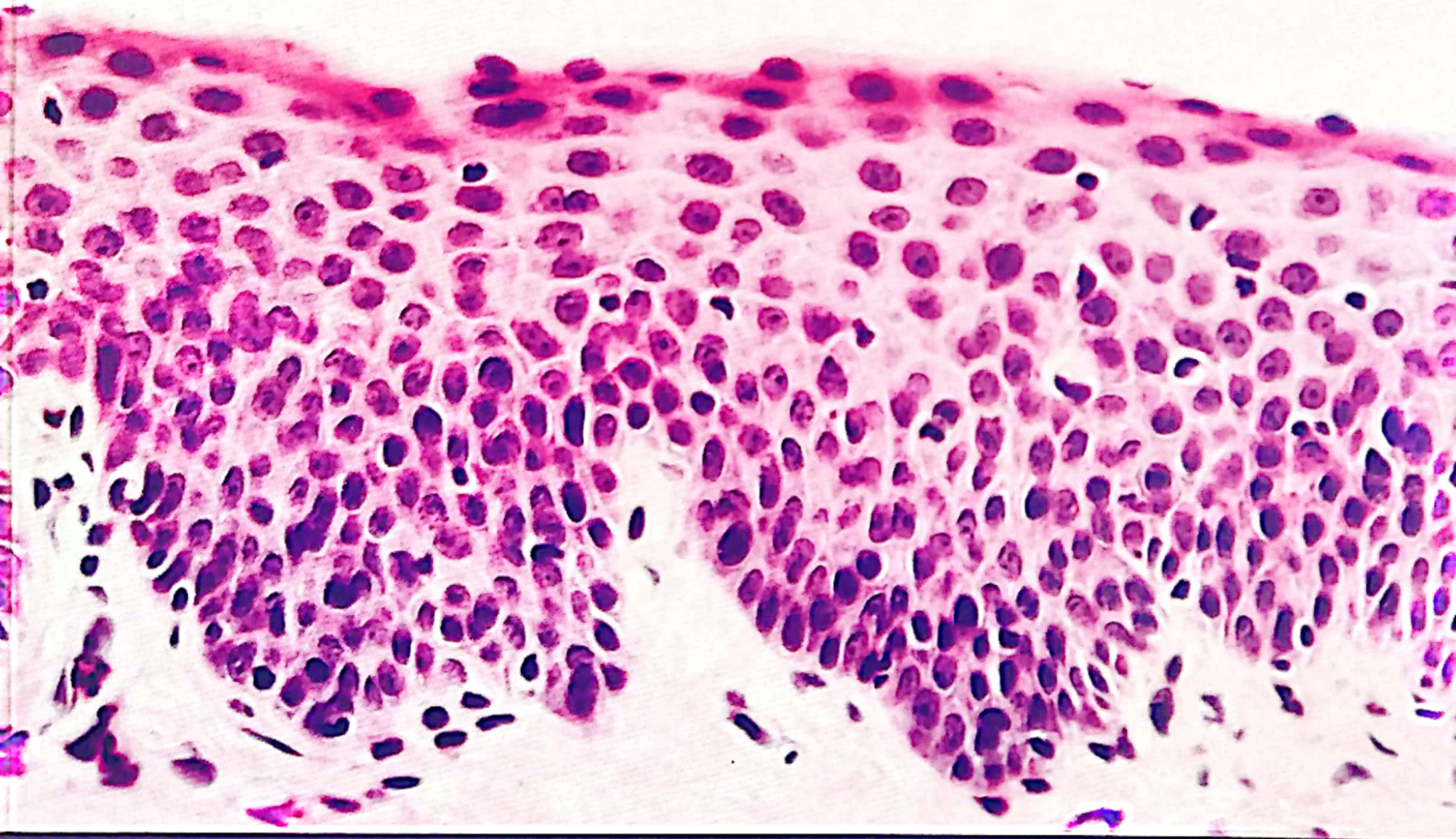
Medical Histology

Text & Atlas

Revised 6th Edition



Laiq Hussain Siddiqui

MEDICAL HISTOLOGY

Text & Atlas

Revised Sixth Edition

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Introduction 1

Any individual living thing, whether plant or animal, is called an organism. The living substance of all organisms is known as protoplasm. Understandably, the protoplasm is composed of atoms combined to form molecules. Following the progress from atom to organism, different levels of structural organization are revealed. Smallest unit of protoplasm capable of independent existence is called a cell. Simplest plants and animals consist of a single cell. Higher animals, including humans, can be regarded as a complex society of independent cells of many types that are specialized to carry out the functions essential for survival and reproduction of the animal as a whole. Cells serving the same general function/functions are bound together by variable amount of extracellular matrix to form tissues. Two or more tissues are combined to form large functional units called organs. Several organs having interrelated functions constitute an organ system.

Anatomy is a branch of biology which is concerned with the study of the structure of the organisms. Human anatomy is an essential medical science which is concerned with the study of the structure of the human body. The two major subdivisions of this subject are macroscopic anatomy and microscopic anatomy. The macroscopic anatomy, more commonly called gross anatomy, consists of the study of the parts, organs, and structures of the human body by the naked (unaided) eye. The microscopic anatomy, more commonly called histology, is the study of microstructure of the organs, tissues and cells of the body with the help of optical instruments called microscopes.

IMPORTANCE OF HISTOLOGY

The knowledge of histology complements the study of gross anatomy by enabling the students to correlate the microstructure of the organs with their gross structure. A sound knowledge of histology is of paramount importance to understand the subject of physiology (which deals with the study of the normal functions of the cells, tissues, organs, and organ systems). The knowledge of normal histology is also of fundamental importance in understanding the subject of pathology, because one of the major domains of pathology is the study of changes that occur in the structure of the cells and tissues when they become diseased.

MICROSCOPES

Microscopes are optical instruments consisting of a combination of lenses, which provide an enlarged image of small objects and reveal details of structure not distinguishable by the unaided eye. Observation by a microscope is called microscopy.

The usefulness of any type of microscope depends on its two qualities: magnification and resolution.

1. **Magnification** of a microscope means its ability to enlarge the image of the object being viewed.
2. **Resolution** (also called resolving power) of a microscope implies its ability to allow the observer to distinguish between two small objects situated close together. Resolution is inversely related to the wavelength of the source of illumination employed, i.e., shorter the wavelength, greater the resolution.

TYPES OF MICROSCOPES

Taking into account the type of illumination used, the microscopes are divided into two major groups: (i) the light microscopes, also called optical microscopes, which use visible light (or ultraviolet light in the case of fluorescence microscope) to make an image, and (ii) the electron microscopes in which a beam of high velocity electrons is used as a source of illumination.

THE LIGHT MICROSCOPES (OPTICAL MICROSCOPES)

Many types of light microscopes are available. Among these, the bright-field microscope is most commonly used by the students and research workers. Other varieties of the optical microscopes include: stereomicroscope, polarizing microscope, phase-contrast microscope, dark-field microscope, fluorescence microscope, and confocal microscope.

THE BRIGHT-FIELD MICROSCOPE

A bright-field microscope generally consists of five components: (Fig. 1.1)

1. A light source (illuminator).
2. A condenser lens.
3. A stage on which the tissue specimen (mounted on a glass slide) is placed.
4. An objective lens.
5. An ocular lens.

The light source or **illuminator** produces a beam of light which illuminates the tissue specimen which is under observation.

The **condenser lens** focuses the light on the specimen to be examined.

The **objective lens** gathers the light which has passed through the tissue specimen. This lens magnifies and projects the illuminated image of the specimen toward

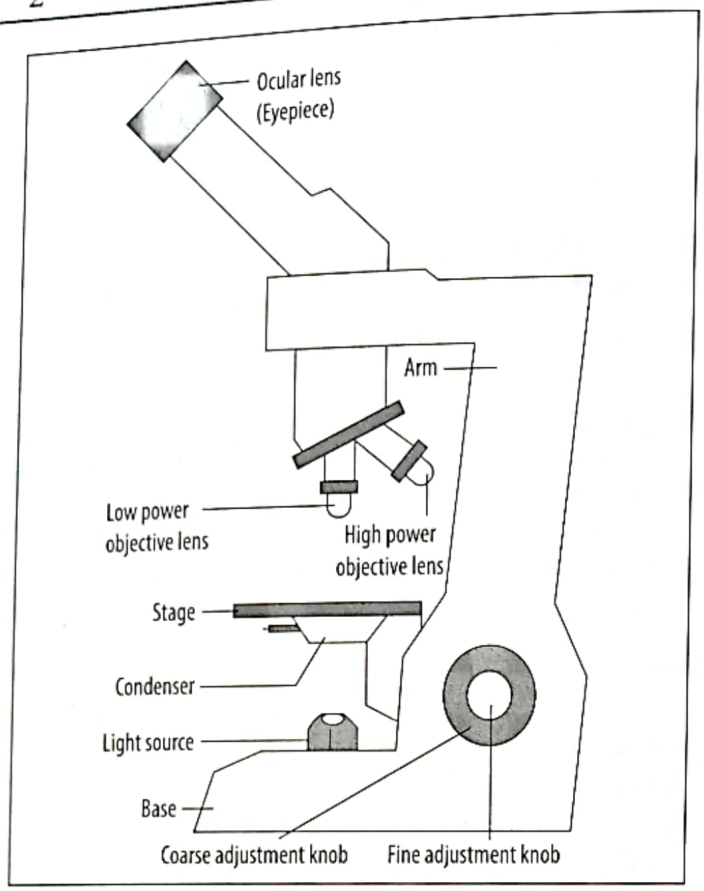

Fig. 1.1 A diagram showing various components of a light (bright-field) microscope.

the ocular lens. The ability to gather light and resolve the specimen detail depends on the numerical aperture of the objective lens. Usually four objective lenses are available in a microscope, which are fixed on a moveable turret located just above the specimen. Out of these, the first three objective lenses magnify the image 4, 10 and 40 times, respectively. The fourth objective enlarges the image 100 times and is also called oil immersion objective. Oil immersion is a technique used to enhance the resolving power of the light microscopes. It involves immersion of the objective lens as well as the specimen in a layer of transparent oil of high refractive index, thereby increasing the numerical aperture of the objective lens.

The **ocular lens**, also called eyepiece lens or simply eyepiece, further magnifies the image 10 times and, focuses the resulting image on the retina of the viewer's eye. Only one ocular lens is present in the *monocular* microscopes, whereas the *binocular* microscopes have two ocular lenses.

The resolving power of the commonly used bright-field microscope is about 0.2 µm and it can magnify the images 1000 to 1500 times.

Preparation of Tissue for Bright-Field Microscopy

The direct microscopic examination of the living cells and tissues is possible only to a limited extent. Most commonly, dead tissue is used for microscopy which, immediately after removal from the animal body, is treated with chemical preservatives and then cut into thin slices called histological *sections*. After staining with various dyes, the sections can be studied with the microscope in the transmitted light. To summarize, the process of preparation of the tissue for bright-field microscopy involves four main steps: fixation, embedding, sectioning, and staining.

Fixation

Purpose of fixation is preservation of the tissue structure with the least possible alteration. This is achieved by the influence of various chemical compounds called *fixatives*, which render the structural components of the cells insoluble. The major action of the fixatives is to bring about stabilization of proteins by causing the formation of cross-links between the protein molecules. The change in the condition of proteins also results in the inactivation of certain cellular enzymes that would otherwise start the process of self-digestion (autolysis). The most commonly used fixative is **formalin**. Pure formalin is 37% solution of formaldehyde gas in water. For general purpose, a 10% solution of formalin is employed as a tissue fixative.

Embedding

Although the tissue obtains some degree of firmness by fixation, it is still far too soft to be cut into thin sections. Therefore, prior to sectioning, the tissue must be embedded in a material which, after hardening, has a consistency that permits it to be sectioned together with the tissue piece. Paraffin wax is the most frequently used embedding agent. Paraffin is solid at room temperature but becomes liquefied when heated to 60° C. The fixed and dehydrated tissue specimens are kept immersed in heated paraffin within small cubical containers which are kept in an oven at 60° C for several hours. This allows the paraffin to infiltrate into the tissue specimens thoroughly. When the containers are removed from the incubator and allowed to cool, the paraffin hardens to form a firm, cubical block containing the embedded tissue specimen.

Sectioning

Sectioning of the tissue block into thin slices is carried out by the help of a machine called *microtome*. The sections are usually 3-10 µm thick and each tissue section is mounted on a glass slide. The mounted tissue sections are allowed to dry in an oven to remove the moisture and help the sections to adhere to the slides. The dried tissue sections are ready to be stained.

Staining

Most tissues of the body are colorless and, as such, it is difficult to study their structural details. To overcome this difficulty, methods of staining tissues have been devised. The purpose of staining is to enhance the natural contrast, so that distinction can be made between different components of the tissues and cells. Stains in general use are considered to be acids or bases, but in fact they are neutral salts having both acidic and basic radicals. When the coloring property of the dye is in the basic radical of the neutral salt, the stain is called a **basic dye** and the

structures that stain with it are called *basophilic structures*. On the other hand, when the coloring property of the dye lies in the acid radical of the salt, the stain is known as **acidic dye** and the structures which stain with it are referred to as *acidophilic structures*. The commonly used basic dyes include hematoxylin, toluidine blue, and methylene blue, etc. The acidic dyes which are in common use include eosin, acid fuchsin, orange G, and picric acid, etc.

It is important to understand that the basophilic substances which attract the basic dyes are themselves acidic in nature, e.g., the nucleus of a cell takes a basic stain because it contains deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Similarly, the ribosomes of the cytoplasm are basophilic because they contain RNA. On the other hand, the acidophilic substances are themselves basic in nature and therefore, make bonds with acidic dyes. The general cytoplasm of the cells stains acidophilic, because the constituent proteins of the cytosol are rich in basic amino acids.

In routine histology, **hematoxylin and eosin** (abbreviated as **H&E**) is the stain of choice. As indicated by its name, this stain consists of a combination of a basic dye – hematoxylin, and an acidic dye – eosin. By the H&E staining technique, the acidophilic substances/structures in the tissues take a pinkish to reddish color, whereas the basophilic substances/ structures are stained light blue to deep blue or purple. In histological descriptions, the terms acidophilic and eosinophilic are usually used synonymously.

