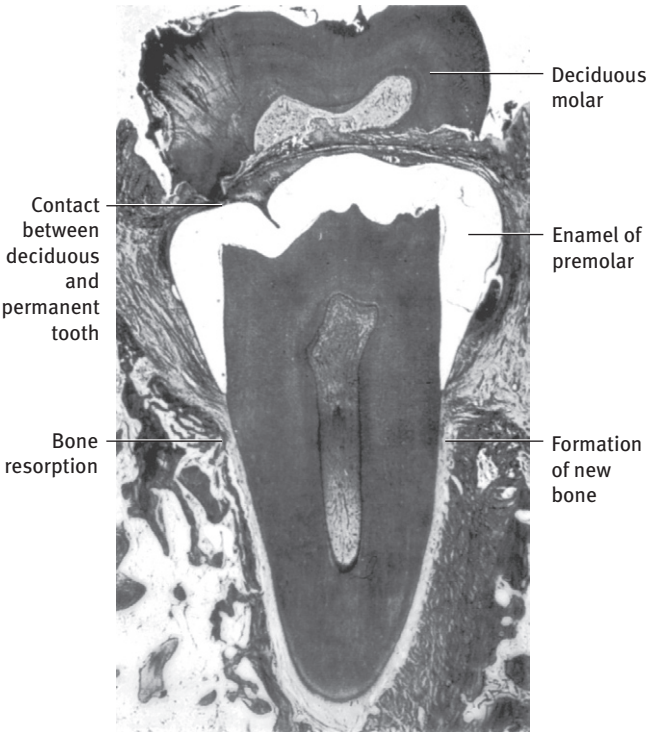

Figure 14.8 Roots of primary molar completely resorbed. Dentin of primary tooth in contact with enamel of premolar. Resorption of bone on one side and formation of new bone on opposite side of premolar caused by transmitted eccentric pressure to premolar (From Grimmer EA: J Dent Res 18: 267, 1939).

the invaginations. Peripheral to the ruffled border is a clear zone (Fig. 14.10) in which the cytoplasm is devoid of organelles but rich in filaments consisting of the contractile proteins actin and myosin. The clear zone represents the attachment apparatus of the odontoclast. The cytoplasm of the odontoclast is characterized by an exceptionally high content of mitochondria and many vacuoles, which are especially concentrated adjacent to the ruffled border. Acid phosphatase activity occurs within these vacuoles (Fig. 14.11). They develop membrane specializations like ruffled border and clear zone after they contact the resorbing surface. The odontoclasts fuse with each other to form a multinucleated giant cell only after they get attached to the resorbing surface. Odontoclasts are able to resorb all the dental hard tissues, including, on occasions, enamel. They resorb all dental tissues in the same way as oseoclasts resorb bone. When dentin is being resorbed, the presence

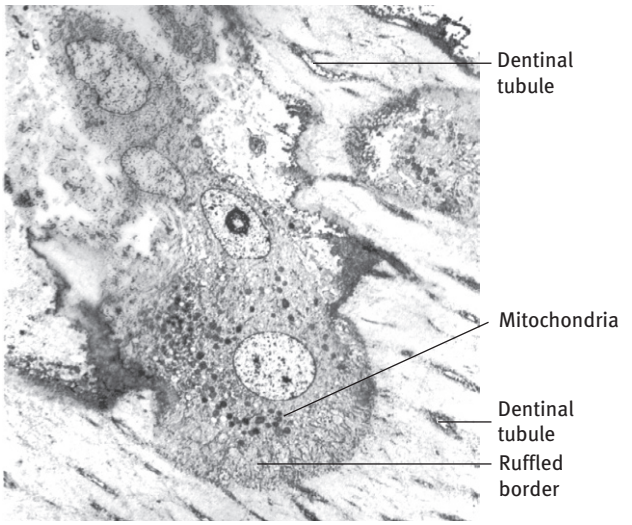

Figure 14.9 Multinucleated odontoclast displays ruffled border that is well adapted to resorption lacuna in root dentin. Dense mitochondria are aggregated toward 'resorptive' (lower) pole of cell, and most of the cytoplasm is highly vacuolated. Dentinal tubules are visible in oblique section (×3000) (From Freilich LS: J Dent Res 50:1047, 1971).

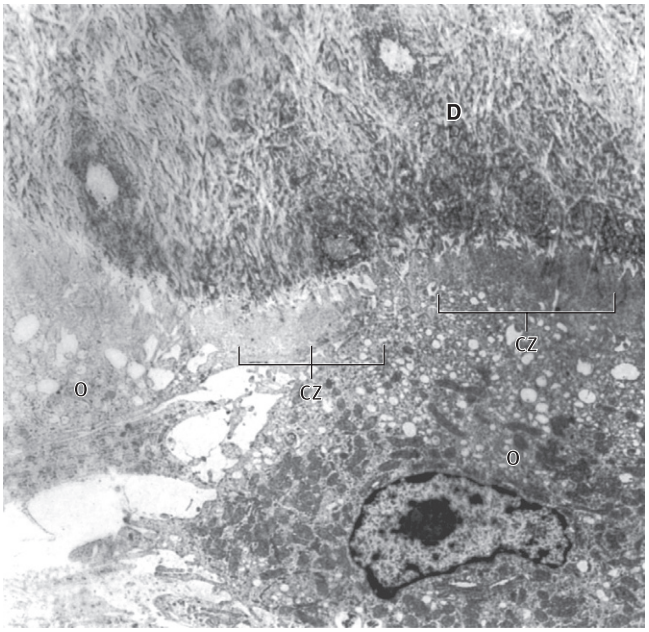

Figure 14.10 Peripheral aspect of two odontoclasts (O) resorbing dentin (D) illustrating clear zone (CZ) thought to be associated with attachment.

of the tubules provides a pathway for the easy extension of odontoclast processes (Fig. 14.12).

Odontoclasts probably have the same origin as osteoclasts. The monocyte, circulating in the blood, originally gives rise to all the different tissue macrophages, including the osteoclast, but what is not certain is whether osteoclasts are further formed from mainly resident tissue macrophages or continuously from circulating monocytes. Odontoclasts, therefore are probably derived from tartrate-resistant acid phosphatase (TRAP)-positive circulating monocytes. The HLA-DR positive cells were found adjacent to odontoclast at the pulp–dentin border during the stage of active eruption. Later it was found to cover the exposed dentin surface after the odontoclast had withdrawn and before the reparative process of cementum deposition occurs. It has been suggested that these cells were involved in the differentiation, migration and activation of odontoclast and cementoblast like cells during resorption of deciduous teeth.

The periodontal ligament cells in teeth undergoing shedding express the tumor necrosis factor (TNF)–family molecule named **R**eceptor **A**ctivator of **N**uclear factor **K**appa B **L**igand (RANKL). RANKL expression is linked to odontoclast formation and activation of shedding process. Odontoclasts derived from resorbing deciduous teeth expressed both RANKL and its receptor, RANK. It was observed that RANKL increased odontoclast actin ring formation and resorbing activity in a dose-dependant manner. Similarly, the expression of osteoprotegerin (OPG), a decoy receptor that prevents RANKL from binding to RANK was found to be less in the periodontal ligament cells of resorbing teeth when compared to nonresorbing teeth. These findings suggest that odontoclast differentiation is promoted by RANKL and is inhibited by OPG, as in the case of osteoclast differentiation in bone resorption.

Debate also exists concerning the distribution of odontoclasts during tooth resorption. Odontoclasts are most commonly found on surfaces of the roots in relation to the advancing permanent tooth. However, they have also been described in the root canals and pulp chambers of resorbing teeth lying against the predentin surface.

Although their location in the pulp chamber has been disputed, the most likely reason is that different patterns of resorption exist for different teeth. For example, single-rooted teeth are usually shed before root resorption is complete (Fig. 14.13); therefore odontoclasts are not found within the pulp chambers of these teeth, and the odontoblast layer remains intact. In molars, however, the roots are usually completely resorbed and the crown is also partially resorbed, before exfoliation. When this happens (Fig. 14.14), the odontoblast layer is replaced by odontoclasts (Fig. 14.15), which resorb both primary and secondary dentin (Fig. 14.16). Sometimes all the dentin is removed, and the vascular connective tissue is visible beneath the translucent cap of enamel.

Similarities and dissimilarities of odontoclasts to osteoclasts are depicted in Boxes 14.1 and 14.2.

The process of tooth resorption is not continuous since there are periods of rest and repair; however, in the long term, resorption predominates over repair. Repair is achieved by cells resembling cementoblasts that lay down a dense collagenous matrix in which spotty mineralization occurs (Figs 14.17, 14.18). The resorbed enamel surface as well as the resorbed dentinal surface becomes coated with cellular cementum like tissue secreted by mononuclear cells. The organic thin layer found on the resorbed surface is reported to aid in cemental deposition.

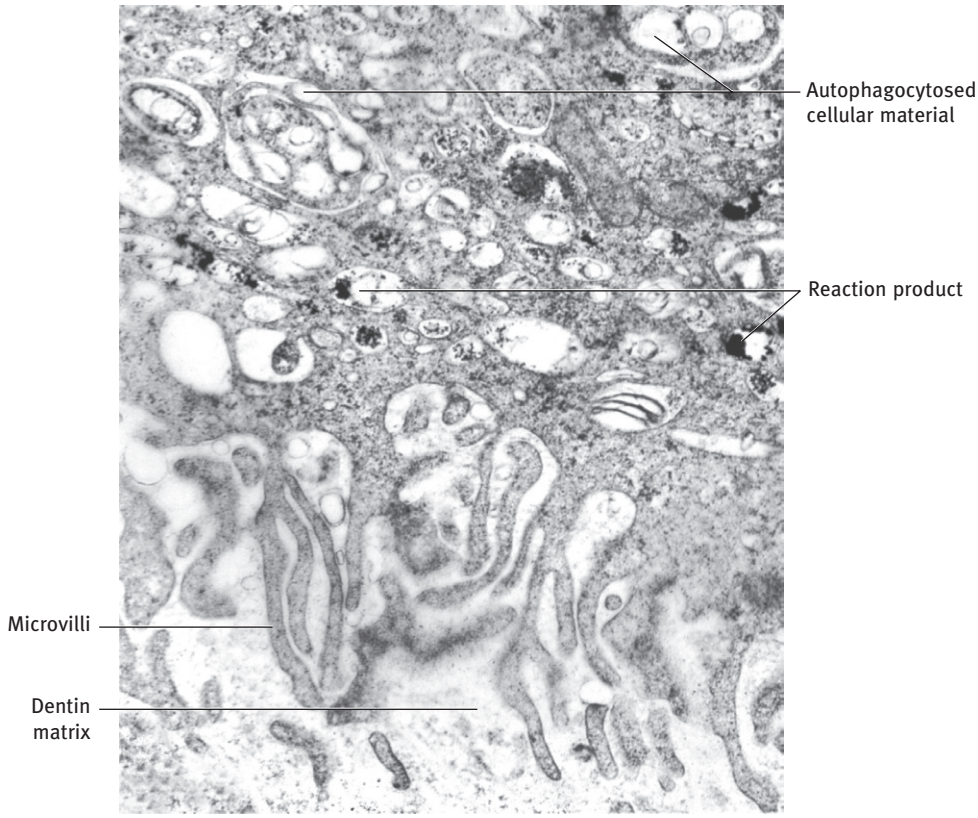

Figure 14.11 Interface between odontoclast ruffled border region (indicated by irregular microvilli) and disintegrated dentin matrix of root surface undergoing resorption. Numerous membrane-bound vacuoles in odontoclast cytoplasm show varied contents, including autophagocytosed cellular material and dense patches of reaction product, indicating acid phosphatase activity (×40,000) (From Freilich LS: J Dent Res 50:1047, 1971).

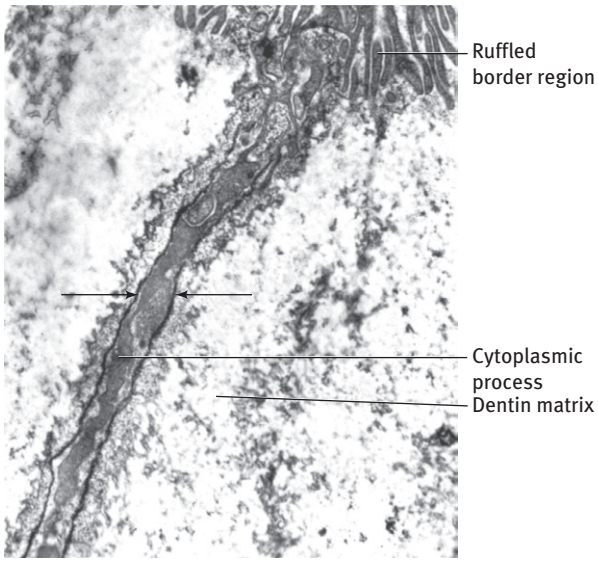

Figure 14.12 Electron micrograph showing cytoplasmic process emanating from ruffled border region of odontoclast and occupying dentinal tubule. Dentin matrix occupies bulk of field (×25,000) (From Freilich LS: J Dent Res 50:1047, 1971).

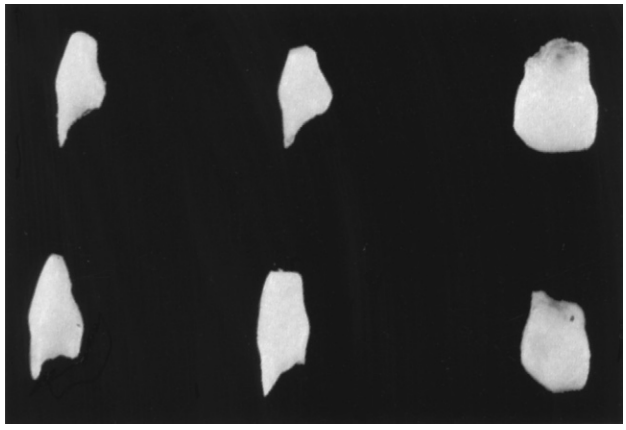

Figure 14.13 Random selection of exfoliated deciduous incisor and canine teeth showing that considerable amount of root dentin remains at time of exfoliation.

A cellular cementum like tissue was deposited in shallow and smaller resorption bays while larger and deeper resorption bays were covered with cellular cementum like tissue. Both types of cementum like tissue showed intrinsic collagen fibers but extrinsic collagen fibers which normally get attached to a cellular cementum as in repair was not seen in resorption. Formation of cementum like tissue may play a role in retention of deciduous teeth until shedding occurs.

After the root resorption was complete, the dentogingival junction migrated apically along the inner resorbing surface and was attached to the residual pulp. Another part of the gingival epithelium partly lined the surface of the erupting permanent teeth. Thus the

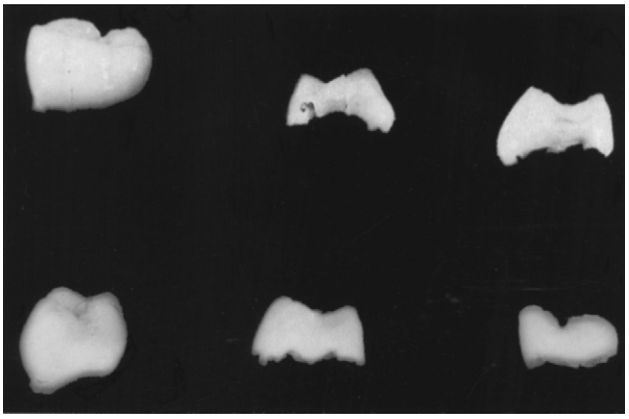

Figure 14.14 Random selection of exfoliated deciduous molars showing that total loss of roots usually occurs before these teeth are shed. This photograph also shows occurrence of enamel resorption.

gingival epithelium holds the tooth in the cervical region just prior to shedding and the final part of shedding occurs by tearing this attachment. Inflammatory process is also involved in the apical migration of the junctional epithelium.

MECHANISM OF RESORPTION AND SHEDDING

The mechanisms involved in bringing about tooth resorption and exfoliation are not yet fully understood. It seems clear that pressure from the erupting successional tooth plays a key role because the odontoclasts appear at predicted sites of pressure.

Pressure exerted by tumors and cysts may be the cause for pathological root resorption. The mechanisms of differentiation and resorption by odontoclasts in these cases may be similar as in shedding.

Unlike osteoblasts, cementoblasts covering the root are not responsive to hormones and cytokines. Therefore prior to resorption, the cementoblastic layer has to be damaged probably by inflammatory processes. The reduced enamel epithelium of the erupting permanent teeth is suggested to release some substances to initiate this process.

How the odontoclast actually resorbs dental hard tissue is not known. In the case of bone resorption it is thought the osteoblasts must first degrade the osteoid, thereby exposing mineralized bone to which osteoclasts can attach. The same may also hold for dentin resorption. Predentin, however resists resorption more than any other dental tissue.

The mechanisms of physiological root resorption and bone resorption were similar. The factors that activate osteoclast and odontoclast were same and odontoclasts express ATPase, cathepsin K and matrix metalloproteinase-9, similar to osteoclast.

Whatever the preliminary steps in hard-tissue resorption, it is clear that the odontoclast attaches to the hard-tissue surface peripherally through the clear zone, thereby creating a sealed space lined by the ruffled border of the cell. In this way, a microenvironment results. The membrane of the ruffled border acts as a proton pump, adding hydrogen ions to the extracellular environment and acidifying it so that mineral dissolution occurs. Primary lysosomes secrete their enzymatic contents into the same environment

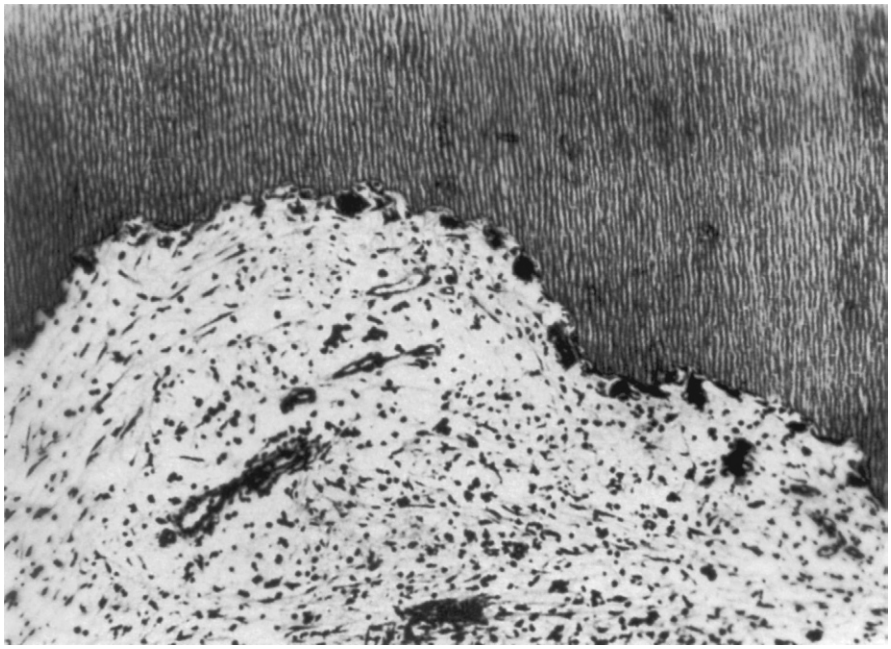

Figure 14.15 Osteoclastic resorption in surface of coronal dentin of deciduous first molar. Odontoblast layer is absent and numerous odontoblasts can be seen lining pulp chamber (From Weatherell JA and Hargreaves JA: Arch Oral Biol 11:749, 1966).

Figure 14.16 Odontoclasts resorbing secondary dentin (From Weatherell JA and Hargreaves JA: Arch Oral Biol 11:749, 1966).

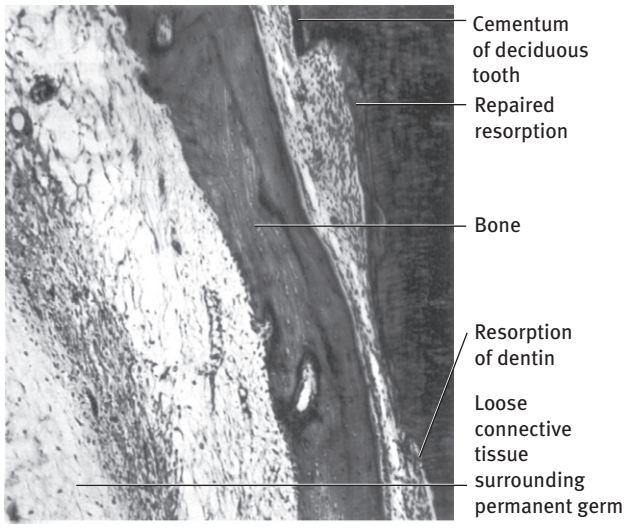

Figure 14.17 High magnification of repaired resorption from area X of Figure 14.6.

Box 14.1 Odontoclasts: Similarities to Osteoclasts

- Large multinucleated giant cell of variable shape
- Occupy resorption bays or lacunae
- Show ruffled border and clear zone peripheral to it
- Cytoplasm adjacent to ruffled border shows high amounts of mitochondria and vacuoles
- Acid phosphate activity within vacuoles
- Process of resorption similar
- Similar origin: from TRAP + circulating monocytes
- Odontoclast differentiation promoted by RANKL and inhibited by OPG

Box 14.2 Odontoclasts: Dissimilarities with Osteoclast

- Smaller and contain fewer nuclei
- Produce smaller resorption bays
- Resorb dental hard tissues: dentin, cementum and enamel
- Seen, therefore on surfaces of dentin, cementum and enamel which are to be resorbed
- Seen also in pulp chamber and root canal of resorbing deciduous teeth

to degrade the organic matrix. Odontoclasts resorbing enamel, phagocytose crystals that have been liberated from the partially demineralized enamel matrix by acids, and subsequently dissolve them intracellularly.

Although pressure obviously has a key role in initiating tooth resorption, other factors are also involved. It is a common clinical observation that when a successional tooth germ is missing, shedding of the deciduous tooth is delayed. Also, experimental removal of a permanent tooth germ delays, but does not prevent, shedding of its deciduous predecessor. The forces of mastication applied to the deciduous tooth are also capable of initiating the resorption. As an individual grows, the muscles of mastication increase in size and exert forces on the deciduous tooth greater than its periodontal ligament can withstand. This leads to trauma to the ligament and the initiation of resorption. That this is so has been established experimentally by placing a splint bridge into the mouth of an experimental animal in such a way as to protect the deciduous tooth from occlusal stress. When this is done, resorption of the deciduous tooth is halted and repair takes place. In practice a combination of both factors likely determines the rate of resorption. As resorption of the roots initiated by pressure of the underlying tooth occurs, there is a progressive loss of surface area for attachment of the periodontal ligament fiber bundles. This weakening of tooth support occurs because it has to withstand increasingly greater occlusal forces generated by the growing muscles of mastication.

Although the resorption of the dental hard tissues has been studied extensively, much less is known about the resorption of the dental soft tissues, the pulp and the periodontal ligament. In case of the periodontal ligament it has been demonstrated that apoptotic cell death is involved (Fig. 14.19). This form of cell death involves shrinkage of the cells so that they can be phagocytosed by

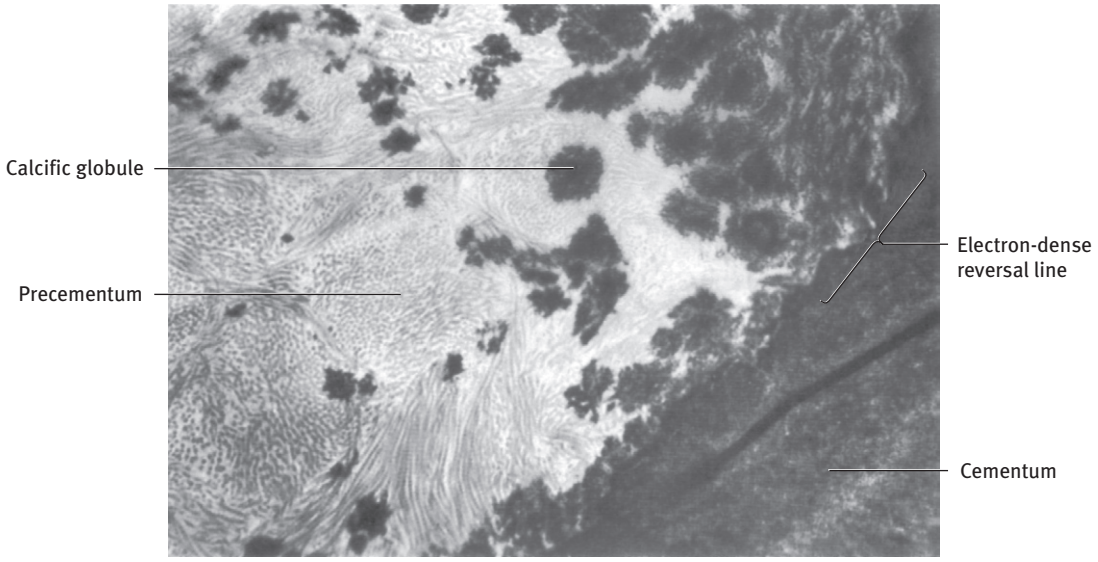

Figure 14.18 Electron micrograph of resorption lacunae where repair of cementum is taking place. Newly deposited repair tissue is not as electron dense as underlying cementum. Note electron-dense reversal line and calcific globules in precementum (From Furseth R: Arch Oral Biol 13:417, 1968).

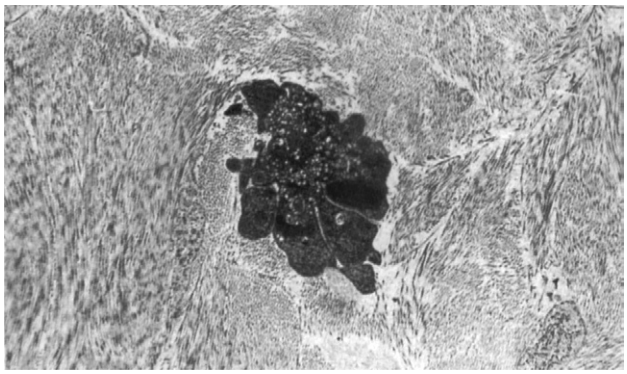

Figure 14.19 Apoptotic cell death in periodontal ligament fibroblast of shedding tooth (From Ten Cate AR: Oral histology, development, structure, and function, St Louis, 1989, The CV Mosby Co).

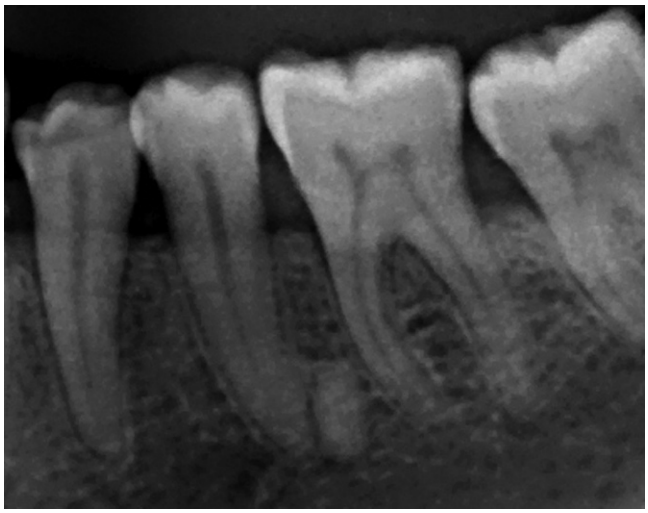

Figure 14.20 Remnants of roots of deciduous molar embedded in bone (*Courtesy* Maxillofacial diagnostics).

neighboring cells. Apoptotic cell death is a normal feature of embryogenesis and is programed so that cells die at specific times to permit orderly development. The occurrence of apoptotic cell death in the resorbing periodontal ligament, together with the observation that in monozygotic twins the eruption pattern is largely (80%) determined by genetic factors, suggests that shedding is a programmed developmental event influenced by local factors.

CLINICAL CONSIDERATIONS

Remnants of Deciduous Teeth

Sometimes parts of the roots of deciduous teeth are not in the path of erupting permanent teeth and may escape resorption. Such remnants, consisting of dentin and cementum, may remain embedded in the jaw for a considerable time. They are most frequently found in association with the permanent premolars, especially in the region of the lower second premolars (Fig. 14.20). The reason is that the roots of the lower second deciduous molar are strongly curved or divergent. The mesiodistal diameter of the second premolars is much smaller than the greatest distance between the roots of the deciduous molar. Root remnants may later be found deep in the bone, completely surrounded by and ankylosed to the bone (Fig. 14.21). Frequently they are cased in heavy layers of cellular cementum. When they are close to the surface of the jaw (Fig. 14.22), they may ultimately be

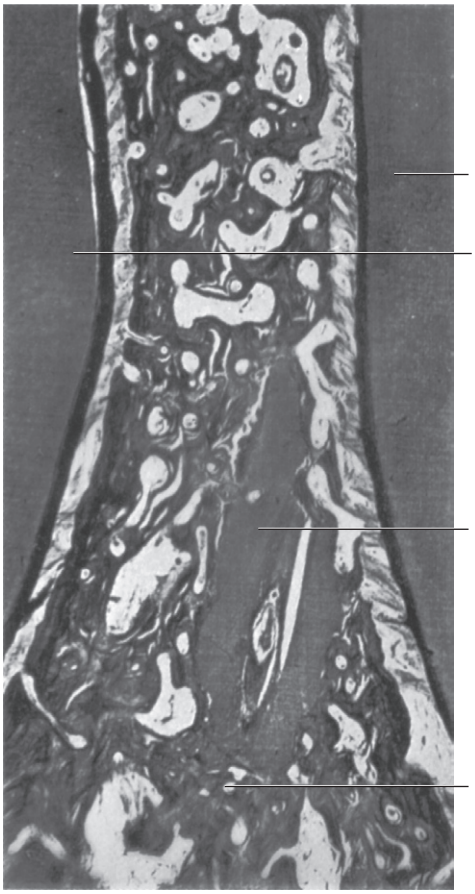

Figure 14.21 Remnant of deciduous tooth embedded in and ankylosed to the bone (From Schoenbauer F: Z Stomatol 29-892, 1931).

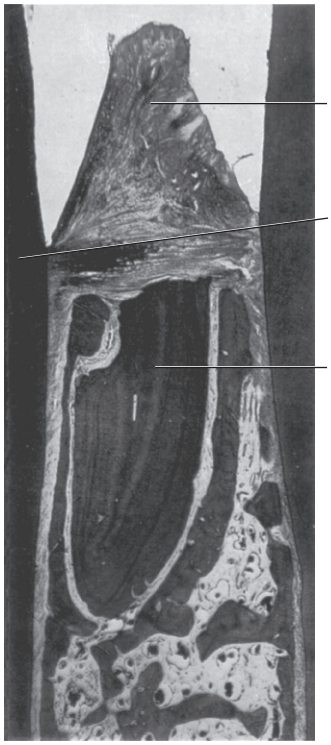

Figure 14.22 Remnant of deciduous tooth at alveolar crest.

exfoliated. Progressive resorption of the root remnants and replacement by bone may cause the disappearance of these remnants.

Retained Deciduous Teeth

Deciduous teeth may be retained for a long time beyond their usual shedding schedule. Such teeth are usually without permanent successors, or their successors are impacted. They are invariably out of function. Retained deciduous teeth are most often the upper lateral incisor (Fig. 14.23A), less frequently the second permanent premolar, especially in the mandible (Fig. 14.23B), and rarely the lower central incisor (Fig. 14.23C). If a permanent tooth is ankylosed or impacted, its deciduous predecessor may also be retained (Fig. 14.23D). This is most frequently seen with the deciduous and permanent canine teeth.

If the permanent lateral incisor is missing, the deciduous tooth is often resorbed under the pressure of the erupting permanent canine. This resorption may be simultaneous with that of the deciduous canine (Fig. 14.24). Sometimes the permanent canine causes resorption of the deciduous lateral incisor only and erupts in its place. In such cases the deciduous canine may be retained distally to the permanent canine. A

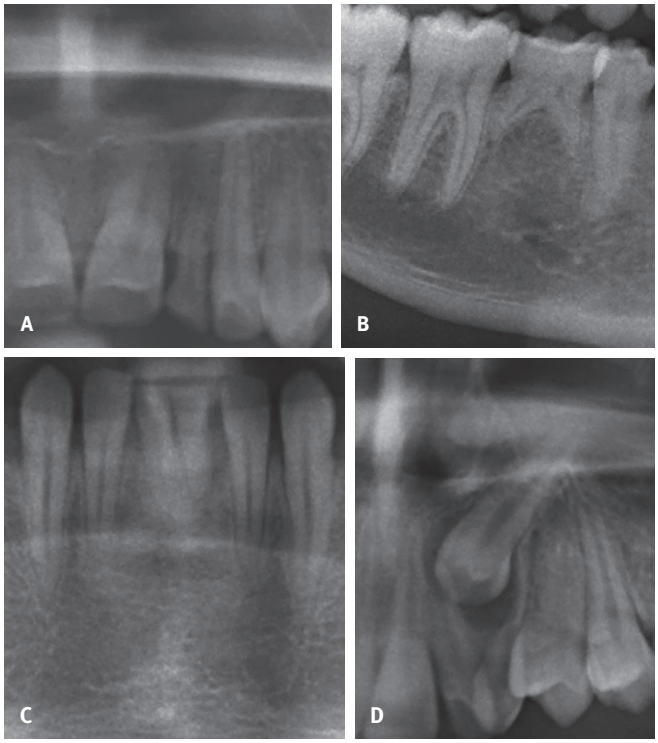

Figure 14.23 Radiograph of retained deciduous teeth (**A**) Upper permanent lateral incisor missing, deciduous lateral retained (**B**). Mandibular deciduous second molar retained due to absence of second premolar. (**C**) Permanent lower central incisors missing and deciduous teeth retained. (**D**) Upper permanent canine embedded and deciduous canine retained (*Courtesy* A,C and D Department of Orthodontics, B, Department of Oral Medicine and Radiology, KSR Institute of Dental Science and Research).

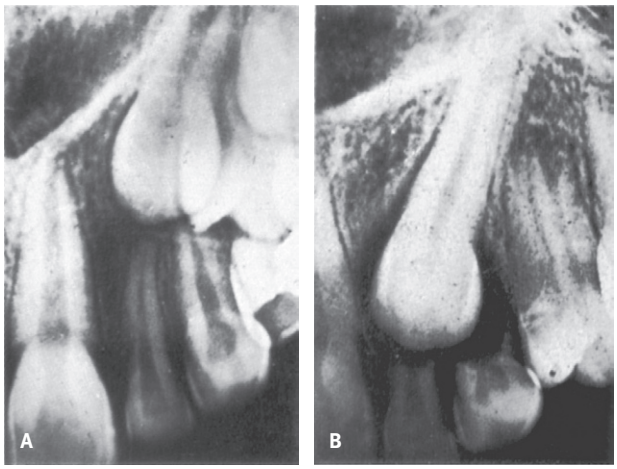

Figure 14.24 Upper permanent lateral incisor missing. Deciduous lateral incisor and deciduous canine are resorbed because of pressure of erupting permanent canine. (**A**) At 11 years of age. (**B**) At 13 years of age.

supernumerary tooth or an odontogenic tumor may occasionally prevent the eruption of one or more of the permanent teeth. In such cases, ankylosis of the deciduous tooth may occur.

Submerged Deciduous Teeth

Trauma may result in damage to either the dental follicle or the developing periodontal ligament. If this happens, the eruption of the tooth ceases, and it becomes ankylosed to the bone of the jaw. Because of continued eruption of neighboring teeth and increased height of the alveolar bone, the ankylosed tooth may be either 'shortened'or submerged in the alveolar bone. Submerged deciduous teeth prevent the eruption of their permanent successors or force them from their position. Submerged deciduous teeth should therefore be removed as soon as possible.

SUMMARY

Definition

Shedding is a physiological process by which deciduous teeth roots and parts of crown undergo resorption by multinucleated giant cells called odontoclasts.

Causative Factors in Shedding

Pressure exerted by growing permanent successor tooth germ and increased masticatory forces are the factors responsible for shedding.

Pattern of Shedding

Resorption of anterior teeth roots begin in the lingual surfaces whereas in the case of molars the area between the roots get resorbed first. This is largely due to the position of the underlying tooth germ which later on lies directly beneath the roots.

Resorption is not a continuous process. During rest periods cementum like tissue is deposited by cementoblasts like cells. The process continues on to the crown with dentin and enamel getting resorbed. The dentogingival junction migrates along the inner resorbed surface and holds the tooth in the cervical region just prior to shedding. The cells of the periodontal ligament undergo apoptotic cell death.

Odontoclasts and Mechanism of Resorption

Odontoclasts are similar to osteoclasts in that apart from being multinucleated it has numerous vacuoles and mitochondria especially adjacent to the ruffled border. The clear zone peripheral to the ruffled border shows contractile proteins—actin and myosin. Odontoclasts and osteoclasts have similar origins in that they are derived from monocytes. Odontoclasts resorb all dental tissues in a similar way like osteoclasts resorb bone. Hydrogen ions released from the ruffled border dissolve the inorganic portion and liberated lysosomal enzymes degrade the organic component. The cells of periodontal ligament in teeth undergoing resorption express RANKL. RANKL is linked to odontoclast formation and activation.

Clinical Considerations

Remnants of Deciduous Teeth, Retained Deciduous Teeth and Submerged Teeth

Parts of the roots of the teeth not lying in the path of erupting successor teeth escape resorption and get embedded in the bone, or if found close to surface get exfoliated. Sometimes whole of the deciduous teeth is retained. This is commonly seen in maxillary lateral incisor due to the absence of permanent lateral incisor. Trauma to the deciduous teeth results in ankylosis. These ankylosed deciduous teeth cannot continue to erupt like adjacent teeth hence lie below occlusal plane and are referred to as submerged teeth.

REVIEW QUESTIONS

1. Define shedding. What are the factors responsible for shedding?
2. Enumerate the differences in the shedding pattern between anterior and posterior teeth.
3. Describe odontoclasts. Explain their role in shedding.
4. List out the similarities between osteoclasts and odontoclasts.
5. Explain the occurrence of retained deciduous root, retained deciduous teeth, and submerged teeth.

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Temporomandibular Joint 15

CHAPTER CONTENTS

GROSS ANATOMY

The temporomandibular joint (TMJ) is formed by the articulation between the articular eminence and the anterior part of the glenoid fossa of the squamous part of temporal bone above and the condylar head of the mandible below (Fig. 15.1A, B).

Like most diarthrodial synovial joints, the TMJ contains a fibrous intra-articular disk that is interposed between the articular surfaces and functions as a shock absorber. The disk also increases the type and range of movements and probably contributes to the stability of the joint. It is an oval, fibrous, avascular, noninnervated plate that is firmly attached to the medial and lateral poles of the condyle by medial and lateral collateral ligaments. The disk is biconcave in sagittal section, with a thin intermediate zone, a thick anterior band, and a thick posterior band.

The anterior band continues into loose fibroelastic connective tissue, which is avascular and innervated, known as the anterior foot extension or anterior ligament. The latter attaches to the ascending slope of the articular eminence and become contiguous with the anterior capsule laterally and the superior head of the lateral pterygoid muscle medially (Fig. 15.1A). Some fibers of the superior head of the lateral pterygoid muscle attach to the anterior band. The posterior band is continuous with a loose connective tissue rich in elastic fibers called the bilaminar zone, which is highly vascular and richly innervated. The superior stratum, or lamina, of the bilaminar zone attaches to the posterior wall of the glenoid fossa and the squamotympanic suture, and the inferior stratum attaches to the posterior aspect of the mandibular condyle.

The joint capsule is a fibroelastic sac that attaches to the ascending slope of the articular eminence anteriorly and to the lips of the squamotympanic fissure posteriorly. Between these two attachments it is attached to the margins of the glenoid fossa superiorly and to the neck of the condyle inferiorly.

The posterior capsule is highly vascular and sometimes is called pis vasculosa. Parotid gland tissue is usually found in the posterior portion of the glenoid fossa between the posterior capsule of the joint and the postglenoid tubercle. The lateral aspect of the capsule is strengthened by the temporomandibular ligament. The anterior surface of the capsule, unlike other surfaces, is usually ill defined. The inner surface of the capsule is smooth and glistening because of the presence of a synovial membrane lining. The latter does not extend over the articular surfaces of the disk, the articular eminence, or the condyle.

The disk divides the joint space into two compartments: a lower one between the condyle and the disk (condylodiskal) and an upper one between the disk and the temporal bone (temporodiskal). The disk acts as a third bone and provides a movable articulation for the condyle. In the lower joint space rotational movement about an axis through the heads of the condyles permits opening of the jaws; this is designated as a hinge movement. In the upper joint space, because of the firm attachment of the disk to the lateral and medial poles of the condyle and the contraction of the inferior head of the lateral pterygoid muscle, a translatory movement occurs as the disks and the condyles traverse anteriorly along the descending slopes of the articular eminences to produce an anterior and inferior movement of the mandible. In a healthy joint the condyle, a disk, and temporal components are contiguous with each other during all movements, and the superior and inferior joint spaces are reduced to the thickness of a synovial film except for small pockets or recesses at the most anterior, posterior, medial, and lateral limits of the joint spaces (Fig. 15.1A).

The posterior part of the disk gets compressed and the blood is squeezed out when the disk moves posteriorly as in closure of jaws. When the disk is drawn forward during mouth opening, the posterior part of the disk gets filled up with blood. The presence of elastic

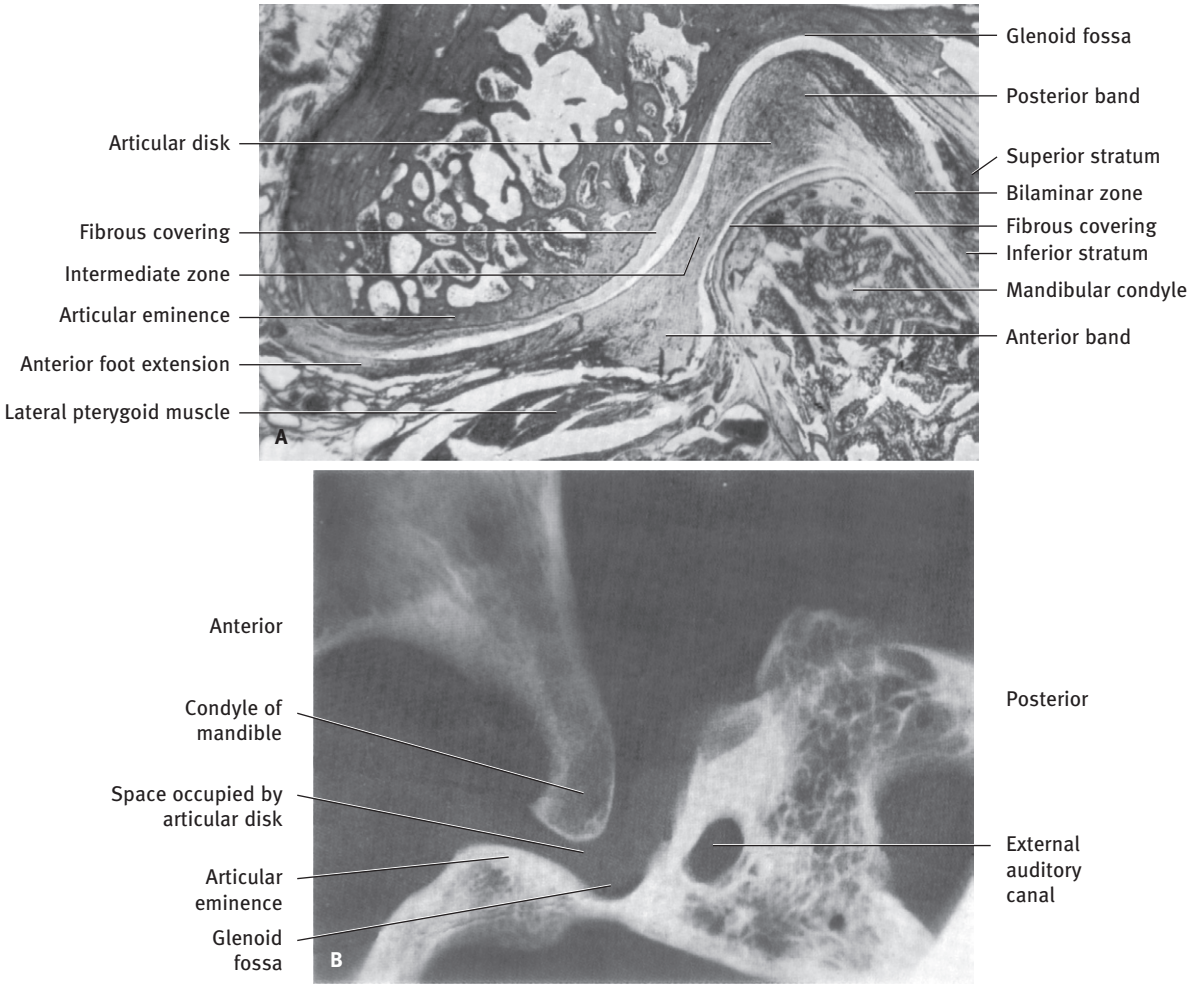

Figure 15.1 (**A**) Sagittal section through temporomandibular joint. (**B**) Lateral radiograph of TMJ showing condyle of mandible, articular eminence and glenoid fossa.

fibers in the superior lamellae, is said to recoil and help in the posterior movement of the disk.

Except for the avascular disk, the joint tissues are innervated by branches of the auriculotemporal branch of the mandibular nerve of the fifth cranial or trigeminal nerve. Proprioceptor fibers from the joint are carried by masseteric nerves and perhaps other muscular branches of the mandibular nerve.

There are four types of nerve endings in the TM joint. The Ruffini's corpuscles, present in the capsule are the proprioceptors and sense the changes in the joint when the joint is static. The pacinian corpuscles, also present in the capsule, act as mechanoreceptors to signal the rapidity and slowness of the joint movement. The Golgi tendon, present in the TM joint ligament, functions as a mechanoreceptor to protect the joint when joint movements become excessive. The free nerve endings which are nociceptors (receptors for pain), are the most numerous and widely distributed; protect the joint from excessive movements, by causing pain and curtailing the movement (Box 15.1).

The arterial supply to the joint is through branches of the maxillary and superficial temporal arteries. Large venules are consistently seen close to the anterior ligament of the disk, the bilaminar zone, and the posterior capsule. The details of the anatomy and physiology of the macrocirculation and microcirculation of this important joint need further study.

DEVELOPMENT OF THE JOINT

At approximately 10 weeks the components of the fetus' future joint become evident in the mesenchyme between the condylar cartilage of the mandible and the developing temporal bone. Two slit-like joint cavities and an intervening disk make their appearance in this region by 12 weeks. The mesenchyme around the joint begins to form the fibrous joint capsule. Very little is known about the significance of newly forming muscles in joint formation.

The developing superior head of the lateral pterygoid muscle attaches to the anterior portion of the fetal disk. The disk also continues posteriorly through the petrotympanic fissure and attaches to the malleus of the middle ear. This connection is usually obliterated by the growth of the lips of the petrotympanic fissure and does not exist in the adult joint.

Box 15.1 Types of Nerve Endings

- Ruffinii's corpuscles-proprioception-sense changes in joint when it is at rest
- Pacinian corpuscles-mechanoreceptor-signals-pace of joint movement
- Golgi tendon-mechanoreceptor-signals excessive movement
- Free nerve endings-nociceptors-cause pain to prevent excessive movement

HISTOLOGY

Bony Structures

The condyle of the mandible is composed of cancellous bone covered by a thin layer of compact bone (Fig. 15.1A). The trabeculae are grouped in such a way that they radiate from the neck of the mandible and reach the cortex at right angles, thus giving maximal strength to the condyle. The large marrow spaces decrease in size with progressing age as a result of noticeable thickening of the trabeculae. The red marrow in the condyle is of the myeloid or cellular type. In older individuals it is sometimes replaced by fatty marrow.

During the period of growth a layer of hyaline cartilage lies underneath the fibrous covering of the condyle. This cartilaginous plate grows by apposition from the deepest layers of the covering connective tissue. At the same time its deep surface is replaced by bone (Fig. 15.2). Remnants of this cartilage may persist into old age (Fig. 15.3). Unlike metaphyseal primary cartilage of long bones, the hyaline cartilage of the condyle is not organized in parallel rows of cells at the interface between the forming bone and the cartilage. Therefore the cartilage is usually referred to as secondary cartilage.