THE POLARIZING MICROSCOPE

When light passes through a polarizing filter, it comes out vibrating only in one direction. In a polarizing microscope two polarizing filters are used. The first filter is located below the condenser lens and is known as **polarizer.** The second filter, known as **analyser,** is placed between the objective and ocular lenses. When the polarizer and analyser are so disposed that their main axes are perpendicular to one another, no light can pass through, resulting in a dark field effect. However, if substances or structures containing asymmetric molecules which are oriented in a highly ordered manner are located between the polarizer and analyser, their repetitive molecular structure allows them to rotate the axis of light emerging from the polarizer. Therefore, such things appear as bright structures against a dark background. The capacity to rotate the direction of the vibration of polarized light is called *birefringence* (double refraction). The substances or structures exhibiting birefringence are called *anisotropic.* The substances or structures possessing a single refraction are referred to as *isotropic*. Under the polarizing microscope, the isotropic structures do not appear bright.

THE PHASE-CONTRAST MICROSCOPE

This variety of microscopes is used for the study of unstained fresh tissues or living cells. The phase contrast microscope is based on the fact that light waves passing through regions with different refractive indices are deflected differently and put out of phase with one another. Different cytoplasmic and tissue constituents produce phase differences because they vary in thickness and refractive index.

The phase contrast apparatus consists of optical plates placed within the condenser and objective lenses, which convert the phase differences into differences of light intensity. Thus, differences in refractive index are rendered directly visible.

THE DARK-FIELD MICROSCOPE

This microscope uses a strong, oblique light that does not enter the objective lens. This is achieved by using a special dark field condenser in which no light passes through the center of the lens. The light thus reaches the object to be viewed at an angle so oblique that none of it normally enters the objective lens. Therefore, the viewer sees the field being examined as a dark background. However, when the specimen to be examined is placed between the condenser and objective lenses, the light scattered by some of the structures in the specimen manages to reach the objective lens. Such structures appear bright against a dark background and can be seen easily. The dark-field microscopy is employed to render unstained and transparent specimens clearly visible.

THE FLUORESCENCE MICROSCOPE

In fluorescent microscopy, fluorescent compounds having affinity for specific cell components are u

sed as stains, e.g., the fluorescent stain acridine orange binds with DNA & RNA. In a fluorescent microscope, the treated tissue specimens are irradiated with ultraviolet light upon which the fluorescent structures emit light having longer wavelengths which are in the visible range. The fluorescent microscope contains special filters that select the rays of specific wavelengths allowing the viewer to see only that which is fluorescing.

THE CONFOCAL MICROSCOPE

In the ordinary optical microscopes, the beam of light is quite large and, consequently, the excess light produces a flare which decreases the resolving power of the objective lens. The confocal microscopes are specially designed to eliminate the out-of-focus light, so that a sharp focus with a high resolution is produced. This microscope employs two major technical improvisations: (i) a small point of high intensity light obtained from a laser, and (ii) a plate with a pinhole aperture which is placed in front of the image detector lens.

THE ELECTRON MICROSCOPES

In an electron microscope a beam of high velocity electrons is passed through the tissue specimen to be examined. The wavelength of the electron beam is 1/2000 of the visible light beam. Because the resolution is inversely proportional to the wavelength of the illuminating source used, the

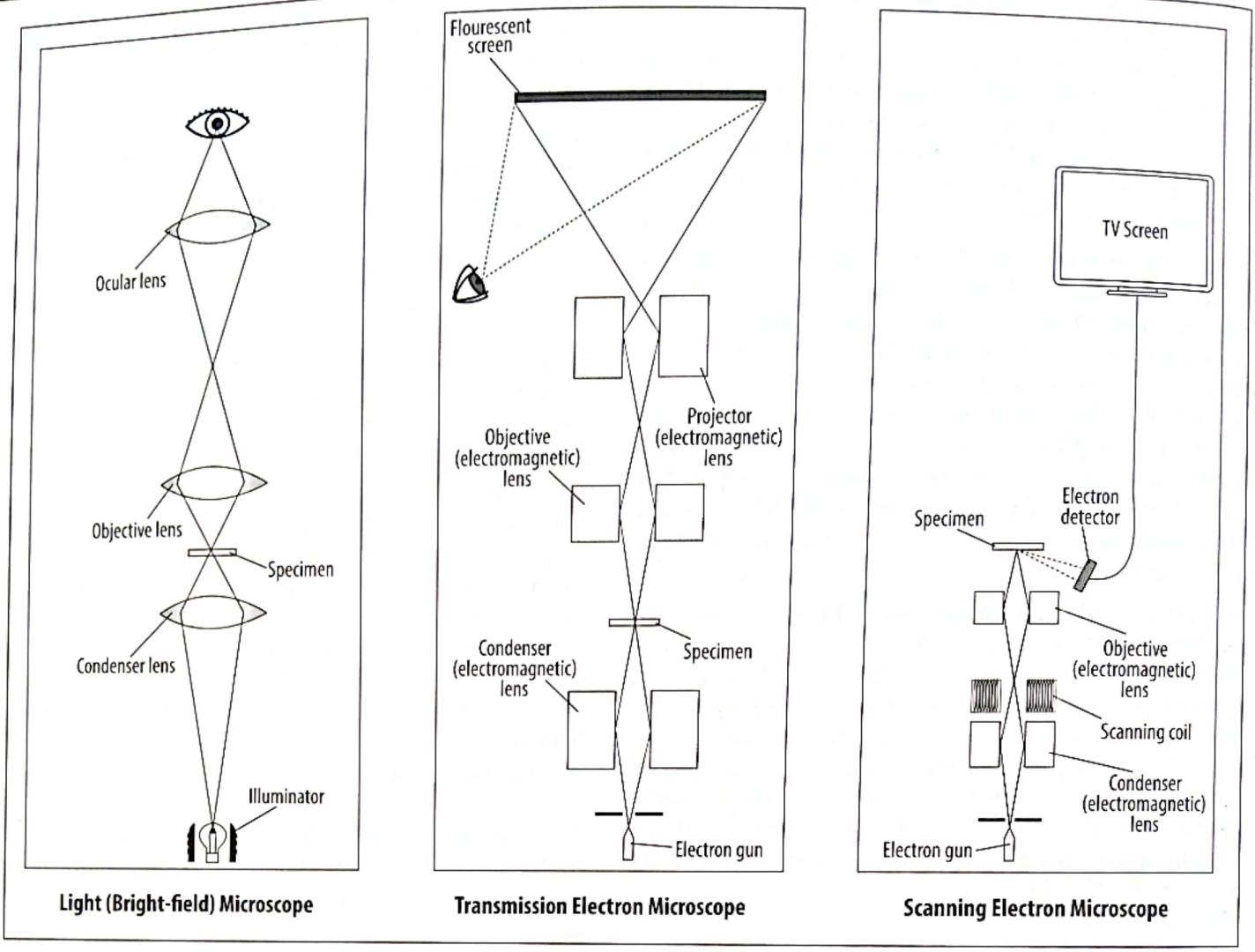

Fig. 1.2 Comparison of the optical paths of different types of microscopes.

resolution of the electron microscope is correspondingly higher than that of the light microscope. The magnifying power of the EM is also much greater than that of the light microscope. The high resolving and magnifying power of the electron microscope allows study of the structure of the body tissues at subcellular level; the structure visible only under the electron microscope is called *ultrastructure* or fine structure.

It should, however, be noted that, although being superior to the light microscopes in resolution and magnification, the electron microscopes have certain limitations as well. To be specifically mentioned is the fact that quite large areas of a tissue specimen (generally several cm^2) can be examined under the LM, whereas in EM the specimen area available for examination is very small, usually being less than 1 mm^2. In addition, the EM shows only black and white images, whereas a number of staining techniques are available for imparting different colors to various components of the tissues being examined by the LM.

Two varieties of electron microscopes are generally used for histological purposes:

1. Transmission electron microscope.
2. Scanning electron microscope.

TRANSMISSION ELECTRON MICROSCOPE

The transmission electron microscope (TEM) functions on the principle that a beam of electrons can be deflected by electromagnetic fields in a manner similar to deflection of light in the glass lenses. In this microscope, the illuminating source is a beam of high velocity electrons accelerated in a vacuum. The beam is passed through the tissue specimen to be studied and is focused upon a fluorescent screen or photographic plate by a series of electromagnetic fields (Fig. 1.2). The usual resolution of a transmission electron microscope is about 2 nm, while the ultrathin tissue sections can be magnified up to 120,000 times.

SCANNING ELECTRON MICROSCOPE

The scanning electron microscope (SEM) is used to view the surface of a solid specimen. It differs from the tem in that the electrons do not pass through the specimen under examination. The fixed specimen is introduced into an electron beam that scans the surface of the specimen, which has been coated with a very thin layer of a heavy metal (like gold or palladium). Electrons that are reflected or ejected from the heavy metal coat are captured by electron detectors and finally focused to produce a highly magnified 3-D view on a high resolution monitor (Fig. 1.2). The image

can also be made permanent, either by photographing it or by digitalizing it for storage in a computer.

Preparation of Tissue for Electron Microscopy

For electron microscopy the commonly used fixatives are glutaraldehyde and osmium tetroxide. After fixation the tissue is embedded in epoxy resins (e.g., araldite). The tissue blocks so obtained are subjected to sectioning at machines called *ultramicrotomes* which produce ultrathin sections ranging from 40 to 100 nm in thickness. These ultrathin sections are stained to enhance the inherent contrast of the tissue/cell structures by passing them through a solution of lead citrate and uranyl acetate.

IMPORTANT NOTE: In histological descriptions given in the following chapters, the term light microscope (abbreviated as LM) generally refers to the bright-field microscope. Similarly, the term electron microscope (abbreviated as EM) commonly refers to the transmission electron microscope.

SMALLER UNITS OF MEASUREMENT

The following table shows the small units of measurement which are commonly used to describe the microscopic structure of the tissues and cells:

Unit	Symbol	Value
Micrometer	μm	0.001 mm (1/1000 mm)
Nanometer	nm	0.001 μm (1/1000 μm)

The Cell 2

The smallest unit of protoplasm capable of independent existence is called a cell. The cells form structural and functional units of all living organisms. Biologically, the cells can be grouped into two fundamentally different varieties: prokaryotic cells and eukaryotic cells.

In the *prokaryotic* cells, the genetic material consists of a double-stranded DNA molecule which is called *nucleoid*. The nucleoid is not enclosed by a membrane and lies amongst the other cellular constituents. Also, no organelles are present in the cytoplasm of a prokaryotic cell. The bacteria and archaea belong to the prokaryotic variety of cells.

In the *eukaryotic cells*, the genetic material exists as chromosomes that lie within the *nucleus* of the cell which is surrounded by a definite *nuclear membrane* which separates the nuclear contents from the other cellular components. Special, organized structures, called *organelles*, are a characteristic feature of the cytoplasm of the eukaryotic cells. As already stated, the eukaryotic cells form the structural and functional units of all living organisms except bacteria and archaea. The human body is composed of trillions of eukaryotic cells.

A human cell may be defined as a small mass of protoplasm enveloped by a cell membrane and containing at least one nucleus*. The protoplasm inside the nucleus is known as *karyoplasm,* while the remaining protoplasm of the cell is called *cytoplasm*. More than 200 different types of cells are found in the human body.

Each living cell is capable of producing energy to carry out its normal functions. Each cell synthesizes macromolecules for its own use, while many cells also synthesize macromolecules meant for export from these cells. Generally, the human cells are capable of communicating with each other.

Shape and Size of the Human Cells

Shape. Free cells (e.g., white blood cells and ova) have a spherical or ovoid shape. In most issues, however the shape of the cells is modified, e.g., cuboidal, columnar, polygonal, pyramidal, cylindrical, fusiform, or irregular, etc.

Size. The human cells generally range from 5 to 50 µm in diameter. However, certain cells may be exceptionally large, e.g., the mature ovum (which has a diameter of 120 µm) and some large neurons (which may measure up to 150 µm in diameter).

* The mature red blood cells (erythrocytes) neither contain a nucleus nor any cell organelles; therefore, these cells are not considered to be true cells.

STRUCTURE OF A HUMAN CELL

Each human cell is enveloped by a membrane called cell membrane. Enclosed within the cell membrane are two major compartments: (i) the nucleus, and (ii) the cytoplasm.

THE CELL MEMBRANE

This membrane, also called *plasma membrane* or ***plasmalemma***, envelops the cell completely and acts as a selective structural barrier between the contents of the cell and contents of the extracellular space. It must be noted that the cell membrane is much more than just a boundary structure and participates in numerous functions of the cell.

Being very thin, the plasmalemma cannot be seen in tissue sections under the light microscope. However, it can very clearly be seen in highly magnified electron micrographs which are prepared from sections of those tissue samples that were fixed in osmium tetroxide. In such micrographs, the cell membrane generally seen to have an average thickness of 7.5 nm and a trilaminar (3-layered) structure consisting of two electron dense lines separated by an electron-lucent central zone; each layer is roughly 2.5 nm in thickness (Fig. 2.1 A). It must however be remembered that the thickness of the cell membrane is variable and ranges from 7.5-10 nm in different cells.

Biochemical analysis of the cell membrane reveals that it is composed mainly of phospholipids, proteins and cholesterol. Studies have revealed that the cell membrane consists of a bimolecular layer of phospholipids (called lipid bilayer) in which are embedded proteins and cholesterol. Because the lipid bilayer is fluid in nature and the large protein molecules suspended in it exhibit a mosaic pattern, this model of the cell membrane structure is known as the ***fluid mosaic model*** (Fig. 2.1 B).

It is to be noted that the intracellular membranes, which enclose the nucleus and organelles of the cell, are also have the same trilaminar structure as that of the cell membrane.

Each phospholipid molecule of the lipid bilayer of the cell membrane consists of a polar (hydrophilic) head and a nonpolar (hydrophobic) tail. The head of each phospholipid molecule is composed of glycerol conjugated to a nitrogenous compound by a phosphate bridge. The nitrogenous compound may be choline, serine or ethanolamine. The nonpolar tail of each phospholipid molecule is made up of two long chains of fatty acids which are covalently linked to the glycerol component of the polar head of the phospholipid molecule. In the bimolecular layer the hydrophilic heads of the phospholipid molecules

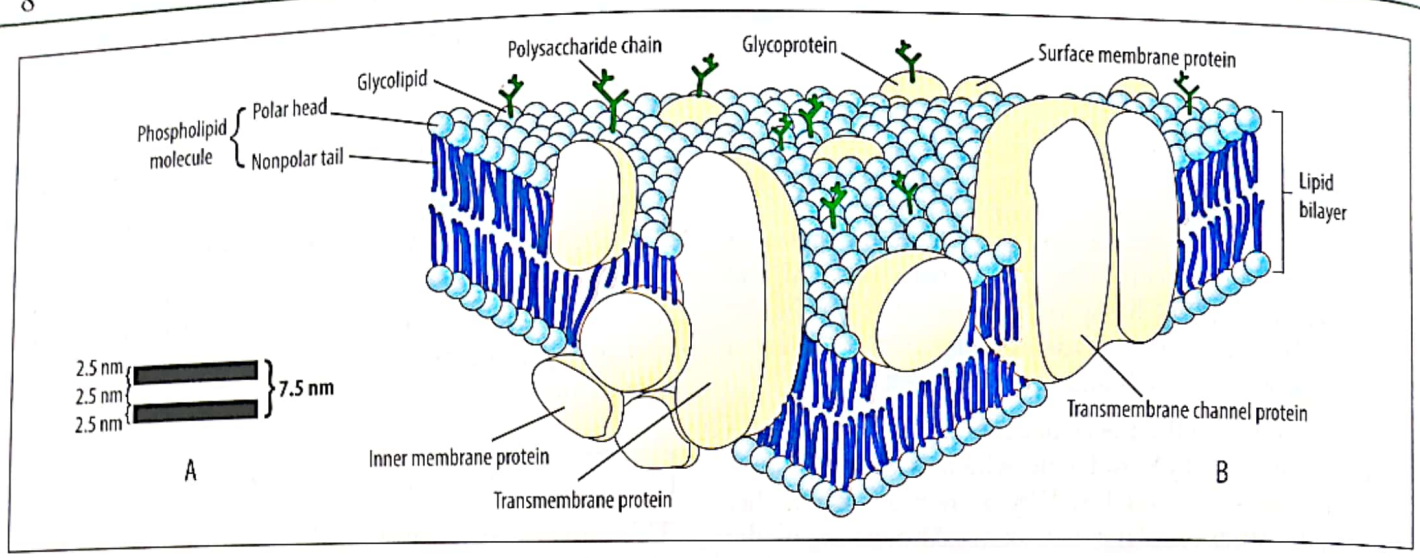

Fig. 2.1 A. Diagrammatic representation of the trilaminar structure of cell membrane (as portrayed in electron micrographs). B. Schematic diagram depicting the of fluid mosaic model of the molecular organization of the cell membrane.

lie at the outer and inner faces of the cell membrane, while the hydrophobic tails are directed toward the middle of the bilayer.

The cholesterol molecules lie between the long fatty acid chains of the phospholipids. Cholesterol stiffens the cell membrane and, therefore, the membrane fluidity is diminished by a high cholesterol content.

The peculiar double layered arrangement of the phospholipid molecules explains the trilaminar appearance of the cell membrane which is seen in the electron micrographs of the osmium fixed tissues. The two dense lines result from the deposition of osmium on the hydrophilic heads of the phospholipids molecules, while the intervening pale zone represents their unstained hydrophobic fatty acid chains.

Protein molecules make up approximately 50% of the total membrane mass. The membrane proteins can be divided into two groups: (1) integral proteins, and (2) peripheral proteins. The **integral proteins** are either embedded within the lipid bilayer or pass through the bilayer completely. The **peripheral proteins** are not embedded in the lipid bilayer but, instead, stay loosely associated with the integral proteins at the internal and external surfaces of the cell membrane; weak electrostatic forces bind the peripheral proteins to the integral proteins.

Most of the membrane proteins are integral proteins. Some of these proteins are embedded in the cell membrane in such a way that one of their ends is anchored in the lipid bilayer, while the other end projects on the internal (cytoplasmic) or external (extracellular) surface of the cell membrane. However, majority of the integral proteins pass through the entire thickness of the cell membrane and hence are called *transmembrane proteins*. As indicated by their name, the transmembrane proteins span the lipid bilayer from one surface of the cell membrane to the other. Some of the transmembrane proteins span the membrane only once and, are therefore, known as **one-pass proteins.** However, many of the transmembrane proteins are quite long and folded so that they make several passes through the membrane and hence are called **multipass proteins** (Fig. 2.2).

Chains of carbohydrate residues (sugars), which are generally oligosaccharides, are attached on that surface of the plasma membrane which faces away from the cytosol. Therefore, they are located on the outer surface of the cell membrane and on the inner surface of the membranes enclosing the cytoplasmic organelles. These carbohydrate residues are bound either to the heads of the phospholipid molecules to constitute glycolipids, or they are attached to the membrane proteins to form glycoproteins.

Most of the component proteins of the plasmalemma as well as intracellular membranes are of basic nature. Therefore, the cell membrane and all the intracellular membranous structures, e.g., the mitochondria, Golgi apparatus, and smooth endoplasmic reticulum, attract acid dyes and are stained eosinophilic in the ordinary H&E stained tissue sections.

On some cells, especially on the epithelial cells of the body, the glycoproteins and glycolipids of the cell membrane constitute a layer called glycocalyx. Under the EM, the glycocalyx appears as a fuzzy coat over the outer surface of the cell.

The **glycocalyx**, also called **cell coat**, performs several functions which may vary in different cells. Generally, the molecules of the glycocalyx enable the cell to recognize the other cells, help in cell association and adhesion, and serve as receptor sites for hormones. The glycocalyx covering the luminal surface of the absorptive cells (enterocytes) of the small intestine (Fig. 2.3) plays an important role in the digestion of the food materials. In this location the glycocalyx contains enzymes that bring about the final degradation of the sugars and proteins, so that the simple end products of digestion can be absorbed by the enterocytes readily. The glycocalyx covering the endothelial cells lining

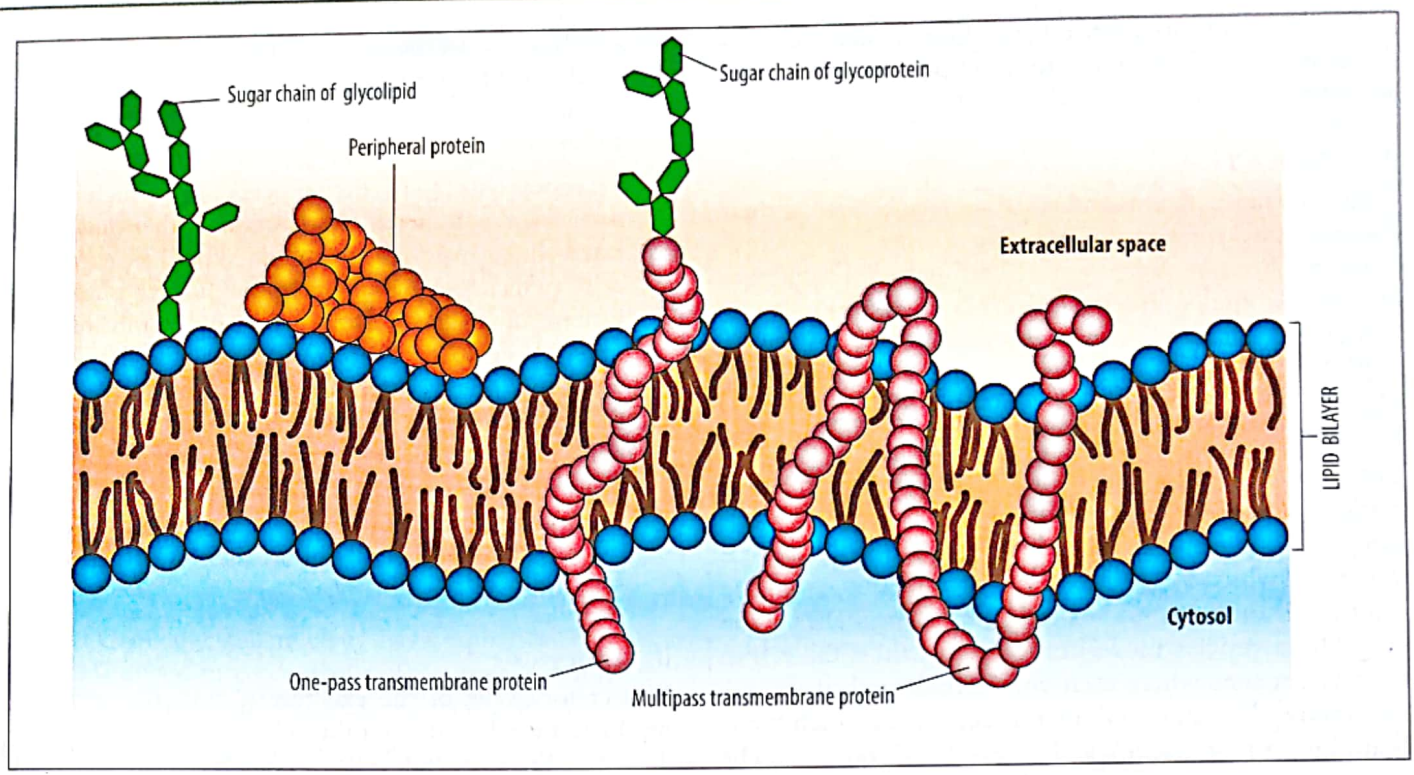

Fig. 2.2 Diagrammatic representation of a section through the cell membrane showing its molecular organization

the blood vessels also contains many important enzymes like angiotensin-converting enzyme (ACE), antithrombin III, and lipoprotein lipase.

TRANSPORT ACROSS THE CELL MEMBRANE

The cell membrane controls the interaction of the cell with the extracellular environment. All the materials that enter or leave the cell must pass through the cell membrane. Different substances are transported across the cell membrane by three major mechanisms: (1) passive transport, (2) active transport, and (3) vesicular transport.

1. Passive Transport

In this type of transport, the substances cross the cell membrane down their concentration gradient (i.e., from higher concentration region to low concentration region). No input of energy is involved in this process. Passive transport involves two types of mechanisms: passive diffusion and facilitated diffusion.

i. Passive Diffusion

Certain substances can easily pass through the lipid bilayer of cell membrane by simple diffusion down their concentration gradient; this is called passive diffusion or ***simple diffusion***. Fats and fat-soluble molecules, small uncharged (hydrophobic) molecules, and dissolved gases (O_2 and CO_2) cross the cell membrane by passive diffusion.

ii. Facilitated Diffusion

Water and water-soluble molecules like glucose and amino acids, and various ions (Na^+, K^+, Ca^{2+} and Cl^-) also cross the cell membrane down their concentration gradient

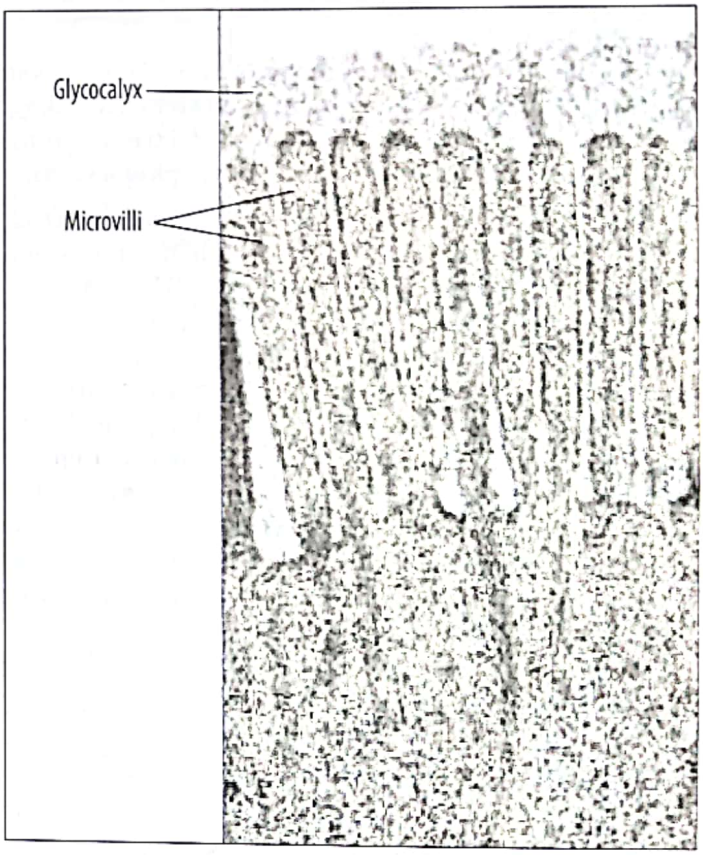

Fig. 2.3 An electron micrograph of the apical parts of the absorptive cells (enterocytes) of the small intestinal mucosa, showing thick layer of glycocalyx on the plasmalemma covering the microvilli of these cells.

but require the aid of a special variety of transmembrane proteins called **channel proteins.** These proteins form hydrophilic channels that regulate the movement of ions

and specific molecules across the cell membrane. Some of these channels are gated, i.e., they can be opened or closed according to the physiological need of the cell.

2. Active Transport

Cells can transport ions and small molecules against their concentration gradients by spending energy. Such energy-requiring *active transport* is mediated through a special variety of transmembrane proteins called **carrier proteins.** The best example of active transport is the continuous extrusion of Na^+ out of the cell by the sodium pumps.