In addition to the appositional subperiosteal mandibular bone growth, the growth of the secondary cartilage and its replacement with bone contribute to the downward and forward growth of the mandible. This process can be stimulated internally by growth hormone or externally by mechanical forces such as the use of functional appliances designed for this purpose by orthodontists.

The roof of the glenoid fossa (Fig. 15.1) consists of a thin, compact layer of bone. The articular eminence is composed of spongy bone covered with a thin layer of compact bone. Areas of chondroid bone are commonly seen in the articular eminence, and in rare cases islands of hyaline cartilage are found.

Articular Fibrous Covering

In synovial joints, the articular surfaces are covered with hyaline cartilage. TM joint, unlike other synovial joints, the articular surfaces are covered with fibrous tissue. This is because the mandible is formed from membranous ossification and there are no cartilages present to cover the articular surface.

The condyle and the articular eminence are covered by a rather thick layer of fibroelastic tissue containing fibroblasts and a variable number of chondrocytes.

The fibrous covering of the mandibular condyle is of fairly even thickness (Fig. 15.3). Its superficial layers consist of a network of strong collagenous fibers. Chondrocytes may be present and have a tendency to increase in number with age. They can be recognized by their thin capsule, which stains heavily with basic dyes. The deepest layer of the fibrocartilage is rich in small undifferentiated cells as long as growing hyaline cartilage is present in the condyle. It contains only a few thin collagenous fibers. In this zone, called the reserve cell zone, appositional growth of the hyaline cartilage of the condyle takes place (Fig. 15.2). The presence of fibrocartilage increases the capacity to withstand the mechanical stress and this type of cartilage does not tend to calcify, thus it resists ageing process to some extent.

The fibrous layer covering the articulating surface of the temporal bone (Fig. 15.4) is thin in the articular fossa and thickens rapidly on the posterior slope of the articular eminence (Fig. 15.1A). In this region the fibrous tissue shows a definite arrangement in two layers, with a small transitional zone between them. The two layers are characterized by the different courses of the constituent fibrous bundles. In the inner zone the fibers are at right angles to the bony surface. In the outer zone they run parallel to that surface. As in the fibrous covering of the mandibular condyle, a variable number of chondrocytes are found in the tissue on the temporal surface. In adults only the deepest layer shows a thin zone of calcification.

No continuous cellular lining is on the free surface of the fibrocartilage. Only isolated fibroblasts are situated on the surface itself. They are characterized by the formation of long, flat cytoplasmic processes.

Opinions differ as to whether TM joint is a weight bearing joint. The presence of wavy collagen fibers both in the articular disk and in the fibrous tissue covering the articular surface, are suggested to be due to compression and are cited in support of weight bearing nature of the joint. The finding of chondroitin sulfate as the principal

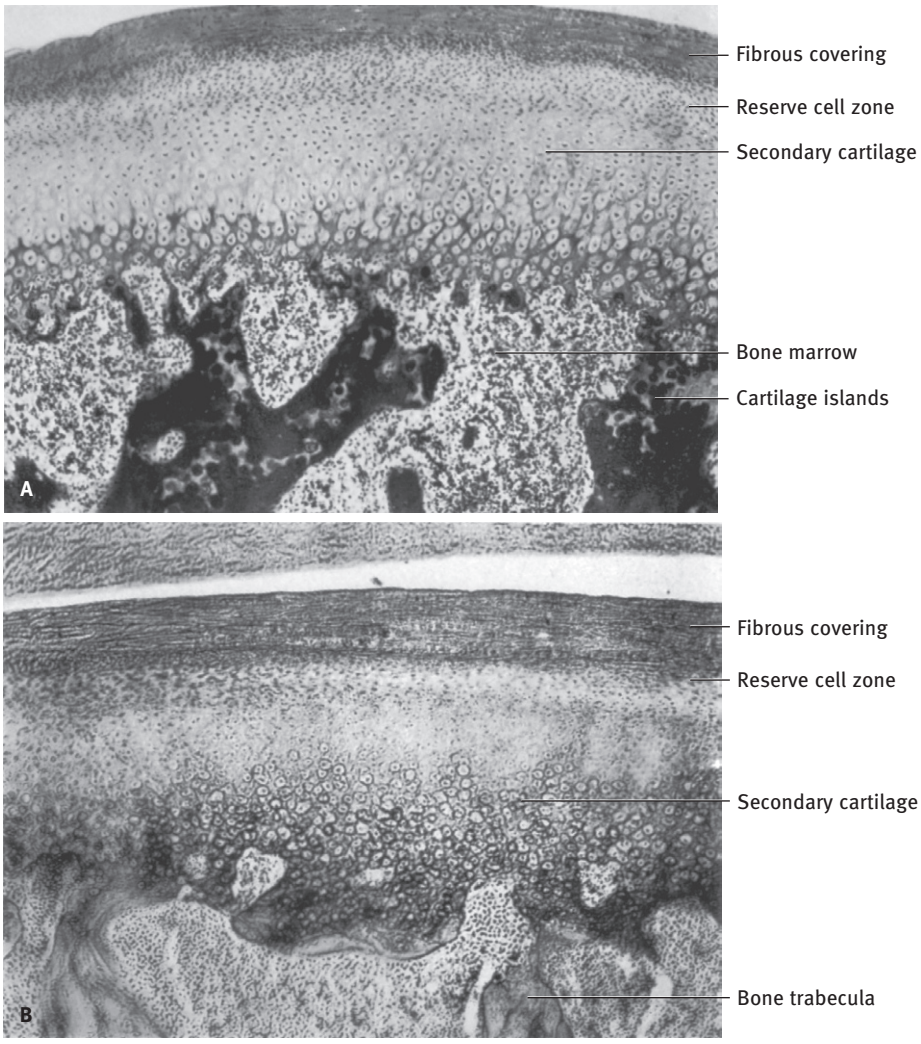

Figure 15.2 Sections through mandibular head. (**A**) Newborn infant. (**B**) Young adult. Note transitional zone (also known as reserve cell zone) between fibrous covering and cartilage, characteristic for appositional growth of cartilage.

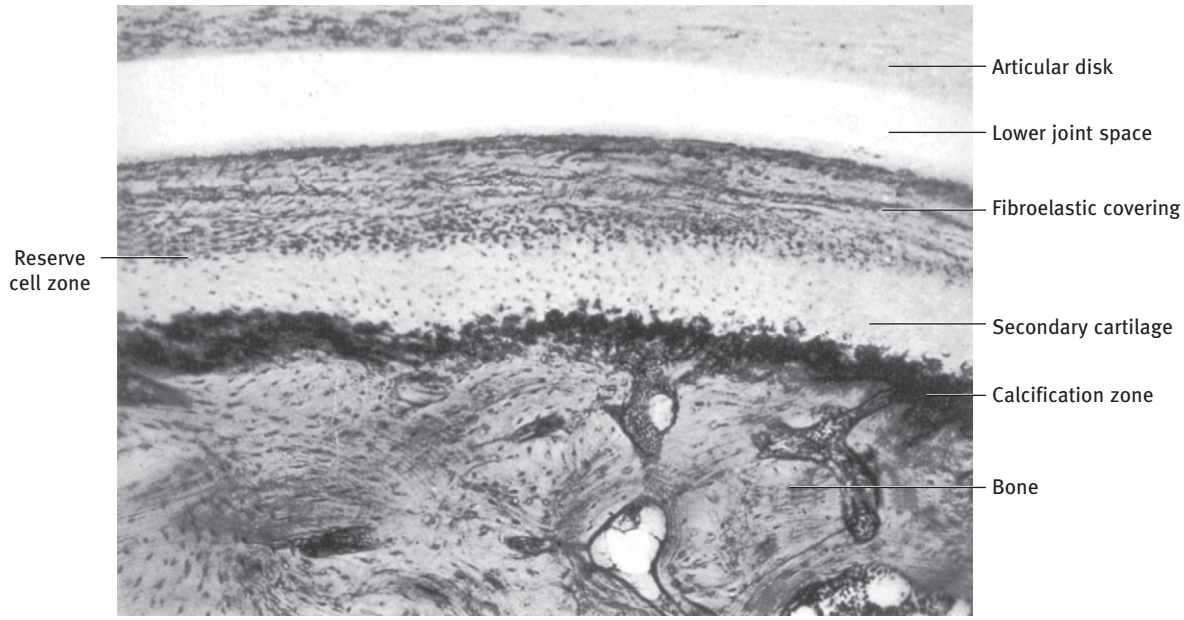

Figure 15.3 Higher magnification of part of mandibular condyle shown in Figure 15.1A.

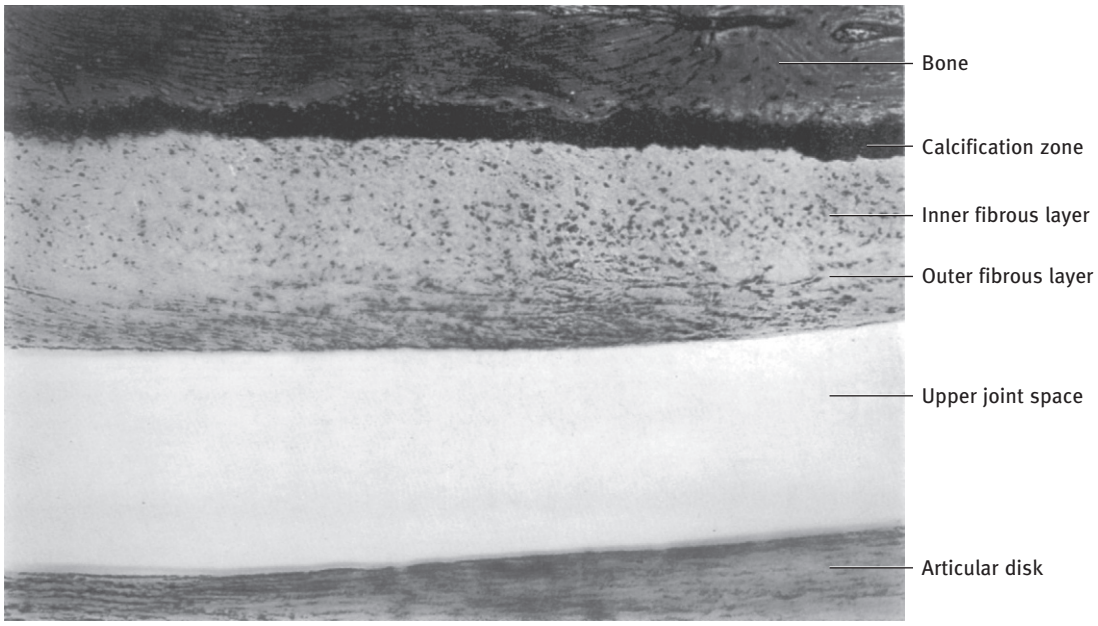

Figure 15.4 Higher magnification of articular tubercle shown in Figure 15.1A.

glycosaminoglycans (GAG) in articular disk of TM joint, as seen in cartilage, suggests that the disk is subjected to compressional loads, as in weight bearing joints. However, the absence of cartilage covering the articular surface is cited as a point against the weight bearing nature of the joint.

Articular Disk (Box 15.2)

In young individuals the articular disk is composed of dense fibrous tissue. The interlacing fibers are straight and tightly packed (Fig. 15.5). Elastic fibers are found only in relatively small numbers, although their number markedly present in the upper lamina of bilaminar region which probably helps during retraction or during initial phase of elevation of the mandible by means of elastic recoiling. The fibroblasts in the disk are elongated and sent flat cytoplasmic processes into the interstices between the adjacent bundles.

With advancing age and in areas of the disk subjected to excessive mechanical stress, some cells appear rounded and arranged in pairs similar to chondroid cells. The presence of rounded cells in the articular disk are not true chondrocytes, because they lack a capsule, surrounding them. However the presence of chondrocytes is considered to be a pathological change.

Box 15.2 Articular Disk

- Articular disk is an oval fibrous plate
- Separates joint cavity into two compartments
- Has no blood or nerve supply
- It is thin in the middle and thicker at the ends
- It is attached to the condyle
- It allows condyle to rotate and moves with it during gliding movement of the joint

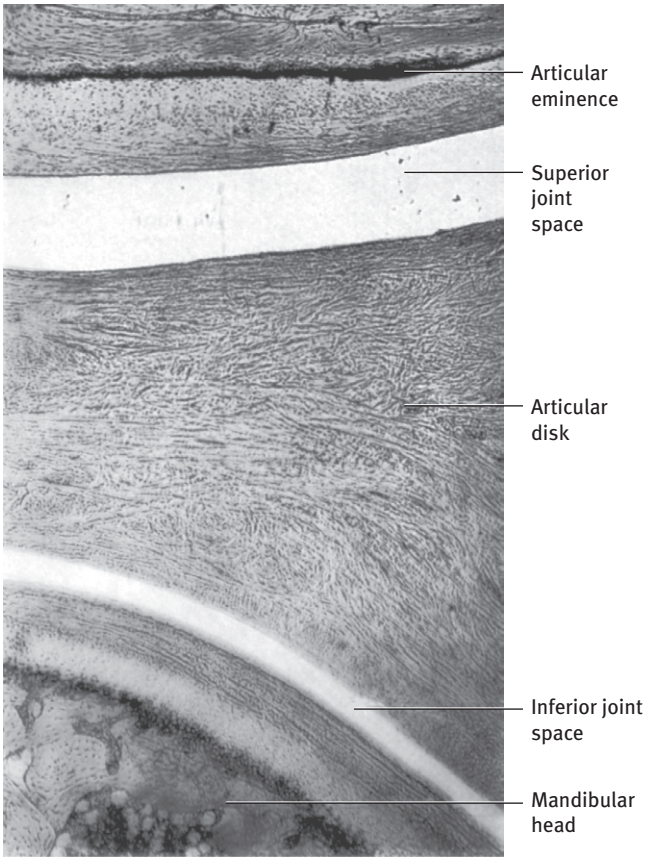

Figure 15.5 Higher magnification of articular disk shown in Figure 15.1A.

Chondrocytes, with typical territorial matrices that stain heavily with basic dyes, can be observed in the articular disk of many species, including humans (Fig. 15.6). The presence of chondrocytes may increase the resistance and resilience of the fibrous tissue.

The fibrous tissue covering the articular eminence, the mandibular condyle, and the large central area of the disk, is devoid of blood vessels and nerves and thus has limited reparative ability.

Synovial Membrane

As in other synovial joints, the articular capsule is lined with a synovial membrane that folds to form synovial villi. Synovial villi project into the joint spaces (Fig. 15.6). The synovial membrane is stretched and flattened during joint movements, while at rest it shows foldings. The synovial membrane consists of internal cells, which do not form a continuous layer but show gaps between the cells, and the subintimal connective tissue layer, rich in blood capillaries. The intimal cells are of three types. The first is rich in rough endoplasmic reticulum (RER) and is called the fibroblast like, or B cell. It is sometimes called a secretory S-cell. The second type is rich in Golgi complex and lysosomes and contains little or no RER. It is called the macrophage like, or A cell. The third type has a cellular morphology between cell types A and B.

A small amount of a clear, straw-colored viscous fluid (synovial fluid) is found in the articular spaces. It is a lubricant and also a nutrient fluid for the avascular tissues

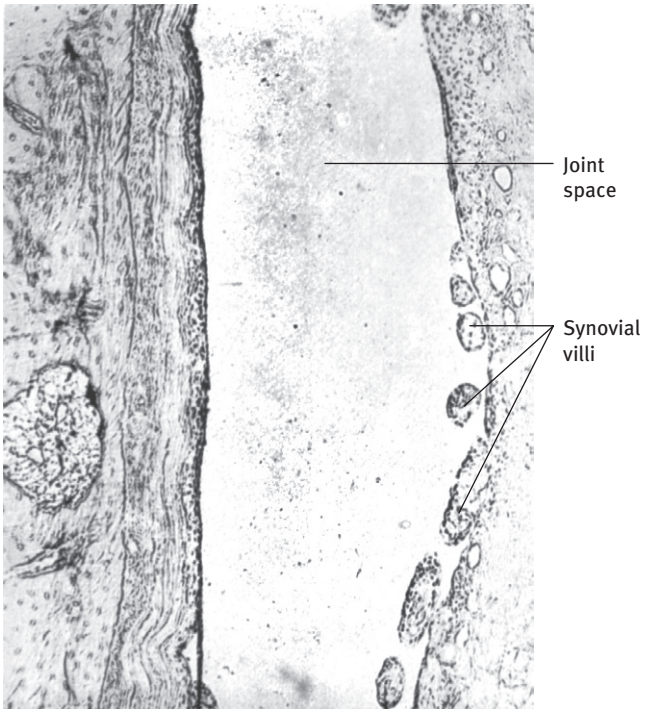

Figure 15.6 Synovial villi lining capsule of temporomandibular joint.

covering the condyle and the articular eminence and for the disk. It is elaborated by diffusion from the rich capillary network of the synovial membrane that is augmented by mucin, possibly secreted by the synovial cells. As an age change, the amount of synovial fluid decreases and villous projections are seen to increase in number. The synovial membrane has considerable ability to regenerate.

CLINICAL CONSIDERATIONS

The thinness of the bone in the articular fossa is responsible for fractures if the mandibular head is driven into the fossa by a heavy blow. In such cases injuries of the dura mater and the brain have been reported.

The finer structure of the bone and its fibrocartilaginous covering depends on mechanical influences. A change in force or direction of stress, especially after loss of posterior teeth, may cause structural changes. These changes may include fibrillation (separation between collagen bundles) of the fibrous covering of the articulating surfaces and of the disk. Abnormal functional activity may also produce injury to the articular bones. Compensation and partial repair may be accomplished by the development of cartilage on the condylar surface and in the disk. In severe trauma the articular bone is destroyed, and cartilage and new bone develop in the marrow spaces and at the periphery of the condyle. When this occurs, the function of the joint is severely impaired.

The articular surface is capable of remodeling due to functional demands. It shows changes due to loss of teeth or due to attrition of teeth. It also gets remodeled due to orthodontic treatment.

Normally, in the open position of the mandible the interincisal distance is approximately 48 mm in males

and 45.5 mm in females. In approximately 18% of the population the mandible deviates on opening, and in almost 86% of this group deviation is to the left. In approximately 35% of the population the TMJ produces sounds during opening movements. The joint has palpable irregularities and produces popping and clicking noises. However, use of a stethoscope reveals that approximately 65% of TMJs produce some kind of sound. This feature by itself, especially if not a sign of disease may not require treatment.

The term *myofacial pain dysfunction syndrome* is used to indicate a dysfunction of the TMJ. It is characterized by: (1) masticatory muscle tenderness (most frequently, the lateral pterygoid and then, in order, the temporalis, medial pterygoid, and masseter); (2) limited opening of the mandible (> 37 mm); and (3) joint sounds. This symptom complex is seen more often in females than in males. Its cause is usually spasm of the masticatory muscles. Since the condition may be related to stress, treatment should be as conservative as possible.

Dislocation of the TMJ may take place without the impact of an external force. The dislocation of the jaw is usually bilateral, and the displacement is anterior. When the mouth is opened unusually wide during yawning, the head of the mandible may slip forward into the infratemporal fossa, causing articular dislocation of the joint.

Recently diagnostic techniques such as computerized tomography (CT) and magnetic resonance imaging (MRI), which permit the visualization of the TMJ disks in patients, are being applied increasingly in the diagnosis of internal disk dislocation or derangement. The disk, for reasons not yet determined, becomes displaced anteromedially and creates one or more of the following signs and symptoms: pain, clicking, limitation of jaw movement, deviation of the jaw or opening, and locking. If the condition remains untreated, it could lead to osteoarthrosis.

Diagnosis of cases of the TMJ disk perforation is also on the increase, partly because of the use of arthroscope, MRI, and arthrographic techniques in the investigation of TMJ diseases. Recently research has shown that experimentally produced disk perforation in rhesus monkeys leads to secondary osteoarthrosis. Consequently, treatment of human disk perforation will require more serious consideration than it receives at present.

SUMMARY

Anatomy

The articulation of the TM joint is between the parts of temporal bone (articular eminence and glenoid fossa) and condylar head of the mandible. The articular surfaces are covered by the fibrous tissue. An intra-articular disk is interposed between the articular surfaces. The disk is an oval fibrous plate and it is noninnervated and avascular. The disk is biconcave in sagittal section with thin intermediate zone and thicker anterior and posterior bands. It increases the types and ranges of movements. In the meniscomandibular compartment (lower compartment) mainly rotational movements take place and in the meniscotemporal compartment (upper compartment) of the joint gliding movements take place.

Microscopic Features

The structure of the condyle consists of cancellous bone covered with compact bone and subserves the function of absorbing the mechanical stress. Trabeculae become thicker to reduce the spaces between them and red marrow is replaced by yellow bone marrow with increasing age.

The disk consists of fibroelastic tissue with few chondrocytes. It is attached to poles of condyles by collateral ligaments. Margins of the disk are attached to the capsule of the joint. The disk is attached anteriorly to the capsule and lateral pterygoid muscle and posteriorly by means of bilaminar region to the squamotympanic fissure and to the neck of the mandible. Between two laminae there are rich vascular plexus. The upper lamina with abundant elastic fibers helps to initiate the elevation of the mandible. The structure of the articular surfaces and the intra-articular disk is capable of withstanding the stress better. The intra-articular disk with advancing age shows chondroid cells; but the presence of chondrocytes is pathological. The intra-articular disk acts as a shock absorber. When the disk is drawn forward as in mouth opening the posterior part of the disk gets filled up with blood and when the disk is drawn backwards, as in closing of the mouth the disk gets compressed and blood is squeezed out. The presence of elastic fibers in the superior lamellae helps in the posterior movement. The joint capsule and temporomandibular ligament steady the mandible during the movements. Unlike other synovial joints (where the articular surfaces are covered with hyaline cartilage) the TM joint articular surfaces are covered with fibrous tissue.

The synovial membrane secretes the synovial fluid which lubricates and nourishes the avascular tissues of the joint. The membrane has a very good capacity for regeneration. The synovial membrane is absent on the articular surfaces. The cells of the membrane are named as B cells (or secretory S cells), A cells (macrophage like) and cells having features in between B and A cells. With advancing age the quantity of synovial fluid decreases and the synovial villous projections increase.

Blood and Nerve Supply

Branches of superficial temporal and masseteric arteries supply the joint. The nerves supplying the capsule and the disk are auriculotemporal and masseteric nerves. The nerve endings present are Ruffini's corpuscles in capsule, which act as proprioceptors, Pacinian corpuscles in the capsule and Golgi tendon organs in temporomandibular ligament act as mechanoceptors and free nerve endings, which are widely distributed pain receptors limiting the excessive movement by causing pain.

Development of TM Joint

During 10th week of intrauterine life condylar cartilage of mandible and development of temporal bone are noticed. Two slit-like joint cavities and intra-articular disk is demonstrable during the 12th week. Lateral pterygoid muscle gets attached to the disk.

Clinical Considerations

In trauma, glenoid fossa being thin may give way making head of the mandible to enter into the cranial cavity. The articular surfaces get remodeled and the knowledge of which is made use of in moving the teeth by using

orthodontic appliances. The dislocation of the joint occurs mostly anteriorly, as occurring due to excessive mouth opening (as in yawning). Displacement of the disk anteromedially occurs due to unknown causes causing pain, clicking and limitation of movement. This leads to osteoarthrosis. In osteoarthrosis, perforation of the disk can take place. The clicking sound can be heard on auscultation, and the left side deviation on opening of the mouth are seen normally in 18% of cases. Limited mouth opening, clicking of the joint and masticatory muscle tenderness are features of stress induced condition, called myofacial pain dysfunction syndrome.

REVIEW QUESTIONS

1. In what way the articular surfaces of TMJ differ from other synovial joints?
2. What purpose does the rich nerve supply of the joint serve?
3. What are the different types of nerve endings present in the ligaments of the TMJ?
4. What are the advantages of the presence of the intra-articular disk?

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16 Maxillary Sinus

CHAPTER CONTENTS

DEFINITION

The maxillary sinus is the pneumatic space that is lodged inside the body of the maxilla and that communicates with the environment by way of the middle nasal meatus and the nasal vestibule.

DEVELOPMENTAL ASPECTS

The initial development of the maxillary sinus follows a number of morphogenic events in the differentiation of the nasal cavity in early gestation (about 32 mm crown-rump length [CRL] in an embryo). First, the horizontal shift of palatal shelves and subsequent fusion of the shelves with one another and with the nasal septum separate the secondary oral cavity from two secondary nasal chambers (see Chapter 2). This modification presumably influences further expansion of the lateral nasal wall in that the wall begins to fold; thus three nasal conchae and three subjacent meatuses arise. The inferior and superior meatuses remain as shallow depressions along the lateral nasal wall for approximately the first half of the intrauterine life; the middle meatus expands immediately into the lateral nasal wall. Because the cartilaginous skeleton of the lateral nasal capsule is already established, expansion of the middle meatus proceeds primarily in an inferior direction, occupying progressively more of the future maxillary body.

The maxillary sinus thus established in the embryo of about 32 mm CRL expands vertically into the primordium of the maxillary body and reaches a diameter of 1 mm in the 50 mm CRL fetus (at this time the first glandular primordia from the maxillary sinus epithelium are apparent), 3.5 mm in the 160 mm CRL fetus, and 7.5 mm in the 250 mm CRL fetus. In the perinatal period the human maxillary sinus measures about 7 to 16 mm (standard deviation [SD] 2.64) in the anteroposterior direction, 2 to 13 mm (SD 1.52) in the superoinferior direction, and 1 to 7 mm (SD 1.18) in the mediolateral direction. According to Schaeffer, these diameters increase to 15, 6, and 5.5 mm, respectively, at the age of 1 year, to 31.5, 19, and 19.5 mm at the age of 15 years, and to 34, 33, and 23 mm in the adult. Although the exact time at which the human maxillary sinus attains its definite size is not known, the sinus appears to expand and modify in form until the time of eruption of all permanent teeth.

DEVELOPMENTAL ANOMALIES

Agenesis (complete absence), aplasia, and hypoplasia (altered development or underdevelopment) of the maxillary sinus occurs either alone or in association with other anomalies, for example, choanal atresia, cleft palate, high palate, septal deformity, absence of a concha, mandibulofacial dysostosis, malformation of the external nose, and the pathologic conditions of the nasal cavity as a whole. The supernumerary maxillary sinus, on the other hand, is the occurrence of two completely separated sinuses on the same side. This condition is most likely initiated by outpocketing of the nasal mucosa into the primordium of the maxillary body from two points either in the middle nasal meatus or in the middle and superior or middle and inferior nasal meatuses, respectively. Consequently, the result is two permanently separated ostia of the sinus.

STRUCTURE AND VARIATIONS

The maxillary sinus is subject to a great extent of variation in shape, size, and mode of developmental pattern. It is inconceivable therefore to propose any structural description that would satisfy the majority of human maxillary sinuses. Usually, however, the sinus is described as a four-sided pyramid, the base of which is facing medially toward the nasal cavity and the apex of which is pointed laterally toward the body of the zygomatic bone. The four sides are

related to the surface of the maxilla in the following manner: (1) anterior, to the facial surface of the body; (2) inferior, to the alveolar and zygomatic processes; (3) superior, to the orbital surface; and (4) posterior, to the infratemporal surface. The four sides of the sinus, which are usually distant from one another medially, converge laterally and meet at an obtuse angle. The identity of each of the four sides is somewhat difficult to discern, and the transition of the surface from one side to the other is usually poorly defined. Thus it is apparent that the comparison of the sinus space to a geometrically well-defined body is of pedagogic value only.

The base of the sinus, which is the thinnest of all the walls, presents a perforation, the ostium, at the level of the middle nasal meatus. In some individuals, in addition to the main ostium, two or many more accessory ostia connect the sinus with the middle nasal meatus. In 5.5% of instances the main ostium is located within the anterior third of the hiatus semilunaris, in 11% within the middle third, and 71.7% within the posterior third; in 11.3% the ostium is found outside and in a posterior position to the hiatus semilunaris. The accessory ostia are found in 23% of these instances in the middle nasal meatus and occur rarely in the inferior nasal meatus.

In the course of development the maxillary sinus often pneumatizes the maxilla beyond the boundaries of the maxillary body. Some of the processes of the maxilla consequently become invaded by the air space. These expansions, referred to as the *recesses*, are found in the alveolar process (50% of all instances), zygomatic process (41.5% of all instances), frontal process (40.5% of all instances), and palatine process (1.75% of all instances) of the maxilla. The occurrence of the zygomatic recess usually brings the superior alveolar neurovascular bundles into proximity with the space of the sinus. The frontal recess invades and sometimes surrounds the content of the infraorbital canal, whereas the alveolopalatine recesses reduce the amount of the bone between the dental apices and the sinus space. The latter development most often pneumatizes the floor of the sinus adjacent to the roots of the first molar and less often to the roots of the second premolar, first premolar, and second molar, in that order of frequency. The fully developed alveolar recess is characterized by three depressions separated by two incomplete bony septa. The anterior depression, or fossa, corresponds to the original site of premolar buds, the middle to the molar buds, and the posterior to the third molar bud.

Structure and topographic relations of maxillary sinus are depicted in Box 16. 1.

MICROSCOPIC FEATURES (Box 16.2)

The maxillary sinus is lined by a mucosa which is firmly bound to the underlying periosteum (Figs 16.1, 16.2). The epithelium, which is pseudostratified, columnar, and ciliated, is derived from the olfactory epithelium of the middle nasal meatus and therefore undergoes the same pattern of differentiation as does the respiratory segment of the nasal epithelium proper. The most numerous cellular type in the maxillary sinus epithelium is the columnar ciliated cell. In addition, there are basal cells, columnar nonciliated cells, and mucus-producing, flask-shaped secretory goblet cells (Fig 16.2). Ultrastructurally (Fig 16.3) a ciliated cell encloses the nucleus and an electron-lucent cytoplasm with numerous mitochondria and enzyme-containing organelles. The basal bodies, which serve as the attachment

Box 16.1 Structure and Topographic Relations

Maxillary sinus is roughly pyramidal in shape
Base of pyramid is towards nasal cavity and apex towards body of zygomatic bone
Related to maxilla; anteriorly to the body, superiorly to the orbital surface, posteriorly to infratemporal surface and inferiorly to alveolar process
Sinus opens into the middle meatus of the nose
Expansion of sinus called recesses, extend to alveolar, zygomatic and frontal process of maxilla
Roots of permanent maxillary first molar or premolars found very close to sinus, only thin plate of bone often separates them

Box 16.2 Microscopic Features

- Lined by pseudostratified epithelium
- Cilia contains 9+1 pairs of microtubules
- Cilia spreads the mucus
- Goblet cells are flask shaped cells, present in the basal layer, secrete mucus
- Subepithelial glands provide serous and mucous secretion to the sinus

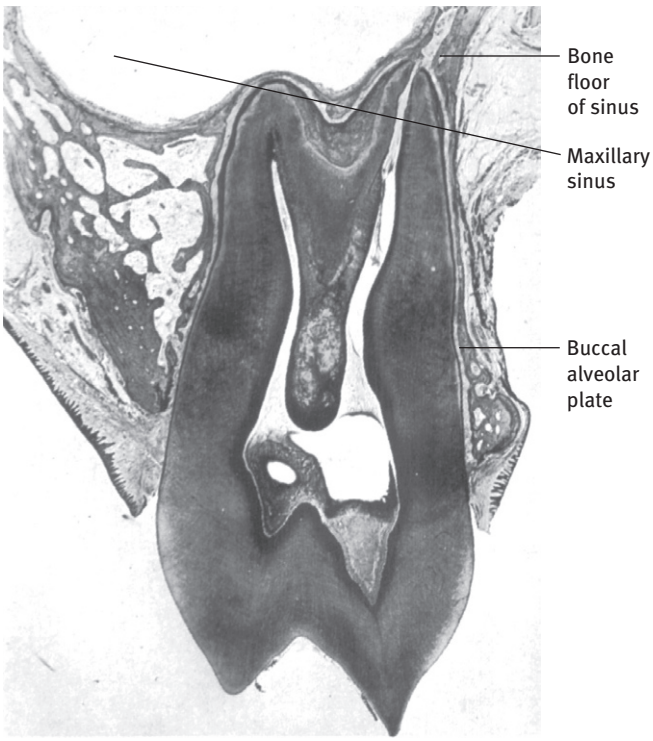

Figure 16.1 Buccolingual section through first upper premolar. Apex is separated from sinus by thin plate of bone.

of the ciliary microtubules to the cell, are characteristic of the apical segment of the cell. The cilia are typically composed of 9 + 1 pairs of microtubules, and they provide the motile apparatus to the sinus epithelium. By way of ciliary beating, the mucous blanket lining the epithelial surface moves generally from the sinus interior toward the nasal cavity. The beating pattern and direction are genetically programmed.

The Goblet cell displays all of the characteristic features of a secretory cell. In its basal segment the cell is occupied by, in addition to the nucleus, the cytocavitary network consisting of the rough and smooth endoplasmic reticulum and the Golgi apparatus, all of which are involved in the synthesis of the secretory mucosubstances. From the Golgi apparatus the zymogenic granules transport the mucopolysaccharides toward the cellular apex and finally release this material onto the epithelial surface by exocytosis (Fig. 16.4). In addition to the epithelial secretion, the surface of the sinus is provided with a mixed secretory product (serous secretion, consisting primarily of water with small amounts of neutral nonspecific lipids, proteins, and carbohydrates, and mucous secretion, consisting of compound glycoproteins or mucopolysaccharides or both) from the subepithelial glands (Fig. 16.5). These are located in the subepithelial layer of the sinus and reach the sinus lumen by way of excretory ducts (Fig. 16.6) after the ducts have pierced the basal lamina.

On the basis of histochemical differentiation and fine structural characteristics it is evident that the acini of subepithelial glands contain in varying proportions two types of secretory cells, serous and mucous. The serous cell is stained with ninhydrin-Schiff and Sudan black B procedures and encloses an electron-dense, homogeneous secretory material. The mucous cell reacts positively with the alcian blue 8GX procedure for acid sialomucin or sulfomucin or both, and produces an electron-lucent, heterogeneous secretory material. The myoepithelial cells (Fig. 16.7) surround the acini composed of either both secretory cells or a pure population of cells of either secretory type.

The secretion from these glands, like that of the other exocrine glands, is controlled by both divisions of the autonomic nervous system (Fig. 16.7). The autonomic axons, together with general sensory components, are supplied to the maxillary sinus from the maxillary nerve complex. Numerous nonmyelinated and fewer myelinated axons are readily observable in the subepithelial layer of the sinus (Fig. 16.8). They are related here to the blood capillaries, fibroblasts, fibrocytes, collagen bundles, and other connective tissue elements.

FUNCTIONAL IMPORTANCE

Very little is known about the participation of the paranasal sinuses in the functioning of either the nasal cavity or the respiratory system as a whole. This is partially

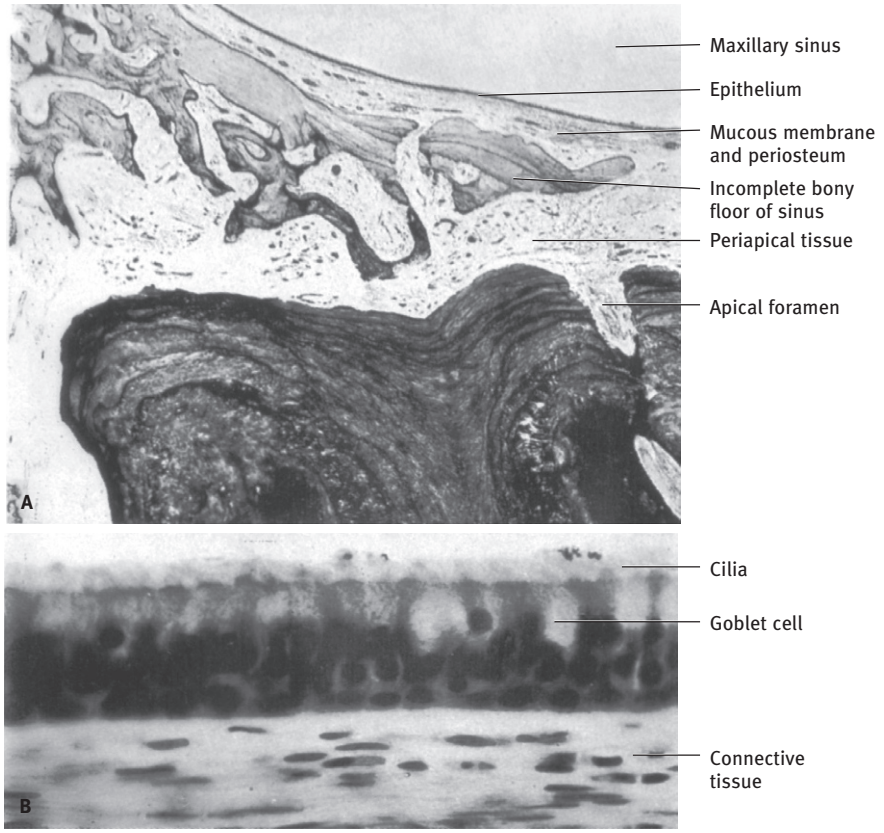

Figure 16.2 Mucous membrane and epithelium of maxillary sinus. **(A)** Apical region of second premolar. Lining of sinus is continuous with periapical tissue through openings in bony floor of sinus. **(B)** High magnification of epithelium of maxillary sinus (From Bauer WH: Am J Orthodont 29:133, 1943).

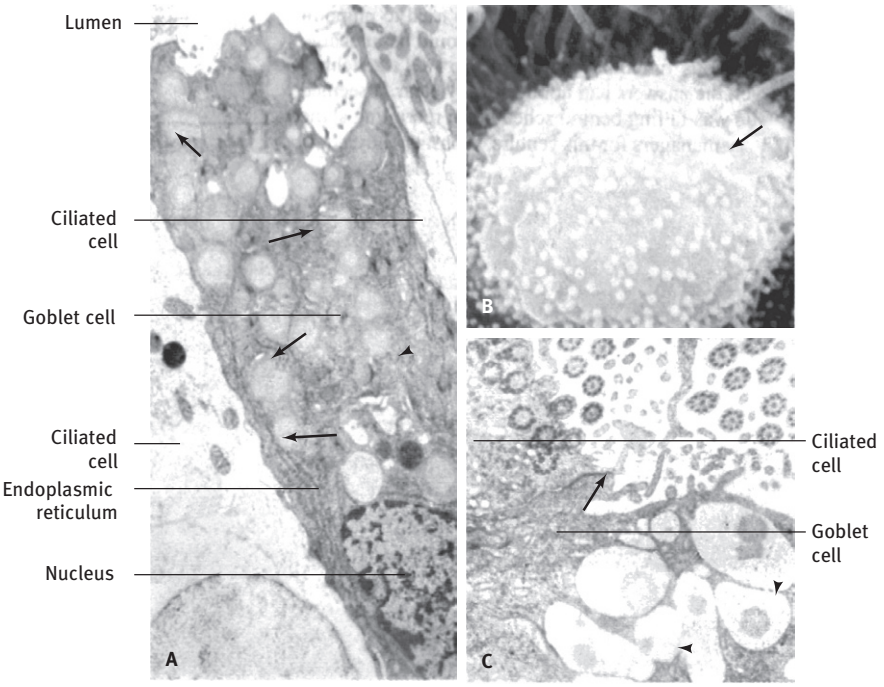

Figure 16.3 **(A)** Electron micrograph of thin section (about 35 nm [350 Å]) taken from rat trachea. Goblet cell is surrounded by two ciliated cells. From nucleus toward lumen, Goblet cell is occupied by endoplasmic reticulum, Golgi apparatus (arrowhead), and numerous secretory granules (arrows). Luminal surface of Goblet cell is covered by short microvilli. **(B)** Scanning electron micrograph taken from rat trachea demonstrates surface view of Goblet cell (arrow) bordered above by numerous cilia from neighboring cells. In addition to microvilli, surface of Goblet cell appears rough because of projection of apically situated secretory granules. (**C**) Electron micrograph of thin section taken from human maxillary sinus demonstrates apical portions and surfaces of ciliated cell and Goblet cell. Several secretory granules in Goblet cell are demonstrated as either individual organelles or coalescing with one another (arrowheads). A junctional complex between the two cells is indicated by arrow (A, Uranyl acetate and lead citrate stain; ×10,400. B, Fixed in aldehyde, dried by critical-point technique, and coated with a layer of gold-palladium about 20 nm [200 Å] thick; ×14,000. C, Uranyl acetate and lead citrate stain, ×22,400).

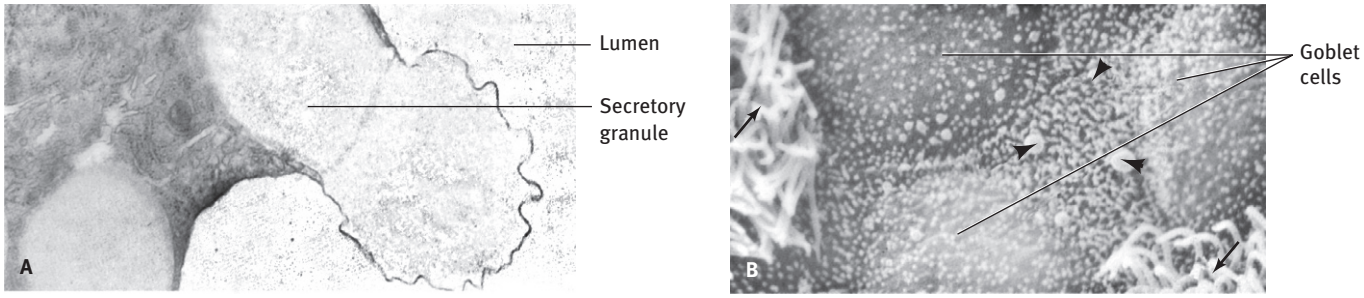

Figure 16.4 Secretory material from Goblet cell is released into lumen by exocytosis. (**A**) Electron micrograph of thin section taken from rat trachea shows a secretory granule in process of extrusion from cell into lumen. (**B**) Scanning electron micrograph taken from rat trachea shows several Goblet cells and parts of two ciliated cells (arrows). Arrowheads indicate surface projection of secretory granules in process of extrusion from cell into lumen (**A**, Uranyl acetate and lead citrate stain; ×48,000. **B**, Fixed in aldehydes, dried by critical-point technique, and coated layer of gold-palladium about 20 nm [200 Å] thick; ×11,600).

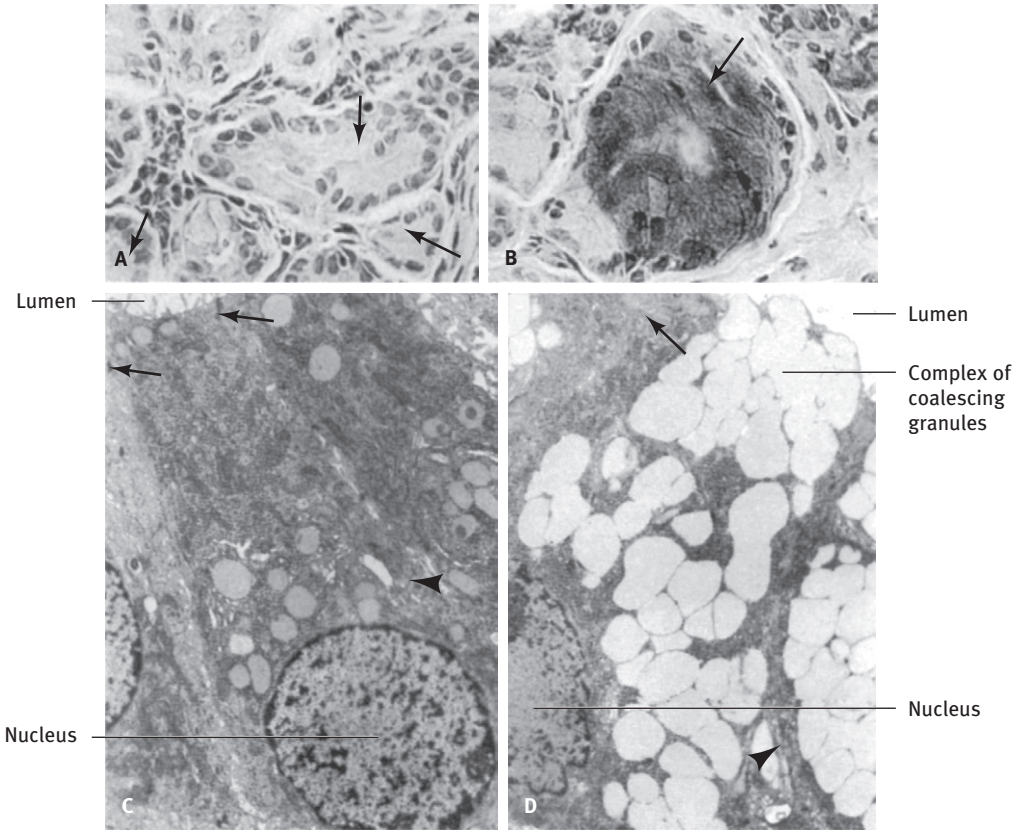

Figure 16.5 (**A**) and (**B**) Micrographs taken from human maxillary sinus demonstrate several serous acini (see arrows in A) and mucous acinus (see arrow in B). Note positive reaction of secretory material with alcian blue in mucous acinus and no reaction in serous gland. (**C**) and (**D**) Electron micrographs illustrate respectively a thin section of several serous and mucous secretory cells taken from human submucosal maxillary gland. In both representative cells, from nucleus toward acinar lumen, cytoplasm is occupied by endoplasmic reticulum, mitochondria, secretory granules, and Golgi apparatus (arrowheads). Note difference in electron opacity between the two types of secretory granules. Serous granules are separated from one another by respective membranes, while mucous granules frequently coalesce among them. (Note the complex of coalescing granules.) Junctional complexes between cells in both illustrations are indicated by arrows (A, Alcian blue and fast red procedure; 31900 for serous acini and 32000 for mucous acinus. B, Uranyl acetate and lead citrate stain; 37200 for serous gland and 36750 for mucous gland).

because of the relative inaccessibility of the sinuses to the systemic functional studies and because of the great variation in size of sinuses and their relationship to and communication with the nasal cavity. It is not surprising then that the theories of the functional importance of the sinuses range from no importance on the one hand to a multitude of involvements on the other hand.

The sinus is regarded by some as an accessory space to the nasal cavity, occurring only as a result of an inadequate process of ossification. In contrast, others report the functional contributions of the maxillary sinus in many aspects of olfactory and respiratory physiology. In individuals in whom the maxillary ostium is large enough and conveniently situated in the hiatus semilunaris the air pressure in

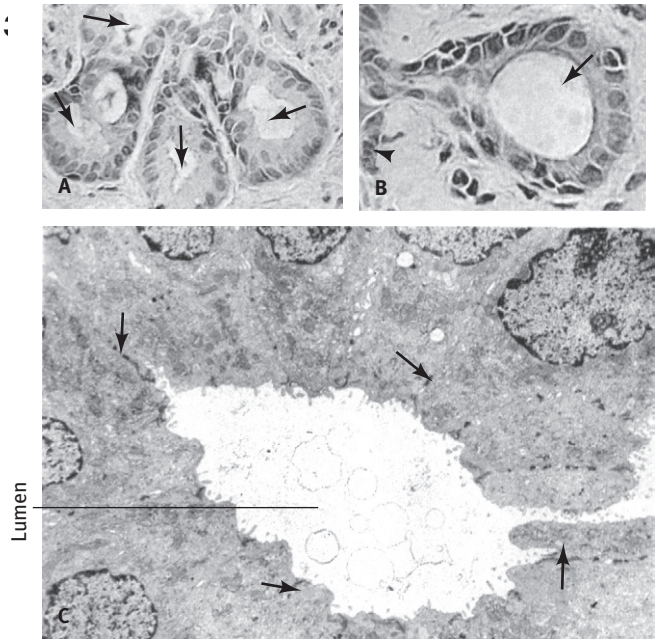

Figure 16.6 **(A)** and **(B)** Micrographs represent excretory ducts of maxillary gland taken from human maxillary sinus. Ductal cells, from cuboid to columnar in shape, surround lumen (arrows), which measures in these instances up to 12.5 μm in radius. B, Duct is demonstrated in a close apposition to epithelium of sinus (arrowhead). **(C)** Thin section of several cells lining lumen of excretory duct from human maxillary gland. In addition to nucleus, these cells contain endoplasmic reticulum, Golgi apparatus, numerous mitochondria, lipid droplets, and occasional lysosomes. Arrows, point to many junctional complexes between ductal cells (A and B, Alcian blue and fast red procedure; C, uranyl acetate and lead citrate stain; A to C, ×1600, ×2400, ×6900).