3. Vesicular Transport

The mechanism by which large molecules and small particles enter, leave or move within the cell is called *vesicular transport*. The **vesicles** are membrane-bound spherical structures which are formed either by budding from the cell membrane or from the membranous organelles of the cell. These vesicles move in the cytoplasm of the cell to their target sites where each vesicle fuses with the target membrane (i.e., the cell membrane or the membrane bounding an organelle) and releases its contents. The vesicular transport involving the cell membrane is can either be endocytosis or exocytosis.

i. Endocytosis

The term used for those processes of vesicular transport by which substances enter the cells. Generally, three mechanisms of endocytosis are recognized: (a) pinocytosis, (b) receptor-mediated endocytosis, and (c) phagocytosis.

a) **Pinocytosis*** also called ***fluid-phase endocytosis*** or ***potocytosis***, is the process by which the fluids and small molecules enter the cell. When the material to be endocytosed comes in contact with the cell membrane, a shallow depression is first formed, which soon deepens to form a flask-shaped invagination of the plasmalemma. Narrowing and subsequent closure of the neck of the invagination detaches it from the plasmalemma as a small *pinocytotic vesicle* (about 50-100 nm in diameter) which contains the extracellular fluid along with the entrapped molecules (if any). In the cytoplasm, the pinocytotic vesicles finally fuse with early endosomes.

b) **Receptor-mediated endocytosis** is the mechanism by which specific macromolecules are allowed to enter the cell. This process depends on the presence of receptor proteins, called *cargo receptors*, in the cell membrane. The cargo receptors recognize and bind to specific macromolecules (**ligands**) which come in contact with the cell membrane. Internally the cargo receptors become associated with a special protein called *clathrin*. The assembly of clathrin molecules beneath the cargo receptors creates a pull on the cell membrane, so that a clathrin-coated pit is formed. The coated pit eventually becomes a pinocytotic vesicle which is called a coated vesicle. The *coated vesicle* finally pinches off from the cell membrane and comes to lie in the cell cytoplasm where it usually fuses with an endosome.

c) **Phagocytosis**** is the process by which large particles, e.g., bacteria, cell debris and other unwanted extracellular materials, are engulfed by the phagocytic cells (which belong to the mononuclear phagocyte system of the body). When a bacterium or any other large particulate matter comes in contact with the plasma membrane of a phagocytic cell, the plasmalemma of the cell produces processes that surround the large particle. The edges of these processes fuse leaving the ingested particle in a large membrane-bound vesicle called **phagosome**. Within the cytoplasm of the cell, each phagosome fuses with a lysosome to form a *phagolysosome*.

ii. Exocytosis

In secretory cells of the exocrine glands, the secretory products are released from the cell surface by exocytosis. The membrane-bound vesicles containing the secretory product originate in the cytoplasm of the cell and move toward the cell membrane to fuse with it. The cell membrane then opens at the site of fusion and the contents of the vesicle are discharged into the extracellular space.

CELL SURFACE RECEPTORS

The plasma membrane of many cells contains *receptors* which play important role in communication of cells with each other. These receptors respond to signaling molecules (ligands) which come in contact with the external surface of a target cell (i.e., the cell receiving the signal). The cell surface receptors are generally the integral glycoproteins of the plasmalemma which function in recognizing signaling molecules and in transducing the signal into an intracellular action. The cell surface receptors can be classified into three main types: ion channel-linked receptors, enzyme-linked receptors, and G protein-linked receptors.

The **ion channel-linked receptors** are gated channel proteins, which require the binding of a ligand to open their gate. These channels remain open until the ligand dissociates from the receptor.

The **enzyme-linked receptors** are one-pass transmembrane proteins whose outer (extracellular) surfaces serve as receptors for specific ligands. When a ligand binds to an enzyme-linked receptor, the intracellular region of the transmembrane protein produces enzymes which carry out the required action in the cell.

The **G protein-linked receptors** are multipass transmembrane proteins whose extracellular surfaces function as receptor site for ligands. Their intracellular regions are linked to trimeric GTP-binding proteins (G proteins). These receptors activate a chain of intracellular

* In Greek, *pinein* = to drink

** In Greek, *phagein* = to eat

events either through the adenylate cyclase pathway or through the phospholipase-c pathway.

NUCLEUS

The nucleus contains the genetic material of the cell and functions as the command center of the cell which control metabolic activities occurring in the cell. It sends directions to the cell to grow, mature, divide, or die. There is a constant exchange of materials between the nucleus and cytoplasm. At least one nucleus is present in each cell of the body except in the mature erythrocytes (which are incapable of protein synthesis, have limited metabolic activity, and are not considered to the true cells).

Size. In the human cells, the nuclear diameter usually ranges from 3 to 10 µm, averaging 6 µm in most of the cells.

Shape. Generally, the nucleus has a spherical (round) shape. However, in certain cells of the body the nucleus may be oval, fusiform, lobulated, or irregular in shape.

Position in the cell. Position of the nucleus generally depends on the shape of the cell. In spherical and polygonal cells, it is normally located in the center of the cell, but may be present in an eccentric position in certain cells. In the cuboidal, fusiform, and squamous (flat) cells, the nucleus generally occupies a central position in the cell. In the columnar and pyramidal cells, the nucleus usually lies in the basal region of the cell.

Number. Generally, the human cells are mononucleate i.e., each cell contains only one nucleus. However, some cells of the body are binucleate (i.e., having two nuclei) or multinucleate (i.e., having many nuclei); for example, some of the hepatocytes (liver cells) are binucleate, and all skeletal muscle cells are multinucleate.

STRUCTURE (Fig. 2.4)

The nucleus of a resting (nondividing) cell is surrounded by a *nuclear envelope* which is composed of two parallel unit membranes. Granules and particles of a basophilic material known as *chromatin* occupy most of the space within the nuclear envelope. In addition, each nucleus contains a round, non-membrane-bound, dark-staining body called *nucleolus*; in some cells, the nucleus may contain more than nucleoli. A semifluid material called nuclear ground substance or *nuclear sap* fills that space within the nucleus which is not occupied by the chromatin or nucleoli. In stained sections, very pale-staining or almost clear zones represent the nuclear sap.

NUCLEAR ENVELOPE

When ordinary (H&E) stained sections are seen under LM, the nuclear envelope is visible as a dark blue or purple line. EM reveals that the nuclear envelope is about 40 nm thick and consists of two parallel unit membranes separated by a narrow space called the *perinuclear cisternal space*. On the internal aspect of the inner nuclear membrane is present a

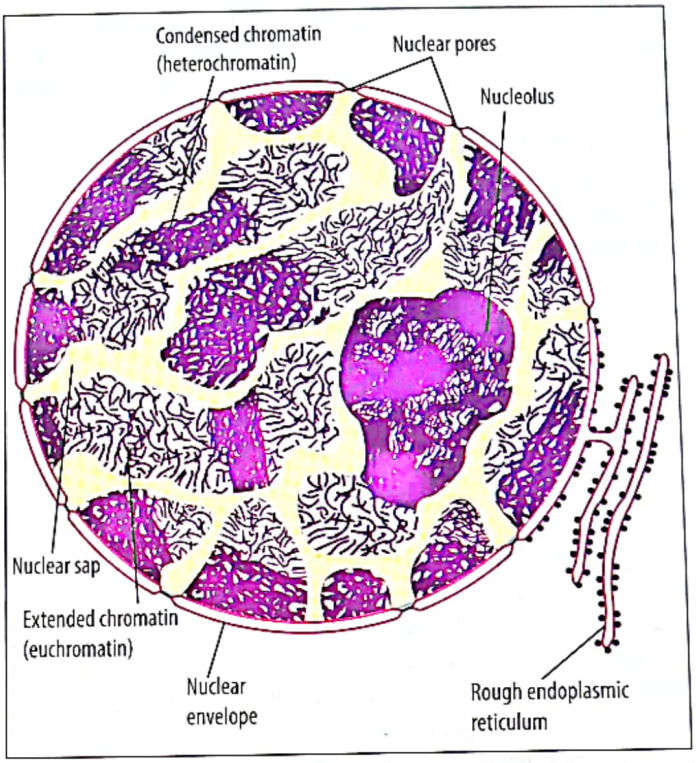

Fig. 2.4 Schematic interpretation of the state of the chromatin in the nucleus of a nondividing cell.

network of protein filaments which is called *nuclear lamina*. Because of its rigid network of protein filaments, the nuclear lamina supports and stabilizes the nuclear envelope. The outer membrane of the nuclear envelope is studded with ribosomes. At several places, this membrane is fused with the membranous wall of the rough endoplasmic reticulum of the cytoplasm. Thus, the perinuclear cisternal space is connected to the cisternae of the rough endoplasmic reticulum of the cytoplasm.

At numerous points over the surface of the nucleus, there are small circular openings in the nuclear envelope which are called *nuclear pores*. These pores are formed by the fusion of the outer and inner membranes of the nuclear envelope. The nuclear pores average 70 nm in diameter and serve as channels through which exchange of materials between the nucleus and cytoplasm occurs; for example, the mRNA passes from the nucleus into the cytoplasm and the histones pass from the cytoplasm into the nucleus. Each nuclear pore contains a *nuclear pore complex,* which consists of eight special proteins called *nucleoporins* which are arranged in an octagonal symmetry around the lumen of the nuclear pore.

CHROMATIN

When the routine H&E-stained sections are examined under LM, the chromatin appears in the form of fine to coarse granules distributed irregularly within the nucleus. The chromatin has been found to consist of **DNA** (deoxyribonucleic acid) and associated basic proteins called **histones.** The DNA exists in the form of flexible rod-like structures called ***chromosomes***. The chromosomes

may be folded, coiled or crumpled at various sites along their course, so as to form little masses, which are large enough, when stained, to be visible under the light microscope. These masses are called condensed chromatin or **heterochromatin**. Those parts of chromosomes, which are relatively straight or uncoiled, are invisible under LM and constitute what is called extended chromatin or **euchromatin**. The euchromatin is metabolically active with regard to RNA synthesis, while the heterochromatin is the relatively inactive part of the chromatin.

Compact Nuclei and Vesicular Nuclei. A nucleus which contains an abundance of heterochromatin appears compact and takes an intensely basophilic stain. On the other hand, a nucleus containing abundant euchromatin gives a vesicular appearance and stains poorly. The vesicular appearance of such a nucleus is due to the fact that it exhibits a large number of clear (i.e., poorly-stained) areas which appear as vesicles or sacs. The poorly stained vesicular spaces are separated from each other by thin dark-staining partitions, which represent the scanty heterochromatin of these nuclei. The inference of the foregoing discussion is that, in stained sections, the nuclei that predominantly contain heterochromatin appear compact and darkly-stained, whereas the nuclei containing larger amount of euchromatin give a vesicular and pale-staining appearance under the microscope.

The EM reveals that the smallest structural units of the chromatin are the **nucleosomes**. Each nucleosome is a 10 nm diameter particle that consists of a core (made up of 8 histone molecules) which is wrapped by two complete turns of DNA molecule (containing about 150 base pairs). The DNA extends to the next nucleosome as a 1.5 nm thick filament which is called *linker DNA*. This structural organization of the chromatin is often referred to as *beads on a string*.

The next higher order of organization of the chromatin is a **chromatin fibril** which is about 30 nm in diameter. A chromatin fibril is formed by coiling of a long strand of nucleosomes. Each turn in the coil of the chromatin fibril consists of six nucleosomes. In the heterochromatin, the chromatin fibrils are highly folded and tightly packed on each other. In the euchromatin, the chromatin fibrils are less folded and more loosely arranged, allowing the access of the enzyme RNA polymerase to the DNA (for the purpose of transcription of DNA to mRNA).

The number of chromosomes is constant and characteristic for each species. In the somatic cells of the humans this number is 46, which is called *diploid* number (indicating that the cell possesses two chromosomal sets, each set consisting of 23 chromosomes). The mature sex cells have only 23 chromosomes; this number is referred to as *haploid* number (*haplo* is a Greek word meaning single, indicating that the cell has only one set of 23 chromosomes). Each chromosome in a haploid cell has its own distinctive size and shape. One of these chromosomes is a sex chromosome; the other 22 are known as autosomes. In every oocyte (ovum) the sex chromosome is an X chromosome, while in a spermatozoon it can either be an X or a Y chromosome. Each diploid somatic cell of an individual has two chromosomes of each type, forming 23 homologous pairs (22 pairs of autosomes + 1 pair of sex chromosomes).

One member of each homologous pair of chromosomes is contributed by the spermatozoon and the other by the ovum at the occasion of fertilization. The nucleus of a somatic cell of a female contains 44 autosomes and two X chromosomes, while that of a male has 44 autosomes and an XY pair of sex chromosomes. It should, however, be noted that a few cell types in the human body have a chromosome number that is more than twice the haploid number; such cells are called *polyploid cells*. The number and characteristics of chromosomes encountered in an individual are known collectively as *karyotype*.

In females, one of the sex (X) chromosomes remains condensed and may be seen as a small deeply stained body called ***Barr body***. In the vesicular nuclei of the neurons, the Barr body may be seen as a small rounded dot near the nucleolus. In the cells of the oral mucosa (easily obtained by scraping the inner surface of the cheeks), the Barr body is usually seen to be located adjacent to the nuclear membrane. In the neutrophil variety of the white blood cells, the Barr body may be seen as a drumstick-shaped appendage on one of the nuclear lobes.

NUCLEOLUS

The nucleolus is a dense, roughly spherical intranuclear structure which is not surrounded by a membrane. It can only be observed in a resting cell because it disappears during the cell division. The nucleolus is the site of ribosomal RNA (rRNA) synthesis and assembly of ribosomes. It is variable in size but is specifically well-developed in cells engaged in active protein synthesis. Some cells may contain more than one nucleolus. In the routine (H&E) staining, the nucleolus takes a basophilic stain.

Components of the nucleolus include DNA, rRNA, and proteins. The DNA of nucleolus consists of portions of those chromosomes that contain genes which encode for rRNA; this DNA is also called *nucleolar organizer*. The rRNA genes are transcribed by RNA polymerase-I. The freshly transcribed rRNA molecules become associated with proteins (which are imported from the cytoplasm) to form ribosomal subunits. The ribosomal subunits pass back into the cytoplasm (via the nuclear pores) to aggregate into complete ribosomes.

Upon electron microscopic examination, the nucleolus appears as a sponge-like network of electron-dense material. Most of this material has a granular appearance and is called **pars granulosa.** The pars granulosa represents maturing ribosomal subunits. The other component of the electron-dense material consists of fine, densely packed filaments and is called **pars fibrosa.** The pars fibrosa represents newly transcribed rRNA still un-associated with proteins. The network formed by the granular and the filamentous

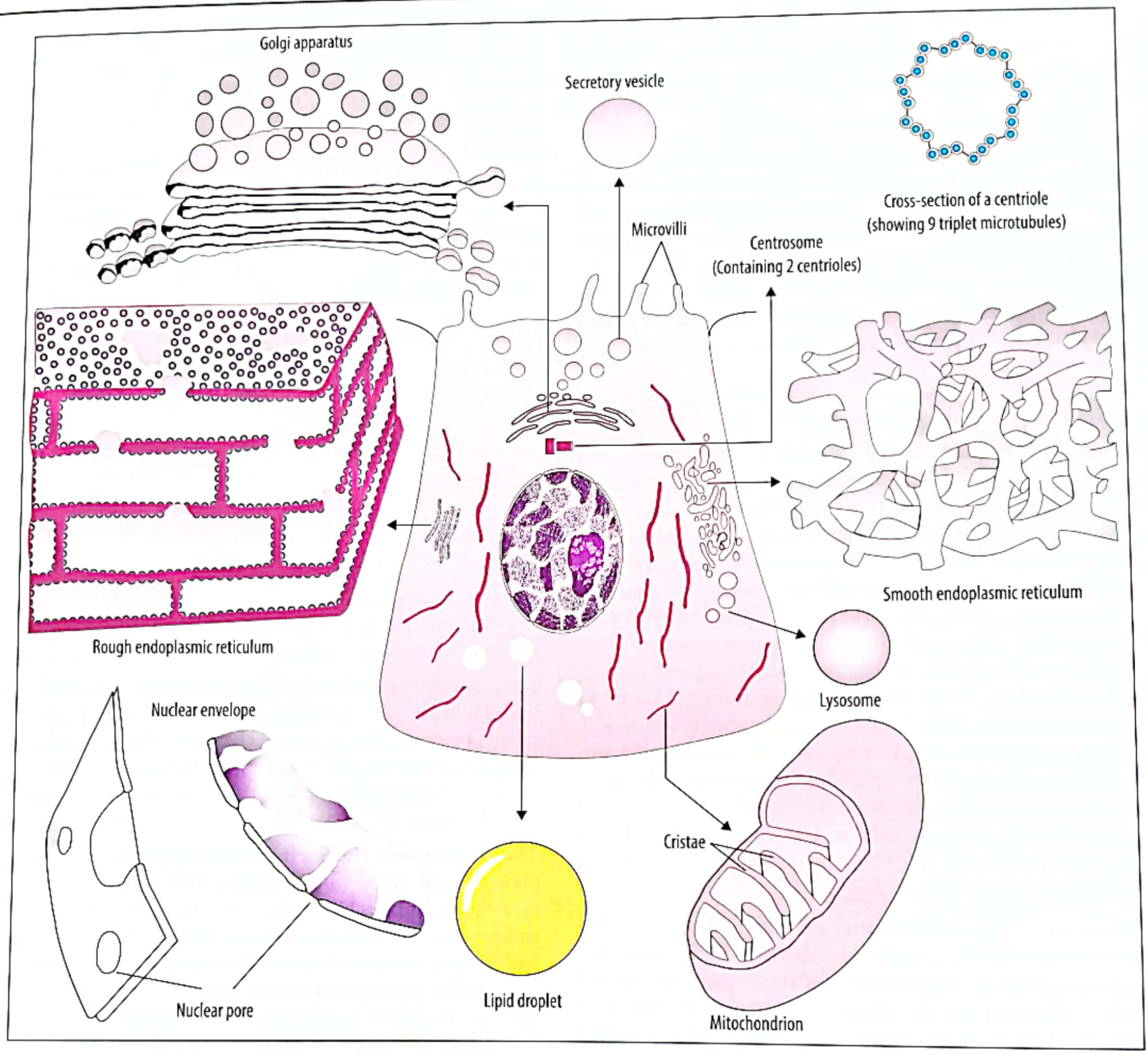

Fig. 2.5 Diagrammatic representation of the microscopic structure of a hypothetic human cell.

materials is known as *nucleolonema*. The interstices of this network consist of lighter-staining regions which represent the nucleolar organizer DNA. This DNA consists of portions of those chromosomes which contain the gene loci that encode rRNA. In humans, the nucleolar organizer DNA exists on five pairs of chromosomes which are chromosomes 13, 14, 15, 21 and 22.

CYTOPLASM

The cytoplasm occupies the space between the cell membrane and the nuclear envelope. Most of the metabolic processes of the cell occur in the cytoplasm but are controlled essentially by the nucleus. The cytoplasm consists of cytosol and cytoskeleton. The **cytosol** is a fluid matrix in which are suspended cytoplasmic ***organelles*** and cytoplasmic ***inclusions***. The **cytoskeleton** is a system of tubules and filaments.

CYTOSOL

The major component of the cytosol is water. In addition, it contains soluble enzymes, soluble proteins, electrolytes, nutrients, metabolites, and RNA, etc.

Previously, the cytosol was regarded to be soluble, unstructured part of the cytoplasm, being semifluid or gel-like in nature. Studies with more advanced microtechniques have revealed that the cytosol is not unstructured and contains a delicate network consisting of very fine interconnected microtrabeculae. This network holds the cytoplasmic organelles in position and plays an important role in their redistribution within the cytoplasm. The soluble unpolymerized constituents of the

cytosol, e.g., the enzymes, are also supposed to be bound to the microtrabecular network in a manner not yet fully understood.

CYTOPLASMIC ORGANELLES

The organelles are organized structures having distinctive morphology and function which are found in the cytoplasm of all eukaryotic cells. Most varieties of the organelles are membrane-bound, but a few types are not surrounded by a membrane. The cytoplasmic organelles include: mitochondria, ribosomes, rough endoplasmic reticulum, smooth endoplasmic reticulum, Golgi apparatus, endosomes, lysosomes, peroxisomes, proteasomes, and centrioles.

MITOCHONDRIA

The mitochondria are double membrane-bound organelles which produce energy for the cell. The size, shape and number of mitochondria vary considerably depending on the tissue type and physiological state of the cell examined. These organelles are very dynamic structures and, in a living cell, they constantly undergo fission (i.e., division) and fusion. They also change their shape and size frequently. They may vary in shape from a small granule-like sphere to a thread-like or rod-like cylinder. Their diameter varies from 0.75 µm to 2 µm, whereas their length is more variable and ranges from 1 µm to 10 µm. The number of mitochondria also varies greatly in different cells or in the same cells under different physiological conditions. Metabolically inactive cells usually contain only a few mitochondria but a metabolically active cell, e.g., a liver cell, may contain 1000 to 2000 mitochondria.

Generally, the mitochondria are distributed evenly in the cytoplasm of the cells. However, in many cells they are found to be concentrated in those parts of the cell where demand for the supply of energy is higher, e.g., the basal parts of the epithelial cells lining the proximal convoluted tubules of kidney (which are involved in active ion transport across the cell membrane). That region of the cytoplasm which is rich in mitochondria stains eosinophilic (because of the large amount of membrane contained in the mitochondria).

Electron microscopic studies reveal that each mitochondrion is surrounded by two unit membranes. The outer membrane has a smooth outline, whereas the inner membrane invaginates as a series of characteristic extensions called **cristae**. (Fig. 2.6) The cristae serve to increase the surface area of the inner mitochondrial membrane. The cristae are generally shelf-like in appearance but in some cells the mitochondrial cristae may be tubular in shape. The number of cristae in the mitochondria is also variable; they are more numerous in the mitochondria of those cells which are engaged in high metabolic activity.

The narrow space between the outer and inner membranes of the mitochondria is known as **intermembrane space.**

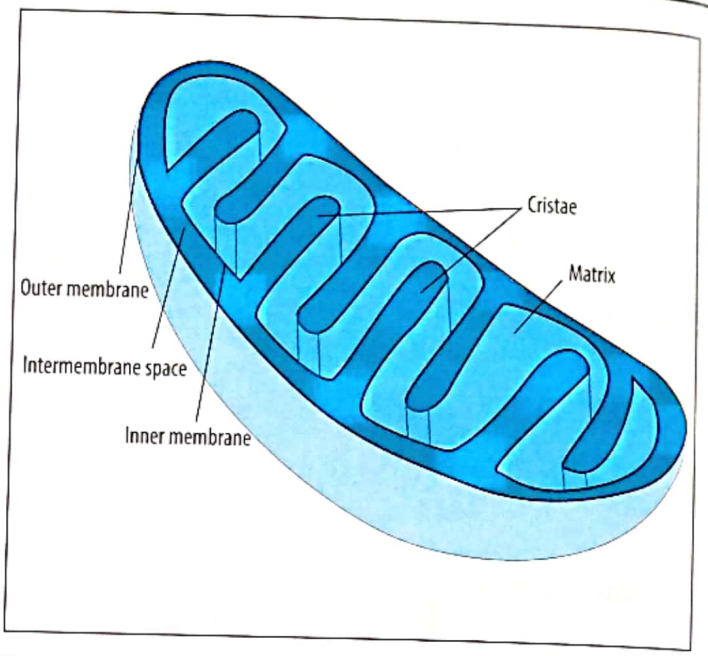

Fig. 2.6 Diagram showing the internal structure of a mitochondrion.

The large space enclosed by the inner membrane is referred to as the **matrix space** (also called *intercristal space*); this space contains mitochondrial matrix.

The **outer mitochondrial membrane** contains a large number of multipass transmembrane proteins called **porins**, which form channels through which ions, metabolites and small molecules can enter the intermembrane space; hence, the contents of this space resemble those of the cytosol.

The **inner mitochondrial membrane** is rich in a phospholipid called **cardiolipin**, which makes the inner mitochondrial membrane impermeable to ions, electrons, and protons. The inner membrane and cristae contain the biochemical enzyme systems for the electron transport chain and oxidative phosphorylation. One of these enzymes, called *ATP synthase,* occurs as large transmembrane proteins that protrude from the inner mitochondrial membrane and cristae as racquet-shaped structures, each of which has a globular head (measuring about 10 nm in diameter) and a cylindrical stalk (measuring about 5 nm in length and 4 nm in width).

The mitochondrial **matrix** is a slightly electron-dense material that fills the internal cavity (matrix space) of each mitochondrion. It consists mainly of proteins which represent the enzymes involved in the beta oxidation of fatty acids, amino acid oxidation, and tricarboxylic acid cycle. The matrix also contains mitochondrial ribosomes, tRNA, mRNA and circular molecules of DNA. The mitochondrial matrix also contains spherical, electron-dense bodies (30-50 nm in diameter) which are called *matrix granules*. These granules have been found to be composed of phospholipoproteins. The matrix granules can store Ca^{2+} and other cations.

The DNA of mitochondria is double-stranded and has a circular structure. The circular strands of DNA are

synthesized within the mitochondrial matrix and their duplication is independent of nuclear DNA replication. The mitochondrial DNA encodes only 13 mitochondrial proteins which are synthesized over the mitochondrial ribosomes by the activity of mitochondrial mRNA, and tRNA (which are also encoded by the mitochondrial DNA). However, most of the mitochondrial proteins are encoded by the nuclear DNA and synthesized in the polysomes of the cytoplasm and then imported into mitochondria. The mitochondria are self-replicating organelles. Following the cell division, the mitochondria reproduce and increase in number by fission. They enlarge in size, replicate their DNA and divide into two nearly equal halves.

Functions of the Mitochondria

1. The primary function of the mitochondria is production of energy for the cell's activities and, therefore, they are also called powerhouse of the cell. The mitochondria synthesize the high energy-carrying compound ATP by the process of oxidative phosphorylation which involves the oxidation of glucose, fatty acids and amino acids and phosphorylation of ADP (adenosine diphosphate) to ATP (adenosine triphosphate). The energy so produced is provided to the cell to carry out its energy-requiring functions.
2. The mitochondria also perform the function of keeping the cytoplasmic concentration of Ca^{2+} ions at a low level. Whenever the cytosolic concentration of Ca^{2+} rises to a dangerously high level, the matrix granules pump the calcium ions into mitochondria to maintain an optimal calcium ion concentration in the cytosol.

RIBOSOMES

The ribosomes appear as small, electron-dense particles measuring 25-30 nm in diameter. They are composed of ribosomal RNA (rRNA) and a specific type of proteins called *ribosomal proteins*. The ratio of rRNA and ribosomal proteins is nearly 60:40. The ribosomes are not bounded by a membrane.

Each ribosome consists of two subunits of unequal size (Fig. 2.7); there is a **large subunit** (having a size of 60 Svedberg units*) and a **small subunit** (size 40S). Each of these subunits is composed rRNA and ribosomal proteins. The rRNA is formed within the nucleus in the nucleolus, while the ribosomal proteins are synthesized in the cytosol. From the cytosol, the ribosomal proteins are transported into the nucleus to become associated with the rRNA. The ribosomal subunits leave the nucleus through the nuclear pores and reach the cytoplasm. When synthesizing a new protein, a large and a small subunit join to form a ribosome, with a messenger RNA (mRNA) strand locked between the two subunits.

The ribosomes generally occur as groups known as **polysomes** (also called ***polyribosomes***). A polysome

* The Svedberg unit (S) is used to express the size of a particle based on its rate of sedimentation in a centrifuge machine.

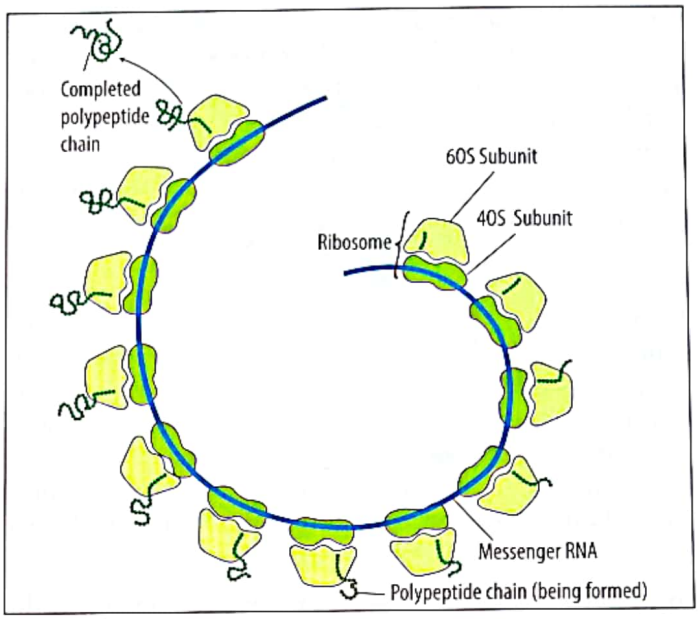

Fig. 2.7 Diagram depicting the structure of ribosomes of a polysome. The process of synthesis of polypeptide chains (proteins) is also shown.

consists of many ribosomes attached to a spiral strand of mRNA. Mostly, the ribosomes are found to be lined up on the cisternae of the rough endoplasmic reticulum. However, the ribosomes may also be found to be randomly distributed in the cytosol as free ribosomes. Individual ribosomes cannot be seen in the ordinary H&E-stained tissue sections examined under the light microscope. However, because of their high RNA content, the ribosomes impart basophilia to that region of the cytoplasm where they are present in abundance.