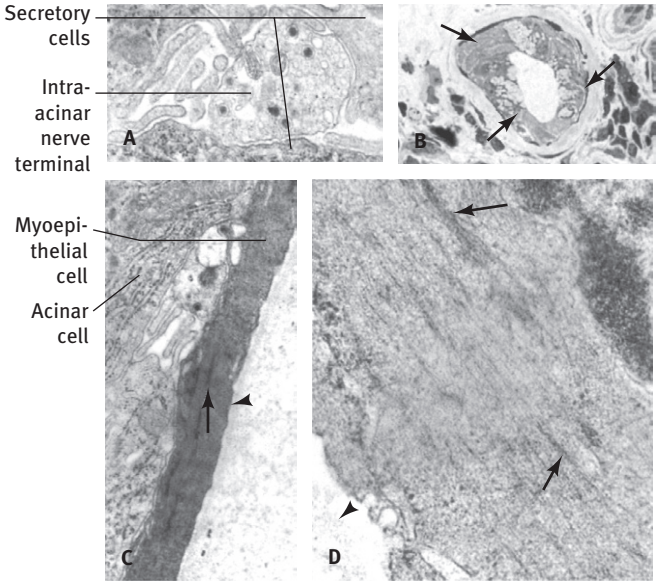

Figure 16.7 **(A)** Electron micrograph of intra-acinar nerve terminal in juxtaposition to two secretory cells taken from human maxillary gland. Note, in addition to mitochondria, two populations of small vesicles, dense and translucent, inside nerve terminal. **(B)** Thin section (0.5 μm) of several mucous and ductal cells from human maxillary gland. Periphery of this acinus is surrounded by dark-appearing myoepithelial cells (arrows). **(C)** and **(D)** illustrate relationship between acinar cells and myoepithelial cell and numerous bundles of filaments (arrows) that occupy most of the cytoplasm of the myoepithelial cells, respectively. In both instances basal lamina adjacent to myoepithelial cell is indicated by arrowhead (A, C, and D, Uranyl acetate and lead citrate stain; B, toluidine blue stain; A to D, ×37,500, ×1600, ×27,000, ×96,000).

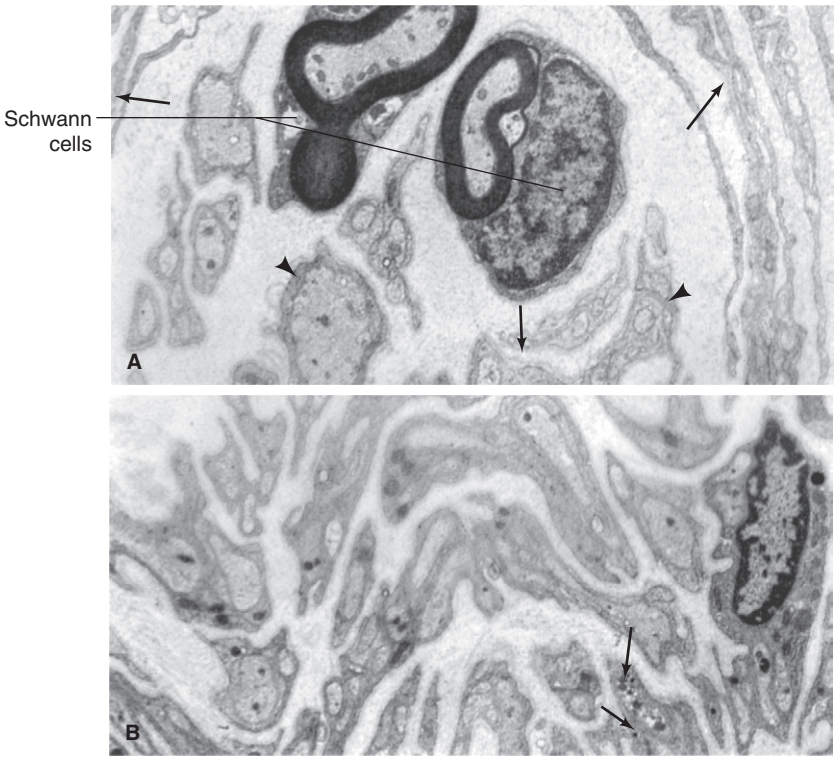

Figure 16.8 **(A)** Electron micrograph of two myelinated and several nonmyelinated axons in mucoperiosteal layer of human maxillary sinus. Schwann cells (labeled and at arrowheads) are intimately related to axons, which contain individual mitochondria and microtubules or microfilaments cut in different planes. Arrows, point to connective tissue elements surrounding either individual axon or entire nerve. **(B)** Nonmyelinated axons isolated from human mucoperiosteal layer of maxillary sinus. Most of them contain the same organelles as in A. However, some (arrows) are occupied by dense or translucent vesicles (**A** and **B**, Uranyl acetate and lead citrate stain; A, ×11,200; B, ×19,200).

the sinus fluctuates from ±0.7 to ±4 mm of water between the nasal expiration and inspiration. This dependence of the pressure in the sinus on the wave of respiration is, however, less probable in instances of either the small maxillary ostium or the ostium hidden in the depth of the hiatus semilunaris. On the basis of the same two conditions related to the structure and topography of the ostium, the functions attributed to the sinus like humidification and warming of inspired air and contribution to the olfaction, for instance are subject to controversy. However, it is possible that if air is arrested in the sinus for a certain time, it quickly reaches body temperature and thus protects the internal structures, particularly the brain, and probably the eyeball as well, against exposure to cold air. The other contributions by paranasal cavities to the resonance of voice, lightening of the skull weight, enhancement of faciocranial resistance to mechanical shock, and the production of bactericidal lysozyme to the nasal cavity have been reported.

CLINICAL CONSIDERATIONS

The section on developmental anomalies discusses several modifications of genetic and other origins in the developmental pathways of the maxillary sinus (agenesia, aplasia, hypoplasia, and supernumerary sinus). Some other criteria that correlate the extent of pneumatization by sinuses with the general dysfunctions of the endocrine system are by now developed. In case of pituitary gigantism, for example, all sinuses assume a much larger volume than in healthy individuals of the same geographic environment. It is also known that in some congenital infections such as by spirochetes in congenital syphilis the pneumatic processes are greatly suppressed, resulting in small sinuses.

In most respects the pathogenic relationship of the maxillary sinus to the orodental complexes is the result of topographic arrangement and of the functional and systemic association between the two territories. The transfer of a pathologic condition from the sinus to the orodental apparatus, or vice versa, is achieved either by mechanical connections or by way of the blood or lymphatic pathways. Since the upper first molar tooth is most often closest to the floor of the maxillary sinus, surgical manipulation on this tooth is most likely to break through the partitioning bony lamina and thus to establish an oroantral fistula (2.19% of all such fistulas are caused by first molars, 2.01% by second molars). If untreated, the lumen of such fistulas might epithelialize and permanently connect the maxillary space with the oral cavity. A similar condition might arise as a result of either a molar or a premolar radicular cyst, granuloma, or abscess. Hypercementosis of root apices and subsequent extraction of the affected tooth may also lead to a perforation. It is necessary therefore to consider on a radiograph the relationship between any such premolar or molar tooth with the floor of the maxillary sinus prior to surgical intervention.

The chronic infections of the mucoperiosteal layer of the sinus, on the other hand, might involve superior alveolar nerves if these nerves are closely related to the sinus and cause the neuralgia that mimicks possible dental origin. In this instance the diagnosis must be based on a careful inspection of all the upper teeth as well as of the maxillary sinus to differentiate cause and eventual result of this condition. It is important to note that walls of the sinus are with the nerves coursing through them. Infraorbital nerve in the roof, anterosuperior alveolar nerve in the anterior wall, middle superior alveolar nerve in the lateral aspect, posterosuperior alveolar nerve in the posterior wall and greater and lesser palatine nerves on posteromedial aspect run very closely. The neuralgia of the maxillary nerve (tic douloureux) could also have an etiologic origin in the superior dental apparatus or the mucoperiosteal layer of the sinus or both. For the diagnosis and treatment of this condition, it is most important to determine precisely the causal focus. Because of overlap of innervated territories and close topographic relationships between the teeth and the sinus, however, the causal focus is often difficult to assess.

The pathogenic association of the sinus with the orodental system, or vice versa, is based, in addition to a close topographic relationship, on an extensive vascular connection between these two regions by the superior alveolar vessels. As a consequence of this vascular arrangement, nonspecific bacterial sinusitis may be followed by some oral manifestations. Also the infections caused by the streptococci, staphylococci, pneumococci, or the virus of the common cold are likely to spread from either of the two regions to involve the other one. Finally, malignant lesions (e.g. adenocarcinoma, squamous cell carcinoma, osteosarcoma, fibrosarcoma, lymphosarcoma) of the maxillary sinus may produce their primary manifestation in the maxillary teeth. This may consist of pain, loosening supraeruption, or bleeding in their gingival tissue.

SUMMARY

Definition

Maxillary sinus is one of the paranasal sinuses found in the body of the maxilla. It is also known as maxillary antrum.

Development

The maxillary sinus is established at 32 mm CR stage. It is formed by expansion of middle meatus into the nasal cavity. It increases in size from1mm (50 CR stage) to about 15 mm (anteroposteriorly) just before birth. In the adult its size increases further and the average dimensions are 34 mm anteroposteriorly, 33 mm superoinferiorly, and 23 mm mediolaterally.

Developmental Anomalies

Agenesis, aplasia, and hypoplasia are very rare and usually they occur in association with anomalies involving face or palate. Supernumerary sinuses also occur. In pituitary gigantism

the sinuses are large and in congenital syphilis they are very small.

Structure

Maxillary sinus varies in size and shape and this is linked to the development and eruption of teeth. It is generally described as a four-sided pyramid with its base towards nasal cavity medially and apex pointing towards zygomatic bone laterally. Its four sides are related to the maxilla in the following manner; anterior to the facial surface, posterior to the infratemporal surface, superior to the orbital surface, and inferior to the alveolar process. The bony wall in the base of the sinus is the thinnest. The sinus opening called the ostium, is located near the base and it opens into the middle meatus of the nose. The sinus often expands beyond its normal anatomy and they are known as recesses. These extend most often to the alveolar processes and to a lesser extent to frontal, zygomatic, or palatine processes of the maxilla.

Functional Importance and Clinical Considerations

Many functions are attributed to maxillary sinus. Being an air space it lessens the weight of skull, gives resonance to the voice and protects the skull against mechanical shock. It warms and moistens the inspired air. The lysozyme it produces is bactericidal and thus contributes to the defense against bacterial infections.

The close proximity of the roots of maxillary I molar and to a lesser extent the maxillary I and II premolars to the sinus floor is of importance to dentist. Infections in the periapical area of these teeth erodes the already thin bone in this region and it spreads to the sinus causing sinusitis and in this process causes a communication between the oral cavity and the nasal cavity, which is known as oroantral fistula. Surgical procedures to retrieve a broken fragment of a root or difficult extractions may also result in oroantral fistula if adequate care is not taken to protect the thin bony wall. Extensive vascular connection between the sinus and teeth may cause infection to spread from teeth to sinus and vice versa. Pain due to sinusitis may present as pain involving maxillary posterior teeth as superior alveolar nerve supplies both maxillary posterior teeth and the sinus. Malignant tumors from the sinus like squamous cell carcinoma may erode the floor and present as loosening of teeth or cause gingival bleeding often leading to erroneous diagnosis and treatment.

Microscopic Features

The sinus is lined by pseudostratified ciliated columnar epithelium like all other respiratory passages. The epithelium shows four types of cells namely the basal cells, the nonciliated columnar cell, the ciliated columnar cell, and the Goblet cells. The ciliated columnar cell shows cilia which helps to spread the mucus over the lining and to the nasal cavity. Ultrastructurally the cilia are composed of 9 + 1 pairs of microtubules, which are attached by basal bodies to the cell. The Goblet cell is a flask-shaped cell producing mucus and the mucus is released into the sinus cavity by exocytosis. In the subepithelial connective tissue mucous and serous glands are present. Their secretions reach the surface through ducts. The secretions from these glands are under the control of autonomic nervous system. The connective tissue is firmly attached to the periosteum of the bone, and is referred to as mucoperiosteum.

REVIEW QUESTIONS

1. Describe the anatomy of the maxillary sinus. Add a note on its anatomical variations.
2. Describe the histology of the maxillary sinus.
3. What are the ways in which maxillary sinus is of importance to the dentist?

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17 Age Changes in Oral Tissues

CHAPTER CONTENTS

Aging is a continuous, detrimental and innate phenomenon in an organism. It is a time related process, which happens in a constant and steady manner right from birth and continues till death. Aging should not be confused with senescence. Senescence occurs after maturation, and it increases the amenability of an organism to death.

THEORIES OF AGING

Various theories have been proposed to explain the process of aging with little success. A few of these appear to have a more concrete scientific basis and are better accepted than the others. One such widely accepted theory is the concept of **Free radicals**. First postulated by Harman in 1956, this theory suggests that free radicals cause oxidative damage to cells and their products, resulting in aging. Another theory proposed by Leo Szilard in 1959 puts forth the idea that genes involved in DNA metabolism and repair become faulty with time. This leads to accumulation of **DNA damage** that manifests as aging. Similarly, Watson in 1972 suggested that telomeres may be involved in aging. **Telomeres** are small repetitive sequences seen at the ends of chromosomes. During every cell division, some of this telomere is lost. Although it can be replenished by the enzyme telomerase, over time, the length of telomere becomes short and cell division no longer happens. The main drawback of these theories is that, it is very difficult to prove whether each characteristics is a cause for aging, or is the result of aging. **Lipofuscin**, an intracellular pigment seen in many organisms, was initially thought to be a cause for aging. However, experiments have shown that lipofuscin is rather a product of aging process than being its cause.

AGE CHANGES IN ENAMEL

The most apparent age change in enamel is attrition or wear of the occlusal surfaces and proximal contact points as a result of mastication. This is evidenced by a loss of vertical dimension of the crown and by a flattening of the proximal contour. The earliest clinical change is the loss of mamelons. In addition to these gross changes, the outer enamel surfaces themselves undergo posteruptive alterations in structure at the microscopic level. These result from environmental influences and occur with a regularity that can be related to age (Fig. 17.1).

The surface of enamel is rough and irregular in unerupted and newly erupted teeth. This is due to the presence of perikymata visible to the naked eye and at the ultrasructural level the uneven surface is due to enamel pits, rod ends, enamel caps and enamel brochs. However, as soon as the tooth erupts into the oral cavity at the points of highest contour of the surfaces, lose their characteristic morphology; the uneven surface becomes even, and to the naked eye, the perikymata are lost gradually. The rate at which structure is lost depends on the location of the surface of the tooth and on the location of the tooth in the mouth. Facial and lingual surfaces lose their structure much more rapidly than do proximal surfaces, and anterior teeth lose their structure more rapidly than do posterior teeth.

Age changes within the enamel proper have been difficult to discern microscopically. The fact that alterations do

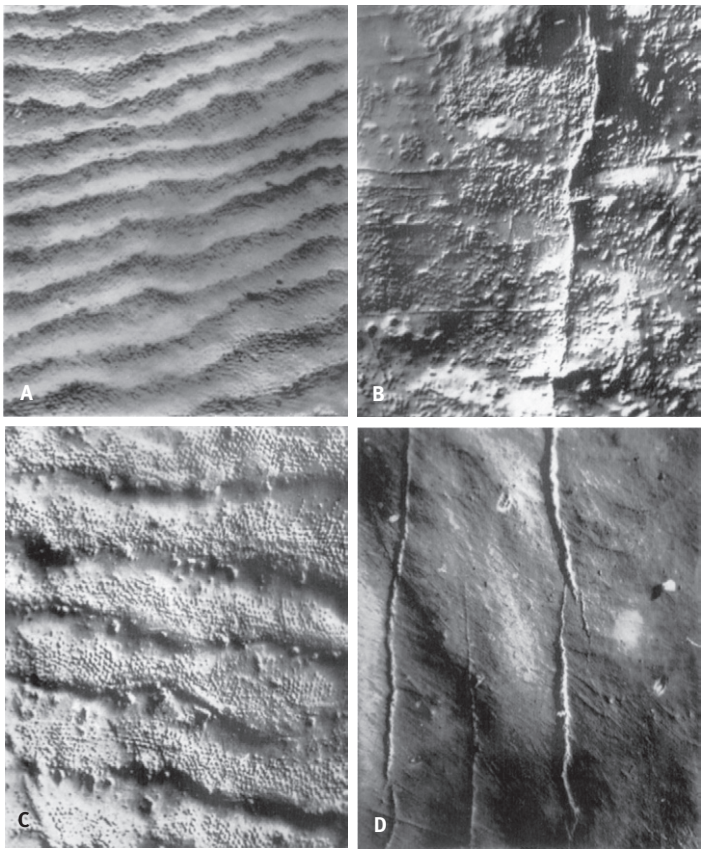

Figure 17.1 Progressive loss of surface structure with advancing age. (**A**) Surface of recently erupted tooth showing pronounced enamel prism ends and perikymata. Patient is 12 years of age. (**B**) Early stage of structural loss that occurs during first few years (wear is more rapid on anterior teeth than on posterior teeth and more rapid on facial or lingual surfaces than on proximal surfaces). Note small regions where prism ends are worn away. Patient is 25 years of age. (**C**) Later stage. Here elevated parts between perikymata are worn smooth, while structural detail in depths of grooves is still more or less intact. Eventually wearing proceeds to point where all prism ends and perikymata disappear. Patient is 52 years of age (Since these are negative replicas, surface details appear inverted. Raised structures represent depressions in actual surface). (**D**) Surface worn completely smooth and showing only 'cracks,' which actually represent outer edges of lamellae. Patient is 50 years of age, (All magnifications ×105) (From Scott DB and Wyckoff RWG: J Am Dent Assoc 39:275, 1949).

occur has been demonstrated by chemical analysis, but the changes are not well understood. For example, the total amount matrix is said by some to increase, by others to remain unchanged, and by still others to decrease. Localized increases of certain elements such as nitrogen and fluorine, however, have been found in the superficial enamel layers of older teeth. This suggests a continuous uptake, probably from the oral environment, during aging. As a result of age changes in the organic portion of enamel, presumably near the surface, the teeth may become darker, and their resistance to decay may be increased. Suggestive of an aging change is the greatly reduced permeability of older teeth to fluids. The decrease in permeability of enamel due to age is due to increase in the size of the crystal. The crystal size increases due to ions acquired by it from the oral fluids. The increase in size of the crystal decreases the pores between them causing a reduction in permeability.

Though there is insufficient evidence to show that enamel becomes harder with age, recent studies have suggested that the superficial layers of enamel become extremely hard with age and that both hardness and modulus of elasticity of enamel increases by about 12% by 55 years. This has been attributed to the uptake of fluoride by the surface layers. Though the hardness of enamel increases with age, it has been suggested that it tends to become more brittle in nature.

AGE AND FUNCTIONAL CHANGES IN DENTIN

As changes are seen in the overlying enamel, the dentin also begins to show certain important changes with increasing age. Such changes in dentin take place to ensure that the function of protection is taken over from enamel as the enamel wears with aging.

The two most important changes seen with advancing age are: (i) increase in the thickness of dentin due to continuous, gradual deposition of secondary dentin, and (ii) increased sclerosis or obliteration of dentinal tubules during which minerals are added into the tubules with an associated decrease in the amount of dentinal fluid, reduced sensitivity and permeability. These changes namely the continuous formation of dentin and sclerosis is interlinked with functional demands placed on dentin. The stimuli for their formation may be either physiological or pathological. Therefore, these age changes and functional changes are considered together.

Pathologic effects of dental caries, abrasion, attrition, or the cutting of dentin of operative procedures cause changes in dentin. These are described as the development of *dead tracts*, *sclerosis*, and the addition of *reparative dentin*. The formation of reparative dentin pulpally underlying an area of injured odontoblast processes can be explained on the basis of increased dentinogenic activity of the odontoblasts. The mechanisms underlying the series of events that occur in the development of reparative dentin, dead tracts, and sclerosis are not yet fully understood although the histology has been clearly described.

Reparative Dentin

If by extensive abrasion, erosion, caries, or operative procedures the odontoblast processes are exposed or cut, the odontoblasts die or survive, depending on the intensity of the injury. If they survive the dentin that is produced is known as *reactionary* or *regenerated dentin*. Those odontoblasts that are killed are replaced by the migration of undifferentiated cells arising in deeper regions of the pulp to the dentin interface. It is believed that the origin of the new odontoblast is from cells in the cell-rich zone or from undifferentiated perivascular cells deeper in the pulp. The newly differentiated odontoblasts then begin deposition of *reparative dentin*. This action to seal off the zone of injury occurs as a healing process initiated by the pulp, resulting in resolution of the inflammatory process and removal of dead cells. The hard tissue thus formed is best termed reparative dentin although the terms tertiary dentin, response, or reactive dentin are also used. Reparative dentin is characterized as having fewer and more twisted tubules than normal dentin (Fig. 17.2). Dentin-forming cells are often included in the rapidly produced intercellular substance, termed osteodentin, and is seen, especially in response to rapidly progressing caries. In other instances, a combination of osteodentin and tubular dentin are seen (Fig. 17.3). It is due to the irregular nature of the dentinal tubules, these types of dentin are also referred to as *irregular secondary dentin*, in order to differentiate from the regular secondary dentin formed not as a result of any external stimuli. It is suggested that transforming growth factor-beta (TGF-β) is involved in the production of tubular dentin, while bone morphogenetic protein (BMP) is involved in the production of osteodentin.

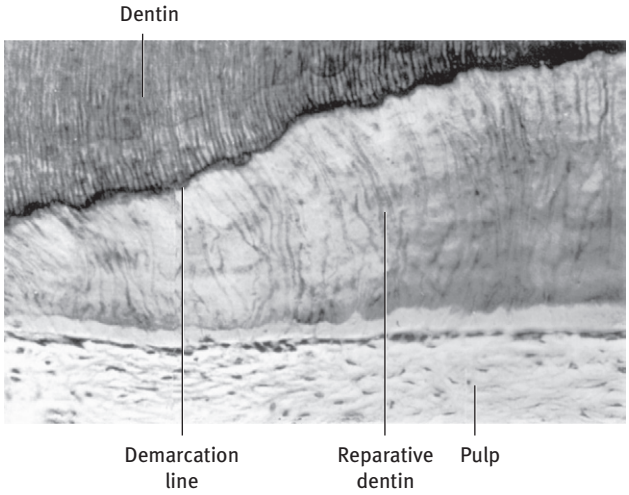

Figure 17.2 Reparative dentin stimulated by penetration of caries into dentin. Dentinal tubules are irregular and less numerous than in regular dentin (Decalcified section).

It is believed that bacteria, living or dead, or their toxic products, as well as chemical substances from restorative materials, migrate down the tubules to the pulp and stimulate pulpal response, leading to reparative dentin formation. All of the events in this process are not yet known. In tertiary dentin formation, interglobular dentin, incremental lines with varying mineral content were seen suggesting that its formation is influenced by biological rhythms. Ultrastructural studies showed that the dentinal tubules varied in size, shape, had irregular circumference with projections of mineralized tissue into them and were tubules with a high mineral content. Tertiary dentin differed from other forms of dentin in that dentin phosphophoryn is not present.

Many similarities exist between development and repair, including matrix-mediation of the cellular processes and the apparent involvement of growth factors as signaling molecules despite the absence of epithelium during repair. While some of the molecular mediators appear to be common to these processes, the close regulation that was seen in primary dentinogenesis may be less ordered during tertiary dentin formation.

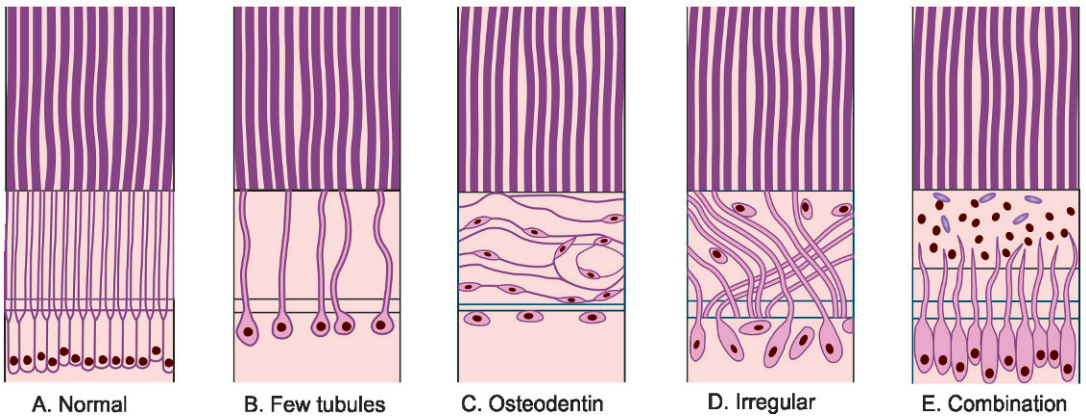

Figure 17.3 Diagrammatic illustration of normal (**A**) and other types of reparative dentin (B to E). Reparative dentin contains fewer than normal tubules (**B**), or it includes cells within its matrix (**C**), shows irregularly arranged tubules (**D**), or is a combination of different types (**E**).

Several growth factors and extracellular matrix molecules which are expressed during odontogenesis are re-expressed under pathological conditions. Nestin and Notch protein which are expressed in young odontoblasts and in subodontoblastic layer during odontogenesis, are absent in adult tissue, but are re-expressed during reparative dentin formation.

Dead Tracts

In dried ground sections of normal dentin the odontoblast processes disintegrate, and the empty tubules are filled with air. They appear black in transmitted and white in reflected light (Fig. 17.4A, B). Loss of odontoblast processes may also occur in teeth containing vital pulp as a result of caries, attrition, abrasion, cavity preparation, or erosion (Figs 17.4, 17.5). Their degeneration is often observed in the area of narrow pulpal horns (Fig.17.5) because of crowding of odontoblasts. Again, where reparative dentin seals dentinal tubules at their pulpal ends, dentinal tubules fill with fluid or gaseous substances. In ground sections, such groups of tubules may entrap air and appear black in transmitted and white in reflected light. Dentin areas characterized by degenerated odontoblast processes give rise to dead tracts. These areas demonstrate decreased sensitivity and appear to a greater extent in older teeth. Dead tracts are probably the initial step in the formation of sclerotic dentin.

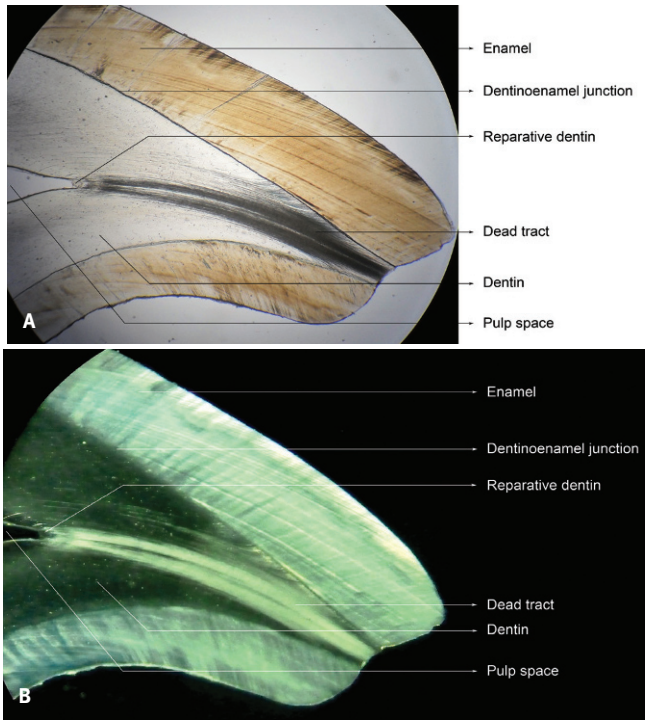

Figure 17.4 Photomicrograph of ground section of teeth showing dead tracts in vital tooth caused by attrition and exposure of dentinal tubules (**A**). Dead tracts appear dark in transmitted light (x40) (**B**). Dead tracts appear bright in reflected light. (x40). Reparative dentin underlies dead tracts in both the sections.

Sclerotic or Transparent Dentin

Stimuli may not only induce additional formation of reparative dentin but also lead to protective changes in the existing dentin. In cases of caries, attrition, abrasion, erosion, or cavity preparation, sufficient stimuli are generated to cause collagen fibers and apatite crystals to begin appearing in the dentinal tubules. This condition is prevalent in older individuals. In such cases, blocking of the tubules may be considered a defensive reaction of the dentin. Apatite crystals are initially only sporadic in a dentinal tubule but gradually the tubule becomes filled with a fine meshwork of crystals (Fig. 17.6). Gradually,

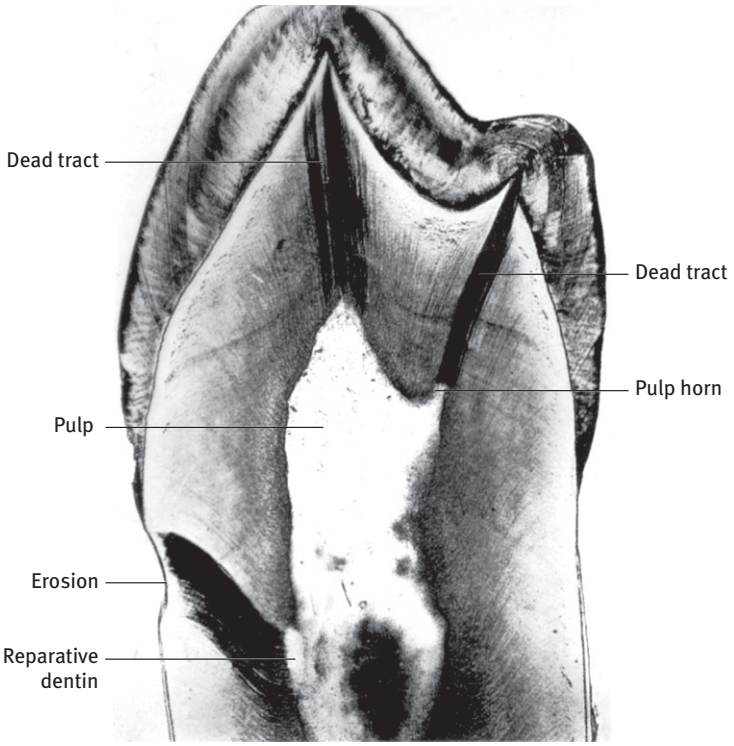

Figure 17.5 Dead tracts in dentin of vital human tooth caused by crowding and degeneration of odontoblasts in narrow pulpal horns and by exposure of tubules to erosion.

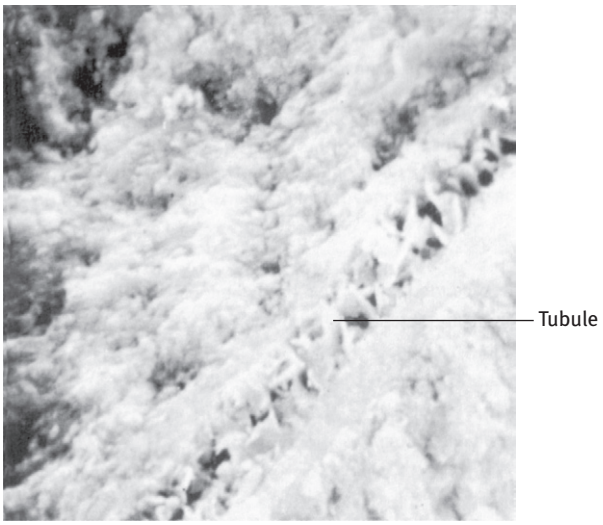

Figure 17.6 Scanning electron micrograph of partially sclerosed longitudinal sectioned dentin tubules. Plate-like crystals form a meshwork occluding tubule lumen (×9700) (From Lester KS and Boyde A: Virchows Arch [Zell-pathol] 344:196, 1968).

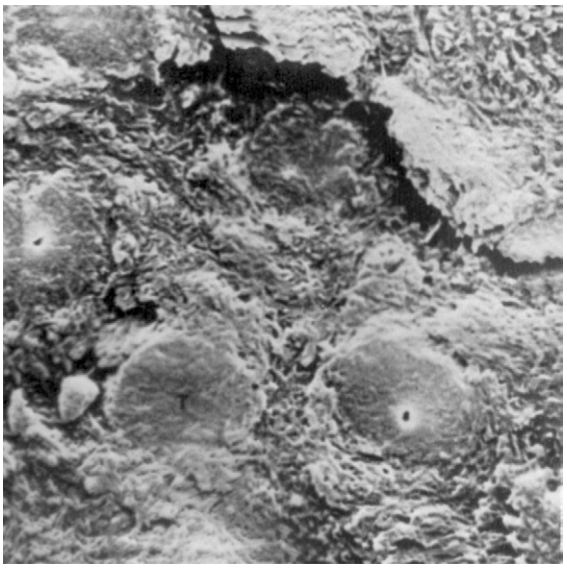

Figure 17.7 Scanning electron micrograph of fractured cross-section of dentin located between attrited surface and the pulp. Various degrees of closure of the tubule lumen are seen. Complete obliteration (T) is seen as well as a minute lumen in other tubules. (×5800) (From Brannstrom M: Dentin and pulp in restorative dentistry, London, 1982, Wolfe Medical Publications Ltd).

the tubular lumen is obliterated with mineral, which appears very much like the peritubular dentin (Fig. 17.7). The refractive indices of dentin in which the tubules are occluded are equalized, and such areas become *transparent*. Transparent or sclerotic dentin can be observed in the teeth of elderly people, especially in the roots (Fig. 17.8). This may be regarded as an age change. Sclerotic dentin may also be found under slowly progressing caries. Sclerosis reduces the permeability of the dentin and may help prolong pulp vitality. Mineral density is greater in this area of dentin, as shown both by radiography and permeability studies. The hardness of sclerotic dentin varied, those formed as a result of aging were harder than those found below carious lesions.

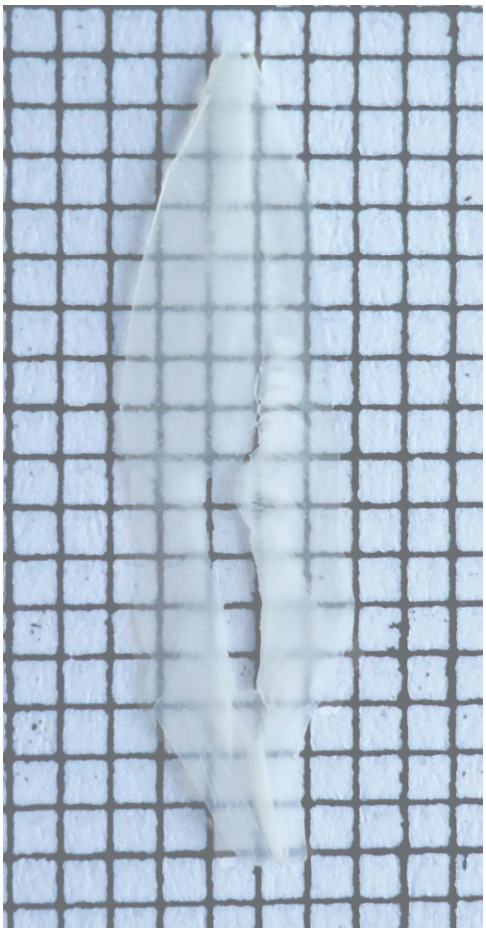

Figure 17.8 Sclerotic dentin makes the tooth appear transparent.

Though the sclerotic dentin was harder than normal dentin, its elastic properties were not altered, but its fracture toughness was reduced. The crystals present in the sclerotic dentin were smaller than those present in the normal dentin. It appears transparent or light in transmitted light and dark in reflected light (Figs 17.5, 17.8).

The elastic modulus of intertubular, peritubular, transparent and healthy dentin did not differ appreciably.

AGE CHANGES IN PULP

Cell Changes

The volume of pulp decreases with age which may be attributed to continuous deposition of secondary dentin throughout the life. Young pulp differs from those seen in elderly individuals. The regressive changes begin immediately after the tooth erupts into the oral cavity. The changes may be seen in both cellular and extracelluar components of the pulp. The number, nature, properties and capabilities of the cells change. However, there seems to be no remarkable loss in vitality of the pulp. The density of odontoblasts and the pulpal fibroblasts decreases with age in general. But the degree of age- related changes may not be same in both crown and root. The decrease in pulp cell density is greater in root compared to crown and at all ages the pulp cell densities including odontoblasts is greater within the crown as compared to the root.

However, it is interesting to note that the rate of deposition of dentin in root is greater as compared to crown.

In addition to the appearance of fewer cells in the aging pulp, the cells are characterized by a decrease in size and number of cytoplasmic organelles. The typical active pulpal fibrocyte or fibroblast has abundant rough-surfaced endoplasmic reticulum, notable Golgi complex, and numerous mitochondria with well-developed cristae. The fibroblasts in the aging pulp exhibit less perinuclear cytoplasm and possess long, thin cytoplasmic processes. The intracellular organelles, mainly the mitochondria and endoplasmic reticulum are reduced in number and size.

Fibrosis

In the aging pulp accumulations of both diffuse fibrillar components as well as bundles of collagen fibers usually appear. Fiber bundles may appear arranged longitudinally in bundles in the radicular pulp, and in a random more diffuse arrangement in the coronal area. This condition is variable, with some older pulps showing surprisingly small amounts of collagen accumulation, whereas others display considerable amounts (Fig. 17.9). The increase in fibers in the pulp organ is gradual and is generalized throughout the organ. Any external trauma such as dental caries or deep restorations usually causes a localized fibrosis or scarring effect. Collagen increase is noted in the medial and adventitial layers of blood vessels as well. The increase in collagen fibers may be more apparent than actual, being attributable to the decrease in the size of the pulp, which makes the fibers present occupy less space, and hence they become more concentrated without increasing in total volume.

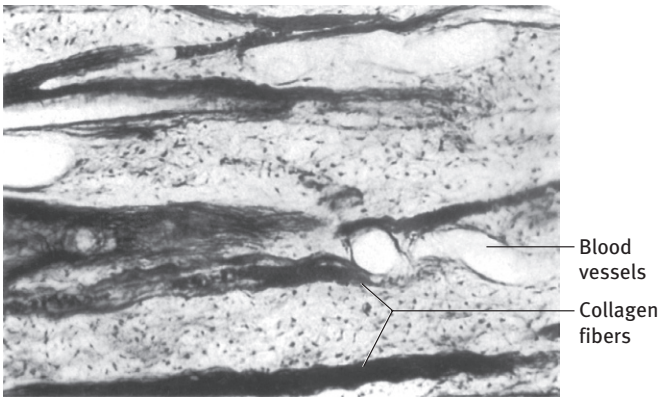

Figure 17.9 Bundles of collagen fibers around and among blood vessels of pulp.

Vascular Changes

Vascular changes occur in the aging pulp organ as they do in any organ. Blood flow decreases with age. This is due to decrease in the number of blood vessels and due to formation of atherosclerotic plaques within pulpal vessels. In other cases, the outer diameter of vessel walls becomes greater as collagen fibers increase in the medial and adventitial layers. Also calcifications are found that surround vessels (Fig. 17.10). Calcification in the walls of blood vessels is found most often in the region near the apical foramen.

The capillary endothelium shows changes due to age. Ultrastructurally, certain changes are seen in capillaries of pulp and in the endothelial cell lining of these capillaries with increasing age. Only continuous capillaries with irregular thickness were noted. The basement membrane

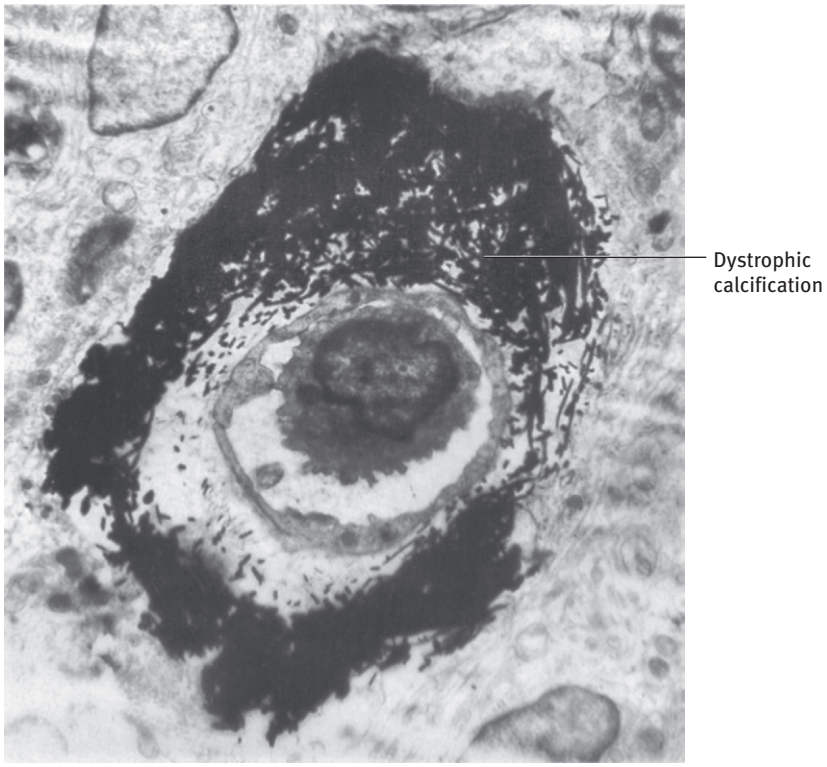

Figure 17.10 Small vessel containing lymphocyte. Its wall exhibits no basement membrane around endothelial cells. It is probably a lymphatic capillary. Calcification appears around periphery of vessel.

was noted to be discontinuous in old pulp vessels and appeared more thick and homogeneous. Cytoplasm of endothelial cells showed certain remarkable changes like an increase in the pinocytic and micropinocytic vesicles, particularly towards the abluminal side of the cell membrane as compared to luminal side. Extensive Golgi complex with distended cisternae, spherical lipid-like droplets, nongranular glycogen molecules, abundant ribosomes, RER, multivesicular bodies and Weibel-Palade bodies were also noted. Weibel-Palade bodies are intracytoplasmic organelles seen in endothelial cells in all vertebrates and contain von Willebrand factor and P-selectin. Hence, probably play an important role in inflammatory process. Abundant microfilaments in association with these pinocytic and micropinocytic vesicles were also noted which were arranged in a dense network in the cytoplasm, forming the predominant cytoplasmic component.

Pulp Stones (Denticles)

Pulp stones or denticles are nodular, calcified masses appearing in either or both the coronal and root portions of the pulp organ. They often develop in teeth that appear to be quite normal in other respects. They usually are asymptomatic unless they impinge on nerves or blood vessels. They have been seen in functional as well as embedded unerupted teeth. Pulp stones are classified, according to their structure as true denticles or false denticles. True denticles are similar in structure to dentin in that they have dental tubules and contain the processes of the odontoblasts that formed them and that exist on their surface (Fig. 17.11A). True denticles are comparatively rare and are usually located close to the apical foramen. A theory has been advanced that the development of the true denticle is caused by the inclusion of remnants of the epithelial root sheath within the pulp. These epithelial remnants induce the cells of the pulp to differentiate into odontoblasts which then form the dentin masses called true pulp stones.

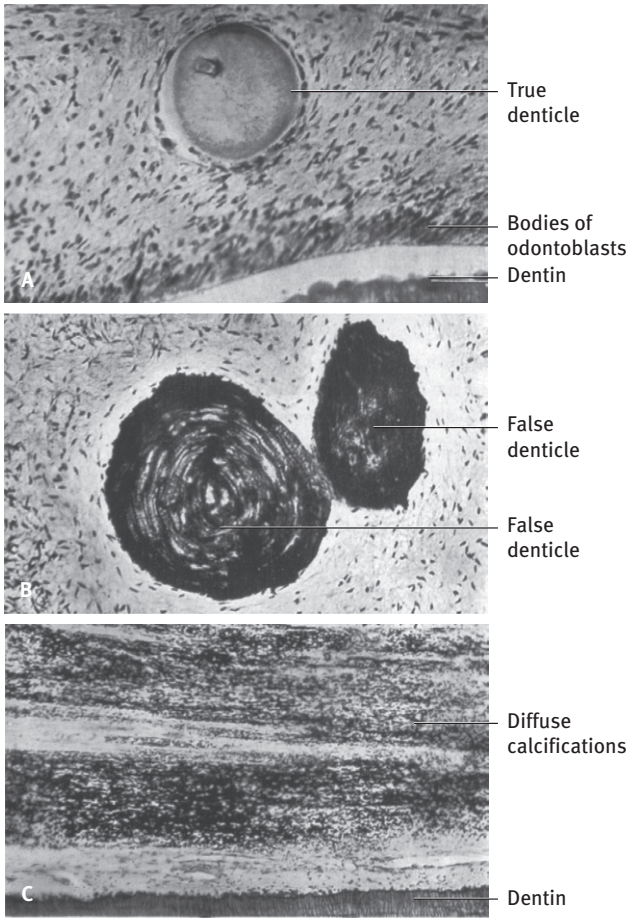

Figure 17.11 Calcifications in the pulp. (**A**) True denticle. (**B**) False denticle. (**C**) Diffuse calcifications.

False denticles do not exhibit dentinal tubules but appear instead as concentric layers of calcified tissue (Fig. 17.11B). In some cases, these calcification sites appear within a bundle of collagen fibers (Fig. 17.12). Other times they appear in a location in the pulp free of collagen accumulations

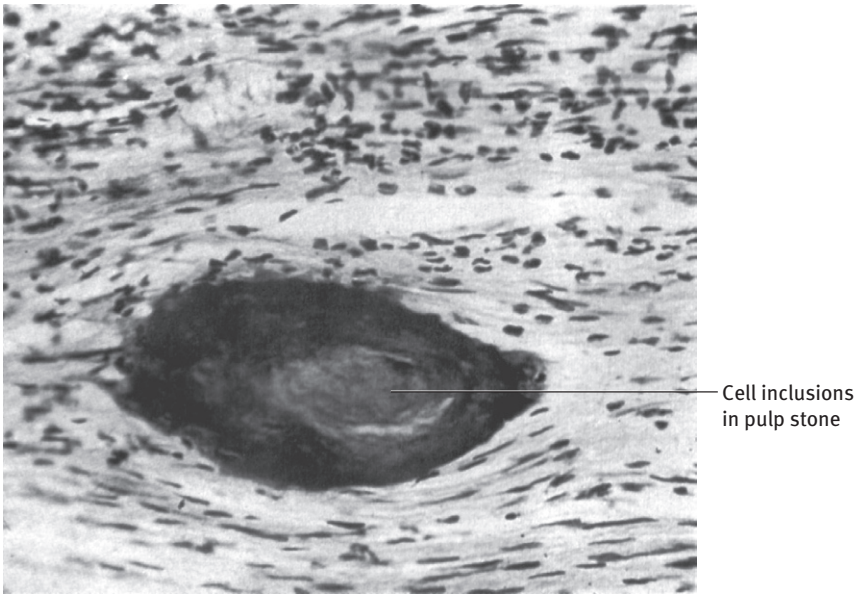

Figure 17.12 Free, false pulp stone within collagen bundle of coronal pulp.

(Fig. 17.11C). Some false pulp stones undoubtedly arise around vessels as seen in Figure 17.10. In the center of these concentric layers of calcified tissue there may be remnants of necrotic and calcified cells (Fig. 17.12). Calcification of thrombi in blood vessels, called phleboliths may also serve as nidi for false denticles. All denticles begin as small nodules but increase in size by incremental growth on their surface. The surrounding pulp tissue may appear quite normal. Pulp stones may eventually fill substantial parts of the pulp chamber.

Pulp stones may also be classified as free, attached, or embedded, depending on their relation to the dentin of the tooth (Fig. 17.13). The free denticles are entirely surrounded by pulp tissue, attached denticles are partly fused with the dentin, and embedded denticles are entirely surrounded by dentin. All pulp stones are believed to be formed free in the pulp and later become attached or embedded as dentin formation progresses. Pulp stones may appear close to blood vessels and nerve trunks (Fig. 17.14). This is believed to be because they are large and grow so that they impinge on whatever structures are in their paths. The occurrence of pulp stones appears more prevalent through histologic study of human teeth than found by radiographic study. It is believed that only a relatively small number of them are sufficiently enough to be detected in radiographs. The incidence as well as the size of pulp stones increases with age. According to one estimate, 66% of teeth in persons 10 to 30 years of age, 80% in those between 30 and 50 years, and 90% in those over 50 years of age contain calcifications of some type. A statistically significant relationship has been found between patients with cardiovascular disease and presence of pulp stones. This finding suggests the usefulness of dental radiographs to identify patients with cardiovascular diseases for further screening and evaluation.