Functions

Ribosomes are sites where translation of mRNA into an amino acid sequence occurs, which results in the formation of polypeptides chains (proteins). In simple words, the ribosomes are the sites of protein synthesis.

Each of the two ribosomal subunits has a specific function assigned to it. The small subunit reads the mRNA code, while the large subunit joins the amino acids according to the encoded sequence and, thus, a polypeptide chain is formed.

The ribosomes associated with the rough endoplasmic reticulum are involved in the synthesis of integral proteins of membranes of the cell, lysosomal enzymes, and secretory proteins. The free ribosomes synthesize proteins and enzymes that function in the cytosol to ensure cell's own normal activity.

ENDOPLASMIC RETICULUM

The endoplasmic reticulum is the largest membrane system of the cell. It consists of an anastomosing network of intercommunicating membranous tubes and sacs known as *cisternae*. Two morphologically and functionally distinct components of this organelle are recognized:

(1) rough endoplasmic reticulum (RER), which bears ribosomes (as polysomes) on the outer surface or its wall, and (2) smooth endoplasmic reticulum (SER) which does not have any ribosomes associated with its surface. The relative proportions of the amount of RER and SER vary in different cells, depending on the function/functions performed by a particular cell.

Rough Endoplasmic Reticulum (RER)

When tissue sections are studied under the EM, the rough endoplasmic reticulum is seen to exist in the form of parallel stacks of flattened membranous cisternae (sacs). The outer (cytosolic) surface of these cisternae is studded with ribosomes which give this organelle a rough (granular) appearance. Generally, the RER is distributed throughout the cytoplasm but its density is usually higher near the nucleus and Golgi apparatus.

In stained tissue sections examined under LM, the presence of RER in any part of the cell can be detected by the presence of basophilia in that particular part of the cytoplasm. This basophilia is imparted by the RNA of the ribosomes associated with the RER.

The amount and distribution of the RER in the cytoplasm depends on the cell type and its physiological state. Rough endoplasmic reticulum is most abundant in those cells which are engaged in the synthesis of secretory proteins, e.g., the acinar cells of the salivary glands and pancreas. Similarly, two varieties of the connective tissue cells, which secrete proteins, also contain large amounts of RER; these cells are: (i) fibroblasts which secrete the protein collagen, and (ii) plasma cells which secrete immunoglobulins.

Functions

The chief function of the RER is synthesis and segregation of proteins which are not destined for the cytosol; such proteins can have several destinations. The lysosomal enzymes synthesized by the RER are transferred to the Golgi apparatus where they are packaged into lysosomes. Some proteins are incorporated into the plasma membrane and other membranes of the cell as integral proteins. The secretory proteins are also transferred to the Golgi apparatus to be packaged into secretory granules, which release these proteins to the cell exterior by exocytosis. The proteins needed for the formation of different components of the cytoskeleton are also synthesized in the RER.

The proteins and enzymes are synthesized at the polysomes of the rough endoplasmic reticulum, from where they enter the cisternae of reticulum and, thus, are isolated from all other cytoplasmic contents. While within the cisternae of the RER, the newly synthesized proteins undergo some post-translational modifications which include folding of the large-sized proteins, glycosylation of the glycoproteins, and hydroxylation of some proteins.

Smooth Endoplasmic Reticulum (SER)

The cisternae of the SER are generally tubular in shape. These membranous tubules usually form a complicated network in the cytoplasm. No ribosomes are associated with the membranous cisternae of the smooth endoplasmic reticulum.

In most of the cells, the SER is inconspicuous because, in general, the cells do not have a large amount of SER. However, in some cells of the body, the SER is very abundant and occupies a large part of cytoplasm. The cytoplasm of such cells exhibits distinct eosinophilia when viewed under the light microscope (this eosinophilia is imparted by the membrane of the SER cisternae).

Functions

Principal functions of the SER include: (1) lipid synthesis, (2) contribution to glycogenolysis, (3) xenobiotics detoxification, and (4) sequestration of calcium ions.

1. **Lipid Synthesis.** The SER is the site of synthesis of phospholipids for all membranes of the cell. The SER also synthsizes cholesterol, which is an essential component of the membranes of the cell. As the cholesterol also serves as the raw material for the synthesis of steroid hormones, the SER is abundantly found in those cells which are involved in the secretion of steroid hormones, e.g., cells of the adrenal cortex and interstitial cells of the testes.
2. **Contribution to Glycogenolysis.** Glycogen, the principal storage polysaccharide of the body, is stored mainly in the liver and skeletal muscle. When needed, the glycogen is degraded to glucose by the process of glycogenolysis. In the liver cells (hepatocytes), glycogenolysis occurs when the blood sugar level falls below the minimum normal level, and in the skeletal muscle cells it occurs during active exercise. In the hepatocytes, the final step of glycogenolysis (conversion of glucose 6-phosphate into glucose) occurs within the cisternae of the SER (which contain the enzyme glucose 6-phosphatase). From the SER, the glucose passes into the cytosol of the hepatocytes which finally release it into the blood.
3. **Xenobiotics Detoxification.** Detoxification of xenobiotics (foreign chemicals) takes place in the smooth endoplasmic reticulum of the liver cells. The xenobiotics include several drugs and many pollutants, such as pesticides and petroleum products.
4. **Calcium Ion Sequestration.** In the cardiac and skeletal muscle tissue, the SER controls the intracellular distribution of calcium by sequestration (and release) of the calcium ions, and thus, plays a very important role in the regulation of muscle contraction.

GOLGI APPARATUS

The Golgi apparatus, also called Golgi complex, consists of stacks of parallel, flattened, membrane-bound, fluid filled sacs called cisternae (Fig. 2.8). The cisternae of the Golgi stacks are slightly curved and their peripheral parts are dilated. Intercommunications are present between the adjacent cisternae.

The size and location of the Golgi complex varies from one cell to another. It is well developed and large in cells that secrete proteins, e.g., the plasma cells and osteoblasts, and in cells that synthesize large amounts of membrane and membrane-associated proteins, e.g., the nerve cells. Usually a single Golgi complex is present in a cell, but some cells of the body contain several Golgi complexes, e.g., the liver cells (hepatocytes) and osteoclasts. Under LM, the secretory cells which have a large Golgi apparatus typically show a clear, poorly stained area which represents the Golgi apparatus. This poorly stained area is usually surrounded by deeply basophilic cytoplasm (representing the RER). In the polarized secretory cells, e.g., cells of the pancreatic acini, the Golgi apparatus occupies a characteristic position in the cell, always lying between the nucleus and the apical cell membrane.

The Golgi apparatus is a highly polarized organelle and the stack of its membranous cisternae are divided into three compartments, which differ from each other biochemically (each compartment has a distinct enzymatic content). The three compartments (or segments) of the Golgi apparatus are: (i) cis compartment, (ii) medial compartment, and (iii) trans compartment.

The cis compartment is the receiving department of the Golgi apparatus and lies close to the RER. The materials synthesized in the RER are carried to the Golgi apparatus in small membrane-bound transport vesicles which are coated by protein called coat protein-II (COP-II). These vesicles fuse with the cisternae of the cis face of the Golgi apparatus and deliver their contents to these cisternae. From the cis compartment, the materials pass to the cisternae of the medial compartment and are finally delivered to the trans compartment, which is the shipping department of the Golgi apparatus. It is to be noted that retrograde transport between different departments of the Golgi apparatus as well as between the Golgi cisternae and the RER also takes place; this transport is mediated by vesicles coated by coat protein-I (COP-I).

The cisternae on the trans face of the Golgi complex form an extensive tubular network which is called *trans Golgi network* (TGN). Large, saccular protuberances are formed at the TGN which bud off as membranous vesicles that carry the completely processed protein products away from the Golgi apparatus to their destinations. Generally, these vesicles remain within the cytoplasm as primary lysosomes or they become secretory granules which travel to the cell periphery, fuse with the cell membrane and release their contents to the cell exterior by exocytosis.

Functions of the Golgi Apparatus

The major functions of the Golgi apparatus are: (i) Post-translational modifications of proteins and (ii) sorting, packaging, and distribution of proteins.

1. **Post-translational modification of proteins.** In the Golgi apparatus some proteins undergo certain structural modifications which are called post-translational modifications. During the process of translation on polysomes, some proteins are synthesized as precursors which are much larger in size than the functional proteins. The active proteins are liberated from their large precursors by a process called ***trimming***. This process involves removal of unwanted parts of the polypeptide chains by proteolysis. In the Golgi apparatus the polypeptides are also subjected to ***covalent modifications*** like glycosylation, phosphorylation, and hydroxylation.

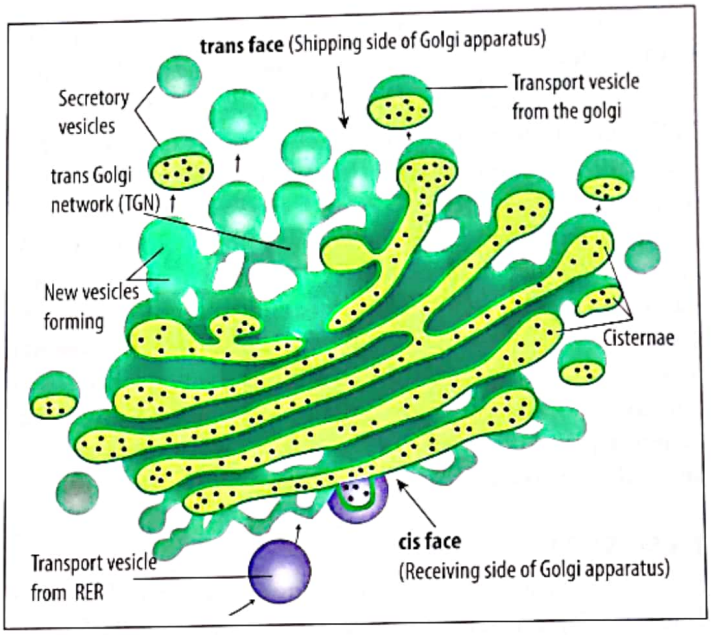

Fig. 2.8 A diagrammatic representation of the structure of the Golgi apparatus.

2. **Sorting, packaging, and distribution of proteins.** In the TGN the proteins are sorted and packaged into shuttling vesicles that bud off from the trans face of Golgi apparatus and distribute proteins to various locations. The secretory proteins are packaged into clathrin-coated vesicles which eventually fuse with the cell membrane to release their contents to cell exterior by the process of exocytosis. The membrane proteins are packaged into vesicles which fuse either with the plasmalemma or with the membranes of organelles within the cell, resulting in renewal of the membrane proteins. The acid hydrolases are packaged into vesicles which leave the TGN to lie within the cytoplasm as *primary lysosomes* (described below).

ENDOSOMES

The endosomes occur as a system of membrane-bound vesicles and tubules that constitute a pre-lysosomal sorting compartment. The membrane enclosing the endosome contains proton pumps which pump h ions into the endosome, and thus, the pH of the endosomal interior becomes much lower than that of the cytosol. After phagocytosis or receptor-mediated endocytosis, the endocytosed macromolecules are processed in endosomes which are classified into two categories: (i) early endosomes, and (ii) late endosomes.

other proteins. Centrosome is the region where most of the microtubules of the cell are formed. The pericentriolar material of the MTOC consists of more than 200 proteins, including the λ-tubulin (lambda-tubulin), α-tubulin, β-tubulin, and pericentrin, etc. In the spherical and polyhedral cells, the centrosome is usually located close to the nucleus, indenting it slightly. In the columnar and pyramidal cells, the centrosome is located between the luminal surface of the cell and the nucleus.

Under low magnifications of EM, each centriole appears as a short rod, averaging 200 nm in diameter and 500 nm in length. Long axes of the two centrioles are at right angle to each other. Under higher magnifications of EM, a centriole appears as a hollow cylinder, the wall of the cylinder consisting of a ring made up of 9 evenly spaced subunits. Each of the 9 subunits forming the wall of a centriole is actually a microtubule triplet consisting of three microtubules running parallel to each other and having common intervening walls. Each triplet is set at an angle of 40° to the tangent of circle of triplets, forming a pinwheel-like arrangement (Fig. 2.9 A). In each triplet the innermost microtubule is designated A, the middle one is named B, and the outermost tubule is labelled C. The A-microtubule has a complete wall formed by 13 protofilaments of the protein tubulin. The tubules B and C share tubulin dimers with each other and with the microtubule A. The microtubule A of a triplet is seen to be connected to the microtubule C of the adjacent triplet by protein links which appear as linear densities (Fig. 2.9 B). The centrioles are not surrounded by any membrane.

An ordinary, nondividing cell contains only one pair of centrioles. In the dividing cells, the number of centrioles is doubled during the S phase (synthesis phase) of the cell cycle. Each new centriole arises close to one end of a pre-existing centriole. Firstly, immature centrioles are formed which are called *procentrioles*. Each procentriole, which lies perpendicular to the mature (i.e., mother) centriole, has nine single tubules in its wall which later give rise to nine triplets. Following the duplication, the two original centrioles migrate (each accompanied by its daughter centriole) to the opposite poles of the cell. Here they induce the generation of microtubules which form the mitotic spindle.

In those epithelial cells which bear kinocilia, each kinocilium is seen to originate from a *basal body* located just under the cell membrane. EM shows that a basal body has the same structure as that of a centriole. In a freshly produced cell in which ciliogenesis (formation of cilia) is going to occur, cylindrical structures called *centriolar organizers* form near the original pair of centrioles. Numerous procentrioles then assemble around the centriolar organizers. All these newly produced centrioles migrate to a position just under the cell membrane of the luminal (i.e., free) surface of the cell and become basal bodies. Each basal body serves as an organizing center for a cilium. Microtubules grow outward from the basal body, push the plasmalemma over them and elongate to form a cilium.