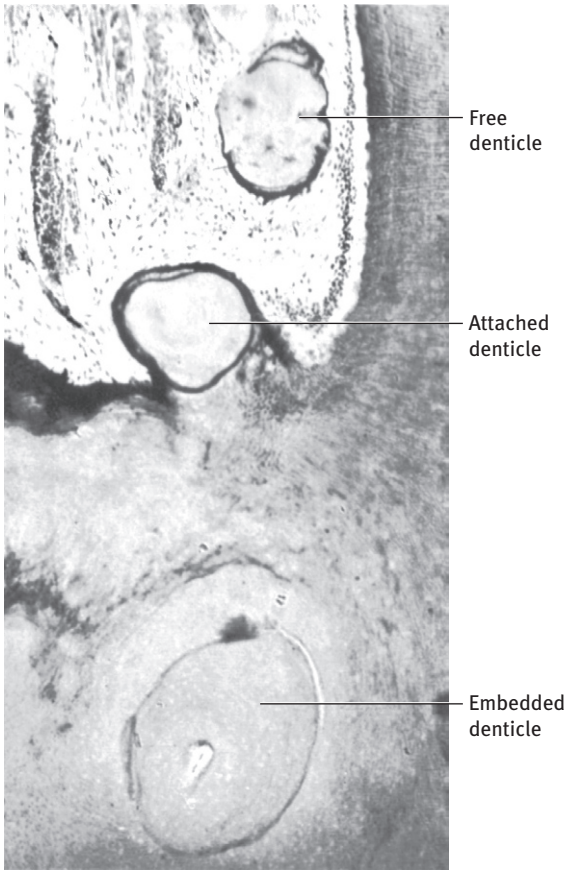

Figure 17.13 Examples of the typical appearance of pulp stones as free, attached, and embedded.

Diffuse Calcifications

Diffuse calcifications appear as irregular calcific deposits in the pulp tissue, usually following collagenous fiber bundles or blood vessels (Fig. 17.11C). Sometimes they develop into larger masses but usually persist as fine calcified spicules. The pulp organ may appear quite normal in its coronal portion without signs of inflammation or other pathologic changes but may exhibit these calcifications in the roots. Diffuse calcifications are usually found in the root canal and less often in the coronal area, whereas denticles are seen more frequently in the coronal pulp. Diffuse calcification surrounds blood vessels, as in Figure 17.10. These calcifications may be classified as dystrophic calcification.

In conclusion, with increasing age the volume of pulp and reparative capabilities of pulp decreases. The blood flow is reduced due to decrease in the number of blood vessels. There is increase in the number of calcified tissues in pulp. All the changes contribute to a decrease in pulp resiliency and its ability to sense and react to insult (Box 17.1).

AGE CHANGES IN PERIODONTIUM

Periodontium includes all those tissues that support the tooth within the socket. Gingiva, periodontal ligament, cementum and alveolar bone constitute periodontium. Since these tissues collectively perform a common function, periodontium may be considered as a specialized connective tissue organ. Like any other connective tissue in the body, tissues constituting periodontium also show changes with aging. The changes seen in different tissues of periodontium are discussed separately.

Changes Seen in Gingiva

The visible clinical changes noted in gingiva are it appears dry and atrophic which is satin-like and friable. Gradually there is a decrease in the width of the gingiva and apical migration of the junctional epithelium. It becomes less resilient and more sensitive to external stimuli. Though it has been suggested that stippling becomes more prominent and pronounced as the age increases, in elderly individuals there is loss of stippling. This loss of stippling is probably due to associated inflammation and may not be because of aging alone.

Histological examination of gingiva shows certain characteristic features in elderly individuals.

Changes seen in the epithelium

There is thinning of epithelium (epithelial atrophy), acanthosis (increase in number of prickle cell layers), probably as an adaptation to counter multiple irritating

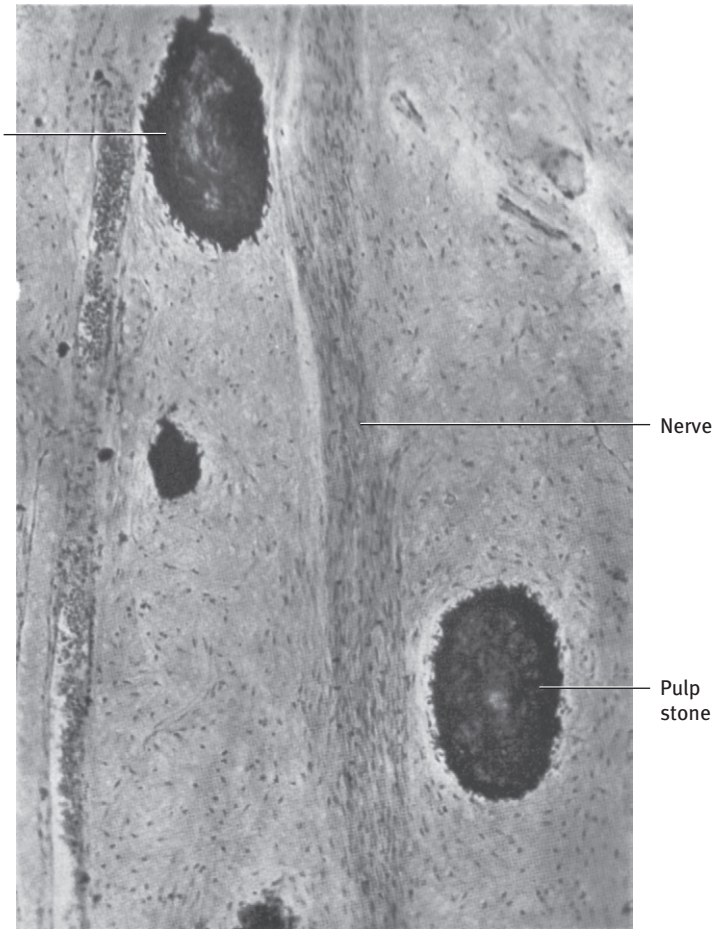

Figure 17.14 Pulp stones in proximity to nerve.

Box 17.1 Age Changes in Dental Tissues

Enamel

Loss of perikymata
Disappearance of mamelons
Surface hardness increase
Increased uptake of fluoride ions
Reduced permeability because crystals become bigger with ions adsorption

Dentin

Secondary dentin formation causes increased thickness of dentin
Sclerotic dentin at apical third
Dead tract
Reduced sensitivity
Reparative dentin formation

Pulp

Decrease in pulp volume due to secondary dentin formation
Decrease in odontoblasts and fibroblasts
Fibrosis
Reduced blood flow
Pulp calcifications—pulp stones and diffuse calcifications

factors and loss of equilibrium between keratinization and cellular proliferation resulting in parakeratosis that is associated with underlying inflammatory infiltrate.

But conflicting results with respect to the shape and height of rete ridges have been published. Few studies have shown that the junction is flat without any presence of epithelial rete ridges and connective tissue papillae, while few other studies have indicated that there is an increase in the height of epithelial rete ridges. Variable results were also published with regard to the regeneration time of the epithelium. While certain studies have shown that the mitotic activity increases with age, few studies have suggested that it remains constant throughout, and few other studies have indicated that it decreases with age. Exfoliated cells of gingival epithelium show a gradual decrease in the diameter of the nucleus and cell, and nuclear-cytoplasmic ratio.

Changes in the connective tissue

In general, with increasing age the skin shows wrinkling mainly because of loss of subcutaneous fat. However, in gingiva since there is no submucosa such wrinkling may not be evident. But like any other connective tissue in the body, it may also show certain changes like there may be a gradual change of finely textured prepubertal

connective tissue to a coarsely textured tissue in elderly individuals. A histomorphometric study has shown that there is a decrease in the number of cells with a decrease in the fibrous component. *In vivo* and *in vitro* studies have indicated that the fibroblasts are both morphologically and functionally altered. The production of collagen by the gingival fibroblasts is decreased 5 fold with increasing age, probably because there is intracytoplasmic phagocytosis. In addition, degradation of connective tissue may be associated with the presence of chronic inflammatory cells, chiefly lymphocytes and plasma cells as gingiva is always in a state of inflammation. It has also been shown that there is an alteration in the composition of extracellular matrix, the proteoglycans. This is because the old gingival fibroblasts may secrete more heparin sulfate and less chondroitin sulfate.

In conclusion, as a result of atrophy of epithelium and disturbances in the equilibrium between keratinization and epithelial cell proliferation, the protective function of the gingiva is reduced and the healing process gets slower with age. In addition, immunosenescence also compounds the situation further.

Location of junctional epithelium

In healthy teeth, the junctional or attachment epithelium is at cementoenamel junction. However, the position of the epithelium on tooth surface is not constant and varies with age and that it migrates progressively apically with advancing age. Though it has been suggested that such migration is partly a physiological process and partly a pathological phenomenon, there is no sufficient evidence to suggest physiological apical migration is due to aging process. Moreover, it appears to be more plausible that it occurs due to periodontal inflammation either due to mechanical trauma or bacterial plaque. Though it has been shown that the composition of the bacterial plaque changes with age, whether such change dictates the onset and course of periodontal breakdown is not clear.

CHANGES IN PERIODONTAL LIGAMENT

The periodontal ligament ages as in all other tissues of the body. The connective tissue of periodontal ligament in young individuals is regular and well-organized. But with aging, it becomes irregular. The most remarkable change in the periodontal ligament is decrease in cell density and the fibrous component. The mitotic activity or proliferation rate of the cells of periodontal ligament also decreases. The cells of the periodontal ligament may show reduced chemotaxy and motility which may be due to reduced expression of C-fos ligand by the senescent cells. The reduced proliferative capacity of cells may be related to decreased expression of osteocalcin by periodontal ligament fibroblasts because it is responsible for failure to progress in cell cycle particularly G1 to S phase. There is also an overall decrease in the production of the organic matrix. Few studies have demonstrated the loss of acid mucopolysaccharides with aging. The periodontal ligament in older teeth show an increase in the number of elastic fibers and a decrease in the number of epithelial cell rests of Malassez. Conflicting reports have been published in literature regarding the width of the periodontal ligament space. While few studies have suggested that the width of the periodontal ligament space decreases with age, other studies have indicated that it increases. However, both may be possible, but depends upon the number of teeth present in nonfunctioning teeth, i.e. teeth without antagonists, the width of periodontal ligament space may be narrow and at the same time when forces act on the remaining existing teeth, there is an increase in the masticatory load resulting in the increase in the width of periodontal ligament space. There is also another explanation that with aging the masticatory forces decreases resulting in narrow width of periodontal ligament space.

The cell number and the cell activity decreases with aging. One of the prominent age changes is seen in the calcified tissues of the periodontium, the bone (alveolar) and the cementum, is scalloping and the periodontal ligament fibers are attached to the peaks of these scallops than over the entire surface as seen in a younger periodontium. This remarkable change affects the supporting structures of the teeth. With aging the activity of the periodontal ligament tissue decreases because of restricted diets and therefore normal functional stimulation of the tissue is diminished. Any loss of gingival height related to gingival and periodontal disease promotes destructive changes in the periodontal ligament.

AGE CHANGES IN CEMENTUM

The thickness of cementum increases with age, particularly at the apex. This may probably be due to passive eruption. Secondly, there is also an increased deposition on the lingual surface as compared to other surfaces. Cementum triples its thickness from 10 years to 75 years. However, in those areas where it is exposed due to gingival recession, its thickness decreases. Cementocytes have the lowest proliferative capacity and gradually die due to decreased accessibility to nutrition as the width of the cementum increases and due to poor elimination of waste products of cementocytes. Hence, cementum becomes acellular except at the apex with increasing age.

AGE CHANGES IN ALVEOLAR BONE

With age there is a gradual decrease in bone formation with a resultant significant decrease in the bone mass. This is either because of a decrease in osteoblast proliferating precursors or decreased synthesis and secretion of essential bone matrix proteins. The extracellular matrix also plays an important role in bone metabolism and there might be a dysfunction of the extracellular matrix with age. Fibronectin present in the matrix plays an important role in osteoblastic activity and that fibronectin damaged by oxygen-free radicals during the aging process might be responsible for reduced bone formation. Animal studies particularly in rats and monkeys have suggested that the periodontal surface of the alveolar bone becomes jagged and that less number of collagen fibers get inserted into bone. The width of cribriform plate and interdental alveolar septum decreases with aging. However, such findings could not be confirmed in human beings. It was also noted that the alveolar bone gradually transform from immature trabecular bone to a

Box 17.2 Age Changes in Periodontium

Cementum

Increased thickness due to passive eruption
Increased degeneration of cementocytes

Periodontal Ligament

Decreased cells and their activity
Decreased collagen turnover
Decreased elastic fibers
Generally ligament width decrease due to decreased masticatory load

Alveolar Bone

Decreased bone formation
Fatty marrow
Greater distance between alveolar crest and CEJ

Gingiva

Apical shift of junctional epithelium
Coarse collagen fibers
Collagen formation decreased, degradation increased

dense lamellated bone with age in rats. However, in human beings there was an increase in the number of interstitial lamellae. The marrow spaces show fatty infiltration. The alveolar process in edentulous jaws decreases in size. Loss of maxillary bone is accompanied by increase in size of the maxillary sinus. Internal trabecular arrangement is more open, which indicates bone loss. The distance between the crest of the alveolar bone and CEJ increases with age, approximately by 2.81 mm.

Box 17.2 depicts age changes in periodontium in brief.

Change in Dental Arch Shape

Studies relating to changes in the dental arch with age have shown that the area of dental arches increased between 3 years and 15 years and that maximum changes were noticed particularly between 5 years and 7 years and 11 years and 13 years probably relating to the periods during which most of the permanent teeth erupt. Though the shape of the dental arch changes with age, it could not be strictly related to age as a variety of factors like pressure from oral and paraoral muscles, position and size of teeth may overlap and influence the changes in shape of dental arch. It was noted that there was an increase in the dental arch index with increasing age suggesting that there is an increase in the dental arch width.

AGE CHANGES IN ORAL MUCOSA

With age the oral mucosa becomes smooth and dry. These are due to epithelium becoming thin mainly due to the reduction in the thickness of epithelial ridges and decrease in the salivary secretion. The filiform papilla becomes reduced and the tongue appears smooth owing to the reduction in the thickness of the epithelium. The number of taste buds gradually decreases with age. In women, it starts slightly ahead as compared to men. It starts between 40 and 50 years in women and between 50 and 60 years in men. They begin to atrophy and most individuals become aware of loss of (or) altered taste perception by 60 years. Initially, the salty and sweet tastes are lost followed by the loss of bitter and sour tastes. Nutritional deficiencies may also be a contributing factor for this change. Varicose veins on the ventral aspect of tongue are often seen and these are termed as lingual varices. Langerhans cells become fewer with age, which may contribute to decline in cell mediated immunity. In the lamina propria, cellularity decreases but collagen content increases. Minor salivary glands show considerable atrophy with fibrous replacement. Nerves and end organs in the oral mucosa may also be affected by age. The effects include a progressive loss of sensitivity to thermal, chemical and mechanical stimuli, and with decline in taste perception.

SALIVARY GLAND FUNCTION AND AGING

It is often assumed that the secretion of saliva decreases with aging. Currently, the effect of aging on salivary gland dysfunction is still not clear. One of the earliest manifestations of salivary gland dysfunction is xerostomia or dry mouth. The causes of dry mouth or xerostomia are many and diverse in nature. The causes can be either physiological or pathological and if pathological, the causes may be local or systemic in nature. Aging may cause organic change in the glands and thus there is a decrease in the number of acini with simultaneous increase in the adipose and fibrous tissue in elderly individuals. The acinar cells become large and eosinophilic, and this change is referred to as oncocytic change. Studies have been carried out to assess the changes with aging in labial salivary glands of healthy individuals, and it was shown that acinar atrophy, ductal dilatation and callus formation were seen more commonly in elderly individuals above 50 years of age. However, foci of inflammatory cells first appeared in the age group of 30 to 39 years with a gradual increase in focus size with increasing age. Though the degree of fibrosis and fatty infiltration increased with age, the amount of fatty infiltration is less compared to fibrosis in individuals of similar ages. Few animal studies have shown that the synthesis of proteins is reduced by 60% in elderly individuals indicating that there is definite change in the concentration and/or activity of organic components of saliva. Studies in human beings have revealed that there is a decrease in the concentration of salivary IgA in labial saliva and that of the mucin in mucous saliva suggesting that oral defense mechanism is compromised to some extent with age.

CLINICAL CONSIDERATIONS

Loss of tooth substance namely enamel, causes exposure of dentin leading to pain like sensation called sensitivity of dentin. Dentin exposure also occurs because of dental caries or trauma. Uptake of fluoride from saliva leading to its adsorption to the surface enamel helps in resisting caries induced demineralization.

Dead tracts formed due to dentin exposure following attrition or below caries is a cause for reduced dentin sensitivity. Sclerotic dentin formation formed as a result of caries in dentin, acts as a seal for the dentinal tubules and thus prevents caries progression. Its formation in the apical third of the root makes the tooth brittle and is a cause for breakage of root in apical third during extraction of teeth in elderly patients.

It may be difficult to locate the root canals because of reduction in pulp chamber due to continuous formation of secondary dentin. Pulp calcifications especially pulp stones interfere with root canal procedures.

The epithelium of oral mucosa becomes thin and may appear red clinically. Reduction in salivary secretion causes mouth to become dry, which is known as xerostomia. This combination of dry mouth and thin epithelium is more prone even for mild trauma leading to epithelium being peeled off causing burning sensation in the mouth. Taste perception is reduced due to loss of papillae of the tongue and reduction in salivary secretion.

SUMMARY

Age change in humans is a continuous and steady process taking place right from birth till death. This should be differentiated from senescence in which changes takes place after maturation and these senile changes increases the amenability of the person to death.

Theories of Aging

Many theories have been put forward to explain the process of aging of which shortening of telomere, injury caused by free radicals and accumulation of DNA damage seems to be more acceptable than others.

Age Changes in Enamel

The age changes in enamel in newly erupted teeth relate to loss of surface structures like enamel caps, enamel brochs and enamel pits at the ultrastructure level, while loss of perikymata and mamelons can be appreciated clinically. Intake of ions from oral fluids like nitrogen, fluorine accumulates at the surface layers, which causes the crystals to become bigger, reducing the permeability of the enamel. Uptake of fluorine increases the hardness of the enamel.

Age Changes in Dentin

The age changes in dentin are intimately related to functional changes, hence they are considered together.

The gradual deposition of secondary dentin and deposition of apatite crystals in the dentinal tubules causing sclerosis or transparent dentin are the important age changes. Sclerotic dentin also occurs as a reaction to seal off the tubules in dental caries. Degenerated dentinal tubules called dead tracts also occur in dental caries or due to exposure of dentinal tubules. Dead tracts and sclerotic dentin reduce the sensitivity and the permeability of the dentin. Dead tracts appear black or dark in transmitted light due to air entrapment in the empty dentinal tubules in the ground section. It appears bright or white in reflected light. The appearance of sclerotic dentin is just the opposite of dead tract, being bright or white in transmitted light and dark in reflected light. Sclerotic dentin is often seen apical to dead tracts. As an age change sclerotic dentin is seen in the apical third of roots.

Reparative dentin forms as a reaction to save the underlying pulp from injurious elements like bacteria and their products and harmful substances from restorative materials. These migrate to pulp through the tubules and cause the undifferentiated cells of the pulp to differentiate into new odontoblast and lay down reparative dentin. This dentin contains fewer and disorganized dentinal tubules.

Age Changes in the Pulp

The important age changes in the pulp are decrease in the volume of the pulp due to secondary dentin formation, decrease in the number of cells, increase in collagen fibers and their aggregation forming fibrosis, reduction in blood flow due to decrease in blood vessels and narrowing of their lumen and occurrence of calcifications.

Pulp calcifications may occur as diffuse calcifications in the radicular pulp or as discrete masses, called pulp stones. The pulp stones are classified based on their structure as true denticles—if they show dentinal tubules or false denticles if they do not contain dentinal tubules. They are also classified depending on their attachment to dentin, as attached, if they are found attached to dentinal wall, or embedded if they are found in the dentin and surrounded by dentin. But most commonly they are found unattached and lying freely in the pulp. They are then called free false pulp stones. Pulp stones are of clinical importance as they can modify root canal procedures.

Age Changes in the Cementum

Cementum increases in thickness, especially at the apex due to passive eruption. More number of cementocytes degenerates, so cementum becomes more of acellular type.

Age Changes in Periodontal Ligament

There is decrease in the number of cells and its proliferative capacity. Decrease in collagen turnover is seen. The elastic fibers decrease. The width of the ligament increases or decreases depending on the amount of masticatory forces the tooth receives. In teeth without function ligament decreases in width.

Age Changes in Alveolar Bone

The alveolar bone shows reduction in bone formation due to decreased activity of osteoblast or reduced activity of stimulating factors. Marrow spaces are increased and fatty infiltration is seen. Maxillary sinus appears bigger due to bone loss in the maxilla. The distance between alveolar crest to CEJ increases.Dental arch width increases maximally during the period of eruption of permanent teeth.

Age Changes in Gingiva

Gingiva being a part of the supporting tissues shows apical migration of junctional epithelium exposing more amount of clinical crown with age. The epithelium becomes thin, dry and friable. The collagen fibers become more coarse, the fibroblastic activity and therefore collagen production is greatly reduced. Collagen degradation

is increased. All these processes are influenced by inflammation in the region.

Age Changes in Oral Mucosa

The oral epithelium becomes thin; the mucosa becomes dry and shiny. The papillae of tongue become reduced leading to a smooth tongue. As the taste buds are reduced, there is progressive loss of taste sensation. Lamina propria shows decreased cellularity but increased collagen content.

Age Changes in Salivary Glands

Acinar cells show reduced activity, which results in reduced salivary secretion leading to xerostomia or dry mouth. Salivary secretion becomes thicker and contains less proteins and IgA. Fibrosis and fatty infiltration are seen in the glands. Acinar cells show oncocytic change.

Clinical Considerations

Exposure of dentin due to attrition or caries causes dentin exposure which leads to pain like sensation called sensitivity. Surface enamel becomes harder due to fluoride uptake from saliva and this helps in protecting teeth from caries. Sclerotic dentin prevents caries progression and it is also a cause for breakage of roots in apical third during extraction of teeth in elderly patients. Pulp calcifications interfere with root canal procedures. Reduction in salivary secretion and loss of papillae of tongue is a cause for reduction of taste perception in the elderly. Burning sensation of the mouth is due to peeling away of thinned out epithelium and dryness of mouth.

REVIEW QUESTIONS

1. Define age changes. How it is different from senescence.
2. Explain briefly the theories of aging?
3. What are pulp stones? Describe the different types of pulp stones
4. When and why reparative dentin forms? How does it differ from normal dentin?
5. What are dead tracts? What causes their formation? Describe their microscopic appearance
6. What is transparent dentin? Why are they called transparent dentin? What causes their formation?
7. Write notes on the age changes of the following tissues:
   Gingiva
   Salivary glands
   Oral mucosa

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18 Histochemistry of Oral Tissues

CHAPTER CONTENTS

Histochemistry is the study of qualitative identification and quantitative assessment of chemical groupings within cells and tissues. Histochemical study of oral tissues are challenging due to the presence of both hard and soft tissues in the oral cavity. Many sophisticated tools employing complex but precise techniques are available nowadays. Another interesting development is the use of a combination of techniques to identify or estimate a particular substance, as for example; autoradiography and electron microscopy as in the estimation of chemical substances during metabolic pathway.

OVERVIEW OF HISTOCHEMICAL TECHNIQUES

Histochemical techniques are based on precise chemical rationales for their ability to identify or stain different biochemical substances. These techniques necessitate using more stringent precautions to preserve the chemical integrity of the tissues than, perhaps, are required in a biochemical or an immunochemical assay. Also, *histochemical techniques provide in situ information that cannot be obtained with biochemical methods.*

Application of immunobiologic principles in the histochemical localization of specific proteins, glycoproteins, or proteoglycans allows detection of a host of biologic molecule that plays important roles in normal tissues during development and in different pathologic conditions. Numerous innovative techniques promoting localization of different chemical residues or components of a large molecule are also available for histochemical application. A case in point is the use of plant lectins as histochemical probes in the characterization of sugar moieties within glycoprotein molecules. Use of these probes has generated much more specific and meaningful in situ information than could be obtained from biochemical assays of total tissue homogenate pools.

In situ hybridization *is the latest histochemical technique that permits identification of a gene (DNA nucleotide sequence) or gene product (messenger RNA [mRNA]) on a tissue section.* The technique draws on the principles and methods of modern molecular biology and genetics wherein synthetic nucleotide probes, complementary DNA (cDNA) probes, or mRNA probes are prepared and allowed to hybridize corresponding/complementary molecular sites in a tissue section. Tissue sections are protected from DNA/RNA degradation, and their proteins and lipids are removed before hybridization. Since the probes are generally radiolabeled with ^{32}P, ^{3}H, or ^{35}S, the loci of hybridization are visualized by standard autoradiographic procedures. To affirm that the hybridized probe (i.e. mRNA) is an appropriate substrate for translation, mRNA can be translated in vitro to its protein product. This protein product can then be analyzed with the appropriate monoclonal antibody, using standard immunohistochemical techniques. *In situ hybridization and the immunohistochemical methods thus complement each other, enhance ultimate specificity, and make for perhaps some of the most powerful investigative and diagnostic tools in biology and medicine.*

Most histochemical techniques have generally been used for qualitative analysis of chemical substances in cells and tissues. However, many *sophisticated techniques are available for quantitative analysis of histochemical reactions.* They include use of the original **microphotocell counter, double-beam recording microdensitometry**, and more recently the **scanning and integrating microdensitometry**. This latter method has been used successfully by Chayen in measuring lysosomal membrane permeability and by Stuart and Simpson in measuring the activity of dehydrogenase enzymes in single cells from bone marrow biopsies of normal and leukemic patients. Phillips and co-workers have done a quantitative analysis of total mineral content in bone by combining microradiography and microdensitometry using a scanning autodensidater attachment.

Many new techniques, not precisely histochemical, are frequently used by histochemists in making qualitative as well as quantitative analysis of tissue substances, particularly mineral elements. These include **X-ray and interference microscopy** for measuring the dry mass of a biologic substance or a reaction product, **X-ray diffraction, X-ray spectrophotometry,** and **electron probe microanalysis**. *Recently, scanning electron microscopy (SEM) has come to occupy an important place in dental research.* SEM has been used in the study of bone morphology and in the analysis of changes in bone architecture induced by the presence of surgically inserted metallic implants (Fig. 18.1). Neiders and co-workers have made conjunctive use of SEM with an electron probe attachment for obtaining a visual surface texture image of tooth cementum analyzed for its mineral content. Reith and Boyde have also used an electron probe with SEM in a study of calcium transport across ameloblasts in the enamel organ (Fig. 18.2). *Techniques of polarized light and X-ray analysis have been used for the study of enamel.* Phosphorescence emitted as a result of tetracycline binding to mineralized tissues has been demonstrated in bone, dentin, and enamel at liquid nitrogen temperatures. **Laser spectroscopy** *has also been used for qualitative and quantitative microanalysis of inorganic components of calcified tissues.* Immunohistochemical techniques using fluorescein tags have been applied to the study of oral tissues.

Light microscopic histochemical techniques have been increasingly adapted for use in electron microscopic histochemistry. The visualization of carbohydrates, specific proteins, and phosphatases are some examples of such adaptive use (Fig. 18.3). **Confocal laser scanning microscope** *is helpful in producing three-dimensional images* with submicron spatial resolution. It is possible to record reflectance signals and/or absorptive transmissions at the wavelength of the argon ion laser preferentially at 488 nm. The reflectance mode of confocal laser scanning microscope is proved to be useful in visualizing under high resolution, the primary histochemical reaction products of oxidases and phosphatases (both cerium-based and DAB/DAB-Ni based products) offering better spatial localization of the enzyme activity.

Radioautographic techniques *play a vital role in histochemistry because of their ability to elucidate the uptake of chemical substances* by the metabolic pathways of different tissues and by different regions of the cytoplasm (Figs 18.4, 18.5). Tissue sections taken from animals injected with a radioisotope are covered with a photographic film or emulsion and left in the dark. Radio waves emitted by the isotope hit the silver halides of the film, and these tracks are later developed by processing of the slide or the metal grid like a photographic film. The radioisotope appears as dark granules in the light microscope and as linear tracks of the radio waves in the electron microscopic autoradiographs.

Histochemical techniques provide in situ information through preservation of tissue architecture unlike the biochemical techniques. The former techniques that have evolved later allow quantitation also.

STRUCTURE AND CHEMICAL COMPOSITION OF ORAL TISSUES

Oral structures are primarily composed of connective tissue and epithelial linings and associated glands. An understanding of these structures and their chemical composition is important in the consideration of biologic

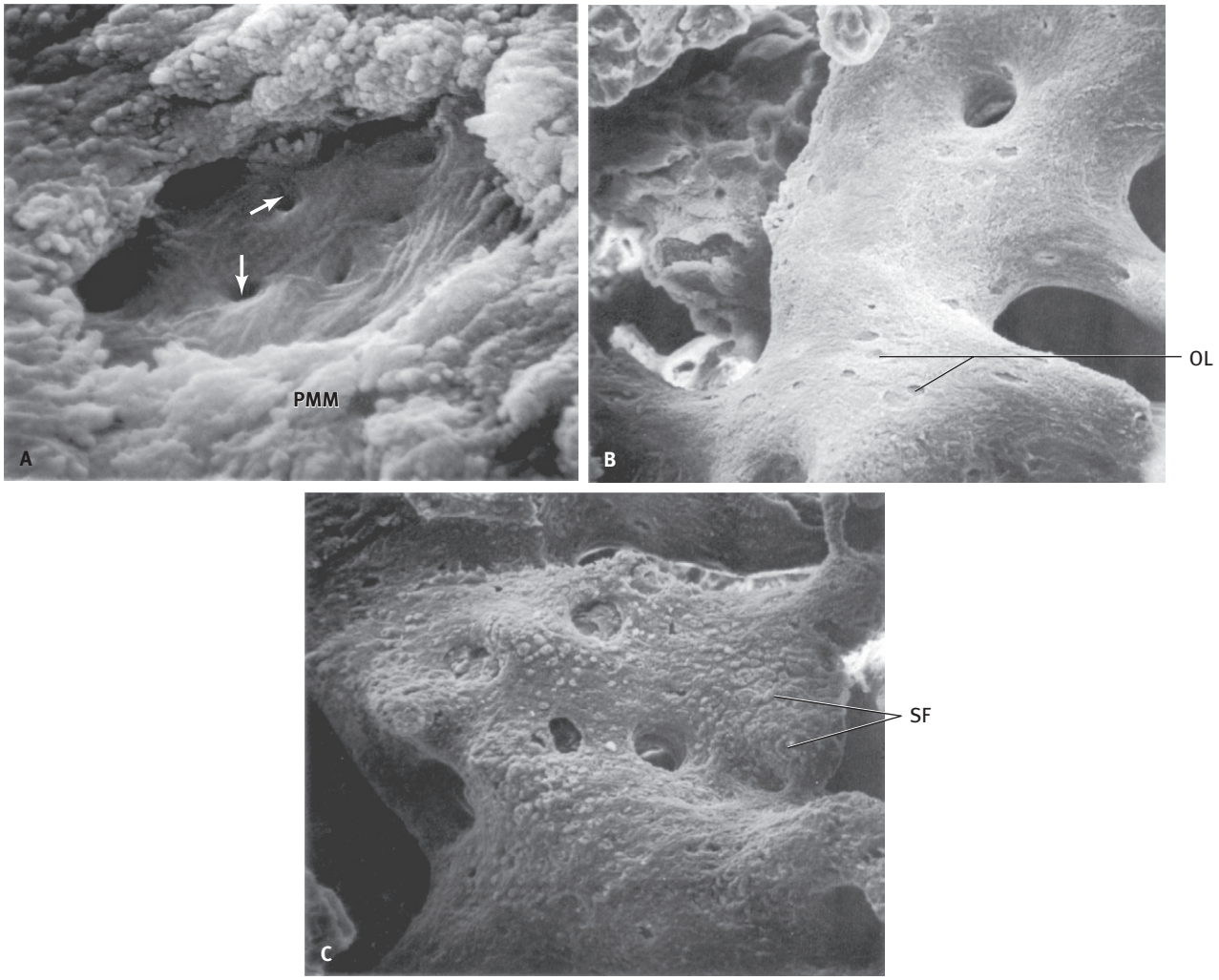

Figure 18.1 Scanning electron micrographs of cancellous bone in deorganified dog mandible. A metallic implant was surgically inserted subperiosteally in previously edentulatized mandible and was kept in place for 24 months prior to removal of bone sample for microscopic analysis. (**A**) A forming osteocytic lacuna with openings of canaliculi (arrows) visible on its inside while the newly deposited perilacunar mineral matrix (PMM) is seen roofing over and around it (×7000). (**B**) A typical bone trabeculum revealing several developing osteocytic lacunae (OL) on its forming surface (×280). (**C**) An atypical bone trabeculum, which reveals a rough surface studded with fully or partially mineralized Sharpey fibers (SF) (×280) (From Russell TE and Kapur SP: J Oral Implantol 7:415, 1977).

problems related to oral health. Significant chemical constituents of these tissues are proteoglycans, glycoproteins, mucins, and enzymes.

Connective Tissue

Connective tissue is derived from the mesenchyme and consists of various types of ***cells and fibers*** that are embedded in an amorphous, semi-gel, colloidal ***ground substance***.

Ground Substance

The ground substance is a mixture of macromolecules. They show ability to interact with cells and fibers in the matrix, thereby playing a role in adhesion and signaling events. The connective tissue ground substance is primarily composed of *proteoglycans* and *glycoproteins*.

Proteoglycans

Proteoglycans are large molecules *consisting of both cell surface associated and extracellular macromolecules*. The former type mediates polyvalent interactions with the cell matrix constituents and with the molecules of the neighboring cell surfaces. They also modulate the activity of the receptors implicated in the recognition of these components. Thus they participate in the perception and convergence of growth-and motility-promoting cues contributed by soluble factors. Through such interactions several proteoglycans found in promotile cells are able to transduce crucial intracellular signals that are likely to be essential for their motility. The matrix associated proteoglycans govern cell migration by structuring permissive and non-permissive migratory paths and when directly secreted by moving cells, may alternatively create a favorable or hostile microenvironment. Such pathways impose directionality to the moving cells, allowing them to be led to the given tissue location and immobilizing them at these sites.

Proteoglycans are formed of a protein core to which a large number of glycosaminoglycan (GAG) chains composed of repeating disaccharide units consisting of hexosamine and uronic acid is attached. The acidic nature of the GAG is attributed to its carboxyl or sulfated

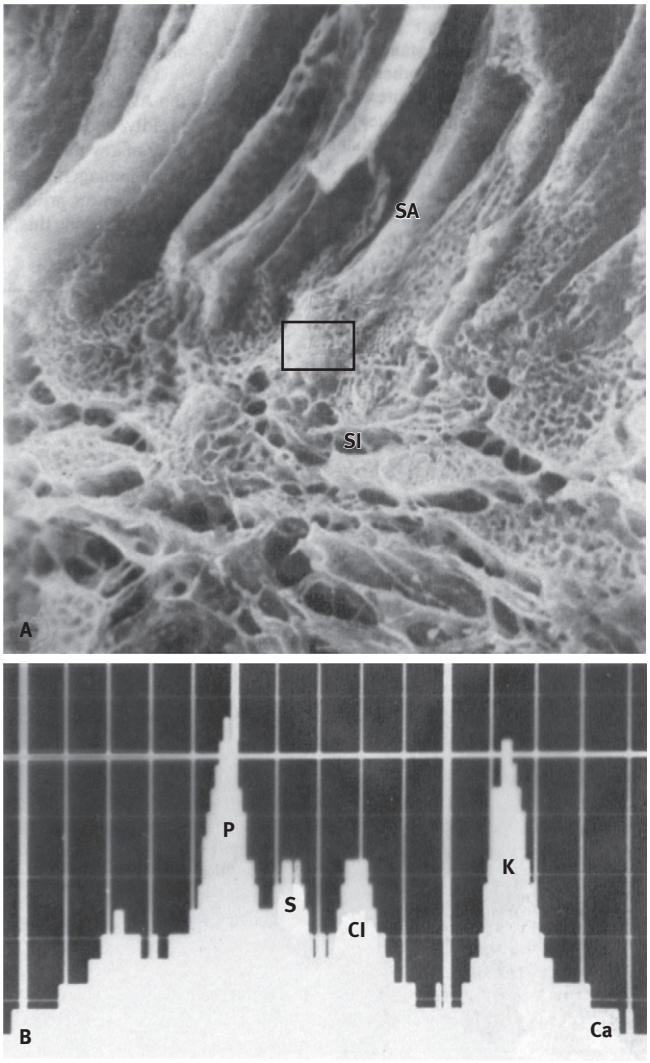

Figure 18.2 (**A**) Scanning electron microscopic view of freeze-fractured surface of secretory ameloblasts (SA) and stratum intermedium (SI) from developing rat molar tooth (×2700). Rectangular area marks proximal region of an ameloblast that was bombarded with electrons in order to obtain an electron probe spectrum, (**B**) for an analysis of its mineral content. (**B**) X-ray spectrum for this site reveals notable peaks for phosphorus (P), potassium (K), sulfur (S), and chloride (Cl) in order of descending heights. No significant calcium (Ca) peak is evident (From Reith EJ and Boyde A: Histochemistry 55: 17, 1978).

groups. *The GAGs may be unsulfated (hyaluronic acid) or sulfated (chondroitin sulfates, keratan sulfates, and heparan sulfates).* Heparin, a highly sulfated GAG, is secreted by connective tissue mast cells. Hyaluronic acid is synthesized as a very large, free, unsulfated GAG that does not require a protein core and differs from chondroitin sulfates in having acetyl glucosamines instead of acetyl galactosamines as its constituent. Hyaluronic acid binds proteoglycan molecules along its length to form large proteoglycan polymers (***aggregating proteoglycan***). The numbers and types of GAGs attached to the protein core in a proteoglycan molecule, as well as the polymeric state of proteoglycans, determine the viscosity of the amorphous ground substance. Hyaluronic acid predominates in the loose connective tissues and, because of its high capacity to bind water, is primarily responsible for transport and diffusion of metabolic substances across tissues. Chondroitin sulfates predominate in the cartilage proteoglycans and are primarily responsible for the supportive and somewhat plastic texture of this tissue. Chondroitin sulfates constitute 1% of the total bone tissue, whereas only 0.5% is present in dentin. There are few proteoglycans which contain only less numerous GAG chains and are called as ***non-aggregating proteoglycans***. These include, *decorin and fibromodulin* which are seen in association with collagen; *perlecan* in association with basal lamina and cartilage; *agrin* in association with basal lamina of neuromuscular junction and glomerulus and *syndecan*, a transmembrane core protein modified by heparan sulfate and sometimes by chondroitin sulfate chains. Syndecans are the major proteoglycans of many organs including vasculature, along with glypican, a membrane proteoglycan and matrix proteoglycans.

Glycoproteins contain fewer associated carbohydrate moieties than those present in the proteoglycans. Also, carbohydrates are not present in the form of regular repeating units. Several glycoproteins secreted into the connective tissue by epithelial cells and fibroblasts have been identified in the last few years. Of them, the most well known and characterized are: (1) *fibronectin* (secreted by fibroblasts, smooth muscle cells, and various other cell types); (2) *laminin* (secreted by epithelial cells and present in all basement membranes); (3) *chondronectin* (secreted by chondrocytes); and (4) *osteonectin* (secreted by osteoblasts). Other molecules which are gaining more importance include *tenascin,* and *thrombospondin.* These glycoproteins promote attachment of cells to their extracellular collagen matrices and therefore not only maintain normal cell morphology but also control cell function.

Fibronectin is a multifunctional adhesive glycoprotein that exists in the extracellular matrix and body fluids. The deposition of fibronectin into the extracellular matrix increases adhesion-dependent cell growth and cell contractility. Fibroblasts adhere to fibronectin in the matrix via α5β1 integrin and syndecan-4. An adhesive phenotype is developed through intracellular cytoskeletal element, actin stress fibers and by activation of focal adhesion kinase and Rho GTPase. Lack of syndecan attachment as it happens in the presence of tenascin-C promotes a motile cell phenotype by downregulation of focal adhesion kinase and Rho GTPase. The polymerization state of fibronectin is an important regulator of composition and stability of extracellular matrix, and also regulates the formation and stability of cell-matrix fibrillar adhesions.

Laminin is a family of large glycoprotein heterodimer present in the basement membranes. The structure consists of three subunits (α, β, γ) linked by disulfide bonds. Laminin-5 is the major adhesive component of epidermal basement membrane. Laminin-5 synthesis is augmented by inflammatory cytokines, growth factors and lysophospholipids. Laminin 8 and 10 are expressed in vascular endothelium.

Tenascin family of glycoproteins displays highly restricted and dynamic patterns of expression in the embryo, particularly during the neural development, skeletogenesis and vasculogenesis. The re-expression in adults occurs during normal process such as wound healing, nerve regeneration and tissue involution and in pathological states including

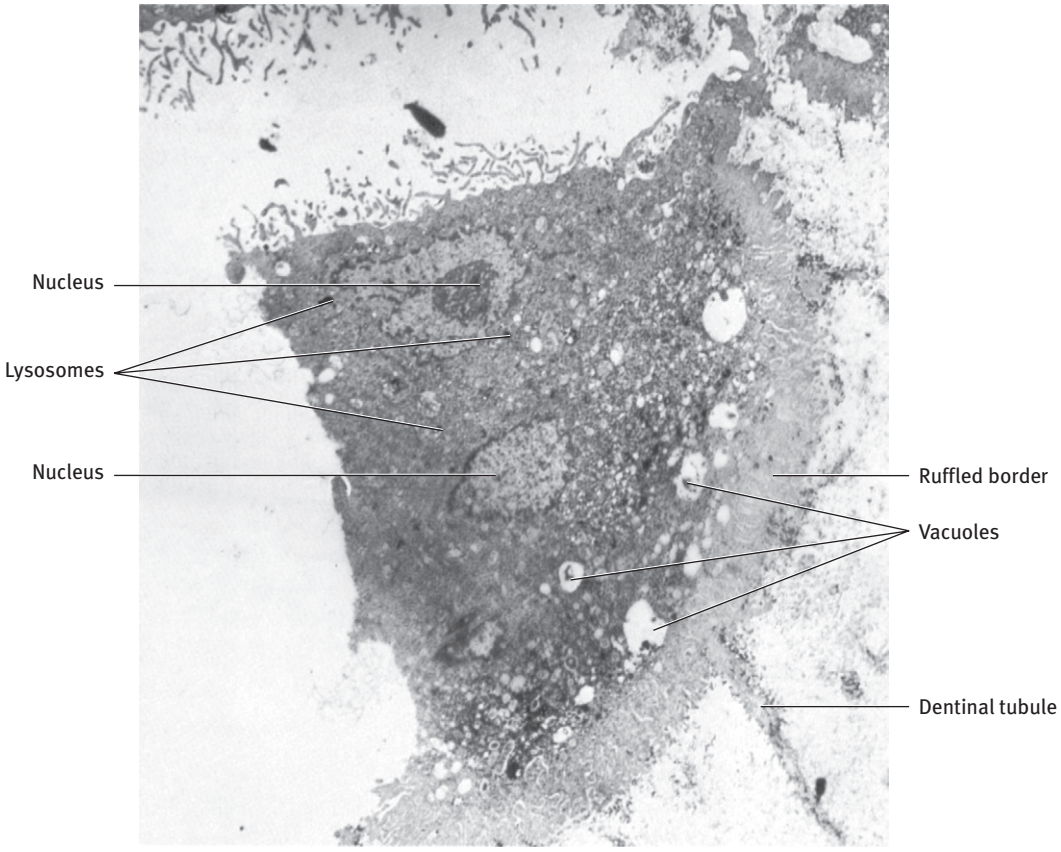

Figure 18.3 Electron micrograph of odontoclast from mongrel-puppy primary tooth undergoing resorption. Notice acid phosphatase reaction product in form of a black precipitate along dentinal tubule, ruffled border, vacuoles, and in lysosomes (Glutaraldehyde fixation, Gomori's metal substitution method; ×6250) (From Freilich LS: A morphological and histological study of the cells associated with physiological root resorption in human and canine primary teeth, doctoral dissertation. Washington, DC, 1972, Georgetown University).

vascular disease, tumorigenesis and metastasis. Four types have been identified until now: -C, -R, -W, and -X.

Thrombospondin is secreted and incorporated into the extracellular matrix by different cells. This is a 450 KD glycoprotein seems to promote proper organization of collagen fibrils in skin and cartilage.

Cells and Fibers

Fibroblasts

Fibroblasts are the most common cell type in the connective tissues. They are responsible for the elaboration of glycoproteins such as fibronectin and proteoglycans that form the amorphous ground substance. They also elaborate the ***fibrous components*** of the ground substance, including different types of ***collagen*** (especially types I and III), ***reticular fibers***, and ***elastic fibers***. When they are activated as during inflammation or any other stimuli, they secrete growth factors, cytokines and many chemical mediators. Thus, they play a major role in developmental process, wound healing and inflammation.

Current biochemical, histochemical, and ultrastructural evidence suggests that ***collagen*** is initially synthesized as much larger preprocollagen polypeptide chains. The prepeptide component is removed during or shortly after translocation in the rough endoplasmic reticulum. Post translational changes include hydroxylation of proline and lysine residues, glycosylation of hydroxylysine residues, formation of disulfide bonds between adjacent chains, and the formation of the characteristic triple helix. Procollagen is secreted on the cell surface where the propeptide sequence is deleted by a specific protease. This is immediately followed by the formation of the collagen microfibrils. The microfibrils serve as a template for initiation and extension of the polymerization and the accretion of more newly secreted monomeric tropocollagen into collagen fibrils. Hydroxylation steps are facilitated by vitamin C and are essential for providing conformation and stability to the triple helix.

The newly elaborated collagen fibrils, formed during development or in wound healing, are equivalent to ***reticular fibers*** in their electron microscopic structure. Both of these fibers stain positively for glycoproteins with silver stains and the periodic acid-Schiff (PAS) method. These reactions indicate the presence of a considerable packing of glycoprotein between aligned microfibrils of tropocollagen macromolecules.

Elastic fibers are elaborated by fibroblasts and also possibly by smooth muscle cells in the walls of blood vessels as elastin. They are composed of a protein component characterized by the presence of the amino acids desmosine and isodesmosine and some glycosaminoglycans. The elastic property results from numerous intermolecular

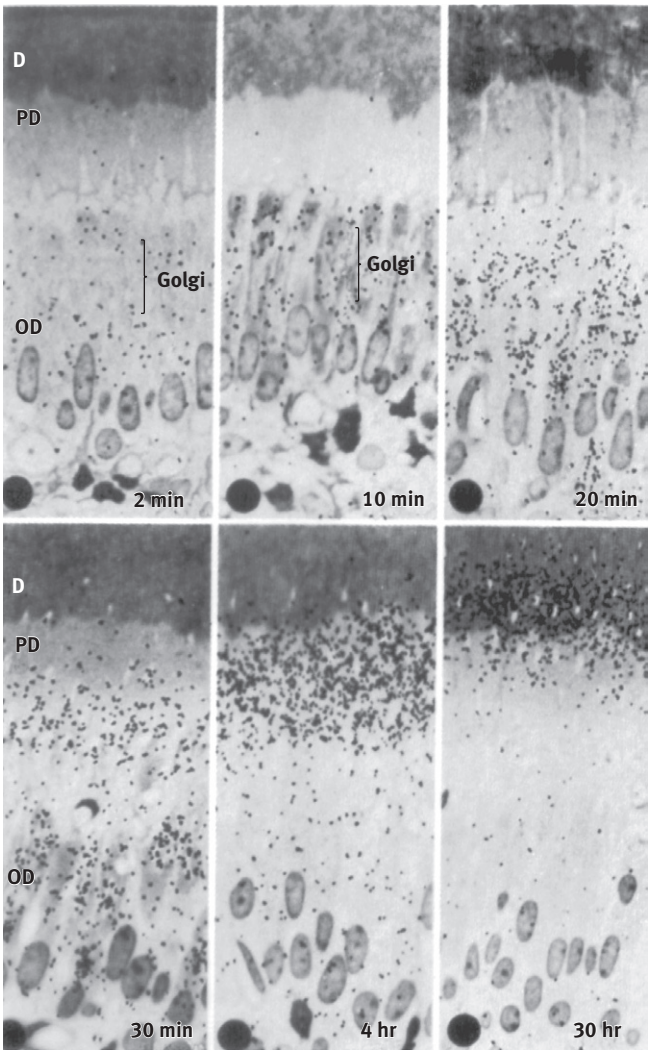

Figure 18.4 Light microscopic radioautographs illustrate path of ^{3}H-proline (injected into a young rat) over odontoblasts, OD; predentin, PD; and dentin, D, at growing end of incisor tooth. Notice that silver grains representing path of ^{3}H-proline appear first in granular endoplasmic reticulum at 2 minutes and subsequently at 10 and 20 minutes in Golgi region of odontoblasts. Thirty minutes after injection silver grains start appearing in odontoblastic processes and predentin, whereas at 4 hours entire radioactivity is located in predentin. Thirty hours after injection, dentin is completely labeled with ^{3}H-proline, now incorporated into collagen fibrils of dental matrix (×1000) (From Weinstock M and Leblond CP: J Cell Biol 60:92, 1974).

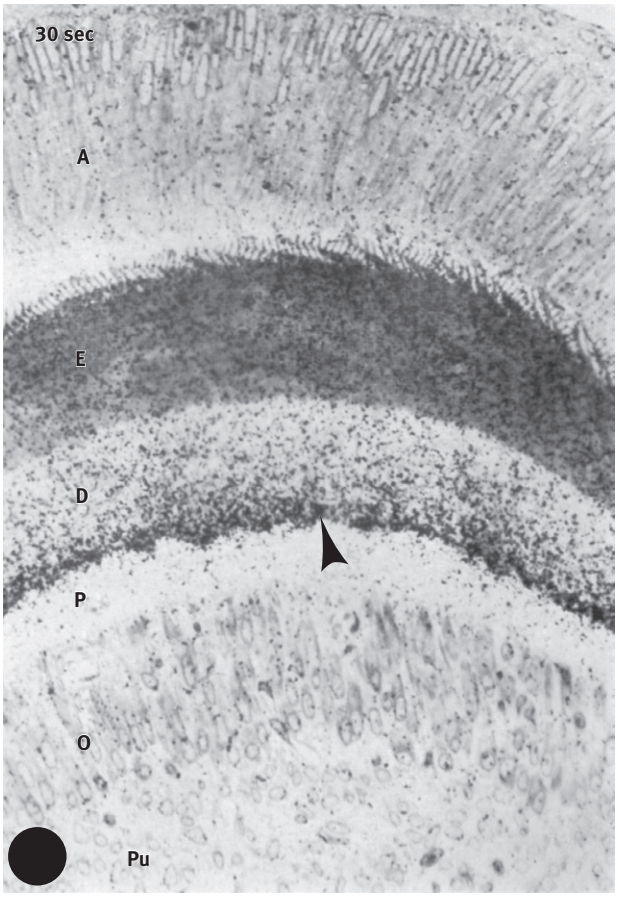

Figure 18.5 Radioautograph of incisor tooth (undecalcified cross-section) at its growing end in young rat killed 30 seconds after intravenous injection of ^{45}Ca. (A) Ameloblasts; (E) enamel; (D) dentin; (P) predentin; (O) odontoblasts; (Pu) pulp. Notice that ^{45}Ca is immediately incorporated into dentin over predentin–dentin junction at arrow. Some ^{45}Ca activity in form of few grains is seen in odontoblasts and predentin (×250) (From Munhoz COG and Leblond CP: Calcif Tissue Res 15:221, 1974).

cross-links between the lysine groups. Glycoproteins like fibrillin-1, fibrillin-2 and microfibril associated glycoproteins are essential for the assembly of microfibrils. Microfibrils exist in the absence of elastin and they are termed as *oxytalan fibers*. During the initial periods of formation of elastic fibers, the microfibril to elastin ratio is greater compared to the later stages of development. The developing fibers are called *elaunin*. Unlike collagen and reticular fibers, elastic fibers are not considered to be important constituents of the fully repaired tissues. Elastic fibers are stained by aldehyde fuchsin, resorcin fuchsin, and specifically by the dye orcein in histologic preparations. A fluorescent staining method, using tetraphenylporphine sulfonate in combination with silver or gold, has been developed by Albert and Fleischer for electron microscopic visualization of elastic fibers. Besides fibroblasts, other cellular elements of connective tissue are ***macrophages***, which scavenge on tissue debris; mast cells, which are rich in the highly sulfated proteoglycan heparin (an anticoagulant) and histamine (a vasodilator); and plasma cells, which elaborate immunoglobulins.

Epithelial Tissues and Derivatives

Salivary glands elaborate the so-called mucins or mucoids. The definition of these substances is exclusively chemical from the biochemical standpoint, but from a histochemical point of view this definition is in part based on color reactions. Histochemical detection of mucins is generally based on their glycosaminoglycan content, which affects certain staining reactions. The acidic nature is attributable to the presence of glucuronic acid, sulfate, or sialic acids. Histochemical observations show that a number of acid mucins lack sulfate esters. Histochemical characteristics of the oral epithelium, the epithelial components of the tooth germ, and the salivary glands are considered in another section of this chapter.

Several histochemical studies have been made on the structural proteins of the salivary gland leukocytes or the so-called salivary corpuscles. Histochemical techniques are also being used in oral exfoliative cytology for the detection of oral cancer. Identification of lung carcinoma by analysis of normal and abnormal cells present in sputum is used clinically.

Enzymes

Histochemistry has enabled histologists to demonstrate the actual sites of cellular enzymatic activity. The topographic distribution of enzymes may be ascertained by the quantitative microchemical techniques developed by the Linderstrøm-Lang group or by techniques that result in the formation of visible reaction products in tissue sections. The latter approach is widely used in histochemical demonstration of enzymes.

The most frequently studied enzymes in oral tissues are those related to the transfer of phosphate esters (***specific and nonspecific phosphatases***) in the organic matrix of bone, dentin, and enamel (***alkaline phosphatase***) and to resorption of bone and of dentin (***acid phosphatase***). ***Oxidases*** and ***dehydrogenases***, reflecting the metabolic activity of different tissues in oral structures, have also been studied extensively. Esterases, generally associated with the hydrolysis of carboxylic acid esters of alcohol, have been studied in salivary glands and in the taste buds. More recently, studies on lysosomal sulfatase and on adenyl cyclase involved in the formation of cyclic adenosine monophosphate (cAMP) have been reported.

Demonstration by histochemical methods requires understanding the composition of tissue components since careful preservation is essential.

- ***Connective tissue*** is composed of cells and fibers. They are supported by ground substance. Cells that form the connective tissue are fibroblasts. They produce both the fibers and ground substance.
- The fibers formed by fibroblasts include collagen fibers, a polypeptide, reticular fibers, and elastic fibers, a polypeptide with some glycosaminoglycans.
- Ground substance is composed of proteoglycans and glycoproteins. While proteoglycans exhibit a protein backbone to which a large number of glycosaminoglycan chains are attached, a glycoprotein contain only a fewer carbohydrate moieties associated.
- **Epithelial tissues and derivatives:** Salivary glands elaborate mucin which is a glycosaminoglycan which is acidic and is attributed to glucuronic acid, sulfate or sialic acid
- **Enzymes** like phophatases, oxidases and dehydrogenases can be studied in the tissues.

HISTOCHEMICAL TECHNIQUES

Fixation Procedures

For histochemical study, a tissue block must be preserved in such a way that it causes minimal changes in the reactivity of the cytoplasmic and extracellular macromolecules, for example, enzymes, structural proteins, protein–carbohydrate complexes, lipids, and nucleic acids. This is *accomplished by using optimum osmotic conditions, cold temperatures, controlled pH of the fixing solutions, and the minimum possible exposure to the fixative.*

Formaldehyde *is considered to be one of the ideal fixatives, especially for enzymes and other proteins.* This is because of its ability to react with major reactive groups of proteins to form polymeric or macromolecular networks, without affecting their native reactivity to histochemical procedures. Formaldehyde has a preservative effect on lipids by altering their relationship with the proteins. Use of electrolytes such as calcium or cadmium in formaldehyde or chromation of tissue blocks subsequent to fixation prevents dissolution of phospholipids. Formaldehyde is generally used as a 10% solution buffered to pH 7 at cold temperatures in the range of 0 to 4°C.

Acrolein and ***glutaraldehyde*** are other frequently used aldehydes, with the latter being routinely used for electron microscopy. Conjunctive use of colloids such as sucrose, ficoll, polyvinylpyrrolidone, and dextrans in the fixing solutions is often made to prevent osmotic rupture of cell organelles. This helps to improve the in situ localization of the histochemical reactions.

Other fixatives used for the study of glycogen, glycoproteins, proteoglycans, and nucleic acids are frequently mixtures of many chemical ingredients. ***Rossman's fluid***, used for visualization of glycogen, glycoproteins, and proteoglycans, contains formaldehyde, alcohol, picric acid, and acetic acid. ***Carnoy's mixture***, used for histochemical staining of nucleic acids, is composed of ethyl alcohol, acetic acid, and chloroform. Alcohol denatures proteins without causing irreversible chemical changes in the active groups but, being a poor fixative, is used in combination with acetic acid and chloroform. Feulgen's reaction, used for visualizing DNA, requires acid hydrolysis of the DNA polymers to expose the deoxyribose sugar residues of DNA molecules. The aldehyde groups thus exposed (on the deoxyribose sugar residues) are then chemically reacted with leucofuchsin (Schiff's reagent) to form a reddish purple reaction product.

The rationale of secondary fixation (postfixation) of the tissue is that the primary fixation being not effective has to be supplemented by another fixative to yield better staining quality. The primary fixative has to allow the penetration of the secondary fixative and also make the tissue withstand this process. The term, *postfixation*, is specifically used when secondary fixation is done on lipid rich tissues and more appropriately for freeze-dried tissues.

Tissue post-fixed with ***imidazole-buffered osmium tetrachloride*** allows localization of lipids rich in unsaturated fatty acids. Cold methanol fixation alone or cold acetone extracts cellular phospholipids and total cellular lipids respectively. Fixation with ***paraformaldehyde*** is preferred study of lipids. Study of ultrastructural elements should optimally preserve the cell membranes integrity and the surface antigens. In such techniques ***postfixation with urany lacetate*** preserves membrane phospholipids and dehydration with acetone minimizes the extraction of phospholipids.

Nakan's ***periodate-lysine-paraformaldehyde*** and ***periodate-lysine-glutaraldehyde*** are found to be superior fixatives to the classical glutaraldehyde/paraformaldehyde double

fixation procedure for cerium-, lead-based techniques of enzyme demonstration.

Some enzyme systems such as cytochrome oxidases are highly labile and therefore cannot be preserved by chemical fixation. Visualization of such enzymes is performed on ***fresh frozen*** (cryostat) sections. However, to prevent diffusion and to preserve the in vivo status of the tissue macromolecules, one must fix the tissue blocks by a ***freeze-drying*** procedure. Tissues are frozen rapidly at very low temperatures, usually in liquid nitrogen, and then placed in a refrigerated vacuum chamber where ice formed in the tissues, is removed by sublimation, that is, by direct transformation into vapor without going through a liquid phase. After dehydration in vacuum, tissue blocks are embedded in paraffin and sectioned routinely with a microtome. Freeze-dried tissues exhibit optimal enzyme activity, show excellent histologic characteristics, and do not show any shrinkage artifacts that are seen with routine fixation. Besides oxidative enzymes, freeze-drying is used for visualization of other enzyme systems, for example, phosphatases and dehydrogenases, and also for the precise localization of otherwise diffusible inorganic ions.

New methods are developed to demonstrate enzymes better histochemically, ***freeze-dried tissues were embedded without fixation at lower temperature [4 to –20°C] in glycol methacrylate resin***. Enzymes like oxidoreductases, esterases, peptidases, and phosphatases except glucose-6-phosphatase were demonstrated better. *The freeze-substituted specimens in acetone were embedded at low temperature in glycol methacrylate resin* for successful demonstration of oxidoreductases and hydrolases. These methods were accurate in localizing the enzymes, the preservation of enzyme activity and tissue morphology.

Techniques of ***freeze-fracture*** and ***freeze-etching*** have been devised for use in electron microscope to avoid use of chemicals in tissue preparation. This technique has enabled biologists to obtain excellent three-dimensional images of the surfaces of various cell membranes not previously observed.

Histochemical study of ***teeth*** *and* ***bone*** *requires careful fixation and controlled decalcification procedures.* Simultaneous fixation and decalcification with ***formaldehyde*** or ***glutaraldehyde*** and ethylenediaminetetraacetic acid (EDTA) have been successfully used in the study of teeth and bone for light and electron microscopic histochemistry. Decalcified ground sections have also been used in histochemical studies of teeth and bone. *Techniques have been developed for sectioning* ***freeze-dried****, undecalcified tissues.* Gray and Opdyke have described a saw for the preparation of 10 to 50 mm sections of undecalcified tissues. Such sectioning has been used for histochemical studies of dental decay. Study of bone has been considerably enhanced by the process of deorganification (deproteinization). Swedlow and colleagues and Kapur and Russell have studied bone architecture with SEM to great advantage after deorganification with concentrated hydrazine.

Specific Histochemical Methods

Histochemical techniques primarily used in the study of oral tissues may be categorized as: (1) glycogen, glycoprotein, and proteoglycan methods; (2) protein and lipid methods; and (3) enzyme methods. They are all characterized by a direct staining reaction or by the formation of an insoluble dye or precipitate at the reactive sites.

Techniques used:

1. Chemical fixatives
   - Used singly — Formaldehyde, acrolein, glutaraldehyde (Electron microscopy)
   - Used as a mixture — Rossman's fluid (Glycogen, glycoproteins, proteoglycans); Carnoy's fluid (Nucleic acids)
   - Used after primary agent (Post-fixation) — Imidazole-buffered osmium tetraoxide (lipid rich tissues)
2. Fresh frozen sections (Enzymes)
3. Freeze — drying — Liquid nitrogen (Optimum enzyme demonstration)

Glycogen, glycoprotein, and proteoglycan

The best known and most frequently used technique for detection of ***carbohydrate*** *groupings is the* ***periodic acid-Schiff (PAS)*** *technique.* The chemical basis of this method lies in the fact that periodic acid oxidizes the glycol groups to aldehydes and these in turn are revealed as a reddish-purple dye product on treatment with leucofuchsin (Schiff reagent). Use of fluorescent reagent anthracene-9-carboxyaldehyde carbohydrazone as a substitute for Schiff reagent has been made by Cotelli and Livingston. Treatment of tissue sections with amylase prior to oxidation removes glycogen from the tissues, and this is reflected in a reduced Schiff reaction product. A comparison of the amylase-digested and undigested sections is used in estimating the amounts of glycogen or other carbohydrate–protein molecules. Electron microscopic visualization of carbohydrates has been achieved, among other techniques, by use of phosphotungstic acid and lead citrate after oxidation with periodic acid. The periodate-thiocarbohydrazide-silver proteinate method of Theiry shows high specificity for glycol containing glycoconjugates at the electron microscopic level.

Proteoglycans *are well demonstrated by thiazine dyes such as* ***toluidine blue, azure A, and Alcian blue.*** Toluidine blue produces a metachromatic reaction ranging from a purple to a red reaction product. This change of color (metachromasia) from the original (orthochromatic) blue color of the monomeric form of toluidine blue reflects the extent of polymerization of the dye molecules as they tag onto the anionic residues on the glycosaminoglycan molecule. Thus heparin present in the mast cell granules and chondroitin sulfates present in the intercellular ground substance of the cartilage or developing bone give an intense red metachromasia, demonstrating the highly acidic or sulfated nature of these proteoglycans. Alcian blue staining has been used to considerable advantage in characterizing the specific types of acid-radicals present within proteoglycans. When used at pH 2 to 2.8, Alcian blue stains weakly acid-sulfated proteoglycans. However, when it is used at pH 1 to 1.2, Alcian blue binds to highly sulfated proteoglycans. By incubating tissue sections in the enzyme sialidase prior to staining with Alcian blue, distinction can be made between sialidase-resistant and sialidase-nonresistant molecules.

Several techniques are available for the localization of proteoglycans or sulfated glycoconjugates at the electron microscopic level. The ***high-iron diamine thiocarbohydrazide-silver proteinate*** (HID-TCH-SP) method of Spicer provides high specificity for sulfated glycoconjugates. This method excludes reaction with carboxyl and phosphate groups.

Several cationic dyes, including ruthenium red, silver tetraphenylporphine sulfonate, Alcian blue, bismuth nitrate, and cuprolinic blue, have been used successfully by several investigators in localizing proteoglycans in oral tissue. ***Ruthenium hexamine trichloride*** is used in detecting the anionic groups and hence can demonstrate the presence of proteoglycans and/or sialoglycoconjugates and glycolipids or phospholipids present on the plasma membrane. ***Cuprolinic acid with magnesium chloride at critical electrolyte concentration*** allows a larger surface representation of proteoglycans than other methods. This method is being used in the studies of predentin and dentin. The smaller granules stained by this cationic dye seem to increase in size and become more electron-dense with increasing the concentration of the electrolyte.

A new histochemical method is now available for staining hyaluronic acid using a ***biotinylated hyaluronic acid-binding complex***, prepared by extraction from cartilage proteoglycans, as a probe. Subsequent to incubation of the formaldehyde-fixed tissue sections with this probe, sections are incubated in peroxidase-conjugated streptavidin; then this complex is visualized by its binding to 3-amino-9-ethylcarbazole, which acts as a peroxidase substrate.

Specific ***plant lectins*** have been used in the identification or characterization of specific carbohydrate moieties within a glycoprotein molecule in the study of carbohydrate histochemistry. Fluorescein dyes or horseradish peroxidase techniques are used as tags for the visualization of lectin binding sites on carbohydrate moieties within a glycoprotein molecule. Electron microscopic localization of sugar moieties in the glycoprotein molecules has been made by using ferritin and horseradish peroxidase as lectin tags.

Proteins and lipids

Histochemistry of proteins is based on classic reactions of protein chemistry involving various amino acid groups, that is, ***amino, imino, carboxyl, disulfide, and sulfhydryl groups***. Reagents such as dinitrofluorobenzene, ninhydrin, or ferric ferricyanide are utilized to give insoluble colored reaction products.

Histochemical study of lipids frequently implies use of ***frozen or freeze-dried*** sections. Total lipids are studied by using fat colorant dyes such as Sudan dyes. Chromation of formol-calcium-fixed tissues and their subsequent staining with ***Sudan black*** has been used for the identification of phospholipids. Extraction procedures with various lipid solvents are considered essential to accompany most histochemical staining procedures for lipids.

Iodoplatinate reactions are used in ultrahistochemical demonstration of phospholipids. Iodoplatinate does not react with amino acids and phosphoproteins. Treatment of tissues with acetone does not alter the staining pattern with iodoplatinate while chloroform/methanol or phospholipase-C treatment abolishes or reduces the staining. ***Malachite green aldehyde*** stains also phosphatidyl serine and sphingomyelin. Both iodoplatinate and malachite green aldehyde have been used in the oral tissues to see the role of phospholipids in the mineralization and to see its association with proteoglycans.

Enzymes

The enzyme techniques utilize many different principles. Some of the criteria used in deciding the application of a technique are related to *avoidance of inhibition by the substrate and insolubility of the primary reaction product and its immediate coupling to the capture reagent to prevent diffusion and false localization of enzyme activity.*

Phosphatases

The ***Gomori method*** for phosphatases uses phosphoric esters of glycerol, glucose, or adenosine. The enzymatically liberated phosphate ion is converted into an insoluble salt, which can be visualized by polarized light or phase contrast, or the salt can be transformed into a cobalt or lead compound, which is black. Riboflavin 5'-phosphate has been used as substrate, which at the site of phosphatase activity results in the formation of a fluorescent precipitate. Electron microscopic demonstration of phosphatase is also based, with some modification, on Gomori's original method of metal substitution. A technique using ruthenium red has demonstrated acid phosphatase in electron microscopic studies.

Another procedure used for demonstration of phosphatases is the ***simultaneously coupling azo dye technique***. It uses a naphthol phosphate or other type of ester. The enzymatically released naphthol is coupled in situ with a diazonium salt to form an insoluble colored reaction product. With regard to the original Gomori glycerophosphate technique, it has been shown that the calcium phosphate formed may diffuse and give false localization. Because of this and other considerations in calcified tissues, the azo dye techniques are better suited for the study of phosphatases in teeth and bones. Sophisticated substrates for use with azo dye techniques have been developed. They facilitate precise microscopic localization of alkaline and acid ***phosphatases*** as well as ***esterases***. ***Aminopeptidases*** can also be detected by an azo dye method.

Several shortcomings were faced by the use of lead as capturing agent in Gomori method. These were overcome by the use of lanthanides like cerium and other elements in the family like gadolinium, didymium, praseodymium, and neodymium proved useful in phosphatase demonstration. The reaction product obtained was uniform and consistently reproducible when cerium is used. Furthermore, no nonspecific deposits as encountered in lead-based reactions were encountered. Cerium was found to be a better capturing agent than lead for inorganic phosphates.

The ***cerium-based*** methods work well with both plastic and cryostat sections. The methods use cerium chloride to convert calcium phosphate into cerium phosphate following which it is converted into cerium phosphate. Several techniques have been formulated to transform calcium phosphate into a visible substance which can

be appropriately visualized. The following methods have been used in localizing non-specific alkaline and acid phosphatase, adenosine triphosphatase, myosin adenosine triphosphatase, glucose-6-phosphatase, and 5′-nucleotidase with cerium as capturing agents. In cerium perhydroxide-diaminobenzidine (***Ce-H_2O_2-DAB***) procedure cerium phosphate is converted into cerium peroxide with hydrogen peroxide which in turn decomposes into cerium hydroxide and oxygen radicals. These radicals oxidize diaminobenzidine to DAB brown. Addition of nickel ions generates a bluish black precipitate (***Ce-H_2O_2-DABNi***). In comparison to the classical metal precipitation, azo, azoidoxyl and tetrazolium procedures these methods offer superior results in both catalytic phosphatase histochemistry and immunohistochemistry while using nonspecific alkaline phosphatase as an enzyme label.

A new method is available to stain semi-thin and ultra-thin resin sections wherein the primary reaction product cerium phosphate is amplified in cerium citrate solution and then subsequently oxidized with H_2O_2 containing DAB-nickel medium. This works on perhydroxide induced DAB polymerization principle (***Ce/Ce-H_2O_2-DABNi***). Post-treatment with osmium tetroxide increases the contrast through formation of osmium black, while silver intensification facilitates usage in ultra-thin sections.

A two-step method uses DAB medium consisting of DAB, Ni-sulfate, methanol, and H_2O_2 in acetate buffer at pH 5.2. This modified medium can be used for both Ce-H_2O_2-DABNi, Ce/Ce-H_2O_2-DABNi methods.

Double capture technique using calcium and cerium has also been designed where para-phenylenediamine/pyrocatechol solution is used instead of DAB/DAD-Ni (***Ca-Ce-H_2O_2-PPD/PC***).

New fluorescence-based histochemical method for localizing alkaline phosphate activity has been developed. In these techniques, calcium binding ***fluorochromes like calcein, calcein blue and xylenol orange*** were used in Gomori type reactions and is performed in freeze-dried, resin embedded tissues. Green, blue, red fluorescence is obtained with calcein, calcein blue and xylenol orange respectively.

A yet another fluorogenic substrate identified, successfully localizes endogenous alkaline phosphatase activity and is being used in standard alkaline phosphatase immunohistochemical techniques to visualize a number of antibodies. The substrate is ***2-(5′-Chloro-2′-Phosphoryloxyphenyl)-6-Chloro-4-3[H]-quinazolinone (ELF-97)***. This has been used successfully in identifying the alkaline phosphate activity in tissues, cultured cells and in fixed cells by flow cytometry. It was also noticed that this substrate is more phosphatable than fluorescein-labeled secondary reagents, thus allowing more time to examine the tissue. The advantage of using the enzyme labeled fluorescence is that the omission signals can be easily distinguished from the background signals of autofluorescent tissues.

A method that enables histochemical localization of ***alkaline phosphatase in decalcified, paraffin-embedded bone and cartilage*** has been formulated by Dengshun Miao and Andrew Scutt. Periodate-lysine-paraformaldehyde gives not only optimal cell surface antigenic preservation of enzyme activity and tissue architecture unlike neutral buffered formalin which destroys alkaline phosphatase activity. Decalcification is performed with EDTA-G solution following which the tissue is embedded in paraffin. The paraffin embedding is a significant advantage over frozen or plastic embedded samples because they can be used in localizing many other antigens, RNA and DNA. Such a development means that these can be co-localized with alkaline phosphatase. Preincubation with 1% $MgCl_2$ in 100 mm Tris-maleate buffer was performed which otherwise results in total lack of staining for alkaline phosphatase. This method is highly reproducible and can be performed on large number of blocks in conventional histology laboratories.

Immunohistochemistry

Precise localization of specific biologic molecules in different intracellular compartments, on cell surfaces, or in extracellular matrices is made possible by the application of some basic principles of immunochemistry. The immunohistochemical techniques are based on the premise that protein-based antigens or immunogens bind avidly to their specific antibodies. Antibodies to specific antigens can be prepared by injecting the known antigen into an animal to provoke an immune response. This response results in the production of antibody immunoglobins, and these can be isolated from the serum of the injected animal.

When a solution containing an antibody or an antiserum is directly applied to a tissue section containing the antigen, the antibody binds specifically to that antigen. This antigen–antibody complex is subsequently attached to a second antibody, which is conjugated either to a fluorescent dye, such as rhodamine or fluorescein isothiocyanate (FITC) or to an enzyme-conjugated to its antibody, such as peroxidase-antiperoxidase (PAP). The antigen–antibody complexes bound to a fluorescent dye are examined in a fluorescence microscope. The antigenic sites fluoresce against a dark background and are immediately photographed on high speed film. The enzyme-bound antigen–antibody complexes are further developed histochemically by exposure to an enzyme

- **Carbohydrate groups:** Periodic acid-Schiff stain
  - Proteoglycans: Toluidine blue, alcian blue, azure A
  - High-iron diamine thiocarbazide: Sulfated glycoconjugates (electron microscopic demonstration)
  - Proteoglycans or sialoconjugates, glycolipids or phospholipids: Ruthenium hexamine trichloride
  - Surface proteoglycans: Cuprolinic acid with magnesium chloride
  - Biotinylated hyaluronic acid- binding complex
  - Lectins
- **Proteins:** Dinitrofluorobenzene, ninhydrin, or ferric ferricyanide
- **Lipids:** Sudan black (Use frozen tissues or freeze-tried sections), Iodoplatinate reactions (for electron microscopic demonstration)
- **Enzymes:** Phosphatases - Gomori method (Lead or cobalt based compound); Simultaneously coupling azo-dye (Diazonium based compound) technique (Phosphatases in teeth); Cerium based methods; Calcium binding fluorochrome like calcein, calcein-blue for fluorescent microscopy; Cerium based compound bound to nickel for electron microscopy

substrate. This results in the development of a dark brown to a black color, which allows examination of the antigenic sites by light or electron microscope. Ferritin and colloidal gold are also frequently used as heavy metal markers for the antigen–antibody complexes because of their electron density and also because of their specific particle size.

HISTOCHEMISTRY OF ORAL HARD TISSUES

Carbohydrates and Protein

The PAS method is used more than any other in studying the ground substance of teeth and bones. Under specific conditions, this method is believed to demonstrate the carbohydrate moiety as well as the glycoprotein complexes. The ground substance of normal mature bone and dentin exhibits little or no reactivity with the PAS technique (Fig. 18.6). However, *developing or resorbing bone and dentin stain intensely with PAS.* Newly formed bone and dentin are also rich in PAS-reactive carbohydrates. Proteoglycans have been visualized in predentin in dentin with Alcian blue, bismuth nitrate or using Spicer's high-iron diamine method. The precise location may be obtained by adding cationic dyes like cuprolinic blue, ruthenium hexamine trichloride or any cationic detergent like cetylpyridinium chloride to the fixative. Cuprolinic chloride demonstrated intercellular proteoglycans in predentin as granules and as filaments. Non-aggregating proteoglycans were also observed in the spaces between collagen fibers as an amorphous substance.

The predentin contained a high proportion of dermatan sulfates, with hyaluronan and chondroitin sulfates additionally being identified. The mineralizing zone dentin matrix, i.e. the predentin/dentin interface is rich in chondroitin sulfate with decrease in dermatan sulfate and hyaluronan. The chondroitin sulfates have been recognized as decorin and biglycan. Keratan sulfates like lumican and fibromodulin have been also localized in predentin. Sulfated glycoconjugates accumulate in predentin and are either removed or masked to staining in the dentin. A four fold increase in sulfation was identified in GAGs of predentin/dentin interface compared to that of predentin and dentin fractions. Antibodies recognizing these leucine-rich proteoglycans have shown a decreasing gradient of intensity from pulpal aspect towards the mineralizing front for chondroitin sulfates while the opposite is true for keratan sulfates.

Interglobular, less-calcified dentin exhibits a distinct PAS reaction (Fig. 18.6) *as does abnormally and poorly calcified dentin matrix in dentinogenesis imperfecta and in odontomas.*

Enamel matrix is essentially nonreactive with the PAS method. However, *enamel lamellae* are intensely stained in ground sections (Fig. 18.7). In some areas the rod interprismatic substance exhibits some reactivity. Use of HIDTCH-SP staining, combined with use of testicular hyaluronidase, heparinitase, and chondroitinase ABC, has revealed that sulfated glycoconjugates present in Golgi vesicles of ameloblasts and on the surface layer of developing enamel matrix do not contain heparan sulfate,

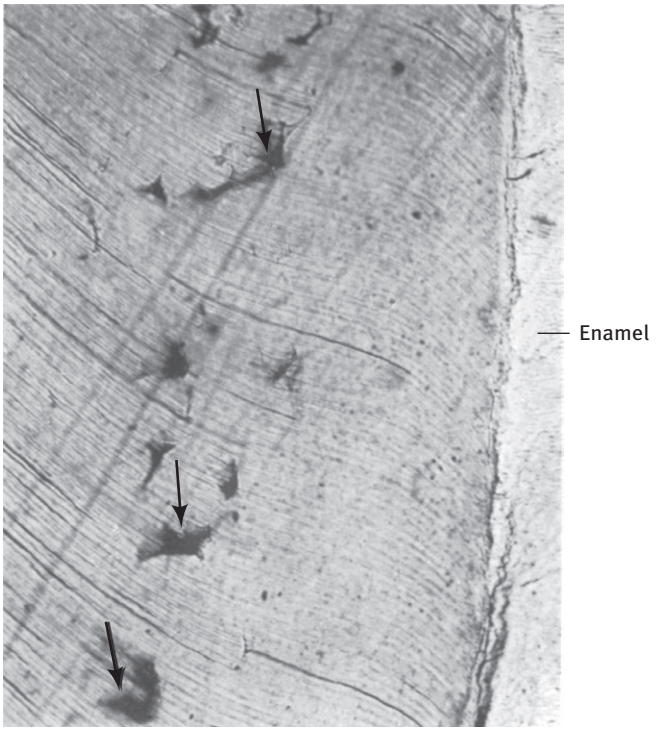

Figure 18.6 Ground section of human tooth showing PAS reactivity of interglobular dentin (arrows) (×143).

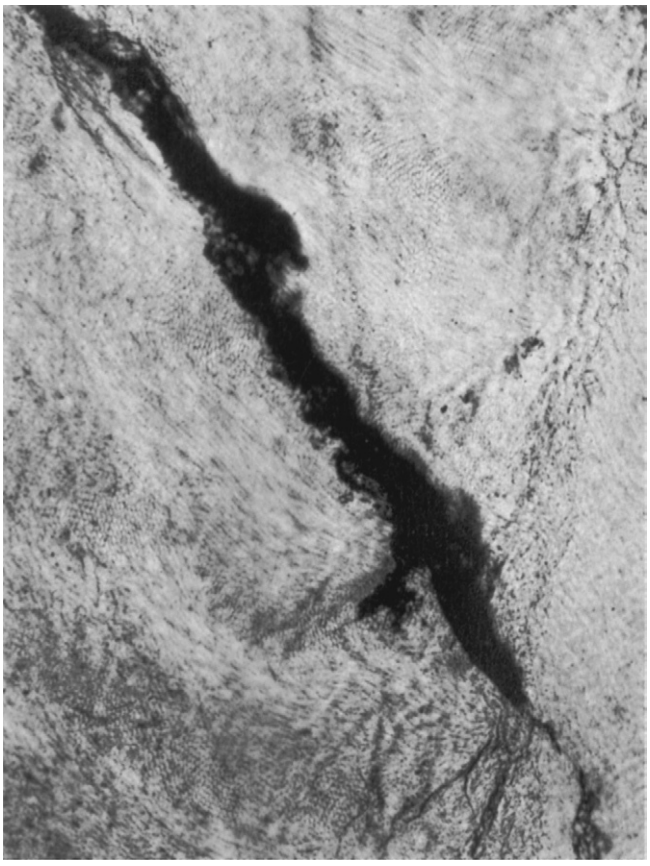

Figure 18.7 Ground section of human enamel. Lamella stains with PAS method (×143).

chondroitin sulfate, and dermatan sulfate, in contradistinction to their presence in the predentin matrix and odontoblasts.

Cementum shows both chondroitin sulfates and keratan sulfates (monoclonal antibodies were used). The keratan sulfates were mainly localized in the mineral unbound matrices. *The chondroitin sulfates were identified as decorin, biglycan and versican while the keratan sulfates were recognized as lumican and fibromodulin.* The chondroitin sulfates were located almost exclusively in nonmineralized portions of the cementum such as precementum and pericementocyte area. Biglycan was localized in the cemental matrix and in the incremental lines of cellular cementum, decorin associated with collagen fibers in the periodontal ligament fibers while versican was found within the lacunae housing cementocytes and osteocytes.

Rat molars subjected to immunohistochemistry have shown intense fibromodulin staining in the extracellular matrix where periodontal fibers insert into the alveolar bone and cementum, the staining intensity increases with the maturation of the fibers.

The chondroitin sulfate was identified as the major glycosaminoglycans species of bovine dental pulp. The dental pulp contains hyaluronan. Versican was found distributed in peripheral areas just below the odontoblastic layer. Versican binds to hyaluronan through a link protein. This complex forms large hydrated proteoglycan aggregates that fill the extracellular space, support odontoblasts, and/or facilitate the transport function of metabolites and nutrients within the tissue.

Specific protein methods identify certain amino acids or their groupings, that is, amino, carboxyl, or sulfhydryl. Only a few of these techniques have been applied in the study of teeth and bone. Of interest are the ***dinitrofluorobenzene (DNFB) and ninhydrin-Schiff methods.*** The DNFB reagent combines with α-amino groups of proteins in tissue sections to form a pale yellow complex. An intense reddish color is subsequently revealed by a reduction and diazotization technique, which results in the formation of an azo dye. The pattern of staining is essentially the same as seen with the PAS method in both normal and abnormal dentin. A modification of DNFB method wherein the final reaction product is a mercaptide of lead or silver has been used for electron microscopic histochemistry of amino groups. The ninhydrin-Schiff method is dependent on the formation of imino groups that decompose to a keto acid to form aldehyde groups, and these are reacted with leucofuchsin (Schiff reagent) to form a final red-colored reaction product. Some of these techniques have been applied to the study of *dental caries in bone and in dentin resorption.*

Histochemical reactions imply a need for some specific protein groups to initiate the *mineralization of predentin and osteoid.* The osteoid consists of the type X collagen, partially broken down proteoglycans and noncollagenous proteins like bone sialoprotein and osteopontin all of which provide the microenvironment required for mineral deposition. Similarly, dentinal matrix consists of type I collagen, non-collagenous proteins like phosphophoryn, dentin sialoprotein, osteocalcin, bone sialoprotein and dentin matrix protein. Everett and Miller have noted the absence of carboxyl and amino complexes in predentin and osteoid in contradistinction to the presence of these complexes in dentin and bone. Sulfhydryl groups are present optimally at the mineralizing front in predentin and osteoid while being present minimally in the mineralized regions of these tissues.

Immature, *newly formed enamel* in rats shows histochemical staining for sulfhydryls and tyrosine residues characteristic of keratin. However, these protein residues are not demonstrable in the mature enamel.

In the last few years, three major classes of calcium-binding proteins have been identified in dentin and developing enamel. Phosphophoryn, a 60–70 KD acidic phosphoprotein, has been identified by immunochemical and immunohistochemical methods in actively synthesizing odontoblasts and in preameloblasts and in dentin, particularly in the intertubular dentin. It has high affinity for calcium and type I collagen. Phosphophoryn is localized in the gap regions of collagen and thus initiates apatite crystal formation by binding with calcium in conformation that promotes this process. Phosphophoryn at the same time aggregates collagen fibrils at the mineralizing front. Molecular modeling has further demonstrated that the spacing of carboxyl and phosphate groups present might be essential for dictating crystal orientation relative to collagen substrate. Because of its chemical properties, phosphophoryn is considered to play a significant role in regulating the ordered deposition of hydroxyapatite crystals within the preformed dentinal matrix.

Amelogenins and enamelins, two major classes of calcium binding proteins, are now known. This is made possible through the use of chemical and immunohistochemical techniques using monoclonal antibodies. Amelogenins secreted by the ameloblasts are relatively low-molecular weight proteins and are of predominant proteins in the developing enamel matrix. During enamel maturation they undergo degradation and disappear at a much faster rate than enamelins do. Enamelins have very high affinity for binding apatite crystals and remain until late stages of enamel maturation.

Several immunohistochemical studies mapping distribution of basement membrane components such as *Type IV collagen, laminin, proteoglycans, and fibronectin during tooth development have shown close association of these proteins with different stages of cell differentiation and matrix secretion* (Figs 18.8, 18.9). Disappearance of type III collagen and confinement of fibronectin surrounding preodontoblasts to the epitheliomesenchymal junction after the polarization of odontoblasts during tooth development are other interesting observations emphasizing the significance of these proteins in the regulation of the complex developmental changes occurring in the tooth anlage.

Lipids

Biochemical studies indicate rather low lipid content in the organic matrix of dentin. Lipids have been demonstrated by the sudanophilic reaction in the *odontoblast processes and enamel rod sheaths.* Sudanophilia is based on the solubility of Sudan dyes with lipids of varied description. Sudanophilia is widespread in the developing tooth, being present in the *zone of mineralization and predentin and in the basal zone of the ameloblasts.* Ultrahistochemical studies have shown presence of phospholipids in the cell

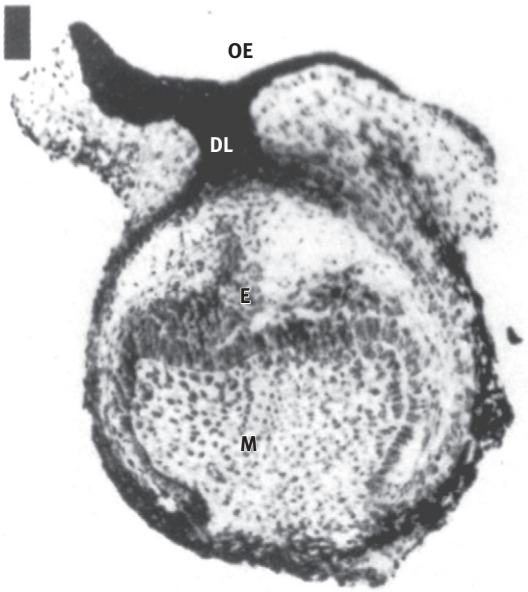

Figure 18.8 Light micrograph of mandibular first molar of day 16 mouse embryo stained with periodic acid-Schiff (PAS). This section corresponds to sections shown in Figure 18.9. Notice epithelial enamel organ (E) surrounds a condensation of dental papilla mesenchyme (M). Dental lamina (DL) connects tooth germ to oral epithelium (OE) (From Thesleff I, Barrach HJ, Foidart JM, et al: Dev Biol 81:182, 1981).

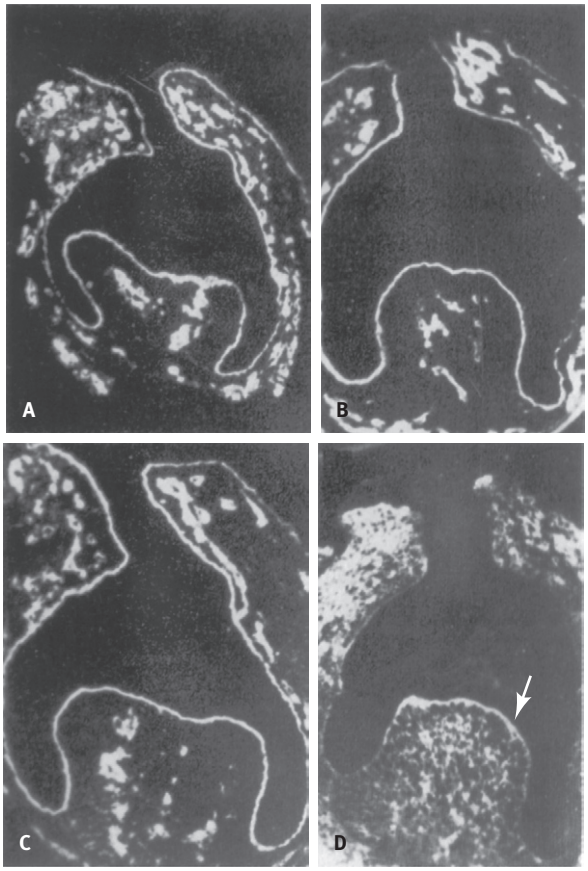

Figure 18.9 Sections of day 16 embryonic molars stained immunohistochemically with, (**A**) antibody to type IV collagen; (**B**) antibody to laminin; (**C**) antibody to basement membrane proteoglycan; and (**D**) with antiserum to fibronectin (see Fig. 18.8 for reference). Linear deposits are seen in oral and dental basement membranes and in walls of blood vessels. Distributions of type IV collagen, laminin, and basement membrane proteoglycan appear identical. Immunofluorescence of fibronectin is particularly intense in dental basement membrane (arrow) and is prominent in mesenchyme of dental papilla. No stain is observed in enamel organ epithelial cells (From Thesleff I, Barrach HJ, Foidart JM, et al: Dev Biol 81:182, 1981).

membrane and the extracellular matrix during tooth development. A modest increase in cell membrane phospholipids (phosphatidylinositol, phosphatidylcholine) occur due to the lengthening of cell. The matrix associated phospholipids include phosphatidylethanolamine, phosphatidylserine and sphingomyelin. Initial phospholipids in dentin matrix are due to intercellular diffusion from blood. Phospholipids are identified in the spaces located between the collagen fibers in predentin and as needle-like structures along side of individual or group of mineralizing collagen fibers. Cell and membrane associated labeling (phospholipase A2-gold) decreased gradually, whereas the extracellular matrix incorporation was stable. Histochemical data proves the close association of phospholipids and proteoglycans. These reactive zones of the predentin and ameloblasts imply a role of phospholipids in the process of mineralization of dentin and enamel matrices.

Enzyme Histochemistry of Hard Tissue

Histochemical techniques are extremely useful in demonstrating specific enzymes in specific cellular and intercellular locations in bone and teeth.

Alkaline phosphatase

Alkaline phosphatase is capable of hydrolyzing phosphoric acid esters. In hard tissues alkaline phosphatase has been implicated in the process of mineralization. However, some studies have raised doubts concerning this assumption and instead suggest that the enzyme is involved in the synthesis of organic matrix only.

Alkaline phosphatase is observed to be associated with osteogenesis and dentinogenesis (Figs 18.10 and 18.11). The *osteoblasts and odontoblasts give an intense staining reaction for the enzyme* (Figs 18.11, 18.12). No enzyme activity is found in calcified bone or dentin matrices per se, except in close association with the matrix-synthesizing cells. At sites of intramembranous bone development, alkaline phosphatase activity is observed in preosteoblasts, osteoblasts, endosteal cells, subperiosteal cells, and in some newly embedded osteocytes (Table 18.1). The osteoblasts and preosteoblasts exhibit alkaline phosphatase activity which is often used as a cytochemical marker to differentiate fibroblasts from preosteoblasts. Wergedal and Baylink report no enzyme activity at the actual calcification sites. However, conflicting views are reported about endochondral bone formation. The enzyme activity is seen in chondrocytes and cartilage matrix, with the activity being weakest in the proliferative zone, higher in the maturative zone, and the highest in the hypertrophic zone. No

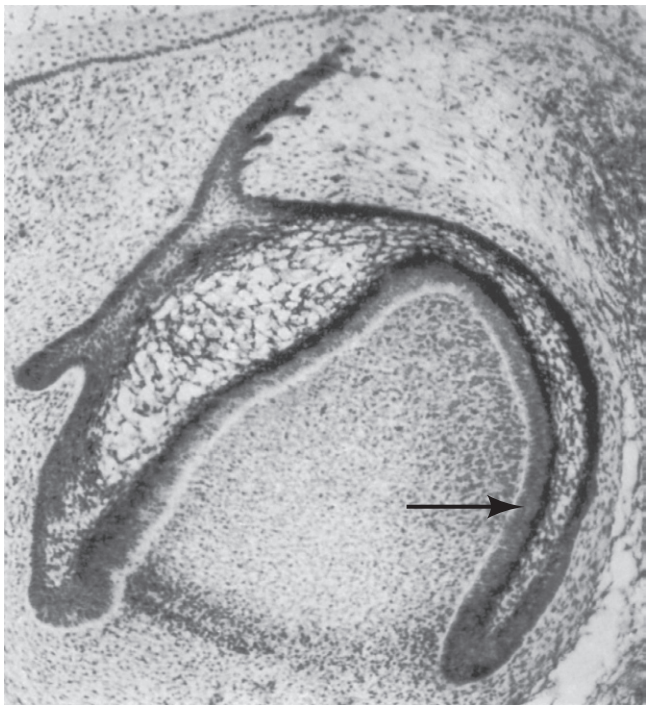

Figure 18.10 Alkaline phosphatase reaction of tooth of monkey embryo. Ameloblastic layer (arrow) is nonreactive (×87).

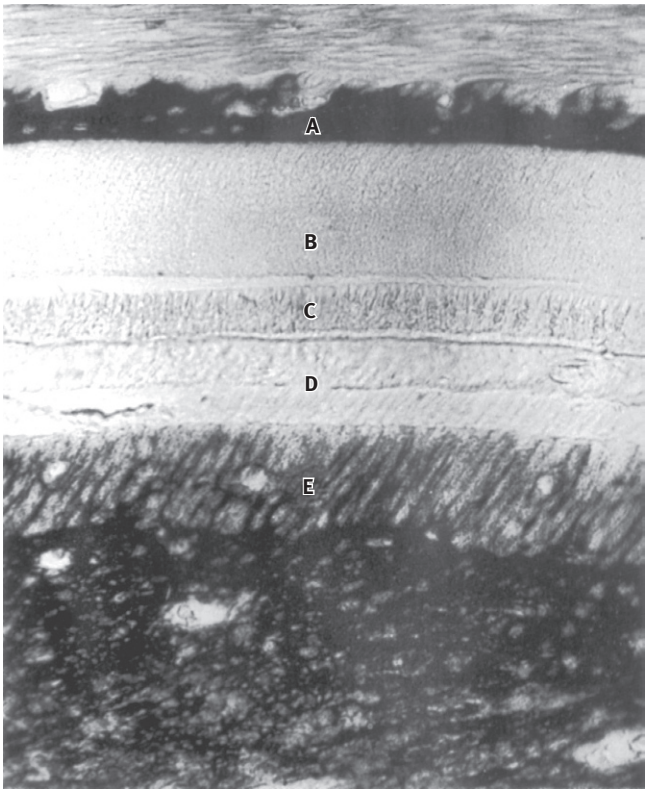

Figure 18.11 Freeze-dried undecalcified incisor of hamster. (A) Stratum intermedium; (B) ameloblasts; (C) enamel matrix; (D) dentin matrix; (E) odontoblasts. Note alkaline phosphatase reactivity of Korff's fibers and subjacent pulp (×87).

activity is detected in the resting chondrocytes and in the matrix of reserve zone. The view that alkaline phosphatase is involved in actual calcification is also strengthened by observations in vitamin D treatment of human rickets wherein the increase in the calcification zone parallels an increase in serum alkaline phosphatase.