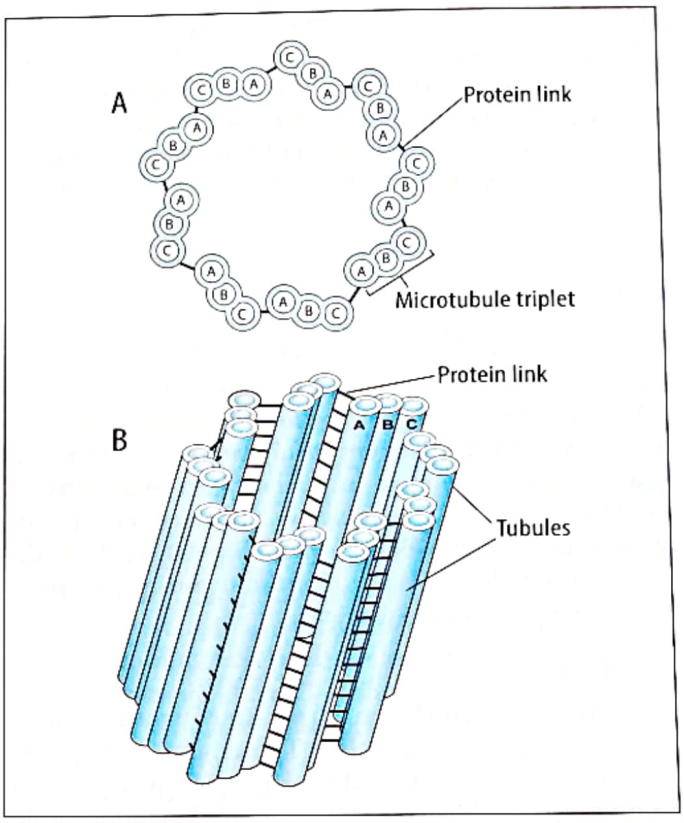

Fig. 2.9 A. Diagram of the cross section of a centriole showing the pinwheel-like arrangement of the nine triplet microtubules. B. A three-dimensional representation of the structure of a centriole.

CYTOPLASMIC INCLUSIONS

Inclusions are considered to be non-living components of the cell which do not possess metabolic activity and are not bounded by a membrane. Cytoplasmic inclusions are classified into two categories: (1) stored food materials, and (2) pigments.

1. Stored Food Materials

i. Lipid

Chief sites of lipid storage are the fat cells (adipocytes), but some other cells of the body, notably the liver cells (hepatocytes), also store lipid in their cytoplasm. The lipid is stored in the cytoplasm of these cells as spherical masses of various sizes called **fat droplets** or *lipid droplets*; these masses are not membrane-bound. Generally, the stored fat consists of triglycerides. In the steroid-secreting cells e.g., cells of the adrenal cortex, the lipid droplets contain cholesterol which serves as the raw material for the synthesis of steroid hormones.

In the routine tissue sections prepared for light microscopy, the lipid is dissolved out and is represented by empty circular spaces in the cytoplasm of lipid-storing cells. The lipid can be demonstrated by using special fixation and staining methods. For example, in frozen sections stained by fat stains, the lipid droplets appear black. Under EM they appear as homogeneous spheres of variable density.

thin filaments, are slender, rod-like structures, averaging 5 nm in diameter. The microfilaments are composed of a protein called *actin.* The free (unpolymerized) actin exists as globular monomers called G-actin. The G-actin monomers polymerize in a linear manner to form actin filaments. Each actin filament consists of a double-stranded helix of filamentous actin (F-actin). The actin filaments are polarized structures having a plus end and a minus end. In ordinary cells, the actin filaments are dynamic structures; G-actin molecules are constantly added at the plus end and removed at the minus end of the filament. Being highly dynamic, the actin filaments can dissociate and re-assemble readily.

In the muscle cells, the actin filaments are structurally stable and are aligned with the thick filaments of the protein myosin in a specific arrangement. Interaction between the thin and thick filaments results in the contractile activity of the muscle cells (for detail on this topic see chapter 10).

The actin filaments perform their functional roles by interacting with different types of **actin-binding proteins (ABPs).** The most commonly occurring ABP is myosin, but several other ABPs have also been identified in different cells of the body; these include α-actinin, β-spectrin, fimbrin, villin, fascin, gelsolin, and adductin.

The microfilaments are shorter and more flexible than the microtubules. In non-muscle cells they are generally found to be irregularly scattered in the cytoplasm to form the structural framework of the cell. However, these filaments are more abundant in the region immediately beneath the cell membrane, which is known as *cell cortex.*

The actin filaments also bind to the intrinsic proteins of the cell membrane and serve to anchor these proteins in position. In those epithelial cells which possess microvilli on their free surface, the microfilaments serve to form the structural core of the microvilli. In motile cells, the microfilaments of the cell cortex play an important role in the cell migratory activity by forming the primary structural component of the pseudopodial processes.

INTERMEDIATE FILAMENTS

Almost all the body cells contain another type of filaments which average 10 nm in diameter and are called intermediate filaments. The intermediate filaments represent a very heterogeneous group of filaments made up of several different proteins. The primary function of the intermediate filaments is to provide structural support for the cell.

Taking into consideration the biochemical nature of the constituent proteins, many types of intermediate filaments have been identified in different cells of the body. The five most common varieties of intermediate filaments are discussed below:

1. **Keratin Filaments.** These filaments are composed of the protein *keratin* and are found in the epithelial cells. They are most abundant in the cells of the stratified squamous epithelium of the epidermis of skin. In these cells, bundles of keratin filaments, called *tonofibrils*, can be seen under the light microscope. In other epithelial cells, keratin filaments usually form a network around the nucleus with bundles radiating to the periphery where they terminate in desmosomes and other specializations of the cell membrane for intercellular junctions. Function of the keratin intermediate filament is primarily a mechanical one; these filaments stabilize the shape of the cell and strengthen its attachment to the basal lamina and neighboring cells.
2. **Vimentin Filaments.** These intermediate filaments are composed of the protein *vimentin* and are found in the cells of mesenchymal origin (fibroblasts, macrophages, and endothelial cells, etc). These filaments may be randomly distributed in cytoplasm in the form of a network or may be gathered in bundles.
3. **Desmin Filaments.** These filaments are composed of the protein *desmin* and are most abundant in the smooth muscle cells where they form a cytoskeleton that transmits the pull of the contractile proteins and ensures a uniform distribution of tensile force across the smooth muscle cell. These filaments are also found in skeletal and cardiac muscle cells where they link the Z discs of the peripheral myofibrils to the plasma membrane of the cell.
4. **Neurofilaments.** These filaments are composed of a special type of protein called *neurofilament protein*. They are found in the neurons where they provide structural support to the cell body and its processes.
5. **Glial Filaments.** These intermediate filaments are made up of glial fibrillary acidic protein and are found in the *astrocytes* which constitute the major variety of the neuroglia (the neuroglia is the supporting tissue of the CNS and is described in detail in chapter 11).
6. **Lamin Filaments.** These intermediate filaments are composed of the protein *lamin* and are chief components of the nuclear lamina, which gives structural support to the nuclear envelope.

Terminal Web

In those epithelial cells that bear microvilli on their free surface, the microfilaments and intermediate filaments of keratin variety form a transversely arranged network in the most apical cytoplasm; this network is called *terminal web.* Many of the intermediate filaments of the terminal web are anchored into the desmosomes of the lateral cell wall. In addition to the filaments, the terminal web contains the actin-binding proteins spectrin and myosin II.

MICROTUBULES

The microtubules are non-branching, rigid, hollow tubes of protein, which can quickly disassemble in one location and reassemble in another. The outer diameter of a microtubule measures about 25 nm, whereas its luminal diameter is about 15 nm. The length of the microtubules

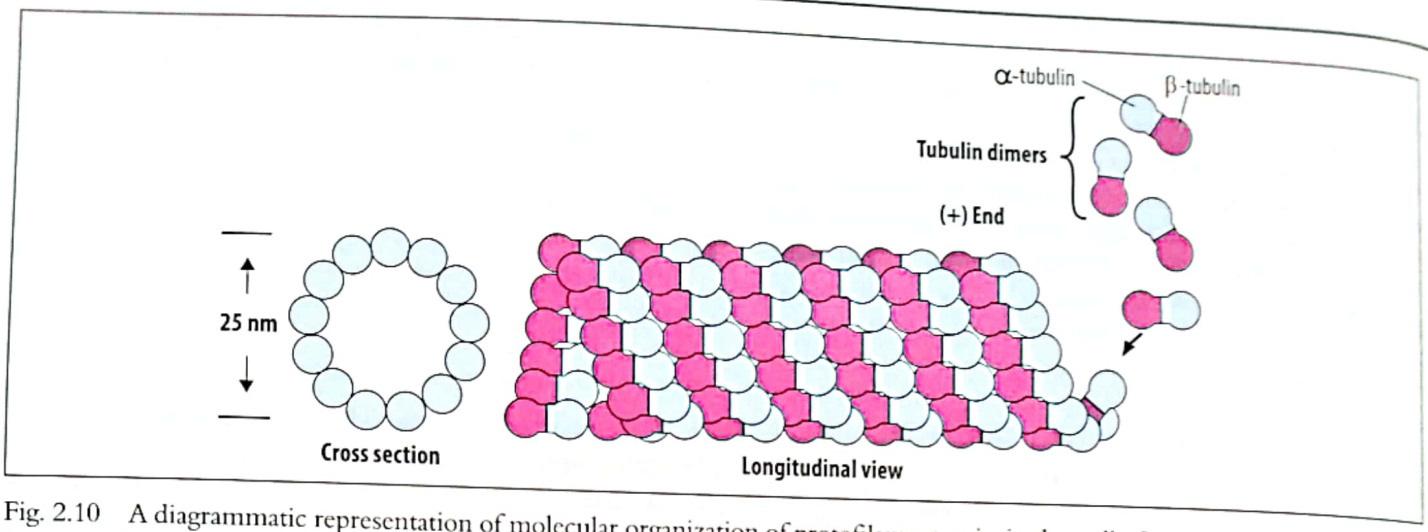

Fig. 2.10 A diagrammatic representation of molecular organization of protofilament units in the wall of a microtubule. The diagram (cross section) on left shows the 13 globular units constituting the wall of the microtubule.

is variable and some of them may be several micrometers long.

The wall of a microtubule consists of *tubulin protofilaments*, each of which is formed by the polymerization of dimeric molecules of tubulin, each of which is composed of an α-tubulin and a β-tubulin molecule. During the formation of a microtubule, tubulin protofilaments join with each other to form a hollow, cylindrical microtubule (Fig. 2.10). In a cross section examined under EM, the circular wall of the microtubule is seen to be composed of 13 globular units (each representing a transversely cut protofilament).

Polymerization of the tubulin dimeric molecules to form microtubules takes place in the *microtubule organizing center* (MTOC), i.e., centrosome of the cell. Polymerization of tubulin molecules requires the presence of magnesium ions and guanosine triphosphate (GTP). Each microtubule possesses a non-growing end (also called *minus end*) which is embedded in the MTOC, and a growing end (*plus end*) which elongates toward the cell periphery.

The microtubules are also associated with special proteins called *microtubule-associated proteins* (MAPs). These proteins are bound to the outer aspect of the microtubules at regular intervals. The major functions of the maps are: (1) to prevent depolymerization of microtubules, and (2) to assist in the intracellular movement of organelles and different types of vesicles along the microtubules.

In the intracellular transport of organelles and vesicles, special MAPs called *motor proteins* play an important role. These proteins become attached to the organelles (or vesicles) and then move along the microtubule track to transport the organelle to the required destination. The motor proteins possess ATPase activity and are capable of hydrolyzing ATP to produce the energy needed for intracellular movement of organelles and vesicles. Two families of motor proteins have been identified: kinesins and dyneins. The *kinesins* move the organelles away from the MTOC toward the plus end of the microtubule, and thus, carry the organelles from the cell center toward the cell periphery. The *dyneins* move the organelles toward the minus end of the microtubules and, therefore, transport the organelles from the periphery toward the cell center.

Functions of the Microtubules

1. Being more rigid than microfilaments and intermediate filaments, the microtubules play a very important for the maintenance of the cell shape.
2. The microtubules form a system of tracks in the cell, along which movement of the cytoplasmic organelles and vesicles occurs.
3. The microtubules form the core of cilia and flagella and impart mobility to these structures (described later in chapter 3).
4. During the mitotic or meiotic cell division, the microtubules give rise to the spindle apparatus.

CELL SURFACE CONTACTS

Normally, the cells maintain contact and with the extracellular matrix either by ***general adhesive contacts*** which have no specialized structural characteristics, or by ***specialized adhesive contacts*** called *cell junctions* which are characterized by special structural features.

The general adhesive contacts between the adjacent cells (or between the extracellular matrix and cells) are maintained due to the presence of **cell adhesion molecules** (**CAMs**) on the surface of the plasma membranes of the cells. These adhesion molecules are transmembrane proteins which have three domains: (i) an intracellular domain which interacts with the cytoskeleton, (ii) a transmembrane domain, and (iii) an extracellular domain which interacts with CAMs of adjacent cells.

Taking into account the dependence of their adhesive properties on the presence of calcium ions, the CAMs are divided into two groups: (i) calcium-dependent adhesion molecules, and (ii) calcium-independent adhesion molecules.

The ***calcium-dependent adhesion molecules*** lose their

adhesiveness in the absence of calcium ions. The most commonly occurring calcium-dependent adhesion molecules are *cadherins.* The cadherin family of proteins is responsible for the general, strong intercellular adhesion in the epithelial and nervous tissues. Another variety of calcium-dependent adhesion molecules includes a family of glycoproteins called *integrins.* These glycoproteins mediate adhesion between the cells and various components of the extracellular matrix like collagen, laminin, and fibronectin.

The calcium-independent adhesion molecules are glycoproteins belonging to the immunoglobulin superfamily. The neural cell adhesion molecules (N-CAMs) are an example of the calcium-independent adhesion molecules. These molecules are found mainly on the surface of the neurons and neuroglial cells.

CELL JUNCTIONS

The cell junctions are specialized adhesive contacts that are characterized by special structural features, which can be studied under the EM.

Classification of the Cell Junctions

The cell junctions are classified upon the basis of two criteria: (1) extent of the areas/areas of the cell membranes involved in the formation of the cell junction, and (2) function of the cell junction.

1. **Classification According to the Extent.** Upon this criterion, the cell junctions are divided into three categories: zonula, fascia, and macula.
   i. A **zonula** is a ***girdle-like*** or belt-like cell junction that encircles the entire perimeter of the cell.
   ii. A **fascia** is a ***band-like*** or strip-like cell junction which has a limited length and does not encircle the whole perimeter of the cell.
   iii. A **macula** is a ***spot-like*** junction which has a very limited extent and is generally circular or oval in shape.
2. **Classification According to the Function.** Depending on the specific function performed by the cell junction, three types of cell junctions are recognized: (i) **occluding junctions**, (ii) **anchoring junctions**, and (iii) **communicating junctions**.