*In the developing molar and incisor teeth, alkaline phosphatase is present in the stratum intermedium, the odontoblasts (*Fig. 18.11*) and subjacent Korff's fibers, and the ground substance.* No activity is observed in the ameloblasts (Fig. 18.11). However, in the incisors of rodents enzyme activity is present in the ameloblasts and the reduced enamel organ at the growing end of the tooth (Table 18.1), with the remaining ameloblasts of the incisor being unreactive, as in the molars. The existence of alkaline phosphatase in the dentin proper has been reported.

Adenosine triphosphatase

A Ca^{++}- and Mg^{++}-dependent adenosine triphosphatase (ATPase) has been *localized at the distal and lateral cell membranes of the ruffle-ended and late transitional (preabsorptive) ameloblasts.* In comparison, *early transitional or smooth-ended ameloblasts show ATPase distribution along the basolateral membranes* but not in the distal region. Such distribution of this enzyme is significant since it is implied to control the access of calcium to the enamel mineralizing the front.

Acid phosphatase

Acid phosphatase is less widely distributed than its alkaline counterpart (Table 18.1). Histochemical localization of intracellular acid phosphatase is generally more discrete than that of alkaline phosphatase because it is localized mainly in specific membrane-bound organelles, the lysosomes. Histochemically two distinct types of acid

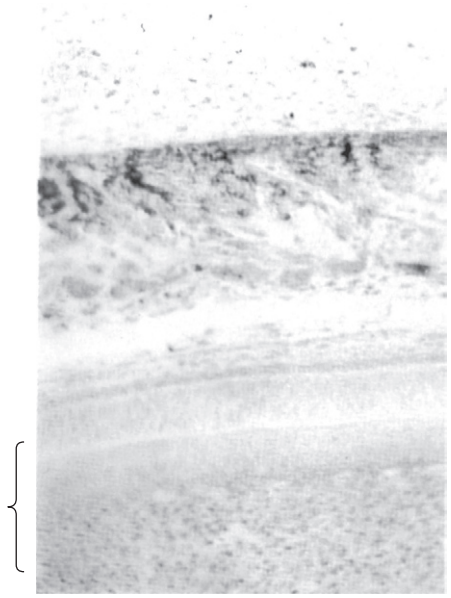

Figure 18.12 Alkaline phosphatase (dark areas) in osteoblasts and acid phosphatase (dark areas) reaction in osteoclasts in resorbing bone adjacent to incisor tooth (brace) in 3-day-old hamster (MX naphthol phosphate and red-violet LB salt incubation for alkaline phosphatase; GR naphthol phosphate and blue BBN salt incubation for acid phosphatase, ×143) (From Burstone MS: In Sognnaes RF, editor: Calcification in biological systems. Washington, DC, 1960, American Association for the Advancement of Science Publications, p 64).

Table 18.1 Enzyme Activity of Cells Associated with Bones and Teeth*

	Alkaline Phosphatase†	Acid Phosphatase†	Amino-peptidase‡	Cytochrome Oxidase‡	Succinic Dehydrogenase‡
Bone					
Osteoblasts	++	0	+	+	+
Osteocytes	++	0	+	+	+
Osteoclasts	0	++	?	++	++
Cartilage					
Active chondrocyte	++	0	++	++	++
Resting chondrocyte	0	0	+	+	+
Hypertrophic chondrocyte	++	0	+	+	+
Tooth					
Stellate reticulum	++	0	+	+	+
Stratum intermedium	++	0	+	+	+
Ameloblasts (molar)	0	0	0	0 or +	0
Odontoblasts	+ or ++	0	+	+	+

*0, No staining; +, less active; ++, more active. †Freeze-dried paraffin-embedded tissues. ‡Fresh-frozen tissues.

phosphatase have been identified in ameloblasts, based on its interaction with substrates—para-nitrophenyl phosphate (pNPP) and beta-glycerophosphate (βGP). Intense activity with pNPP is seen in supranuclear and distal regions of the secretory ameloblasts, and a moderate or slight activity respectively in those regions with βGP. These enzyme activities were less at the late secretory stage of amelogenesis and disappeared at the transitional stage. By electron microscope the activity was seen in the trans side cisternae of Golgi apparatus, in lysosome-like granules and in small vesicles in the Tomes process. The activity with pNPP but not βGP was also localized in the plasma membrane (proximal, lateral, distal surface). The enzyme bound to plasma membrane was tartrate-resistant while the other is not.

Osteoclasts in bone and odontoclasts in resorbing dentin exhibit an intense acid phosphatase activity (Figs 18.12, 18.13). The enzyme is localized in the part of the cytoplasm that lies apposed to the resorbing surface of bone and dentin (Figs 18.13, 18.14). Electron microscopic studies reveal that the enzyme is localized in the lysosomes, although activity is also seen extracellularly between the microvillus-like projections of the ruffled border (Fig. 18.3). Uptake of resorbed mineral, hydrolyzed collagen fibrils, and injected radioactive substances at the site of resorption has been observed.

Acid phosphatase is demonstrated cytochemically using ***naphthol-ASBI phosphoric acid-fast red garnet GBC***, while in tissue sections ***naphthol-ASBI phosphoric acid—para-rosaniline*** is used.

Several studies imply that acid phosphatase may (in addition to its function in bone and dentin resorption) confer 'calcifiability' to the organic matrix by its hydrolytic action on the protein-polysaccharide granules present in the zone of mineralization.

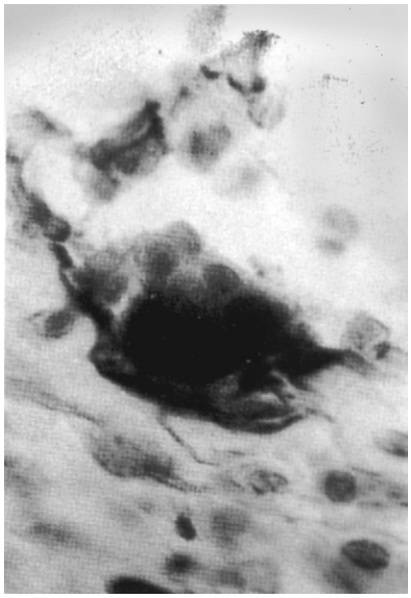

Figure 18.13 Hamster osteoclast showing cytoplasmic acid phosphatase reaction (dark), with some enzyme activity also present in resorbing bone matrix (AS-BI naphthol phosphate and red-violet LB salt incubation. Nuclear counterstain is hematoxylin; ×750) (From Burstone MS: In Sognnaes RF, editor: Calcification in biological systems. Washington, DC, 1960, American Association for the Advancement of Science Publications, p 64).

Esterase

According to histochemical definition, esterases hydrolyze simpler fatty acid esters than do lipases, which hydrolyze complex fatty acid esters.

Most histochemical techniques for esterases do not reveal any activity in bone or dentin. However, with use of specific naphthol esters such as naphthol AS-D acetate, an intense staining reaction is observed in the *calcifying matrices of bone and dentin.* This reactive zone, situated in the tooth between predentin and dentin, is also sudanophilic, indicating the presence of phospholipids.

Considerable esterase activity has also been found in the cells and microorganisms associated with the formation of calculus deposits on teeth.

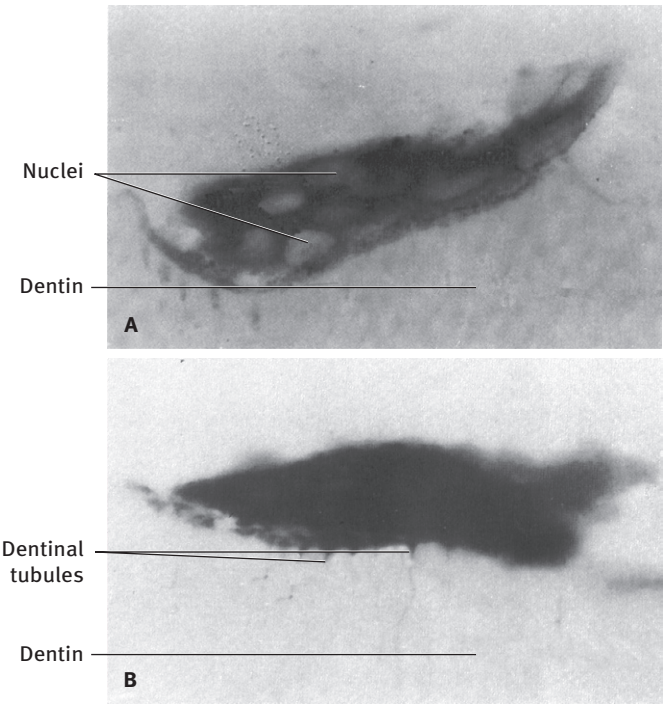

Figure 18.14 (**A**) Acid phosphatase activity in odontoclasts of mongrel-puppy primary tooth undergoing resorption. Notice reaction product is localized in discrete granules in cytoplasm. No reaction is seen in nuclei. (**B**) Acid phosphatase activity in odontoclast and dentinal tubules of mongrel-puppy primary tooth undergoing resorption (Alpha-naphthyl acid phosphatase and fast garnet GBC salt incubation; ×1060) (From Freilich LS: A morphological and histological study of the cells associated with physiological root resorption in human and canine primary teeth, doctoral dissertation. Washington, DC, 1972, Georgetown University).

Aminopeptidase

Aminopeptidases are proteolytic enzymes that hydrolyze certain terminal peptide bonds. Azo dye techniques using L-leucyl-β-naphthylamide or DL-alanyl-β-naphthylamide have been developed for histochemical demonstration of this enzyme. *Human osteoclasts* give a strong reaction, although no staining reaction occurs in the osteoclasts of rodents. *The enzyme is demonstrated in the stratum intermedium and odontoblasts during dentinogenesis.* Some staining reaction is also noticed in the periosteum, perichondrium, and chondrocytes (Table 18.1). It is significant that aminopeptidase has also been *localized in the macrophages and certain sites associated with the breakdown of connective tissues.*

Cytochrome oxidase

Cytochrome oxidase (CO) is an iron-porphyrin protein that enables cells to utilize molecular oxygen. Its histochemical localization therefore reflects the oxygen requirements of the cells and tissues and the levels of their metabolic and physiologic activity.

The original histochemical reaction, the 'nadi' reaction, using α-naphthol and *N,N*-dimethyl-*p*-phenylenediamine, is considered inadequate because of the instability of the substrate solution, lipid solubility, crystallization, and fading of the reaction product—indophenol blue. New techniques using *p*-aminodiphenylamine in conjunction with *p*-methoxy- *p*-aminodiphenylamine or 8-aminotetrahydroquinoline have overcome these technical problems so that the reaction is discretely localized in the mitochondria.

Both *osteoclasts and osteoblasts* show oxidase activity, with the reaction being more predominant in the former. *Stratum intermedium* of both molars and incisors also exhibits oxidase activity (Table 18.1).

In the secretory stage of the ameloblast, the infranuclear accumulation of mitochondria makes this region CO reactive, and this significantly decreases in the later stage when enamel matrix secretion completes. CO reactive mitochondria increased in number in stratum intermedium gradually throughout the secretory phase. In the maturation stage of ameloblasts, CO reactive giant mitochondria in the proximal region and normal sized mitochondria in the distal cytoplasm were localized. The proportion of CO reactive mitochondria in the supranuclear region to those in the infranuclear region was higher in smooth ended ameloblasts as compared to ruffle-ended ameloblasts. Papillary cells possess numerous intensively CO reactive mitochondria throughout maturative stage of ameloblasts.

Succinate dehydrogenase

Succinate dehydrogenase is closely associated with cytochrome oxidase in the mitochondria. It is one of a series of citric acid cycle enzymes that catalyzes the removal of hydrogen, which in turn is removed by a hydrogen acceptor or carrier. This serves as the basis of the histochemical reaction used in demonstrating this enzyme. The enzyme present in the tissues acts on the substrate (usually sodium succinate), causing the removal of hydrogen, which is picked up by a synthetic acceptor (a tetrazolium compound) present in the incubating medium. The reduced acceptor substance, called formazan, appears as a colored reaction product.

The distribution of succinate dehydrogenase in oral hard tissues is essentially similar to that of cytochrome oxidase. The dehydrogenase activity is *higher in osteoclasts*

than in osteoblasts. The *stratum intermedium and the odontoblasts* in developing teeth also reveal a positive reaction (Table 18.1).

Citric acid cycle in osteoblasts and osteoclasts

Besides observations on succinate dehydrogenase, studies on isocitric dehydrogenase, α-ketoglutaric dehydrogenase, and DPN-TPNH-diaphorases have also been reported. It is indicated that osteoclasts maintain a high rate of citrate and lactate production at the expense of glutamate and thereby actively promote decalcification of bone matrix and calcified cartilage.

Calcium-binding sites in enamel organ

Method to localize calcium histochemically has been devised and is being used widely to understand the role of membrane constituents of enamel organ in regulation of calcium during amelogenesis. Localization was possible in rapidly frozen, ***freeze-substituted*** enamel organ tissues stained with ***glyoxal bis 2-hydroxyanil (GBHA)***.

A large number of granular calcium-GBHA is detected in enamel organ, most of which is located along the lateral plasma membrane of ameloblasts. In the ameloblastic layer, the reaction was negative in the presecretory ameloblasts. The reaction becomes intense with the onset of enamel matrix formation and by the end of the transitional stage the reaction diminished. An intense calcium reaction in secretory ameloblasts was exclusively appreciated in the tubulovesicular structures which extend throughout the distal cytoplasm. The intensity of staining increased in concert with enamel matrix formation. In the maturation stage, similar reaction emerged in association with smooth-ended ameloblasts but were absent in the ruffle-ended ameloblasts. Some granular reactions of much smaller diameter were occasionally noted in other cells of the enamel organ at both the secretory and maturative stages.

Summary

A survey of the distribution of various enzymes associated with bone and teeth is given in Table 18.1. It is interesting to note that although acid phosphatase activity is associated with the osteoclasts only, distribution of other enzymes is widespread in hard oral tissues.

HISTOCHEMISTRY OF ORAL SOFT TISSUES

Polysaccharides, Proteins and Mucins

Polysaccharides

The dye carmine is often used to demonstrate glycogen, but it is not as specific as the PAS method. Epithelial glycogen is known to increase during inflammation and repair. Attached human gingiva shows variation in the extent of its keratinization, and this variability is reflected in the glycogen content of the tissue. On the other hand, the nonkeratinized alveolar mucosa virtually always shows constant levels of glycogen. Animal experiments involving benign and malignant epithelial proliferations demonstrate an increase in glycogen.

Proteoglycans with chondroitin sulfate and hyaluronic acid form a major intercellular component of human gingival epithelium. Fibromodulin, biglycan and lumican were identified in basal epithelial cells; prodecorin was distributed in the epithelial basement membrane zone; fibroblasts and myofibroblasts showed procollagen, prodecorin, biglycan, fibromodulin and lumican. The molecular conformation and relatively rapid rate of synthesis and secretion of these macromolecules in the gingiva may explain the lack of susceptibility of this material to the degradative action of specific enzymes. When oral soft tissues are stained with the metachromatic dye like toluidine blue, mast cells become visible in varying numbers in the loose connective tissue. They are seen distributed particularly along the blood vessels. The metachromatic reaction given by the cytoplasmic granules of these cells is caused by the presence of heparin—a sulfated proteoglycan. The cytoplasmic granules also contain histamine—a vasodilator that can be demonstrated by fluorescence microscope. Mast cells are present in particularly large numbers in the tongue and in the gingiva. The lack of these cells in acute necrotizing gingivitis is significant.

Proteins and protein groups

Keratinization is one of the important characteristics of the epidermis. Although under normal circumstances it occurs only in some areas of the oral epithelium, in pathologic conditions it occurs anywhere in the mouth. The mechanism by which the cells of the Malpighian layer are altered to form keratin has been only partly elucidated. The disulfide bridges present in keratin are believed to result from the oxidation of sulfhydryl groups of cysteine. Sulfhydryl groups are demonstrated histochemically by the ferric ferricyanide method in which this compound is reduced to a Prussian blue color by these protein groups. Thus the extent of the blue reaction product reflects the degree of keratinization. Attempts to demonstrate sulfhydryl groups in electron microscope have not been completely successful. However, electron microscopic demonstration of disulfide groups, using alkaline methenamine silver, has been made.

Lipids

Permeability is an important feature of oral mucosa and skin. Although total lipid content showed variations in skin and oral mucosa, different lipid components subserve the function of permeability in keratinized and nonkeratinized oral mucosa and skin. The total covalently bound lipids represent significantly higher amounts in porcine skin compared to that of oral mucosa. Greater permeability of palate relative to skin may be attributed to lower proportion of cholesterol, fatty acids, ceramides of which much lower levels of linoleate containing acylceramide is appreciated. The keratinized mucosa and skin showed O-acetylglyceramides, O-acylceramides and relatively high proportions of ceramide, while the nonkeratinized epithelia showed no acylceramides and a very little ceramide. Depletion of phospholipids coupled with repletion of sterols and sphingolipids (absolute quantity of sphingolipids increases in outer epidermis) occur during differentiation of epithelial cells. The glycolipid to ceramide ratio diminishes in stratum corneum. Neutral lipids like squalene and n-alkalene are

evenly distributed in all the layers with peak occurrence in stratum granulosum.

Mucins

Salivary mucins form semiviscous protective coatings over oral mucous membranes. They are composed of high-molecular weight carbohydrate-protein complexes. Two types of mucins are recognized by the predominant carbohydrate component in their molecules—fucomucins, rich in L-fucose, and sialomucin, rich in sialic acid. The latter is believed to confer acidity on certain types of mucins. Both of these mucins are present together in saliva, with one predominating over the other.

Histochemical techniques have been very useful in our understanding of the salivary mucins present in various salivary glands and their chemical composition. The dye mucicarmine is frequently used for nonspecific staining of mucins. PAS technique is used to identify neutral mucins and they are resistant to diastase (Fig. 18.15). Alcian blue, toluidine blue, colloidal iron, and aldehyde fuchsin methods are used to localize the acid mucins. The acidic mucins may be carboxylated or sulfated. At pH 1, Alcian blue demonstrates sulfated groups and at pH 2.5 the dye demonstrates carboxyl groups. A combination of Alcian blue/PAS is used to differentiate acid and neutral mucins. These techniques reveal species differences in the mucins of different salivary glands.

Enzyme Histochemistry

Alkaline phosphatase

Alkaline phosphatase activity in human gingiva is specifically demonstrable in the *capillary endothelium of the lamina propria* (Fig. 18.16). The reaction product, observed in the gingival epithelium and in the collagen fibers, seems to be a diffusion artifact.

Oral epithelium of the rat exhibits an increased alkaline phosphatase activity during the estrous cycle, correlated to phosphatase changes in the vaginal epithelium. Alkaline phosphatase is implicated in the mechanism of keratinization, although its precise role in this process is still uncertain.

The *basement membranes* associated with salivary gland acini exhibit high alkaline phosphatase activity. Similar activity in taste buds of several species of animals has also been reported.

Acid phosphatase

Acid phosphatase activity in human gingiva seems *related to the degree of keratinization, being very high in the zone of keratinization* and low in nonkeratinized regions. This pattern corresponds with that observed in the skin epidermis. Cells of the functional epithelium in the gingival sulcus have been reported by Lange and Schroeder to be rich in lysosomal enzymes in the normal healthy tissue.

Esterase

Little information is available on the esterase activity of human gingiva. *Superficial layers, including the keratinizing zone, show the presence of some esterase activity.*

High esterase activity is demonstrable in the *salivary gland ducts* and also in the *serous demilunes of the sublingual gland* (Figs 18.17, 18.18). Similar activity is observed in the *taste buds,. basal cell layers of the epithelium and in the underlying connective tissue* of several animal species, and this has been implicated in gustatory discrimination. Mast cells in oral tissues also have esterase activity (Fig. 18.19).

Aminopeptidase

The activity of this enzyme in human gingiva is low and is localized primarily in the *basal layers of the epithelium* and in the underlying connective tissue. An increase in aminopeptidase activity during inflammation and in hyperplasia caused by the drug phenytoin has been

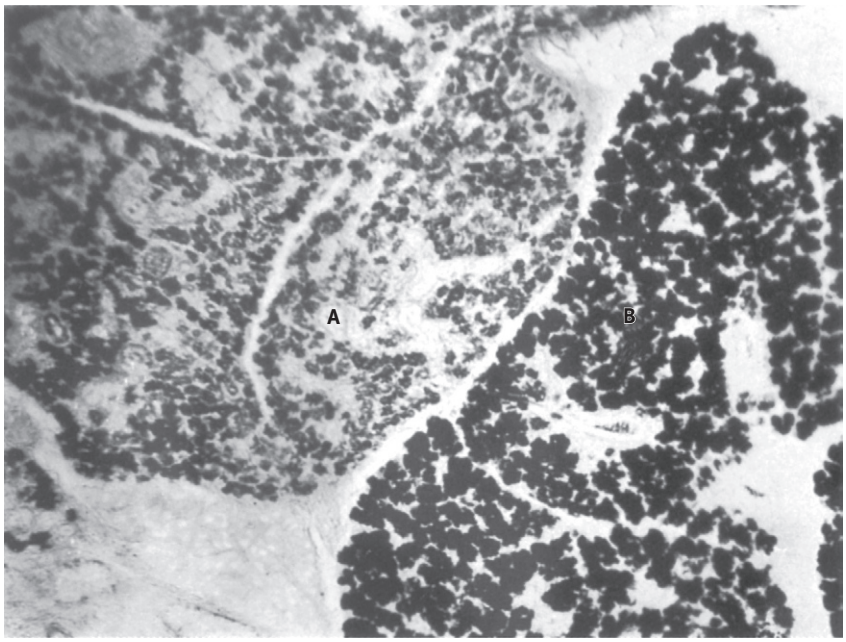

Figure 18.15 Freeze-dried mouse submandibular gland, (A) and sublingual gland, (B) showing PAS reactivity of mucins (×140).

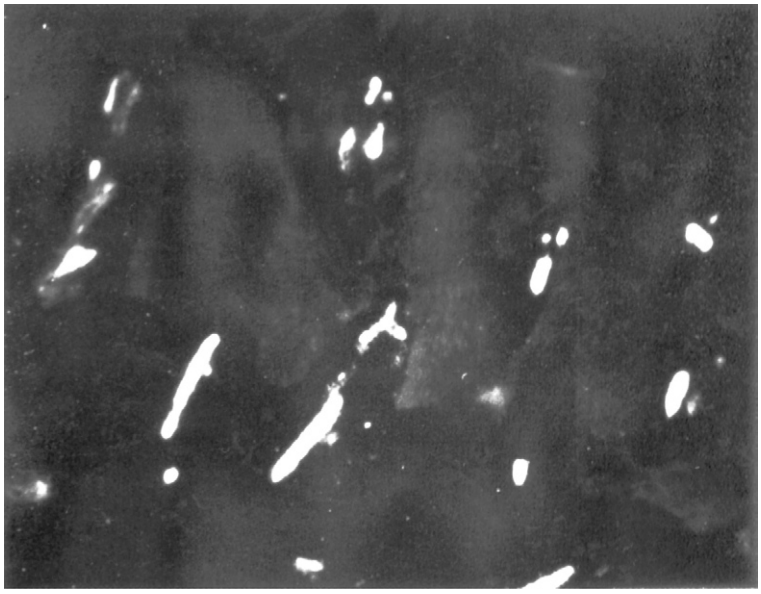

Figure 18.16 Alkaline phosphatase activity of capillaries of lamina propria of human gingiva revealed by ultraviolet fluorescence (×80).

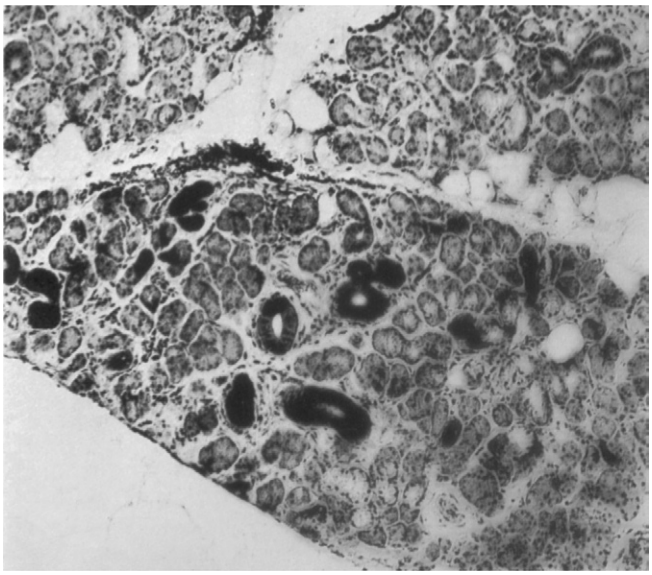

Figure 18.17 Esterase activity of ducts of freeze-dried human parotid gland (Nuclear counterstain; ×110).

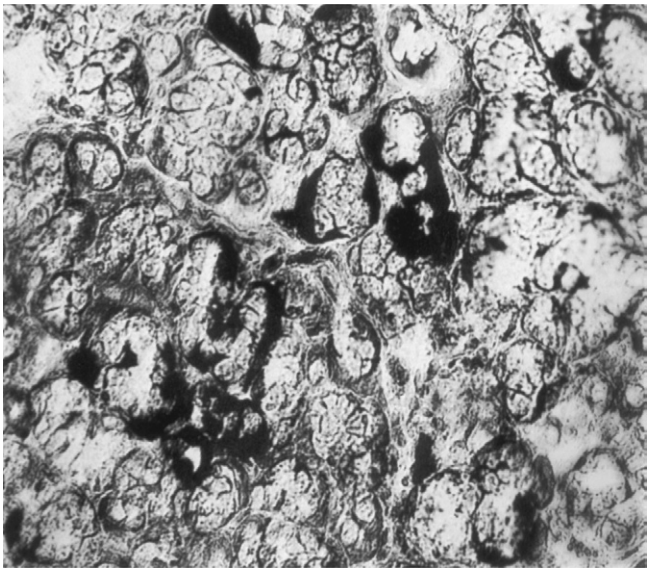

Figure 18.18 Esterase activity of demilune cells of freeze-dried human sublingual gland (×210).

reported. Aminopeptidase is also observed in the *salivary gland ducts.*

β-Glucuronidase

β-Glucuronidase hydrolyzes the β-glycoside linkage of glucuronides. They are involved in conjugation of steroid hormones and in hydrolysis of conjugated glucuronides, and play a role in cell proliferation. The enzyme has been localized in the *basal cell layers of the oral epithelium* in humans and rats.

Cytochrome oxidase

Histochemical techniques demonstrate low levels of cytochrome oxidase activity in human gingiva. Specifically, this cytochrome oxidase activity is localized in the *basal layers of the free and attached gingiva, crevicular epithelium, and epithelial attachment* (Fig. 18.20). In chronic gingivitis, a striking increase in cytochrome oxidase activity is observed in the epithelium from the free gingival groove through to the epithelial attachment. In chronic gingivitis the underlying connective tissue also shows a variable increase in oxidase activity.

Cytochrome oxidase activity is also demonstrated in the salivary glands, especially in the *duct system* (Fig. 18.21).

Succinate dehydrogenase and glucose 6-phosphate dehydrogenase

The distribution pattern of succinate dehydrogenase is similar to that of cytochrome oxidase. This dehydrogenase

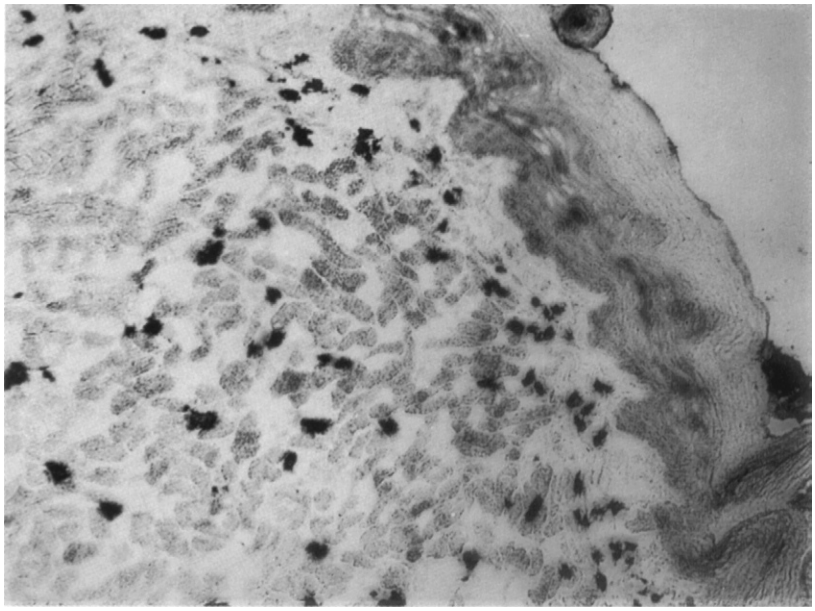

Figure 18.19 Mast cells of rat tongue incubated with substrate solution containing naphthol AS-D chloroacetate (×250).

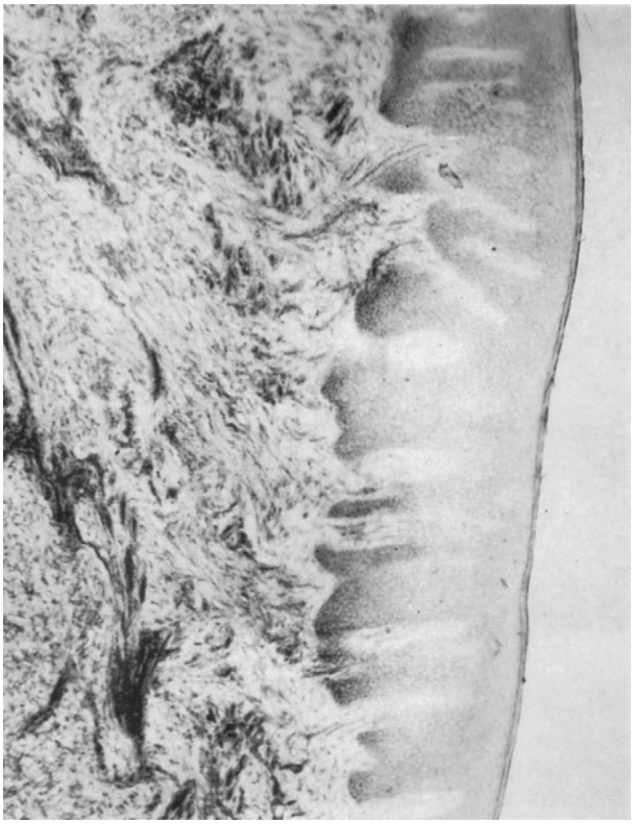

Figure 18.20 Human attached gingiva showing cytochrome oxidase activity of basal cell layer and in connective tissue of lamina propria.

is observed primarily in the *basal cell layers of the gingival epithelium and in the ducts of the salivary glands.* Glucose 6-phosphate dehydrogenase is present in significant quantities in the human oral mucosal epithelium. The levels of this enzyme become highly elevated in malignant dysplastic lesions of the oral mucosa. Elevation of this enzyme is considered 'to assist in the diagnosis of oral cancers'.

Enzyme histochemical detection of lymphatic capillaries

The lymphatics are characterized by a strong 5′-nucleotidase activity, whereas those of blood capillaries reveal a significantly lower or no activity. The alkaline phosphatase or diaminopeptidase activity, on the other hand, is markedly higher in blood capillaries than in the lymphatics. The 5′-nucleotidase activity is localized on the outer surface of the cell membrane of the capillary endothelial cells. Enzyme histochemistry performed on dental pulp revealed lymphatic channels to be distributed more in number in the central part than in the peripheral odontoblastic layer. Also lymph vessels were seen in the root area of the periodontium than in the cervical area.

Angiogenic factor in inflamed gingiva

A novel human angiogenic factor, 67kD protein, obtained from a melanoma cell line has been identified immunohistochemically in inflamed gingival tissue (using a monoclonal antibody to this protein). Specifically, the factor was localized in the macrophages. Such activity was absent in healthy gingival tissue. Similar activity has also been found in the macrophages of inflamed tissue of rheumatoid origin.

Laminin-5

Laminin-5 in its assembled form ($\alpha_3\beta_3\gamma_2$) is speculated to support cell adhesion, unlike its unassembled form. The latter form is a soluble (γ_2) monomeric form. The laminin γ_2 chain is now regarded as one of the typical invasion marker. This form does not contain any integrin binding site. A recent study has shown the release of domain III in the short arm of the γ_2 chain by MT1-MMP, which shows ability to bind with EGFR.

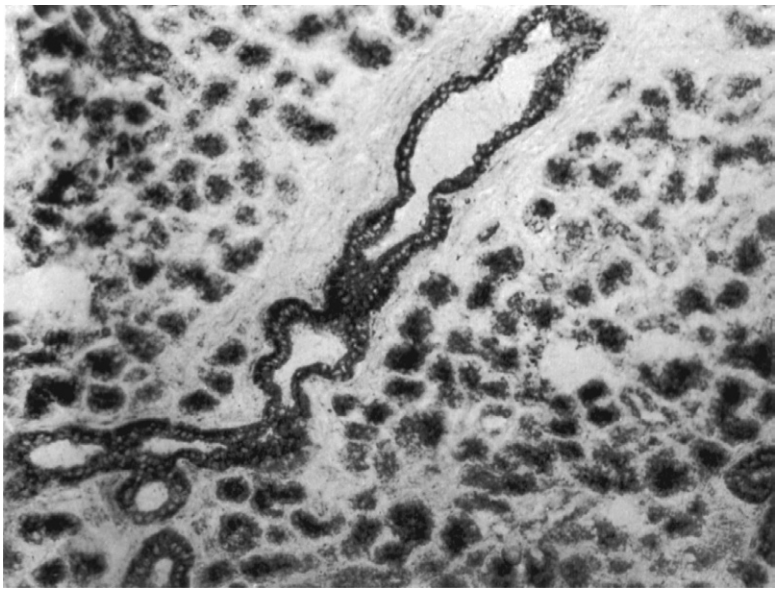

Figure 18.21 Cytochrome oxidase activity in human parotid gland (×143).

CLINICAL CONSIDERATIONS

Histochemical techniques are not only an important tool in dental research but are also frequently used in histopathologic diagnosis. Although the tissue biopsy materials are usually stained with hematoxylin and eosin, there are numerous occasions when this type of staining technique does not permit a definitive diagnosis. In a differential diagnosis of an epithelial tumor in or around the oral cavity, a histochemical stain for mucin may assist the oral pathologist in distinguishing a tumor of salivary gland origin from an odontogenic tumor or a tumor arising from nonglandular epithelium. Because these tumors often require different types of treatment, this distinction is of great practical importance.

Histochemical stains that reveal lipids are of value in correctly diagnosing tumors that arise from the fat cells (lipoma and liposarcoma). They are also an important aid in establishing the identity of vesicles that may appear in tumor cells of various benign and malignant lesions. Since cytoplasmic vesicles may represent lipid, mucin, glycogen, or intracellular edema, their true identity is sometimes important for correct diagnosis and therapy.

Proteoglycans and glycoproteins in connective tissues undergo alterations in various pathologic states. Bacterial infections may occur as a result of the hydrolytic action of the bacterial enzyme hyaluronidase on the polymeric integrity of hyaluronic acid. During *inflammation* or in early stages of *wound healing* there is a histochemically detectable increase in both glycoproteins and proteoglycans. However, as wound healing progresses, there is a gradual decline in the levels of both substances until normal levels are restored. A continuous growth factor stimulation of fibrous components and decreased extracellular matrix degradation results in the formation of scar or keloid.

Proteoglycans are to govern cell movement in numerous physiological and pathological contexts, spanning from early embryonic development to *tumor invasion and metastasis.* These macromolecules elaborate haptotaxis-like mechanisms imposing directionality upon the moving cells. Heparan sulfate chains of syndecan have potential to interact with several ligands including growth factors, cytokines and chemokines and extracellular molecules which are relevant to growth regulation in vascular hypoxia, repair, regeneration and angiogenesis and in immune cell function. Syndecan is incriminated in the maintenance of differentiated epithelial morphology, a well organized actin filament system and normal growth of epithelial cells. In some cancers its expression has been shown to regulate tumor cell function like proliferation, adhesion and motility. Thus they act as a prognostic marker for tumor progression and patient survival. The ectodomains and heparan sulfate chains have been implicated to act as receptors for some bacterial and viral pathogens, mediating *infections.*

Adhesion to fibronectin becomes a predominant interaction during the cell migratory process. In vitro, different fibronectin functional domains have been identified in the attachment, spreading and *migration of neural crest cells.* Laminin-5 synthesis by keratinocytes is augmented by the promoters present in the acute wound fluid. This increase enhances the reparative process by stimulating adhesion and migration of keratinocytes on the wound bed and facilitates basement membrane formation at the dermal-epidermal junction. Levels of fibronectin and its cell membrane receptors are known to undergo a decline in certain forms of cancer. These reduced levels are correlated with the altered or transformed behavior of the cancer cells. Some investigators have suggested that metastatic cancer cells preferentially bind to type IV collagen via laminin, both being components of the basement membranes. In contrast, it has

been suggested that nonmalignant tumor cells do not use laminin for attachment. The presence of tenascin-C in the extracellular matrix promotes a motile phenotype. Tenascin-C has been shown in the connective tissue adjacent to tumors, in wounds, and in inflamed tissues. The basement membrane of fetal oral squamous epithelium contains laminin chains α_2, α_3, α_5, β_1, β_2, β_3, γ_1, γ_2, while the adult normal oral epithelium shows α_3, α_5, β_1, β_3, γ_1, γ_2. Re-expression of α_2, β_2 could be demonstrated in hyperproliferative, dysplastic and carcinomatous lesions. Oncofetal fibronectins could be demonstrated throughout the stromal compartments. The strong association of laminin-5 with integrin $\alpha_6\beta_4$ is critical for stable hemidesmosomal apparatus. The genomic defects occurring in any of the three subunits that form laminin-5 result in a lethal *skin disease* known as Herlitz's junctional epidermolysis bullosa. Defects in the production of tenascin-X affect the collagen deposition in dermis leading to Ehlers-Danlos syndrome. Autoantibodies against laminin-5 are identified in cicatricial pemphigoid. Attachment of various organisms to the host tissue is a critical step in infection. The wall associated protein (Wap-A) in *Streptococcus mutans* has been shown to bind with collagen type I and fibronectin. *E. coli, S. aureus, S. pyogenes, T. pallidum* has been demonstrated to possess components that could bind to laminin. Only few strains of viridian streptococci isolated from the oral cavity showed receptors for laminin while most of those isolated from blood and heart valve expressed them. The bacteria growing in the biofilm shows upregulated surface expression of putative fibronectin and collagen adhesins compared to its plank tonic counterparts growing elsewhere. Organisms like *Porphyromonas gingivalis* is well known for causing periodontitis, and their fimbriae contribute significantly to their virulence. The fimbria is able to achieve this by disrupting the molecular interactions between fibronectin/vitronectin and their receptors α_5 β_1, α_5 β_3 integrins. Thrombospondin regulates angiogenesis and tumor development. There is reduction in this glycoprotein from normal to dysplasia to carcinoma favoring the angiogenic drive that accompanies tumor development. There are evidences to show that the same glycoprotein in the stroma of oral squamous cell carcinoma to be a product of mesenchymal cells and it is believed to enhance tumor cell motility and proteolytic activity in a paracrine fashion.

Poorly calcified dentin matrix as occurring in dentinogenesis imperfecta and odontomas shows an intense PAS reaction. The presence of bluish-brown opalescence and a diminished pulpal chamber in the teeth of patients with dentinogenesis imperfecta type II or in osteogenesis imperfecta is associated with the localization of type III collagen in dentin. Type III collagen is absent in normal adult dentin.

Deficiency of vitamin C results in the loss of molecular stability, resulting in the formation of abnormal, immature collagen and consequent collagen diseases. Mutation of fibrillin-1 gene results in one of the most common inherited connective tissue disorder, Marfan's syndrome.

A variety of fungi that infect humans contain mucopolysaccharides. The hyphae and spores of these fungi are present in the infected tissues, and their correct diagnosis can often be made only after special histochemical stains for mucin have been done. In the human oral cavity diagnosis of histoplasmosis, actinomycosis, blastomycosis, and coccidioidomycosis can often be made only after special histochemical stains.

SUMMARY

Histochemical techniques preserve the chemical nature of the biochemicals that enables not only identification of these substances but also provide their in situ information. An understanding of tissue structure is essential for studying tissue in health and disease, since the chemical constituents of tissue have been shown to alter during disease development. The constituents are primarily carbohydrate–protein complexes (proteoglycans, glycoproteins), enzymes and secretory proteins, lipids, and nucleic acids.

Fixation

The tissue macromolecules must be structurally well preserved in their location for demonstration, which is achieved by using suitable fixatives. Although formaldehyde is used in most situations, other aldehydes are also preferred. Postfixation procedures are used in order to preserve lipids. Fresh frozen sections using cryostat and freeze drying technique are used when enzymes have to be demonstrated. Techniques like freeze fracture and freeze etching are used when three-dimensional images are to be observed.

Specific Histochemical Methods

Demonstration of Carbohydrates

The demonstration of carbohydrate groups is best done using periodic acid-Schiff (PAS) technique. Proteoglycans can be identified using thiazine group of dyes, and for electron microscopic identification high iron diamine thiocarbohydrazide silver proteinate is preferred. The use of biotinylated hyaluronic acid binding complex and plant lectins in characterizing specific carbohydrate moieties gives promising results.

Demonstration of Proteins and Lipids

The demonstration of protein groups involves use of dinitrofluorobenzene ninhydrin or ferric ferricyanide with formation of insoluble colored reaction products. Sudan black, iodoplatinate reactions (ultramicroscopic demonstration), malachite green are used to demonstrate phospholipids and phosphoproteins.

Traditionally Gomori's method and simultaneously coupling azo dye technique are used for phosphatases demonstration. Aminopeptidases can also be demonstrated by azo dye technique. Gomori's method practices the conversion of phosphate ion into insoluble cobalt or lead salt. The newer techniques utilize lanthanides like cerium which not only prevents nonspecific deposits but has also proved as a better capturing agent than lead for inorganic phosphates.
Precise localization of the biological molecules can also be achieved by using immunohistochemistry.

Histochemistry of Oral Hard Tissues

The PAS method is used in studying ground substance of tooth. Newly formed dentin is rich in PAS reactive carbohydrate,

while enamel matrix is non-reactive except for some areas of interprismatic substance. The predentin contains high levels of dermatan sulfates while mineralizing zones of dentin matrix contains more chondroitin sulfates with less dermatan sulfates and hyaluronan. The chondroitin sulfates are seen in precementum and pericementocytic area. The chondroitin sulfates are the major glycosaminoglycans of bovine dental pulp.

The methods like dinitrofluorobenzene (DNFB) and ninhydrin-Schiff are used even in the study of hard tissues. A modified DNFB method where the final end product is in the form mercaptide of lead or silver is used in ultramicroscopic examinations. These techniques have been adapted well to study dental caries and in bone resorption. The sulfhydryl groups are present in the mineralizing front of predentin. In immature enamel both sulfhydryl and thyrosine residue characteristic of keratin are present. The calcium-binding proteins are the major forms of proteins in both enamel and dentin.

The sudanophilia is present in the odontoblast processes, enamel rod sheath, mineralizing zone, predentin, and basal zone of ameloblasts. This indicates increase in cell membrane phospholipids (phosphatidylinositol, phosphatidylcholine) and matrix phospholipids (phosphatidy lethanolamine, phosphatidy lserine, and sphingomyelin) during the process of mineralization and reinforces their role in this process.

The alkaline phosphatase activity is observed at the sites of intramembranous bone development. During endochondral bone formation weak activity of alkaline phosphatase is observed in proliferative zone and high activity is observed in maturative and hypertrophic zone. Alkaline phosphatase activity is seen in stratum intermedium and odontoblasts. The adenosine triphosphatase is localized in the distal and lateral cell membrane of ruffle ended and late transitional ameloblasts explaining the regulation in the access of calcium into the mineralizing front. The acid phosphatase is identified chiefly in membrane bound organelles (lysosomes). Histochemically two types of acid phosphatases have been identified in ameloblasts (pNPP, βGP). Odontoclasts in the resorbing dentin exhibit intense acid phosphatase activity.

Histochemistry of Oral Soft Tissues

The keratinized mucosa shows variation in glycogen according to the extent of keratinization while nonkeratinized mucosa shows constant levels always. The oxidation of sulfhydryl groups of cysteine results in disulfide bridges. Prussian blue reaction reflects the degree of keratinization under light microscope, while demonstration of disulfides by alkaline methenamine silver for electron microscopic study has proved useful.

Lower levels of cholesterol, fatty acids, and ceramides contribute to the increased permeability. The keratinized epithelium shows acylceramides and high levels of ceramides while nonkeratinized epithelium shows no acylceramides and little ceramides. The glycolipids to ceramides ratio diminish in stratum corneum and neutral lipids are the highest in stratum granulosum.

The mucins are of two types—fucomucins and sialomucins. The PAS recognizes neutral mucins and Alcian blue the acidic mucin.

The capillary endothelium and basement membrane associated with salivary gland acini exhibit high alkaline phosphatase activity. The acid phosphatase was demonstrated in relation to zone of keratinization only.

Clinical Considerations

Histochemical methods are valuable tools in dental research and in histopathological diagnosis. Identification of mucin is an indicator of salivary gland tumor. Proteoglycans and glycoproteins show alterations in various pathologic states ranging from infections to tumors. They interact with growth factors, cytokines and govern cell movement; therefore they are important in tumor invasion. Syndecan is shown to regulate cancer proliferation, adhesion and motility. Laminin-5 is shown to support cell adhesion in tumor cells while fibronectin, the sticky protein, decline in certain forms of cancer. In auto-immune diseases like in cicatricial pemphigoid, autoantibodies to laminin-5 have been shown. Certain bacteria like Streptococcus *mutans, E. coli* attach to laminin or fibronectin thus helping them to multiply. Reduction in thrombospondin favors new blood vessel formation and thus helps in tumor growth.

Identification of fungi like histoplasmosis, blastomycosis made by histochemical methods helps to arrive at a correct diagnosis.

Localization of type III collagen helps in diagnosis of osteogenesis imperfecta or dentinogenesis imperfecta type II.

REVIEW QUESTIONS

1. Discuss the fixatives used in histochemical methods.
2. Describe the techniques used in demonstration of carbohydrates.
3. Describe the techniques used in demonstration of enzymes.
4. What are the biochemical changes that occur at the mineralizing zones of hard tissues?
5. Discuss the calcium-binding proteins of dental hard tissue matrices.
6. Discuss the location of various enzymes in dental tissues.
7. Describe the clinical values in understanding the histochemistry of tissues.

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19 Preparation of Specimens for Histologic Study

CHAPTER CONTENTS

The morphologic study of oral tissues involves the preparation of tissue sections for microscopic examination. Knowledge of various types of microscopes and related histologic techniques will assist the student in interpretation of the structure and function of oral tissues.

The fundamental methods of tissue preparation for various types of microscope, although basically similar to those for light microscope, show differences in specific procedures. For example, differences in the tissue preparation for electron microscope are necessitated by the lower penetrating power of electrons compared with the light and the greater resolving power of the electron microscope. Tissues for light microscopic study must be sufficiently thin to transmit light, and its components must have sufficient contrast for the parts to be distinguishable from each other. Routine histologic techniques involve the fixation of tissues in protoplasmic coagulating solution, dehydration in organic solvents, embedding in paraffin or plastics, and cutting of thin sections on a microtome. The sections are mounted on an appropriate supporting structure, stained, and examined under a microscope. The basic procedures are modified depending on the nature of the specimen and the type of microscope to be used for examination of structures of particular interest.

Four methods of preparation of oral tissues for microscopic examination are commonly used:

1. Specimens may be embedded in paraffin and sectioned. The most commonly used method of preparing soft tissues for study with an ordinary light microscope is that of embedding the specimen in paraffin and then cutting sections 4 to 10 mm thick. The sections are mounted on microscope slides, passed through a selected series of stains, and covered with a cover glass.
2. Specimens may be embedded in parlodion and sectioned. Specimens containing bone or teeth require different preparation. Such specimens must be decalcified (the mineral substance removed) and usually embedded in parlodion rather than in paraffin before being sectioned on a microtome. Though pulp is a soft tissue, but being enclosed within hard tissues, decalcified method of study is the only option. Moreover pulp has to be studied in relation to dentin and its volume being too small, practically no other method exists. Similarly, periodontal ligament has to be studied in relation to tooth and bone, therefore, this is the only available method of study.
3. Specimens of calcified tissue may be ground into thin sections. Sections of undecalcified tooth or bone may be obtained by preparing a ground section. This is done by slicing the undecalcified specimen, which is ground down to a section of about 50 mm on a revolving stone or disk. Enamel can be studied only by this method, as decalcification would remove all the inorganic content leaving very little (3 to 5%), of organic matter. Other hard tissues of the tooth can be studied both by ground and decalcified sections due to their appreciable (about 30%) organic content.
4. Specimens of soft tissue may be frozen and sectioned. When it is important that pathologic tissue specimens be examined immediately, or if the reagents used for paraffin or parlodion embedding would destroy the tissue characteristics that are to be studied, the fresh, unfixed, or fixed soft tissue may be frozen and sectioned without being embedded. Such tissue sections are usually referred to as frozen sections.

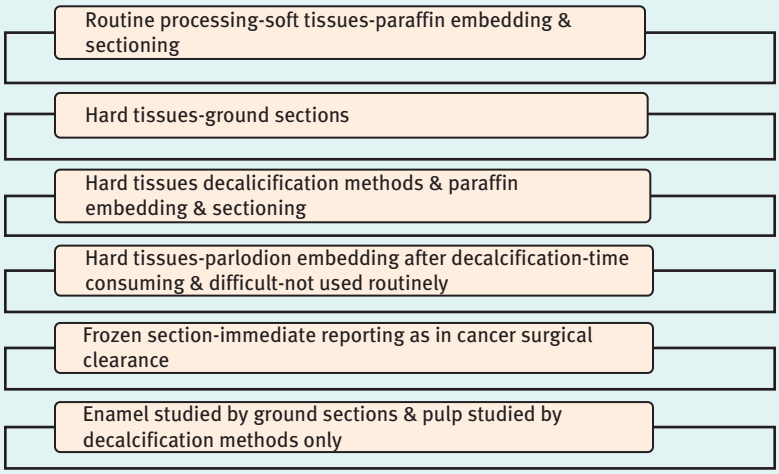
These four methods of specimen preparation are described in more detail.

Box 19.1 provides different methods of tissue processing.

PREPARATION OF SECTIONS OF PARAFFIN-EMBEDDED SPECIMENS

The method of preparing a specimen for sectioning by embedding it in paraffin is suitable for oral specimens such as specimens of gingiva, cheek, and tongue that contain no calcified tissue.

Box 19.1 Methods of Tissue Processing for Different Tissues



Obtaining the specimen Specimens taken from humans or experimental animals must be removed carefully, without crushing, either while the animal is alive or immediately after it has been killed.

Fixation of the specimen

Immediately after removal of the specimen it must be placed in a fixing solution. Specimens that have not been placed in such a solution are seldom any good because of the morphological change that it undergoes due to autolysis and putrefaction. There are many good fixing solutions available. Sometimes the kinds of stains subsequently to be used determine the kind of solution to be chosen. One of the most commonly used fixatives for dental tissues is 10% neutral formalin.

The purposes of fixation are to coagulate the protein, thus reducing alteration by subsequent treatment, and to make the tissues more readily permeable to the subsequent applications of reagents. The fixation period varies from several hours to several days, depending on the size and density of the specimens and on the type of fixing solution used.

After fixation in formalin, the specimen is washed overnight in running water.

Dehydration of the specimen

Since it is necessary that the specimen be completely infiltrated with the paraffin in which it is to be embedded, it must first be infiltrated with some substance that is miscible with paraffin. Paraffin and water do not mix. Therefore after being washed in running water to remove the formalin, the specimen is gradually dehydrated by being passed through a series of increasing percentages of alcohol (40%, 60%, 80%, 95%, and absolute alcohol), remaining in each dish for several hours. The time required for each step of the process depends on the size and density of the specimen. To ensure that the water is replaced by alcohol, two or three changes of absolute alcohol are used. The tissue specimen should never be treated with higher concentration of alcohol after an aqueous fixative as this will induce high shrinkage, due to rapid removal of water.

Clearing of the specimen

Clearing is performed so as to remove the alcohol from the tissue and at the same time allow the permeation of fluid that easily mix with paraffin. This step is used as an intermediate step since paraffin and alcohol are not miscible. The specimen is passed from alcohol through two changes of xylene, a clearing agent which is miscible with both alcohol and paraffin. It also helps in improving the refractive index of the tissue, it allows light to pass through or it becomes clear, hence this procedure is called clearing.

Microwave Processing

Although various methods have been employed thus far to reduce the time required for processing tissues, microwave oven processing has been more successful and easily accepted in many laboratories. Microwaves work on the principle of causing molecules to rotate rapidly and collide, resulting in internal heat production simultaneously throughout the tissue. This heat causes chemicals to infiltrate and act more rapidly. Thus microwave-assisted tissue processing takes much lesser time than conventional methods, and tissues can be sectioned and stained in a matter of 4 to 6 hours. When clubbed with microwave-assisted tissue fixation, time taken can be reduced even further. The quality of stained tissue sections processed thus, have been comparable to those obtained by conventional techniques. Microwaves have been advocated for laboratory use since several years, although they have become popular only in the last couple of decades. Currently, various laboratory grade microwave-assisted tissue processors are also being produced commercially.

Infiltration of the Specimen with Paraffin

When xylene has completely replaced the alcohol in the tissue, the specimen is ready to be infiltrated with paraffin. It is removed from the xylene and placed in a dish of melted embedding paraffin, and the dish is put into a constant temperature oven regulated to about 60°C. The

exact temperature depends on the melting point of the paraffin used. During the course of several hours the specimen is changed to two or three successive dishes of paraffin so that all of the xylene in the tissue is replaced by paraffin. The time in the oven depends on the size and density of the specimen; a specimen the size of a 2 or 3 mm cube may need to remain in the oven only a couple of hours, whereas a larger, firmer specimen may require 12 to 24 hours to ensure complete paraffin infiltration.

Embedding the specimen

When the specimen is completely infiltrated with paraffin, it is embedded in the center of a block of paraffin. A small paper box, perhaps a 19 mm cube for a small specimen, is filled with melted paraffin, and with warm forceps the specimen is removed from the dish of melted paraffin and placed in the center of the box of paraffin. Alternatively, two 'L'- shaped metallic pieces (usually made of brass or aluminum) placed over a metallic platform are used to make a block of paraffin. Attention must be given here to the orientation of the specimen so that it will be cut in the plane desired for examination. A good plan is to place the surface to be cut first toward the bottom of the box. The paper box containing the paraffin and the specimen is then immersed in cool water to harden the paraffin or it may be allowed to bench cool to harden. The hardened paraffin block is removed from the paper box and is mounted on a paraffin-coated wooden cube (about a 19 mm cube) or attached to a circular head of a metallic shaft. The mounted paraffin block is trimmed with a razor blade so that there is about 3 mm of paraffin surrounding the specimen on all four sides so that the edges are parallel. The specimen is now ready to be sectioned on a microtome.

Cutting the sections of the specimen

The wooden cube to which the paraffin block is attached is clamped on a precision rotary microtome and the microtome is adjusted to cut sections of the desired thickness. Although usually 4–6μm is preferred for a cellular tissue like a lymph node 2–3μm is required. The perfectly sharpened wedge-shaped microtome knife is clamped into place for sectioning. Disposable knives are also available for the same purpose.

Mounting the cut sections on slides

Suitable lengths of the paraffin ribbon are then mounted on prepared microscope slides. The preparation of the slides is done by the coating of clean slides with a thin film of Meyer's albumin adhesive (egg albumin and glycerin). A short length of paraffin ribbon is floated in a pan of warm water (about 45°C). A prepared slide is slipped under the ribbon and then is lifted from the water with the ribbon, which contains the tissue sections, arranged on its upper surface. The slide is placed on a constant temperature drying table, which is regulated to about 42°C so that the sections will adhere to the slide. The slide is then allowed to dry on this table.

Staining the sections

There are innumerable tissue stains, methods of using stains, and methods of preparing tissues to receive stains. Some of the many factors that influence the choice of stains are the kinds of tissue to be studied and the particular characteristics of immediate interest.

One combination of stains often used for routine microscopic study is hematoxylin and eosin, commonly known as H&E.

The dried slides are placed vertically in glass staining trays; the trays are then passed through a series of staining dishes that contain the various reagents (Table 19.1). The slides are removed one at a time from the xylene, and the sections are covered with a mounting medium/mountant and a cover glass is affixed. The mountants have low refractive index. Most commonly used mountants are gelatin jelly, fructose syrup, DPX and *Canada balsam*. When the

Table 19.1 Staining of Sections

1. Xylene	2 min	To remove paraffin from sections
2. Xylene	2 min	To remove paraffin from sections
3. Absolute alcohol	2 min	To remove xylene
4. 95% alcohol	1 min	Approach to water
5. 80% alcohol	1 min	Approach to water
6. 60% alcohol	1 min	Approach to water
7. Distilled water	1 min	Water precedes stains dissolved in water
8. Hematoxylin (Harris's)	3–10 min	To stain nuclei
9. Distilled water	Rinse	To rinse off excess stain
10. Ammonium alum (saturated solution)	2–10 min	To differentiate; nuclei will retain stain
11. Sodium bicarbonate (saturated solution)	1–2 min	Makes stain blue
12. Distilled water	1 min	Removes $NaHCO_3$
13. 80% alcohol	1 min	Partially dehydrates
14. 95% alcohol	1 min	Alcohol precedes stains dissolved in alcohol
15. Eosin (alcohol soluble)	1–2 min	To stain cytoplasm and intercellular substance
16. 95% alcohol	Rinse, or longer	Alcohol destains eosin and should be used as long as needed
17. 95% alcohol	Rinse, or longer	To remove excess eosin
18. Absolute alcohol	1 min	To dehydrate
19. Absolute alcohol	2 min	To dehydrate
20. Xylene	2 min	To remove alcohol and clear
21. Xylene	2 min	To clear

mounting medium has hardened, the slides are ready for examination.

Xylene Free Tissue Processing and Staining

A conventional pathology laboratory employs xylene for different purposes (for e.g, clearing in tissue processing, deparaffinization before staining). Although it's carcinogenic nature has not been convincingly proved in humans, prolonged exposure to xylene vapors can cause various health complications. It is therefore essential that xylene use be minimized as much as possible.

Xylene-free tissue processing protocols have already been widely adapted in many laboratories. Use of isopropyl alcohol for dehydration in tissue processing eliminates the need for xylene. Similarly, xylene is not necessary in some microwave processing protocols, as the alcohol is completely vaporized following dehydration. Various mineral and vegetable oils have also been used successfully as xylene substitutes for clearing tissues.

Use of xylene for deparaffinization in staining can also be avoided using some safer alternatives. Mineral and vegetable oils, dishwashing detergent and lemon water have been used for dewaxing, with good results.

PREPARATION OF SECTIONS OF PARLODION-EMBEDDED SPECIMENS

Specimens that contain bone and teeth cannot be cut with a microtome knife unless the calcified tissues are first made soft by decalcification. Furthermore, if a specimen contains any appreciable amount of bone or teeth, the decalcified specimen is better embedded in parlodion (celloidin, pyroxylin) than in paraffin. It is extremely difficult, if not impossible, to get good sections of a large mandible containing teeth in situ if the specimen is embedded in paraffin. Such nitrocellulose embedding media offer spectacular results where the relationship between tissues is essential.

Let us suppose that we have to section a specimen of dog mandible bearing two premolar teeth. One method is as follows.

Obtaining the specimen

The portion of the mandible containing the two premolar teeth is separated as carefully as possible from the rest of the mandible by means of a sharp scalpel and a bone saw. Unwanted soft tissue is removed. If the area of the specimen next to the line of sawing will be seriously damaged by the saw, the specimen should be cut a little larger than needed and then trimmed to the desired size after partial decalcification. It is better to have the mandible cut into several pieces before placing it in the fixative because a smaller specimen allows quicker penetration of the fixing solution to its center. If the tooth pulp is of interest, a bur should be used to open the root apex of the teeth to permit entrance of the fixing solution into the pulp chamber. This operation must be done with care so that too much heat does not burn the pulp tissue.

Fixation of the specimen

The specimen so cut and prepared is quickly rinsed in running water and for fixation is placed immediately in about 400 ml of 10% neutral formalin.

It should remain in the formalin not less than a week and preferably longer. It may be stored in formalin for a long period.

Decalcification of the specimen

When fixation is complete, the specimen is then decalcified. Decalcification may be accomplished in several ways. One way is to suspend the specimen in about 400ml of 5% nitric acid. The acid is changed daily for 8 to 10 days, and then the specimen is tested for complete decalcification.

One way to test for complete decalcification is to pierce the hard tissue with a needle. When the needle enters the bone and tooth easily, the tissue is probably ready for further treatment.

Another way to test for complete decalcification is to determine by a precipitation test whether there is calcium present in the nitric acid in which the specimen is immersed. This is done by placing in a test tube 5 or 6 ml of the acid in which the specimen has been standing and then adding 1 ml of concentrated ammonium hydroxide and several drops of a saturated aqueous solution of ammonium oxalate. A precipitate will form if any appreciable amount of calcium is present. If a precipitate forms, the acid covering the specimen should be changed, and a couple of days later the test for complete decalcification should be repeated. If no precipitate is detected after the test tube has stood for an hour and after several additions of ammonium oxalate, it may be assumed that the specimen is almost completely decalcified. The specimen should be allowed to remain in the same acid for 48 hours longer and the test repeated. Radiographic examination of the specimen to look out for traces of hard tissue is very misleading. The specimen may appear completely radiolucent, yet be hard.

The end point of decalcification is sometimes difficult to determine, but it is important. Specimens left in the acid too short a time are not completely decalcified and cannot be cut successfully, and specimens left in the acid too long a time do not stain well. Because of the adverse effect of prolonged exposure to acid on the staining quality of tissues, specimens should be reduced to their minimum size before decalcification is begun to keep the time necessary for acid treatment as short as possible.

Washing the specimen

When decalcification is complete, the specimen must be washed in running water for at least 24 hours to remove all of the acid.

Dehydration of the specimen

After washing, dehydration is accomplished by the placement of the specimen successively in increasing percentages of alcohol (40%, 60%, 80%, 95%, and absolute alcohol). The specimen should remain in each of the alcohols, up to and including 95%, for 24 to 48 hours, and it should then be placed in several changes of absolute alcohol over a period of 48 to 72 hours. It is necessary to remove, as much as possible, all of the water from the tissues to have good infiltration of parlodion.

From absolute alcohol the specimen is transferred to ether–alcohol (1 part anhydrous ether, 1 part absolute alcohol), because parlodion is dissolved in ether–alcohol. There should be several changes of ether–alcohol over a period of 48 to 72 hours.

Infiltration of the specimen with parlodion

Parlodion is purified nitrocellulose dissolved in ether–alcohol. From the ether–alcohol in which it has been standing, the specimen is transferred to 2% parlodion, covered tightly to prevent evaporation, and allowed to stand for a period of from 2 weeks to a month.

From 2% parlodion the specimen is transferred to increasing percentages of parlodion (4%, 6%, 10%, and 12%). The estimation of the time required for the infiltration of a specimen is a matter of experience, with the determining factors being the size of the specimen and the amount of bone and tooth material present. For the specimen of mandible being described here, the time required for complete parlodion infiltration might vary from several weeks to several months.

Embedding the specimen in parlodion

When infiltration with parlodion is complete, the specimen is embedded in the center of a block of parlodion. A glass dish with straight sidewalls and a lid is a good container to use for embedding. Some 12% parlodion is poured into the dish, and the specimen is placed in the parlodion. Then more parlodion is added so that there is about 13 mm of parlodion above the specimen, the additional amount being necessary to allow for shrinkage during hardening.

Orientation of the specimen at this point to ensure the proper plane of cutting is important. If this piece of dog mandible is to be sectioned in such a way that the premolar teeth are cut in a mesiodistal plane and the first sections are cut from the buccal surface, then the buccal surface of the mandible should be placed toward the bottom of the dish when the specimen is embedded.

The dish is now covered with a lid that fits loosely enough to permit very slow evaporation of the ether–alcohol in which the parlodion is dissolved. As the ether–alcohol evaporates, the parlodion will become solidified and will eventually acquire a consistency somewhat like that of hard rubber.

This process of hardening the parlodion may require 2 or 3 weeks. When the block is very firm, it is removed from the dish and placed in chloroform until it sinks. It is then transferred to several changes of 70% alcohol to remove the chloroform.

Blocks of parlodion-embedded material must never be allowed to dry out. The blocks should be stored in 70% alcohol to allow the parlodion to harden further. Blocks that are to be stored for many months or years should eventually be transferred to a mixture of 70% alcohol and glycerin for storage.

Cutting the sections of the specimen

The hardened block of the parlodion-embedded specimen is fastened with liquid parlodion to a fiber block or to a metal object holder so that it can be clamped onto the precision sliding microtome. This is a different instrument from the rotary microtome used for cutting paraffin. Sections are cut with a sharp planoconcave microtome knife. For the specimen of dog mandible being described here, the sections may have to be cut at a thickness of as much as 15μm. Unlike paraffin sections, these parlodion sections must be handled one at a time since serial sections cannot be made with nitrocellulose media. As each section is cut, it is straightened out with a camel's hair brush on the top surface of the horizontally placed microtome knife and is then removed from the knife and placed flat in a dish of 70% alcohol. It must not be allowed to become dry. If it is important that the sections be kept in serial order, a square of paper should be inserted after every fourth or fifth section as they are stored in the dish of alcohol.

Staining the sections

Ordinarily the parlodion is not removed from the sections, and the sections are not mounted on slides until after staining, dehydrating, and clearing are completed. The sections are passed through the series of reagents separately or in groups of three or four, using a perforated section lifter to make the transfer.

From the 70% alcohol in which they are stored when cut, the sections may be stained with hematoxylin and eosin as follows.

Referring to Table 18.1, omit steps 1 to 3 and start with step 4; that is, transfer the parlodion sections from 70 to 95% alcohol. Follow each step down through step 17, which is 95% alcohol. At this point, for the absolute alcohol specified in steps 18 and 19, substitute carbol xylene (75 ml xylene plus 25 ml melted carbolic acid crystals). This substitution is made because the parlodion is slightly soluble in absolute alcohol. From carbol xylene the sections are transferred to xylene (steps 20 and 21).

The sections should not be allowed to become folded or rolled up during the staining process. When they are put into the carbol xylene, they must be flattened out carefully, because the xylene that follows will slightly harden the parlodion sections so that they cannot easily be flattened.

To mount the stained section on a slide, slip the clean side (no adhesive is used) into the dish of xylene beneath the section, lift the section onto the slide from the liquid, straightening it carefully, and quickly and firmly press it with a small piece of filter paper. The slide bearing the section is then quickly dipped back into the xylene and drained, mounting medium is flowed over the section, and a cover glass is dropped into place. The use of balsam for mounting nitrocellulose section will make mounting easier.

A modification of this embedding method, using acid celloidin instead of parlodion, will preserve much of the organic matrix of tooth enamel during the process of decalcification.

For variations in the hematoxylin and eosin stain and for information on the many other kinds of stains useful for both paraffin-embedded and parlodion-embedded specimens, the histology student must refer to books on microtechnique.

PREPARATION OF GROUND SECTIONS OF TEETH OR BONE

Decalcification of bone and teeth often obscures the structures. Teeth in particular are damaged because

tooth enamel, being about 96% mineral substance, is usually completely destroyed by ordinary methods of decalcification. Undecalcified teeth and undecalcified bone may be studied by making thin ground sections of the specimens.

The equipment used for making ground sections includes a laboratory lathe, a coarse- and a fine-abrasive lathe wheel, a stream of water directed onto the rotating wheel and a pan beneath to catch the water, a wooden block (about a 25 mm cube), some 13 mm adhesive tape, a camel's hair brush, ether, mounting medium, microscope slides, and cover glasses.

Let us suppose that a thin ground section is to be prepared of a human mandibular molar tooth cut longitudinally in a mesiodistal plane. The coarse abrasive lathe wheel is attached to the lathe, water is directed onto the wheel, the tooth is held securely in the fingers, and its buccal surface is applied firmly to that flat surface of the rapidly rotating wheel. The tooth is ground down nearly to the level of the desired section.

The coarse wheel is now exchanged for a fine-abrasive lathe wheel, and the cut surface of the tooth is ground again until the level of the desired section is reached.

At this point a piece of adhesive tape is wrapped around the wooden block in such a way that the sticky side of the tape is directed outward. The ground surface of the tooth is wiped dry and then is pressed onto the adhesive tape on one side of the wooden block. It will stick fast. With the block held securely in the fingers, the lingual surface of the tooth is applied to the coarse abrasive lathe wheel, and the tooth is ground down to a thickness of about 0.5 mm. Then the coarse wheel is again exchanged for the fine-abrasive lathe wheel, and the grinding is continued until the section is as thin as desired.

The finished ground section is soaked off of the adhesive tape with ether and then dried for several minutes. Drying for too long will result in cracking. It is then mounted on a microscope slide. To do this, a drop of mounting medium is placed on the slide, the section is lifted with a camel's hair brush and placed on the drop, another drop of mounting medium is put on top of the section, and a cover glass is affixed for microscopic study.

The teeth used for ground sections should not be allowed to dry out after extraction, because drying makes the hard tissues brittle and the enamel may chip off in the process of grinding. Extracted teeth should be preserved in 10% formalin until used.

Precision equipment (hard tissue microtome) for making ground sections with much greater accuracy is available. The method described here is one in which equipment at hand in almost any laboratory is used. The technical literature contains a number of articles on the preparation of sections of undecalcified tissues.

PREPARATION OF FROZEN SECTIONS

Frozen section provides rapid diagnosis and also permits study of tissue substances like lipid which may be lost during conventional processing procedures. Thus, frozen sections are also being extensively used in the demonstration of labile substances like enzymes and the target molecules for immunohistochemistry.

Fixed soft tissues or fresh unfixed soft tissues may be cut into sections 10 to 15μm thick by freezing the block of tissue with either liquid or solid carbon dioxide and cutting it on a freezing microtome after embedding the friable tissue fragments in gelatin following which it is immersed in 10% formalin. Frozen sections can be quickly prepared and are useful if the immediate examination of a specimen is required. Details of the preparation of frozen sections can be obtained from books on microtechnique.

TYPES OF MICROSCOPY

A thin tissue section has the property to modify the color or intensity of light passing through it. The modified, light-containing information from the section is amplified through the lens system of a microscope and transmitted to the eye. Since the unstained tissues do not absorb or modify the light to a useful degree, tissue staining is used to induce differential absorption of light so that tissue components may be seen.

Many types of microscopes are used for the study of tissues. The most common is the bright-field microscope, which is a complex optical instrument that uses visible light. Modifications of this instrument have provided the phase-contrast, interference, dark-field, and polarizing microscopes. The optical systems that utilize invisible radiations include the ultraviolet microscope, X-ray, and electron microscope. Each of these instruments has been a valuable tool in the study of oral tissues.

SUMMARY

Tissues from the oral cavity, both hard and soft tissues, can be studied microscopically after due preparation. In this chapter preparation of tissues for light microscopic study is described. Tissues have to be made into thin slices so that the light passes through them for visualization. In order to appreciate different components of tissue they have to be stained.

Methods of Study of Hard Tissues—Ground Sections

In routine preparations, hard tissues have to be made soft so that they can be cut easily. However, hard tissues can be made thin by grounding them into slices of about 50 mm thickness. Such sections are called ground sections. Lathe is often used to grind sections using a coarse wheel first and later a fine abrasive wheel so that the rough surfaces are removed. Throughout the preparation the specimens have to be protected from heat and should not be allowed to become dry. The ground tissues have to be washed well, passed through increasing grades of alcohol to ensure complete removal of water. The tissue is then passed through xylene to remove alcohol before it is mounted on a clean glass slide with a mountant medium usually *Canada balsam* or DPX and

covered with a cover glass. For precision work hard tissue microtomes are available to cut tissues into thin sections. For enamel this is the only method of study.

Routine Method of Study of Soft Tissues

Soft tissues have to be washed well to remove blood clots before fixation. In fixation tissue architecture is preserved. Fixation is usually done with 10% formalin for several hours usually overnight. The tissues have to be washed to remove excess of formalin in running water. The tissues are usually embedded in paraffin wax before they are cut into thin sections. Since water and paraffin are not miscible, water is first removed gradually with increasing grades of alcohol to minimize shrinkage. Later alcohol is removed with xylene, so that paraffin wax can penetrate the tissues and replace the xylene. A paraffin wax block is made with the tissue inside using L pieces. The tissue is then sectioned into 4μm sections using a microtome. The commonly used microtome is called rotary microtome. The cut sections are floated in warm water and attached to a clean glass slide, sometimes an adhesive made of egg albumin in glycerin is used for attachment.

Routine Staining Procedure

The commonly used stains are hematoxylin and eosin, both of which are aqueous solutions. Therefore wax has to be removed with xylene and later alcohol in descending grades is used to remove xylene. The sections are hydrated before staining is done. Tissues are first stained with hematoxylin before it is stained with eosin. Details of the procedure are given in the main text. Hematoxylin stains the nucleus violet and eosin stains the cytoplasm pink.

Decalcified Method of Study of Hard Tissues

Hard tissues can be made soft after fixation by decalcifying them usually with 5% nitric acid. The end point of decalcification can be determined mechanically using a pin or radiographically or by chemical methods. After the tissue has become soft it has to be washed well to remove all traces of acid. Thereafter the procedure is like that of a soft tissue. Except enamel all hard tissues can be processed by this method and studied, for enamel contains very little organic material to retain the architecture of the tissue. Pulp enclosed by hard tissue and periodontal ligament surrounded by hard tissue have to be studied by this method only.

Parlodion Embedding of Hard Tissues

Instead of embedding in paraffin wax decalcified tissues can be embedded in parlodion which is a purified nitrocellulose dissolved in ether–alcohol. Though better sections are possible with parlodion it is seldom used, as the procedure is quite cumbersome.

Frozen Sections

For rapid diagnosis as required in cancer surgery and for adipose tissues or for study of enzymes, sections are super cooled in a cryostat, cut and stained. These are called frozen sections. The entire procedure with routine staining takes less than 30 minutes.

Specialized Microscopes

Resolution of light microscopes can be improved by using ultraviolet rays, phase contrast or interference or polarized light. All these types of microscope and electron microscope which have a much higher magnification and resolution are not used for routine work.

REVIEW QUESTIONS

1. What are the different methods of tissue preparation for study under light microscopy?
2. Enumerate various methods for study of different oral tissues.
3. Briefly describe how ground sections are made.
4. Briefly describe the decalcification procedure.
5. Describe the routine staining procedure.
6. What are frozen sections and what are its uses?
7. Explain the terms: Fixation, Clearing and Embedding.

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Molecular Events in Oral Histology

APPENDIX

CHAPTER CONTENTS

CELLULAR AND MOLECULAR EVENTS IN ERUPTION

The various steps in eruption of teeth are the recruitment of the mononuclear cells to the dental follicle, its differentiation into osteoclast and their activation, bone resorption at the coronal half of the dental follicle, and bone formation at the basal end. Thus, dental follicle serves not only as a target tissue for mononuclear cells but also to regulate cellular events of eruption. Abnormalities of dental follicle as seen in certain diseases are therefore associated with failure of eruption. Similarly, failure in the formation of osteoclasts or in its function as seen in osteopetrosis, a bone disorder, is associated with failure of eruption. Experimental increase in the number and size of osteoclast but not their function causes delayed eruption.

Eruption is a localized genetically programed event. The dental follicle contains genes concerned with eruption. The regulatory genes that encode expression of various transcription factors are complex and involve series of signaling interactions between the dental follicle cells and cells of the bony crypt, namely the osteoclasts and the osteoblasts. Paracrine signaling from the stellate reticulum affects gene expression of molecules from dental follicle. Laser capture microdissection which permits specific portions of dental follicle of different ages to be excised and excised portions to be examined using real time polymerase chain reaction (RT-PCR) has enabled the comparative study of different molecules and their role in different regions of the dental follicle.

It has been shown that eruption pathway forms in the bone even if the tooth is stationary and that pressure from erupting tooth is not necessary for bone resorption.

Various molecules regulate eruption, often there are more than one having similar and overlapping functions (redundancy in function). This is to ensure that such a critical event like eruption, does not fail in the absence of a single factor. EGF and TGFα act through the same receptor to increase rate of eruption. Dental follicle cells which secrete colony stimulating factor-1 (CSF-1) and monocyte chemotactic protein-1 (MCP-1) increase the rate of eruption by increasing the recruitment of monocytes and formation of osteoclast. Null mice (knock-out mice) devoid of transcriptor factor gene c-fos or transcriptor genes NFκ (kappa) B1 and NFκB2 show failure of eruption due to lack of osteoclast differentiating factor, and failure of osteoclast formation. Mice lacking PTHrp also showed failure of eruption. Interleukin-1α (IL-1α) increases eruption rate. It increases MCP-1 gene expression, its synthesis and secretion and also CSF-1 formation. NFκB appears to be stimulated only by interleukin-1α. Interleukin formation is enhanced by TGF-β1. Thus, MCP-1, formation is enhanced by IL-1α, TGF-β1, EGF and CSF-1. The molecules CSF-1, c-fos, MCP-1, are expressed maximally just before influx of mononuclear cells and thereafter their numbers decrease.

It was suggested that enamel organ regulates the timing of eruption and that it may function as a 'biological clock'. The presence of IL-1α and PTHrp in the stellate reticulum suggests that stellate reticulum can play such a role.

Many molecules enhance gene expression of CSF-1 and MCP-1. IL-1α and PTHrp by paracrine signaling from stellate reticulum enhance gene expression of CSF-1. CSF-1 has autocrine effect on its gene expression. This leads to a sharp increase in CSF-1 production. Due to

positive feedback mechanism, the recruitment of mononuclear cells is inhibited consequent to reduction of gene expression of its receptor.

In osteoclast formation, RANKL also known as osteoclast differentiating factor (ODF) and osteoprotegerin (OPG) play an important role. OPG interferes with the binding of RANKL to RANK receptor in the alveolar bone by binding to RANKL, thus inhibiting signaling between stromal cells and mononuclear cells, leading to decrease in osteoclast formation. Recent studies in gene microarray showed that secreted frizzled-related protein-1 (SFRP-1) in combination with OPG produced maximal inhibition of osteoclast formation. CSF-1 and PTHrp decrease the expression of OPG. PTHrp increases the production of RANKL also known as both by cementoblast and by osteoblast. Dental follicle cells express vascular endothelial growth factor (VEGF) and a protein kinase C to upregulate VEGF. VEGF promotes the osteoclast formation in its second phase by increasing RANK expression on osteoclast precursors. Osteoblast activates osteoclast by acting through the RANKL/OPG pathway. The complex interrelationship between the various factors involved in the cellular events of eruption, i.e. in the recruitment of mononuclear cells and formation of osteoclast is illustrated in Figure A.1.

Postnatal studies of rat mandibular first molar showed the various genes that were involved in osteoclast formation at different times. At day 3, the major burst of osteoclastogenesis was linked to maximal expression of CSF-1

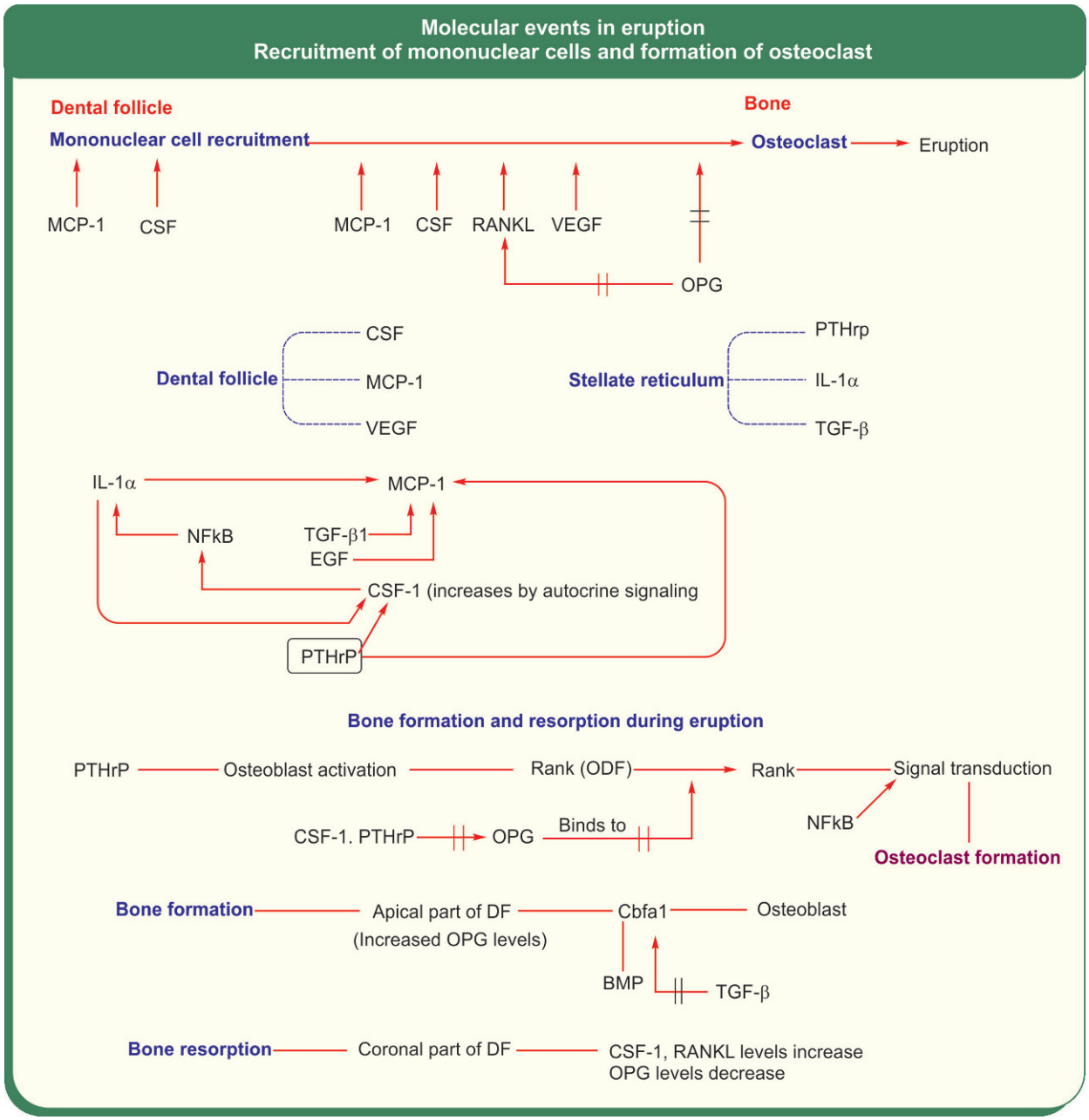

Figure A.1 Outline of molecular events in eruption of teeth. Arrows denote stimulation or enhancement, arrows with crosses denote inhibition, broken lines denote secretion and unbroken lines denote occurrence or results.

and at day 10, the minor burst in osteoclast formation to maximal expression of VEGF.

Bone formation is an equally important event. Core binding factor a1(Cbfa1/Runx2) is considered as the master gene of osteoblast differentiation. Its function is not duplicated. Cbfa1 is expressed by all cells of dental follicle except osteoclast. Growth factors like BMP regulate Cbfa1 expression in osteoblast. TGF-β downregulates Cbfa1 in the apical part of the dental follicle to favor bone resorption.

Dental follicle expresses regional variability in expression of genes regulating bone formation and resorption. It mediates bone resorption in the coronal half evidenced by RANKL expression and bone formation in the basal region, evidenced by BMP-2 expression. Expression of CSF-1, RANKL and OPG are correlated with osteoclastic activity. At the coronal half when the osteoclastic activity was more there was increased expression of RANKL and CSF-1, at the same time in the basal region the increased expression of OPG was correlated with decreased activity of osteoclast.

Extensive degradation and reorganization of extracellular matrix (ECM) components to facilitate eruption occurs, and this is mediated through degradation of ECM by matrix metalloproteinases (MMP) and its inhibition by inhibitors of MMP (TIMPS). MMP-8 is expressed by a wide variety of dental follicle cells and its expression is correlated with collagen types I and III formation.

MOLECULAR BIOLOGICAL FACTORS IN PERIODONTAL LIGAMENT HOMEOSTASIS

Cell Biology of Normal Periodontium

The production and destruction of tissue matrix (turnover) in a healthy state, involves interaction among a myriad of effector molecules that are synthesized and secreted by resident cells themselves.

Growth factors and *cytokines* that are believed to play a role in the pathogenesis of gingivitis and periodontitis, are probably the same ones, that operate to maintain periodontal homeostasis in health. There is evidence that, cytokines secreted by fibroblasts, endothelial cells, epithelial cells and cells of the immune system play an important role in tissue homeostasis, acting along with matrix metalloproteinases and their natural inhibitors, the tissue inhibitors of metalloproteinases.

Cytokines are a series of multifunctional polypeptides and glycoproteins that are secreted by one or several cell types and act locally or systemically. Included in this cytokine group are interleukins, cytotoxic factors, interferons, growth factors, colony stimulating factors and intercrines.

Growth factors have been defined as substances capable of re-initiating proliferation of cells that are in a quiescent state. Once cells enter G1 phase of cell cycle, other factors, i.e. progression growth factors are needed for transition into S phase and subsequent cell division.

The term 'growth factor' is misleading, since it is known that, these compounds display both, stimulatory and inhibitory activities. The resulting cell response is then dependent on the presence of other cytokines, the state of cell activation and the degree of cell differentiation.

In vivo, cytokines play an important role in numerous biological events, including development, homeostasis, regeneration, repair, inflammation and neoplasia.

Few of the cytokines that are important with regard to tissue homeostasis are discussed below.

1. ***Fibroblast growth factor (FGF)*** Two isoforms of fibroblast growth factor have been described in particular, one is acidic and the other basic. The two factors are products of different genes, but are similar in structure and biologic activities. The predominant view is that fibroblast growth factors are not humoral factors, but locally active tissue factors, integrated within the basement membrane.

Acidic fibroblast growth factor has effects on endothelial cell replication and neovascularization. It has been reported that, it stimulates DNA synthesis and cell replication, in bone tissue cultures, which results in increased protein synthesis especially, type I collagen.

Basic fibroblast growth factor has angiogenic properties and is highly chemotactic and mitogenic for a variety of cell types. It stimulates bone cell replication and increases the number of cells of osteoblastic lineage. Fibroblast growth factors bind to heparin sulfate, heparin and fibronectin in the extracellular matrix. One of the major differences between the actions of acidic fibroblast growth factor and basic fibroblast growth factor is that, the effect of basic fibroblast growth factor on cell replication in some cell cultures, is not increased by heparin, as is that of acidic fibroblast growth factor. Fibroblast growth factor is a potent stimulator of periodontal cell migration and mitogenesis, but its effect on matrix production is not clear.

2. ***Platelet derived growth factor (PDGF)*** This factor is a potent growth factor for various connective tissue cells and is released from the α-granules in platelets in conjunction with blood coagulation. PDGF molecule consists of two disulfide-bonded polypeptide chains, denoted A and B. The molecular weight is 28–35 KDa. There is homology between A and B chains with conservation of eight cysteine residues. The heterodimer consisting of A and B chains is the major PDGF isoform in human platelets.

This factor binds to specific high-affinity receptors expressed on the surface of various cell types, including fibroblasts, glial cells and vascular smooth muscle cells.

PDGF is a promoter of cell migration and a potent mitogen for cells bearing PDGF receptors. It acts synergistically with other growth factors as a competence factor and non-reciprocally inhibits EGF (epidermal growth factor) binding to EGF receptors. Progression of cells activated by PDGF from S phase, through rest of the cell cycle into cell division requires the presence of growth factors such as insulin or insulin-like growth factor. PDGF stimulates type V collagen formation and a drop in type III production in gingival fibroblasts. It increases cell proliferation in bone cultures, but also increases bone resorption, a process dependent on prostaglandin synthesis.

3. ***Transforming growth factor (TGF)*** These factors are polypeptides isolated from normal and neoplastic tissues, which are known to cause a change in normal cell growth. TGF has been classified as α and β according to their relationship to EGF.

TGF-α is a 50 amino acid polypeptide with a molecular weight of 5.6 KDa. TGF-α shares extensive amino acid homology with EGF (epidermal growth factor). It causes similar biological effects acting through EGF receptor. However, it is synthesized primarily by malignant cells.

TGF-β was originally purified from human placenta, platelets and bovine kidney. It is synthesized by normal cells, and is found in higher concentrations in the a granules of platelets, where it is present in amounts equivalent to PDGF. It has a molecular weight of 25 KDa and consists of two identical polypeptides of 112 amino acids.

TGF-β stimulates the synthesis of connective tissue matrix components, such as collagen, fibronectin, proteoglycan, and glycosaminoglycans. These effects may be enhanced by reducing the synthesis of proteinases, that are involved in connective tissue degradation such as collagenase. TGF-β reduces the level of collagenase expression, induced by EGF and BFGF (basic fibroblast growth factor) and enhances the induction of tissue inhibitors of MMP (matrix metalloproteinases) by EGF and FGF.

4. ***Interleukin-1 (IL-1)*** Interleukin-1 is a polypeptide, with a great number of roles in immunity, inflammation, tissue breakdown and tissue homeostasis. It is a 33 KDa precursor molecule that is subsequently processed during or after secretion to smaller entities of 15–17 KDa. It is synthesized by various cell types, including macrophages, monocytes, lymphocytes, vascular cells, brain cells, skin cells and fibroblasts, following cellular activation.

Two entities of interleukin-1 are best known as interleukin-1α and interleukin-1β. The two forms bind to the same receptor, which is found on many cell types in varying densities.

Most human interleukin-1 produced by stimulated macrophages is IL-1β. IL-1α remains largely cell-associated, whereas IL-1β is released from the cell.

IL-1 mediates tissue remodeling, repair and inflammation through many physiological and pathological processes. IL-1 plays a key role in the pathogenesis of bone diseases and adult periodontitis. IL-1 enhances fibroblast synthesis of type I procollagen, collagenase, hyaluronic acid, fibronectin and PGE2 (prostaglandin E2). It has also been suggested that interleukin-1 stimulates bone resorption and is identical to osteoclast-activating factors. Unrestricted production of interleukin-1 may lead to severe tissue damage.

5. ***Interferon-γ*** It possesses important immunomodulatory effects and thus is a lymphokine as much as an interferon. It has a molecular weight of 35–70 KDa. The production of interferon-γ is modulated by other cytokines such as interleukin-1. Many biological activities have been ascribed to interferon-γ including action on B and T lymphocytes, antibody production, natural killer cells, macrophages and tumor cells. Antiviral activities have been observed for interferon-γ, but these are less pronounced as compared to interferon-α and interferon-β. It affects collagen production by turning off collagen gene expression.
6. ***Matrix metalloproteinases and their tissue inhibitors (MMPs)*** Connective tissue cells participate in both the formation and breakdown of connective tissue matrix. Such cells are found to synthesize and secrete a family of enzymes known as MMPs (matrix metalloproteinases).

MMP gene family encodes a total of 24 homologous proteinases classified into collagenases, gelatinases, stromelysins, membrane type MMPs depending on their substrate specificity and molecular structures. They are synthesized in a latent non-active form and require activation for enzyme function. These proteinases function at neutral pH and can digest all major matrix molecules. The various MMPs differ substantially in their molecular weight. Collagenase obtained from PMNL cells is larger than collagenase secreted by fibroblasts, but these degrade similar substrates. Neutrophil collagenase is released instantaneously upon challenge, whereas, it takes fibroblasts hours to release the enzyme. Transcription of fibroblast MMP is regulated by a variety of cytokines and growth factors, but a comparable regulation is not known for neutrophil collagenase.

TGF-β can repress MMP production in inflamed tissues. However, it has been observed that expression of MMP-9 is turned on by TGF-β, whereas MMP-2 production is turned off by TGF-β. It has also been proposed that interleukin-1 increases MMP production. Activity of MMP is regulated further by serum derived α2-macroglobulin and by the tissue inhibitors of MMPs.α2-macroglobulin covalently crosslinks with and inactivates target MMPs. Tissue inhibitors of MMPs are expressed by various connective tissue cells, epithelial cells, endothelial cells and macrophages. Tissue inhibitors of MMP-1 gene expression are increased in presence of EGF, TNF-α (tumor necrosis factor), interleukin-1 and TGF-β. In contrast, TGF-β represses gene expression of tissue inhibitor of MMP-2.

Cellular homeostasis in tissues, is the result of a balance among complex interactions of antagonistic and synergistic molecules with cytokines, MMPs and natural inhibitors of MMPs playing major roles. There is a simultaneous existence of four different functional stages and phenotypes of fibroblasts: (1) a cell in proliferation, (2) a cell that participates in matrix synthesis and secretion, (3) a cell that participates in matrix resorption, and (4) a cell in apoptosis.

PDGF, FGF and IL-1 are inducers of anabolic cell activities, whereas interferon-g and PGE-2 are inhibitors of such activities. TGF-β plays an important role in extracellular matrix homeostasis.

BONE: MOLECULAR ASPECTS OF ITS STRUCTURE AND REGULATION OF OSTEOBLASTIC AND OSTEOCLASTIC ACTIVITY

Structure of Bone Matrix

Noncollagenous proteins

Noncollagenous proteins comprise the remaining 10% of the total organic content of bone matrix. Most are ***endogenous proteins*** produced by bone cells, while some like ***albumin*** are derived from other sources such as ***blood*** and become incorporated into bone matrix during osteosynthesis.

Osteocalcin is the first noncollagenous protein to be recognized and represents less than 15% of the noncollagenous bone protein. Osteocalcin is also known as **bone Gla protein** as it contains the amino acid ***γ-carboxy glutamic acid.*** This protein has also been demonstrated in alveolar bone immunohistochemically.

Osteocalcin is a glycoprotein secreted by osteoblasts and is regulated by ***vitamin D_3*** and ***parathyroid hormone.*** The carboxy terminal segment of osteocalcin acts as a chemoattractant to osteoclast precursors, suggesting a role in bone resorption. It is also believed to be involved in bone calcification as it is a calcium binding protein. It is used as a marker of new bone formation.

Osteopontin and **bone sialoprotein** were previously, termed as ***bone sialoproteins I and II*** respectively. These have been demonstrated in alveolar bone, using immunohistochemistry. Both proteins are heavily glycosylated and phosphorylated, with high levels of acidic amino acids. ***Glutamic acid*** is predominant in bone ***sialoprotein*** and ***aspartate*** is predominant in ***osteopontin.*** Despite the structural similarities, these proteins have clearly different functional roles. Bone sialoprotein is restricted to mineralizing tissues, whereas osteopontin has a more generalized distribution. ***Bone sialoprotein*** is thought to function in the *initiation* of mineral *crystal formation in vivo.* In contrast, *osteopontin* is a potent *inhibitor of hydroxyapatite crystal growth,* and is enriched at cell matrix interface, where it can mediate the attachment of bone cells, including osteoclasts. *Osteopontin transcription* is strongly *upregulated by vitamin D_3, whereas bone sialoprotein transcription is suppressed by vitamin D_3.* The expression of both proteins is stimulated by factors that stimulate bone formation, such as ***TGF-β*** family members and ***glucocorticoids. Osteopontin, vitronectin*** and ***fibronectin*** are termed RGD-containing proteins, as they have a specific amino acid sequence (***Arg-Gly-Asp***), which has important implications in the attachment of cells to the bone matrix.

Osteonectin comprises about 25% of noncollagenous proteins. It is bound to collagen and hydroxyapatite crystals. It is a secreted ***calcium binding glycoprotein***, that interacts with extracellular matrix molecules. It has been proposed that, it may play a role in the regulation of cell adhesion, proliferation and modulation of cytokine activity, and in initiating hydroxyapatite crystal formation.

Proteoglycans are also present in the bone matrix. A ***large*** **chondroitin sulfate proteoglycan,** has been extracted from the nonmineralized bone matrix, while two small proteoglycans, ***biglycan*** and ***decorin*** (***chondroitin sulfate proteoglycan I and II*** respectively) have been found in EDTA extracts of bone. Decorin and biglycan comprise $< 10\%$ of the noncollagenous proteins in bone, but this decreases with maturation of bone. A third small proteoglycan (***chondroitin sulfate*** **proteoglycan**) has been found entirely associated with mineral crystals. Biglycan is more prominent in developing bone and has been mineralized to pericellular areas. The precise function of ***biglycan*** is unknown, but similar to decorin, it can ***bind TGF-β*** and extracellular matrix macromolecules, including collagen, and thereby regulate ***fibrillogenesis.*** **Decorin,** as the name suggests, binds mainly within the gap region of collagen fibrils and decorates the fibril surface. The primary calcification in bones is reported to follow removal of decorin and fusion of collagen fibrils.

Lysyl oxidase and **tyrosine rich acidic matrix proteins (TRAMP)** are components of demineralized bone and dentin matrix. Lysyl oxidase is a critical enzyme for collagen cross-linking. TRAMP, also known as ***dermatopontin***, binds decorin and TGF-β, and together these proteins regulate the cellular response to TGF-β.

Procollagen peptides, thrombospondin, fibronectin, vitronectin and alkaline phosphatase are the other proteins found in bone.

Matrix Gla protein and **α2HS glycoproteins** are not secreted by osteoblasts but are of particular interest, with respect to regulation of mineralization. Matrix Gla protein is a mineral binding extracellular matrix protein secreted by vascular smooth muscle cells and chondrocytes that prevent mineralization in vascular tissues and cartilage. The absence of α2HS glycoprotein, which is produced by the liver, compromises the inhibition of apatite formation by serum.

Bone matrix also contains **proteases, protease inhibitors** and a variety of **cytokines** secreted by osteoblasts, that regulate cell metabolism. These cells secrete several members of **bone morphogenetic proteins (BMP) superfamily**, including **BMP-2, BMP-7, TGF-β**, insulin-like growth factors **(IGF-I and IGF-II)**, platelet derived growth factor (**PDGF**) and fibroblast growth factor (FGF). IGF-I, PDGF and TGF-β increase the rapidity of bone formation and bone repair.

Regulation of Osteoblast Activity

The overall integrity of bone is controlled by hormones, proteins secreted by hematopoietic bone marrow cells and bone cells.

Role of parathormone (PTH)

In response to hypocalcemia, the hormone activates a mechanism for the release of calcium from bone, the principal body storehouse. PTH does so, by an indirect effect mediated by PTH receptors on bone stromal cells including osteoblasts, as osteoclasts are devoid of PTH receptors. PTH regulates serum calcium levels by stimulation of bone resorption and can also have anabolic effects in vivo that appear to be mediated through TGF-β and IGF-I. These opposing effects of PTH are consistent with the apparent coupling of bone formation and remodeling.

Cells of mesenchymal lineage that differentiate into osteoblasts were shown to produce PTHrP very early during differentiation, and PTHrP levels decreased with further cell maturation. PTHR1 is expressed at a later stage in differentiation, by committed preosteoblasts. PTHrP (parathyroid hormone related protein) resembles PTH in its amino terminal sequence and the two have similar structural requirements for binding and activation of their common receptor, type 1 PTH receptor (PTHR1). Persistently increased levels of local PTHrP favor increased osteoclast formation through stimulation of RANKL production. Hence, PTHrP needs to be regulated in terms of concentration, location and time, so that it is presented briefly to target cells. PTH is secreted as a hormone in response to a hypocalcemic signal in order to regulate calcium homeostasis by promoting bone resorption, whereas PTHrP functions as a bone cytokine to control bone mass.

While ***vitamin D_3*** stimulates bone resorption, it is also essential for normal bone growth and mineralization. It also

promotes calcium absorption from the intestine. It stimulates synthesis of osteocalcin and osteopontin by osteoblasts and suppresses collagen production. The action of parathormone and vitamin D_3 is that, they enhance bone resorption at high concentrations (pharmacological) and support bone formation at low (physiologic) concentrations.

Growth hormone is required for attaining normal bone mass which is mediated by the local production of ***IGF-I***. It binds to membrane bound growth hormone receptors on activated osteoblasts.

Insulin targets osteoblasts directly and stimulates bone matrix formation and mineralization and indirectly affects bone formation through stimulation of IGF-I (insulin-like growth factor) produced in the liver.

Bone morphogenetic proteins

These are the only factors that can initiate osteoblastogenesis from uncommitted progenitor cells. These proteins are expressed during embryonic development as well as in adulthood. BMPs 2, 4 and 6 direct the pluripotent cells to commit to an osteoblastic pathway. BMPs can also increase the differentiation of committed cells to the osteoblast lineage. BMPs can also upregulate cbfa1 under certain conditions during osteoblast differentiation. cbfa1, in turn, activates osteoblast-specific genes such as osteopontin, bone sialoprotein, type I collagen and osteocalcin.

TGF-β During the early stages of bone formation, the action of transforming growth factor-β is to recruit and stimulate osteoprogenitor cells to proliferate, providing a pool of early osteoblasts. In contrast, during later stages of osteoblast differentiation, TGF-β blocks differentiation and mineralization. These effects appear to be highly dependent on bone cell source, dose applied and local environment, which may be a result of inhibition of DNA synthesis at high TGF-β concentration. Additionally, it inhibits the expression of Runx-2 and osteocalcin genes, whose expression is controlled by cbfa1/Runx-2 in osteoblast-like cell lines.

IGF I and II (insulin-like growth factors) increase proliferation and play a major role in stimulating mature osteoblast function. IGF-1 upregulates osterix but not cbfa1. IGF-1 along with BMP-2 acts synergistically on osterix expression.

FGF (fibroblast growth factors) are a family of structurally related polypeptides that play a critical role in angiogenesis and mesenchymal cell mitogenesis. In normal adult tissues, the most abundant proteins are FGF-1 and FGF-2. FGF-2 is expressed by osteoblasts and is more potent than FGF-1. These factors exert their effect on bone formation, primarily through increased proliferation of osteoprogenitor cells and promotion of osteogenic differentiation.

Glucocorticoids promote differentiation of osteoblasts and stimulate bone matrix formation *in vitro*. But, prolonged treatment with glucocorticoids *in vivo*, results in bone loss, which can be attributed to increased PTH production in response to the inhibitory effects of glucocorticoids on calcium absorption and depletion of osteogenic precursor cells.

PDGF (platelet derived growth factor) The isoforms of this factor have a strong chemotactic effect on osteoblasts and other connective tissue cells and may act to recruit mesenchymal cells, during bone development and remodeling. It has similar effects to fibroblast growth factor in promoting osteogenesis. It acts as a potent mitogen for all cells of mesenchymal origin. PDGF may also have direct and indirect effects on bone resorption by the upregulation of collagenase transcription and an increase in IL-6 expression in osteoblasts.

Vascular endothelial growth factor (VEGF) acts directly on osteoblasts to promote osteoblast migration, proliferation and differentiation in an autocrine manner. It is also a mediator of osteoinductive factors like TGF-β, IGF-1, FGF-2, which upregulate VEGF expression in osteoblasts. VEGF also influences osteoblasts indirectly via its effects on endothelial cells. It stimulates the production of bone forming factors for osteoblasts by endothelial cells.

The ***Wnt/β-catenin signaling pathway*** has been shown to have important roles in the maintenance of self-renewal of stem cells in epidermal and hematopoietic cells and regulation of bone formation. This pathway is proposed to directly promote osteogenesis through actions on Runx-2 gene.

Formation of Osteoclast

Multinucleated giant cells are derived from hemopoietic cells of monocyte macrophage lineage. The earliest identifiable hematopoietic precursor that can form osteoclast is the granulocyte-macrophage colony forming unit (CFU-GM). The early precursor cells proliferate and differentiate to form post mitotic committed precursor cells. These committed precursors then differentiate and fuse to form immature multinucleated giant cells. These are activated to form bone resorbing osteoclasts. The differentiation into osteoclasts is through a mechanism involving cell–cell interaction with osteoblast stromal cells.

The formation of osteoclast requires the presence of ***RANK ligand (receptor activator of nuclear factor κB)*** and ***M-CSF (macrophage colony stimulating factor)***. These two membrane bound proteins are produced by neighboring stromal cells and osteoblasts, thus requiring direct contact between these cells and osteoclast precursors.

M-CSF acts through its receptor on osteoclast precursors c-***Fms*** (colony stimulating factor 1 receptor) and thereby provides signals required for proliferation. M-CSF also enhances osteoclast activity by preventing osteoclast apoptosis.

RANKL belongs to the TNF (tumor necrosis factor) family of ligands. RANKL is also called osteoclast differentiation factor (***ODF***), TNF related induced cytokine (***TRANCE***), or osteoprotegerin ligand (***OPGL***).

RANK is homotrimeric TNF receptor family member. As RANKL binds to RANK on surface of M-CSF triggered osteoclast precursors, a number of signaling pathways are activated committing the cell to osteoclast lineage. These are initially mediated by TRAF 6 TNF (receptor-associated factor), leading to activation of NF-κβ, the (AP)-1 transcription factor complex and ultimately NFATc1 nuclear factor of activated T cells.

RANKL has been implicated in the fusion of osteoclast precursors into multinucleated giant cells, their differentiation into mature osteoclasts, their attachment to bone surface and their activation to resorb bone.

Osteoprotegerin (OPG) is a member of the TNF receptor family and is expressed by osteoblasts. It recognizes

RANKL, and blocks the interaction between RANK and RANKL, leading to an inhibition of osteoclast differentiation and activation. Cbfa1 contributes to the expression of OPG. The balance between RANKL-RANK signaling and the levels of biologically active OPG, regulates development and activation of osteoclasts and bone metabolism.

Regulation of Osteoclast Activity

Estrogen suppresses the production of bone resorbing cytokines including IL-1 and IL-6. Estrogen deficiency results in marked bone resorption by increasing osteoclast activity. It is suggested that estrogen prevents excessive bone loss before and after menopause by decreasing the life span of osteoclast, by promoting apoptosis. Estrogen increases production of TGF-β by osteoblasts which stimulate apoptosis of osteoclasts.

Vitamin D_3 and parathyroid hormone (PTH) Vitamin D_3 promotes the differentiation of osteoclasts from monocyte macrophage stem cell precursors *in vitro. In vivo*, enhanced osteoclastic bone resorption has been observed when vitamin D_3 is applied at high dose. This effect is caused by the stimulation of RANKL production by osteoclasts. PTH is secreted in response to changes in blood calcium and affects bone formation and resorption. PTH binds to osteoblasts and induces the production of M-CSF and RANKL that stimulate the maturation and action of osteoclasts.

Calcitonin is a particularly potent inhibitor of osteoclast activity, but its effects are transient likely, due to downregulation of calcitonin receptor on osteoclast in the sustained presence of hormone. ***Calcitonin*** inhibits proliferation and differentiation of osteoclast precursors. It reduces the dimension of ruffled border and their dissociation into monocytic cells. Calcitonin also promotes osteoclast apoptosis.

Several factors expressed by osteoblasts/lymphocytes have an impact on osteoclastogenesis to enhance (IL-**1,** IL-**6,** IL-**8** and IL-**11**) or limit (IL-**4,** IL-**10,** IL-**12,** IL-**13,** IL-**18**) osteoclast formation.

IL-***6*** is produced by stromal or osteoblast lineage cells in response to PTH and vitamin D_3 and on stimulation by IL-1, TNFα, TGFβ, PDGF and IGF-2. IL-6 alone or in concert with other agents stimulates osteoclastogenesis. But it is a much less potent stimulator of osteoclast generation than IL-1 and TNF-α.

Osteoclast precursors interact with bone matrix to trigger their own differentiation by producing IL-1. IL-1 can also induce osteoclast formation by increasing expression of RANKL on surface of osteoblasts and marrow stromal cells.

TNFα stimulates differentiation of osteoclast progenitors into osteoclasts in the presence of M-CSF independent of RANKL-RANK interaction.

OCIL (osteoclast inhibitory lectin) has been recognized as an inhibitor of osteoclast formation. OCIL and its related proteins ***OCILrP1*** and ***OCILrP2*** are type II membrane bound C-lectins expressed by osteoblasts.

TGF-β ***and interferon***-γ inhibits proliferation and differentiation of committed precursors into mature osteoclasts. TGF-β also promotes apoptosis of osteoclasts. But, TGF-β is also believed to enhance osteoclast differentiation in hematopoietic cells stimulated with RANKL and M-CSF.

IFN-γ interferes with the osteoclast differentiation induced by RANKL and this mechanism is critical for the suppression of pathological bone resorption associated with inflammation.

Bisphosphonates suppress bone resorption via injury to osteoclasts when they solubilize bisphosphonate contaminated bone. These do not suppress osteoclastogenesis. They are capable of inducing osteoclast apoptosis. Bisphosphonates suppress bone resorption without consistent reduction in osteoclast numbers which suggests that these compounds might act through a mechanism that is distinct from OPG. These are used as antibone resorbing agents in various diseases associated with stimulated bone resorption.

PGE_2 (Prostaglandins of E series) can act as powerful mediators of bone resorption and can also influence bone formation. The prostaglandins, induce osteoclast formation through increased expression of RANKL on the surface of immature osteoblasts and stromal cells. PGE_2 can also stimulate bone formation when administered systemically.

MOLECULAR EVENTS FOLLOWING PULP INJURY AND REPAIR

The molecular events that follow injury to the odontoblast are similar to that occurring during primary dentinogenesis. Growth factors, molecular signaling mechanisms and cytoskeletal reorganization all occur in pulp repair as in primary dentinogenesis.

Signals exchanged between odontoblast and progenitors expressing notch receptors influence the fate of the cell differentiation, proliferation or apoptosis. Since bone marrow stem cells also express notch receptors it can be deduced that odontoblast precursors have stem cell like properties. However notch receptors are absent in terminally differentiated odontoblast.

In mild injury, odontoblasts survive to produce reactionary dentin. Molecules present in the cytoskeletal network in the odontoblastic process such as kinesin, actin and myosin play an important role in healing by producing rapidly a dense skeletal network adjacent to the wound site. Similarly nestin, fibronectin and vimentin play an important role to re-establish the normal architecture.

Cell adhesion molecules such as N-cadherin are expressed by differentiating odontoblast but not by terminally differentiated odontoblast. These molecules are involved in cell adhesion, cell recognition, control of cell division, migration and differentiation. Cadherin interacts with catenins which connect them to actin filaments of the cytoskeletal network. Gap junctions that form between odontoblasts facilitate rapid exchange of metabolites, which are necessary during repair.

Growth factors such as TGF-β (transforming growth factor), FGF (fibroblast growth factor), BMP (bone morphogenetic protein), and IGF (insulin-like growth factor) which are secreted by odontoblast and present in dentin matrix, are released when the matrix is demineralized, as occurring in caries or during acid etching. These factors travel through the dentinal tubules to interact with the receptors on the pulp cells and act as mitogens as well as chemotactic agents.

They recruit inflammatory cells, cause migration of odontoblastic process and initiate angiogenesis. TGF-β1 interacts with integrin receptors to facilitate migration. Growth factors have opposite effects on notch-positive and nestin-positive cells. Notch-positive cells are proliferative while nestin-positive cells are differentiated cell populations.

Nerve growth factor released as a result of injury and due to TGF-β1, causes nerves to sprout. Through sprout formation neuropeptides are delivered to facilitate healing. Growth factors and cytokines activate early response genes like c-jun and jun-B which through their protein products like AP-1 regulate cell proliferation, migration and differentiation of odontoblast precursors. Genes homologous to human FIP-2 were shown to play an important role in the healing process.

Macrophages not only remove debris, bacteria and dead cells but also amplify signals by releasing cytokines and growth factors.

Calcium released by injured cells acts as a mediator signaling undamaged cells to coordinate cell migration and regulate cytoskeletal organization.

MOLECULAR INSIGHTS IN TOOTH MORPHOGENESIS

The first morphological sign of tooth development in mammalian embryos is the appearance of localized thickenings of the stomodeal epithelium (E11.5–12.5, tooth germ stage of mice. In this section, all the numbers following 'E' indicate days of development after embryogenesis in mice). Further thickening of dental epithelium and invagination into the underlying mesenchyme leads to the formation of the dental lamina (E13.5, tooth bud stage). The condensation of the mesenchyme around the bud invades the base of the dental lamina, forming the hillock-shaped dental papilla (E14.5, cap stage). The dental papilla deeply invaginates into the dental lamina and constitutes the core of the developing teeth (bell stage).

While the gross histological processes of tooth formation are well documented the governing molecular events are beginning to be elucidated with the continuous application of molecular biological techniques on to the field of developmental biology. Studies on mammalian development are carried out with mice due to its suitability for both genetic and embryological manipulations. Intricate inductive tissue interactions between apposed epithelial and mesenchymal tissue layers are used recurrently throughout vertebrate development to direct the formation of many organs. The morphogenesis of organs requires exact control of proliferation and differentiation of cells in time and space.

The study of tooth development used manipulation of tooth germ explants from wild type and mutant mice. The dental epithelium and ectomesenchyme are separated and recombined with tissues of different origins, developmental stages and altered genetic constitutions. Expression domains of molecules of interest are variously visualized using techniques like in situ hybridization and mRNA labeled probes. Acrylic and heparin acrylic beads can be implanted with proteins to study the downstream gene expression patterns.

Tooth Initiation Potential

Understanding tooth development begins from answering the difficult question as to how the tooth initiation takes place. Experiments conducted combining murine first arch epithelium with neural crest in the anterior chamber of the eye resulted in the formation of tooth, while epithelium from other sites like that of limb or second arch does not produce the same results. When dental epithelial organ is combined with skin, the tooth organ loses its dental characteristics and takes up the features of the epidermis. Thus the primary question that needs exploration is whether the tooth initiation potential harbors within the dental epithelium or dental mesenchyme.

The expression of signaling molecules in the branchial arch ectoderm was expressed in a restricted spatial pattern even after the ablation of the cranial neural crest cells proving the independence of the ectoderm in determining the polarity of the arch although the identity may be determined by neural crest cells.

Up to E9.5, the ectomesenchyme cells remain uncommitted indicating their competency to respond to epithelial signals regardless of their position, but by E10.5, the spatial expression domains have been established in the ectomesenchyme by *Fgf-8* (fibroblast growth factor-8) and *Bmp*-4 (bone morphogenetic protein). These signals are still required to maintain these expression domains but have lost their competency to express any other gene in response to the signals from outside. At E11 stage, the ectomesenchymal cells were shown to be independent of epithelial signals (Fig. A.2). The dental epithelium is shown to stimulate the expression of numerous developmental regulatory genes in the mesenchyme like muscle specific homeobox-like genes in vertebrates (*Msx*-1, *Msx*-2) and distaless gene homologue in vertebrates (*Dlx*). The investigations have shown that these stimulatory effects can be mimicked by signaling molecules including *Fgf*-8, *Bmp*-2, *Bmp*-4, and *Bmp*-7. These experiments have clearly shown us the influence of dental epithelium over the dental mesenchyme during the initial stages of tooth initiation.

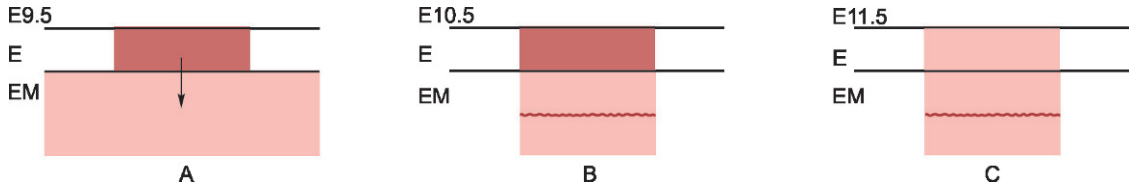

Figure A.2 The schematic representation explains the signaling interdependence between epithelium (E), and ectomesenchyme (EM). **A**, The uncommitted mesenchymal cells are equally responsive and dependent on epithelium for signals. **B**, The domains of ectomesenchymal gene expression become fixed but still depend on epithelium for signals. **C**, The fixed gene expression domains of the ectomesenchyme are no more dependent on epithelium.

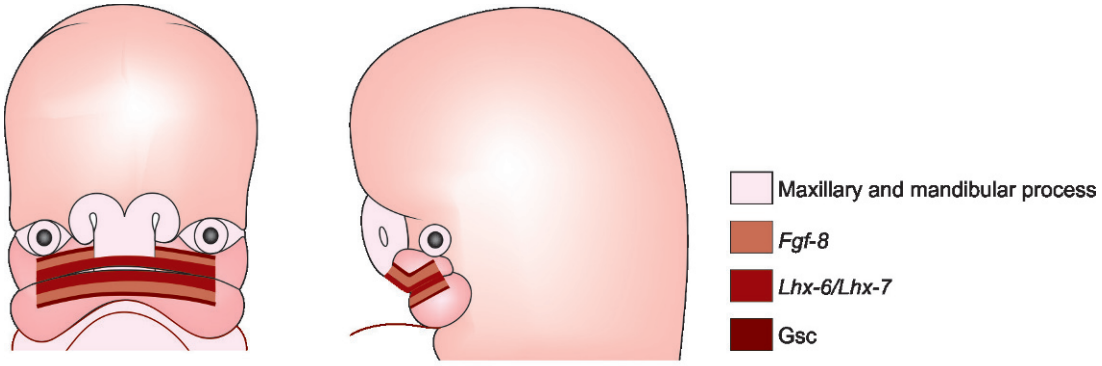

Figure A.3 The schematic representation of head portion of the embryos showing the location of *Lhx*-6/7, *Fgf*-8 and *Gsc* in color coded format. Note the expression of *Fgf*-8 is restricted to the oral epithelium matching very closely to the expression domain of *Lhx*-6/7 in the ectomesenchyme.

Establishment of Oral–Aboral Axis

LIM-homeobox (*Lhx*) genes constitute to that group of gene clusters which are dispersed outside the homeobox gene clusters and they are transcriptional regulators controlling pattern formation. *Lhx*-6 and *Lhx*-7 have been localized during embryogenesis at high levels in overlapping domains in the branchial arch, its derivatives and in the basal forebrain. Both are identically distributed in the ectomesenchyme adjoining the oral epithelium even at E9.5 and later in the mesenchyme adjacent to the epithelial thickenings which constitute the dental primordium. The expression of *Fgf*-8 establishes the anteroposterior axis of the first branchial arch and was shown restricted to the first arch. *Fgf*-8 has been attributed to be regulating the expression of *Lhx*-6 and *Lhx*-7 genes. *Fgf*-8 extends in broad mediolateral domain matching with the expression domains of *Lhx*-6 and *Lhx*-7 (Fig. A.3). The restricted expression of goosecoid (*Gsc*) to establish expression in aboral mesenchyme involves the repression by *Lhx*-6/*Lhx*-7 expressing cells. Although, the mechanism that restricts the oral mesenchyme is independent of *Gsc* expression, the most probable factor that decides this would be the distance from the source of *Fgf*-8. This opens up a cascade of molecular interactions that leads to the establishment of oral–aboral axis and tooth development.

Control of Tooth Germ Position

Fgf-8 has been proposed to act antagonistically with *Bmp*-4 to specify the sites of tooth initiation and the former has been localized in the oral ectoderm as early as E9. It is also shown that mesenchymal *Bmp*-2 and epithelial *Bmp*-4 antagonize the induction of mesenchymal *Pax*-9 (paired box homeotic gene-9) by *Fgf*-8, a member of *Pax* family, which helps to establish the position of prospective tooth mesenchyme. Both *Bmp*-4 and *Fgf*-8 exhibit close interactions with each other (Fig. A.4). *Bmp*-4 and *Fgf*-8 are possibly regulated by transcription factors like *Prx*-1 and *Prx*-2 from neural crest cells since double knock-out mice shows ectopic expression of *Fgf*-8. Another potential regulator of *Fgf*-8 is *Pitx*-2 whose epithelial expression prefigures the location of teeth. *Pitx*-2 has been shown to be required for the progression beyond epithelial thickening or bud stage. Thus *Pitx*-2 seems to regulate *Fgf*-8 in a positive feedback mechanism while *Bmp*-4 in a negative feedback loops (Fig. A.4).

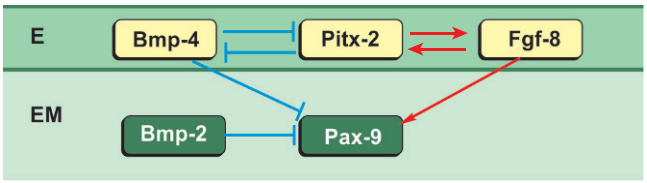

Figure A.4 The schematic representation depicts the fine interactions that control the position of tooth germ. The blue and red arrows indicate the negative and positive feedback mechanisms that exist between various molecules represented.

Functional Redundancy and their Complexities

Presence of multiple signaling molecules, their intricate interactions and their closely knit feedback loops along with their redundant roles increase the complexity of understanding the independent role of molecules involved in tooth initiation.

Of the *Dlx* gene family, targeted mutation in either of the two members, *Dlx*-1 or *Dlx*-2 affects only the skeletal elements derived from the proximal ends of the first and second arches but has no effects on the tooth development. However mice compounded with both *Dlx*-1 and *Dlx*-2 exhibit a selective absence of upper molars. This is because the proximal maxillary and mandibular arch cells are positive for both *Dlx*-1 and *Dlx*-2 while *Dlx*-5 and *Dlx*-6 are positive only in the mandibular arch cells explaining redundant roles of these molecules compensating the loss of *Dlx*-1 and *Dlx*-2 in the mandibular arch and not in the maxillary: thus explaining the absence of maxillary molars.

Sonic hedgehog (*Shh*) has been exclusively identified at the sites of epithelial thickenings and it functions as a mitogen. The *Shh* has been considered crucial for odontogenesis to occur in the regions of the established oral axis. *Shh* knock-out mice have little effect in face development and thus its function in tooth initiation cannot be explained. Hedgehog signaling is mediated by *Gli*-family (glioma-associated oncogene) zinc finger transcription factors. The double mutant embryos of *Gli*-$2^{-/-}$ and *Gli*-$3^{-/-}$ do not produce any recognizable molar tooth buds but just produce a single small incisor tooth germ. Epithelial–mesenchymal signaling interactions involving other well-known pathways of tooth initiation, namely *Lef*-1 pathway, and for the signaling at early bud stage, namely *Bmp*-4, *Msx*-1 and activin-βA were all found to be normal in these mutants.

Thus, the position dependent defects are not only recognized in loss of *Dlx*-1, *Dlx*-2 but also in *Gli*-2 and *Gli*-3.

Activin-βA initially localized in the presumptive tooth ectomesenchyme marking the sites of development of teeth at around E10.5–E11, but the mutant varieties showed development of normal maxillary molars with all the other teeth arrested in bud stage. This phenotype differs from those of *Msx*-1, *Lef*-1 and *Pax*-9 mutants in which all teeth are affected. Unlike *Pax*-9 and *Barx*-1, the domain of activin-βA is not regulated by *Fgf*-8 and *Bmp*-4. Such speculations in the results doubt the role of activin-βA and *Fgf*-8, *Bmp*-4 interactions in tooth initiation. It is noted that the phenotype produced by compounded mutation of both *Dlx*-1 and *Dlx*-2 show a phenotype reciprocal to that of activin-βA, thus it is possible that the *Dlx* genes may activate the same essential genes downstream as that of activin pathway in maxillary molars, thus negating the need for activin during development of these teeth.

Lef-1 (Lymphoid enhancer factor) belongs to the family of T-cell factor proteins. They are transcription factors that associate with beta catenin and activate *Wnt* responsive target genes. *Lef*-1 is initially detected in the dental epithelial thickenings but later during the bud stage shifts into the mesenchyme. *Lef*-1 is identified as a functional link that connects the intraepithelial *Wnt* signal with epithelial to mesenchymal *Fgf* signaling. The requirement of *Lef*-1 in the dental epithelium earlier to bud formation is to be considered since ectopic expression of *Lef*-1 in the oral epithelium results in ectopic tooth formation.

Patterning of Dentition

Tooth patterning namely the determination of tooth type was explained in the earlier years by two theories. The *field theory* states that the ectomesenchyme is only programed to form teeth of one family but it is acted upon by the local factors which modify the shape subsequently. On the other hand, the *clade theory* or *clone theory* proposes that the ectomesenchyme is initially programed into different clones that form the different families of teeth.

Both the models have sufficient support and are earlier discussed throughout the text under different contexts. Thus both the models can be combined—the ectomesenchymal code can be deciphered only after the instructive epithelial signal during tooth initiation and the ectomesenchyme assumes a dominant role during crown patterning.

The extent to which the responses of underlying CNC (cranial neural crest) cells derived ectomesenchymal cells are regionally prespecified has been controversial because of conflicting results obtained from recombination experiments and from the gene targeting experiments. The CNC cells populating the first arch are not prespecified with respect to proximodistal position (i.e. if it would form incisor or a molar tooth) and the odontogenic fate (i.e. if it would form tooth or not). Experiments have shown that the CNC cells when presented to appropriate instructive signals from oral epithelium would be capable of odontogenesis. From the previous paragraphs in the section of tooth initiation, it is clear that recombinations carried out prior to E10.5 will show epithelium determining tooth type, while the same recombination carried out after E10.5 will show ectomesenchyme determining the tooth type (Fig. A.2).

Cell labeling studies have demonstrated that the ectomesenchymal cells of the developing facial processes to participate in tooth development and they form from CNC. These cranial neural crest cells originate at ectodermal/neuroectodermal junction of the developing brain, extending rostrally from the caudal boundary of the hindbrain and neural tube of the midbrain and caudal forebrain (diencephalon). The stream of neural crest cells that migrate into the first branchial arch is derived from the neuroectoderm of the midbrain and the first two rhombomeres of the hindbrain (Fig. A.5). Segment specific combinatorial homeobox (Hox) gene expression specifies the identity of each rhombomere, thus the migrating neural crest cells are expected to carry their Hox code along with them into the branchial arches. However, the Hox genes are not expressed anterior to rhombomeres 3 (Fig. A.6).

At this juncture, the points that require further explanation are if the difference that brings in different forms of teeth in maxillary and mandibular arches is due to the signal from the epithelium or ectomesenchyme and if the Hox code is not present within the ectomesenchyme of the first arch then what is the factor within them that determines the tooth shape.

A subfamily of the homeobox genes has been recognized within the first branchial arch that shows spatial and temporal patterns of expression. They include *Msx* genes and *Dlx* genes (Figs. A.6, A.7). Msx-1, *Msx*-2 and *Alx*-3 are located in the ectomesenchyme in horse shaped fields in the anterior regions (presumptive incisor region) of the first arch while *Dlx*-1, *Dlx*-2 and *Barx*-1 in the proximal/posterior regions (presumptive molar region) of the arch. *Msx*-1, *Msx*-2 and *Dlx*-2 are seen overlapping in the canine region. Thus a model like this imparts the importance of not only the presence of particular code but also the absence of the other code for that region. Secondly, the overlapping code provides morphogenetic cues for many different tooth shapes.

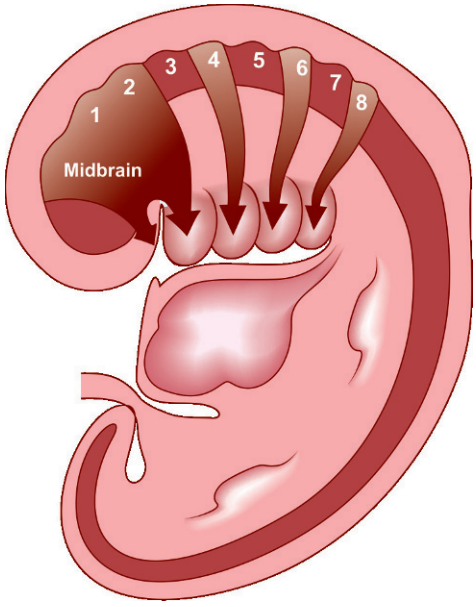

Figure A.5 The colonization of skull vault, branchial arches and facial primordia by neural crest cells.

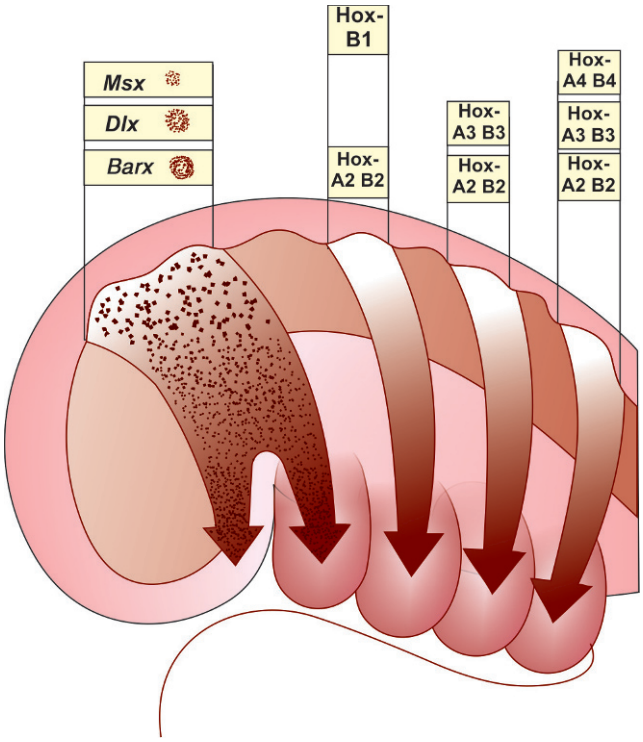

Figure A.6 Migrating neural crest cells express the same homeobox genes as their precursors in the midbrain and rhombomeres 1 and 2 that 'Hox code' is transferred to the branchial arches.

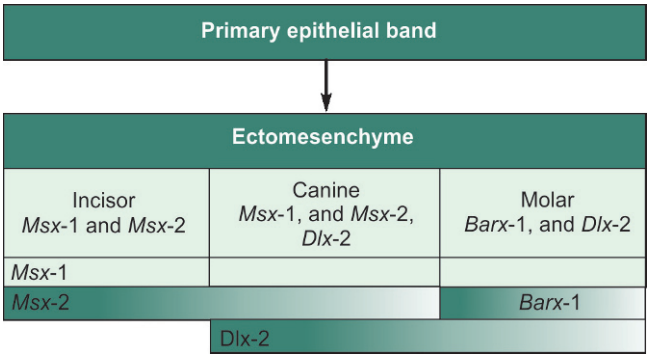

Figure A.7 The homeobox genes *Msx*-1, *Msx*-2, *Dlx*-2 and *Barx*-1 expressed in mandibular mouse ectomesenchyme associated with differing tooth families. Because the mouse does not develop canine, the code given is speculative, but overlap is present in the presumptive canine region.

Recombination experiments have revealed much important information about the rostrocaudal positioning of tooth and arch patterning.

- Primarily it is that the first branchial arch epithelium is unique in containing instructive signals for odontogenesis and these can override the pre-patterning information present in the CNC cells.
- Secondly, the maxillary and mandibular epithelia are interchangeable as regulators of ectomesenchymal gene expression. If this is true, then the instructive signals must produce identical differentiation pathways which are not the case, as it is obvious that different skeletal structures and subtly different teeth are produced in spite of being covered by same epithelium.

A response like this can only be explained by a property intrinsic to the ectomesenchymal cells. In avian and mouse embryos, mapping of cranial neural crest cells showed that the mandibular population is from midbrain and some contribution from rhombomeres one and two while that of the maxilla is from both midbrain and forebrain. Alternatively, the CNC cells in humans might acquire their rostrocaudal specification—mandibular versus maxillary during migration where signals from cranial mesoderm are likely to be involved.

Regulation of Ectodermal Boundaries

The restricted expression of *Shh* at the sites of tooth formation is likely to be required for specifying the sites of where tooth buds will form in future. Comparisons of expression of *Wnt* genes with *Shh* in the early signaling of tooth development revealed that the expression sites of *Wnt*-7b is reciprocal to that of *Shh*. *Wnt*-7b is localized in the oral ectoderm as early as E9.5 but the mechanism of reciprocation still remains obscure. This is because the receptor such as *Mfz*-6 is frizzled (curved) and is expressed throughout the oral ectoderm, and are not excluded from the presumptive areas of dental ectoderm (Fig. A.8).

Expressions of other signaling molecules such as Fgf-8 and Bmp-4 is also highly localized spatially in the ectoderm but their domains remain different from *Shh* and *Wnt*-7b. These signals play an independent role in early epithelial–mesenchymal interactions by primarily acting in defining the anteroposterior axis of the arch.

Stomodeal thickening stage – Dental lamina stage (E11.5–E12.5)

It is interesting to note that *Bmp*-4 which appeared to inhibit tooth development at early stages (Fig. A.4) turns out to be an inducer of molecules required for tooth development at the stage of epithelial thickening. Most of the molecules involved in interactions between the thickened epithelium and underlying mesenchyme appear to be in both molar incisor germs. This includes molecules like *Msx*-1 and *Dlx* that are regionally restricted prior to the appearance of tooth germs (Figs. A.4, A.9, A.10).

The genetic model thus proposed for the early tooth development consists of two independent *Msx*-1 dependent pathways which are triggered by epithelial *Fgf*-8 and *Bmp*-4.

- The cascade places *Fgf*-8 upstream of *Msx*-1 because of the presence of the former in the epithelium as early as E10 and its expression is seen in both *Msx*-1 and *Msx*-1 and -2 deficient models. *Fgf*-3 is placed downstream of *Msx*-1 since *Fgf*-3 expression is not observed in *Msx*-1 mutant models and *Bmp*-4 is not able to induce its expression in the mesenchyme.
- In situ hybridization experiments show that epithelial *Fgf*-8 and mesenchymal *Msx*-1 and *Bmp*-4 expression is preserved in *Dlx* double mutant upper molar mesenchyme. Conversely, in *Msx*-1 mutants, at the initiation stage, epithelial *Fgf*-3 and mesenchymal*Dlx*-1 and *Dlx*-2 expression are

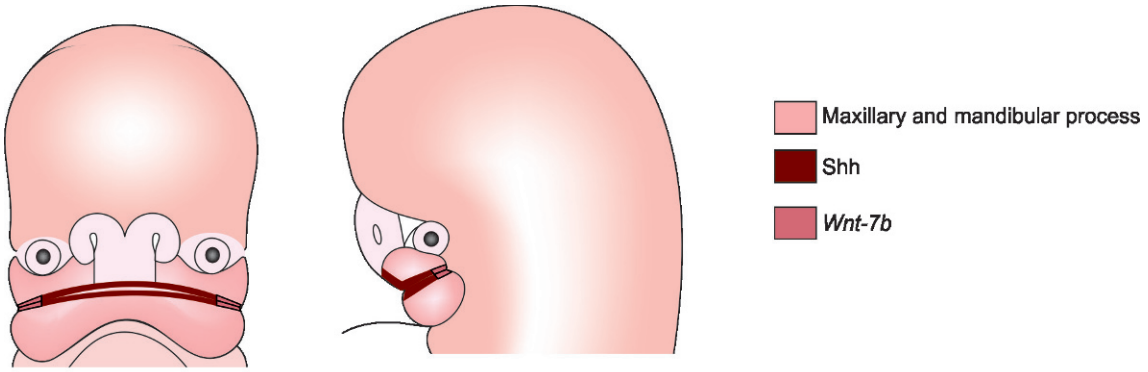

Figure A.8 The color coded schematic representation indicates the location of *Shh* and *Wnt*-7b in the embryo heads. Note the exclusion of *Wnt*-7b from the presumptive dental ectoderm while this region is occupied by *Shh.*

absent. Also, *Bmp*-4 is not induced in the mesenchyme by *Fgfs*. This shows that epithelial *Bmp*-4 can only induce *Bmp*-4 in the mesenchyme in *Msx*-1 dependent manner.

- Moreover, *Bmp*-4 and *Fgf*-8 are able to induce both *Msx*-1 and *Dlx*-2 expression in the dental mesenchyme while *Dlx*-1 expression is induced by *Fgf*-8. These results suggest that *Msx* and *Dlx* might act parallel at the dental lamina stage (Fig. A.9).
- Since *Fgf*-8 induces *Fgf*-3 in the mesenchyme at the transition of lamina–bud stage which also coincides with the transfer of odontogenic potential from epithelium to mesenchyme, *Fgf*'s may additionally be a component of signaling cascade mediating epithelial mesenchymal interactions.

Bud stage (E12.5–E13.5)

At this stage, in contrast to what was proposed in the lamina stage earlier,

- *Dlx*-2 is placed downstream of mesenchymal *Bmp*-4 because of its reduced expression in *Msx*-1 mutant. *Bmp*-4 could rescue the expression of *Dlx*-2 even in the absence of *Msx*-1.

Results of such a model suggest that, while *Dlx*-1 and *Dlx*-2 are likely to function in parallel with *Msx*-1 and *Msx*-2 at lamina stage. *Dlx*-2 expression at the bud stage resides downstream of *Msx*-1. This suggests the requirement of *Bmp*-4 and *Fgf*-3 for the maintenance of *Dlx*-2 expression and not for *Dlx*-1 (Fig. A.10).

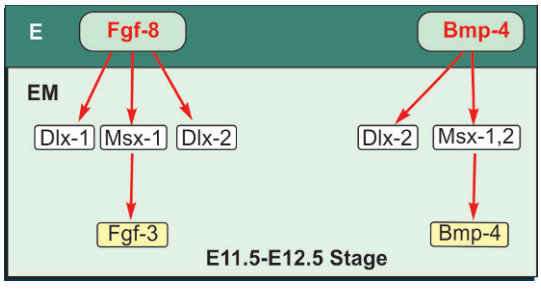

Figure A.9 Flowchart explaining the genetic pathway at E11.5–E12.5 stage where *Bmp*-4 and *Fgf*-8 act through separate *Msx*-1 dependent pathways to induce the expression of downstream genes in the mesenchyme.

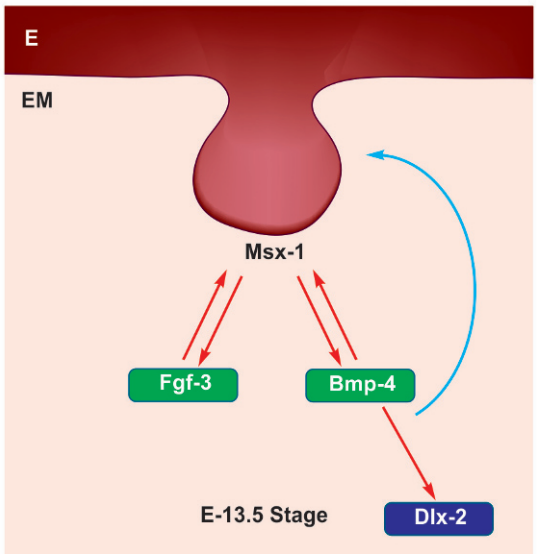

Figure A.10 Flowchart explaining the genetic pathway at E13.5 stage where *Dlx*-2 is induced by mesenchymal *Bmp*-4 and not by epithelial *Bmp*-4 unlike in the E11.5–E12.5 stage thus in a *Msx*-1 dependent manner.

Bud stage–cap stage (E13.5–E14.5)

Analyses of all the factors that appear in the earlier stages reveal few important findings about tooth morphogenesis:

- *Msx*-1 mutants arrest at the bud stage and *Msx*-2 mutants exhibit defects only at later stages of tooth development. *Msx*-1, *Msx*-2 double homozygotes exhibit an arrest at dental lamina stage which affects all molar teeth. *Msx*-1 mutant mesenchyme shows reduced *Bmp*-4 and addition of *Bmp*-4 rescues the arrest at the bud stage.
- *Bmp*-4 is a component of inductive signal that induces the transfer of the tooth inductive potential from dental epithelium to dental mesenchyme. Also *Bmp*-4 is known to induce morphological changes in the dental mesenchyme.
- *Shh* is expressed in the epithelium along with *Bmp*-2 and *Bmp*-4 at E11.5 and remains throughout bud and cap stage. *Bmp*-4 expression shifts to dental mesenchyme at E12.5. *Msx*-1 mutants show loss of mesenchymal*Bmp*-4, downregulation of epithelial *Bmp*-2 and

Shh. Blocking *Shh* expression with suitable antibodies also results in loss of *Bmp*-2. Thus the effect of *Bmp*-4 on *Bmp*-2 is mediated by *Shh*. The requirement of *Shh* in tooth development is time dependent. At E11–E12, *Shh* is needed for dental epithelial proliferation to form bud while at E13 it affects the bud morphology but still the bud proceeds to form tooth.

- *Pax*-9 is expressed in the bud stage mesenchyme and in the domains like that of *activin*-βA and *Msx*-1 in patches of mesenchyme that marks the tooth formation sites. $Pax^{-/-}$ mutants show all teeth arrested at bud stage. Despite the co-expression *activin*-βA expression remains unaffected in $Pax^{-/-}$ and the reverse is also true. Thus these two genes are independently required for the progression of bud stage in tooth development. However, changes do occur in other genes like *Msx*-1, *Bmp*-4 and *Lef*-1 in $Pax^{-/-}$ tooth bud mesenchyme.

Enamel Knot–Signaling Center for Tooth Morphogenesis

Earlier, a transient epithelial structure in the enamel organ known as enamel knot was recognized to play a central role in the control of growth and patterning of the tooth cusps. Enamel knot consists of cells that do not divide but promote the division of adjacent epithelial cells which form the cervical loop and the mesenchymal cells forming the dental papilla. Although a fully differentiated enamel knot can be identified only in the cap stage, the first sign to form this structure begins with cessation of proliferation during the late bud stage at the tip as the epithelium starts to broaden buccolingually.

The development of enamel knot is regulated by signals derived from the mesenchyme and also during the stage at which the enamel knot forms, the potential to induce tooth development resides in the mesenchyme. Though, the most obvious signal in the mesenchyme being *Bmp*-4, it was not able to induce other early markers of knot like *Shh*, and *Fgh*-4 in isolated dental epithelia. Thus it could be concluded that *Bmp*-4 may not be sufficient for enamel knot induction.

- *Bmp*-4 is a potent inducer of p21, a cyclin dependent kinase inhibitor in the enamel knot. Also another *Bmp* molecule, *Bmp*-2 expressed during this period in the dental epithelium was able to induce enamel knot formation. The expression of p21 correlates with withdrawal of cells from cell cycle causing cessation of cell division. The p21 expression has been associated with early cell differentiation in several cell lineages, and also being an early indication of enamel knot differentiation, it was postulated to be regulated in different types of signals.
- The p21 expression is shown to participate in the apoptotic pathway following ultraviolet radiation, where it acts downstream to p53 while it works independent of p53 in association with cell differentiation. Thus p21 is expected to perform such a function in enamel knot and is localized here until the cells disappear by programed cell death.
- *Fgf*s expressed in the enamel knot can stimulate cell division in the enamel epithelium and in the dental papilla which is demonstrated by the incorporation of bromodeoxyuridine (Brdu). The *Fgf* receptors are not present in the enamel knot cells so they do not respond to the mitogenic stimuli. *Fgf*-4 is the only stimulus that strictly is restricted to the primary and secondary enamel knots while others rapidly spread into the surroundings. The *Msx*-2 is involved in regulating *Fgf*-4 and/or genes responsible for cessation of proliferation in enamel knot.
- The extent of enamel knot matched exactly with the area of the non-proliferative cells, but the area of *Fgf*-4 transcripts were slightly smaller. Thus the cessation of cell proliferation in the enamel knot precedes the start of *Fgf*-4 expression.
- Slit-1 is recently identified as another molecule apart from *Fgf*-4 that has been localized in both primary and secondary enamel knot.
- *Bmp*-4 induces *Lef*-1, a transcription factor that conducts the epithelial–mesenchymal interactions at a critical stage of tooth development when the epithelium generates inductive signals to the mesenchyme for the formation of the dental papilla. Ectodysplasin (Eda), a TNF ligand family protein encoded by Tabby gene is identified as a transcriptional target of *Lef*-1. The Tabby mutants show normal shaped enamel knots while the size is smaller the expression levels of enamel knot markers are weak.
- The Downless (Dl) gene encodes Edar, a member of TNF receptor family. The latter has been identified in the oral epithelium from E10, with its highly localized expression later in the enamel knot. A failure in the formation of enamel knot is identified in the Downless mutants, where the knot takes up a form of sheet and the markers show disrupted expression domains while their levels of expression are normal unlike the Tabby mutants.

Thus the enamel knot cells express several signaling molecules like sonic hedgehog, bone morphogenetic proteins-*Bmp*-2, *Bmp*-4, *Bmp*-7, *Wnt* signaling molecules as well as *Fgf*-4. As the same signaling molecules are expressed by well studied vertebrate signaling centers such as notochord, the apical ectodermal ridge and the zone of polarizing activity, it was put forward that the enamel knot represents a signaling center for tooth morphogenesis.

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