The Occluding Junctions

At these junctions, the plasma membranes of the adjacent cells come into contact and fuse, so that no gap remains between them. The fusion of cell membranes seals the intercellular space and makes it virtually impermeable. Thus, the occluding junctions, also called **tight junctions**, create diffusion barriers which prevent the passage of substances via the paracellular route. The occluding junctions function to join the cells of the epithelial sheets tightly together, so that water and other molecules cannot pass through the intercellular space. In addition, the tight junctions prevent lateral migration of specialized cell membrane proteins (e.g., receptors) and thus play a key role in the maintenance of polarity of the cells, especially in the epithelial tissue. The occluding junctions generally occur as zonulae.

The Anchoring Junctions

These cell junctions, also called *adhering junctions,* provide cell-to-cell or cell-to-basal lamina linkage. Four varieties of adhering junctions are recognized: *zonula adherens, fascia adherens, macula adherens,* and *hemidesmosome.*

The Communicating Junctions

These cell junctions not only attach the cells with each other but also allow the exchange of materials between the adjacent cells. The communicating junctions are characterized by the presence of minute tubular passageways that provide direct cell-to-cell communication. These tubular junctions permit the movement of ions and other small molecules between the adjacent cells. The communicating junctions allow coordinated cellular activity by coupling the adjacent cells metabolically as well as electrically. Only one type of cell junctions, called *gap junctions*, belongs to this variety of intercellular junctions.

Structure of Different Varieties of the Cell Junctions

Zonula Occludens

The zonula occludens is a belt-like tight junction which functions to limit the permeability of the intercellular space between the epithelial cells.

EM shows that a zonula occludens actually consists of a series of focal fusions between the plasma membranes of adjacent cells. The adjacent cell membranes approach each other; their outer leaflets fuse, diverge again and then fuse again (Fig. 2.11). One to several of such fusion sites may be present in the belt-like zonula occludens depending on the location and functions of the epithelial sheet. A few fusion sites are seen in the zonulae occludentes between the epithelial cells lining the proximal convoluted tubules of kidney, whereas several fusion sites are observed in the zonulae occludentes between the epithelial cells lining the mucosa of intestine and urinary bladder. The number of fusion sites determines the sealing capability of the zonula occludens; more the number of fusion sites, more effective the occlusion of the intercellular space.

At the fusion sites, specific transmembrane proteins of the adjacent cell membranes bind to each other. Two types of transmembrane proteins, *occludins* and *claudins,* perform the binding function. The extracellular portions of these proteins protrude out from the cell membrane and meet and fuse in the intercellular space with the occludins and claudins projecting out from the plasmalemma of the adjacent cells. On the cytoplasmic side of the cell membranes, the occludins and claudins are reinforced by cytoplasmic *zonula occludens proteins* ZO1, ZO2, and ZO3.

JUNCTIONAL COMPLEX

In many locations in the body (e.g., the epithelial lining of the small intestinal mucosa) the adjacent epithelial cells are bound to each other by a series of cell junctions, which are collectively known as a *junctional complex*. From the apical to the basal side a junctional complex is seen to consist of three components: zonula occludens, zonula adherens, and desmosomes.

CELL RENEWAL

In all multicellular organisms, including humans, the net population of the body cells is determined by the rate of cell proliferation and cell death.

CELL PROLIFERATION

Increase in the number of body cells by mitotic cell division is called cell proliferation. Depending on the frequency of cell division, the cell populations of the body are generally classified into three types: static, stable or renewing.

1. **Static cell populations** include those cells which are incapable of mitotic division in the adult. The two major examples of static cell populations are neurons and cardiac muscle cells.
2. **Stable cell populations** include those cells which do not divide ordinarily but they have the potential to divide to maintain normal structure of the tissue or organs. Usually the stimulus for the division of the stable cells is the loss of cells from a tissue or organ due to cell death occurring as a result of injury or disease. This category of cells includes liver cells, smooth muscle cells, endothelial cells of blood vessels, fibroblasts of the connective tissue, and osteoblasts of the periosteal covering of the bone.
3. **Renewing cell populations** consist of cells that undergo regular mitotic division. These cells are characteristic of those organs which regularly lose cells, e.g., epidermis of skin and epithelial lining of the gastrointestinal tract.

Stem Cells

Most tissues contain a population of relatively undifferentiated cells called *stem cells*. The daughter cells produced by the mitotic division of the stem cells may either differentiate into specialized cells (of the tissue concerned) or remain undifferentiated as stem cells to maintain the pool of stem cells. Some stem cells give rise to specialized cells of more than one type, such cells are said to be multipotent. In contrast, the unipotent stem cells are restricted to the production of only a single cell type. It has been found out that if stem cells from bone marrow are transplanted to other tissues, they may produce cells that will differentiate into mature cells of the host tissue. Currently, the stem cell research constitutes a major field of interest for the researchers.

CELL DIVISION

All cells of the body originate by the division of the pre-existing cells. A cell division comprises nuclear division (karyokinesis) and cytoplasmic division (cytokinesis). There are two mechanisms of cell division: (1) *mitosis*, which occurs in the ordinary somatic cells, and (2) *meiosis*, which is restricted to the developing germ cells.

MITOSIS (Fig. 2.15)

As a result of this type of cell division the parent cell divides and each of the two daughter cells receives a chromosomal karyotype identical to that of the parent cell. Before the onset of the division, the chromosomal DNA is replicated by synthesis of more DNA. This results in the formation of an exact replica of the genetic material of the cell. Each chromosome now consists of two parallel strands called *chromatids*, which are joined to each other at a constricted region called *centromere*. Thus, the cell enters mitosis with twice the normal diploid complement of DNA.

Concurrent with replication of DNA, the centrioles also replicate themselves and subsequently two pairs of centrioles (i.e., two diplosomes) are present in the cell instead of the normal single pair found in a resting cell. The process of mitosis is dynamic and continuous but, to make the understanding of the mechanism easy, it is customarily divided into four phases named, sequentially, as prophase, metaphase, anaphase and telophase. The total time taken by these four phases is 30-60 minutes.

PROPHASE

The onset of prophase is characterized by the moment when the chromosomes become visible under LM as intensely staining fine threads. Due to the progressive coiling of chromosomes, the threads become shorter and thicker and each chromosome can be seen to consist of two sister chromatids joined to each other in the centromere region.

As the chromosomes become visible, the nucleolus decreases in size and eventually disappears. The two pairs of centrioles separate and a pair migrates to each pole of the cell. Soon afterwards, microtubules grow out in a radiating pattern from the fuzzy, electron-dense material surrounding each centriole pair. Collectively, these tubules create a fusiform structure which is called *mitotic spindle*.

METAPHASE

The end of prophase and beginning of metaphase is marked by breakdown and disappearance of the nuclear envelope. This allows the mitotic spindle to take a central position in the cell previously occupied by the nucleus. By this time, most of the microtubules of the spindle extend from pole to pole (i.e., from centriole to centriole). These microtubules are called *interpolar microtubules*.

The most obvious feature of the metaphase is the alignment of all the chromosomes in such a manner that

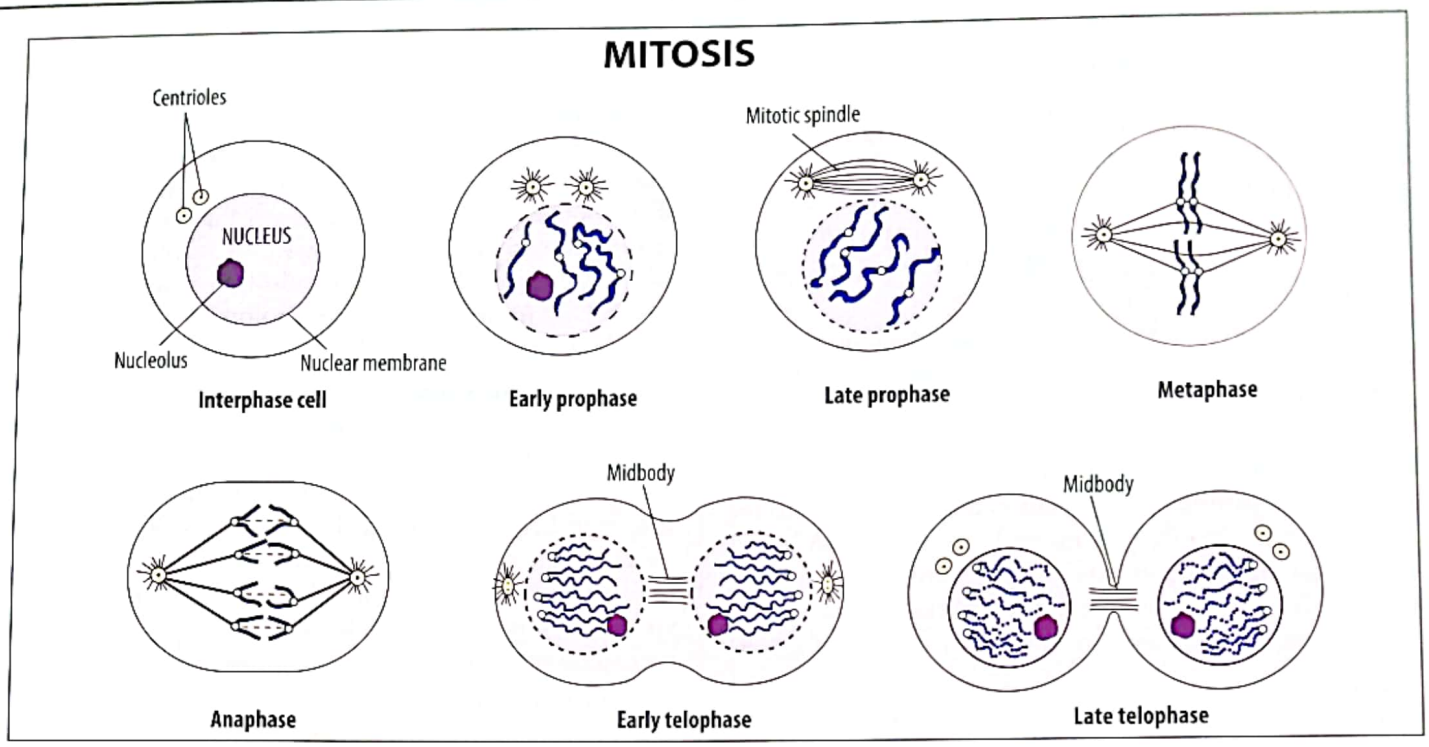

Fig. 2.15 Diagram showing different stages of mitosis.

their centromeres come to lie in a single transverse plane. This plane lies perpendicular to the long axis of the spindle and is known as the equatorial plane of the cell. This orderly assembly of chromosomes, easily seen under LM, is referred to as *equatorial plate* or *metaphase plate.*

A complete condensation of the chromosomes occurs by this time, due to which they stain heavily and each chromosome can now be seen to consist of two chromatids joined at the centromere. An additional set of microtubules is now added to the mitotic spindle. These microtubules become attached to the chromosomes in their centromere region, at a site where each chromatid possesses a plate-like attachment plaque for the microtubules; this plaque is called *kinetochore.* The microtubules attaching to the kinetochore are called kinetochore microtubules. Each metaphase chromosome acquires two groups of kinetochore microtubules (one group for the kinetochore on each of its chromatids). These microtubules exert pull on chromosomes. The opposing poleward forces exerted on the component chromatids keep the chromosomes aligned in the equatorial plane of the cell.

ANAPHASE

During the anaphase, separation of the sister chromatids takes place and they migrate from the equatorial plate to their respective poles. Separation of the sister chromatids is the result of a split in the centromere region. Each chromatid is now free to move to its respective pole as an independent chromosome. In human cells, two identical sets of chromosomes (each consisting of 46 chromosomes) move to their respective poles.

The mechanism of the poleward movement of the chromosomes is still a subject of controversy. The old view that the chromosomes were pulled toward the poles by the contraction of the kinetochore microtubules has been discarded. Recent evidence indicates that as the daughter chromosomes progress toward the poles, the kinetochore microtubules attached to them shorten by undergoing depolymerization at their kinetochore end.

Another factor which contributes to the separation of the daughter chromosomes is the lengthening of the mitotic spindle due to further elongation of the interpolar microtubules. Lengthening of the spindle pushes its two ends further apart, with the result that the chromosomes are pulled apart. During movement of the chromosomes, the centromere of each chromosome remains a little ahead and the arms of chromosomes trail behind. At the end of the anaphase, the chromosomes are clustered at the spindle poles.

TELOPHASE

At the beginning of telophase, a contractile ring appears in the peripheral cytoplasm at the equator of the cell. This ring consists of actin filaments associated with myosins. This ring gradually constricts and produces a *cleavage furrow* around the middle of the elongated cell, foreshadowing its division into two daughter cells. While the cleavage furrow is deepening, a bundle of spindle microtubules still connects the daughter cells; this bundle is called *midbody.*

When the daughter cells are in the process of separation, a nuclear envelope is formed around the chromosomes and nucleoli reappear. The chromosomes then uncoil and lose their stainability except in those regions which are destined to remain condensed as the heterochromatin. At the conclusion of these events, karyokinesis (nuclear division) is completed.

The cleavage furrow deepens further until it encounters the midbody. For a short time, the daughter cells remain connected by a slender cytoplasmic bridge occupied by the microtubules of the midbody. However, these microtubules soon depolymerize and the intercellular bridge is broken, resulting in separation of the daughter cells. This completes the *cytokinesis* (cytoplasmic division).

MEIOSIS

Meiosis is a special type of cell division, which occurs only in the germ cells during the process of their development and maturation to form gametes. It consists of two successive cell divisions, called meiosis-I (first meiotic division) and meiosis-II (second meiotic division). The DNA of the primitive germ cells is replicated prior to the meiosis-I only and there is no replication of DNA before the meiosis-II. The four final cells resulting from the two meiotic divisions are not only haploid (i.e., containing 23 chromosomes) but they also contain 1n amount of DNA.

In addition to causing reduction in chromosomal number and amount of DNA to half (as compared to the ordinary somatic cells), the meiosis is of great importance in contributing to genetic reassortment which ensures genetic diversity in the offspring. The genetic reassortment takes place as a result of the following two mechanisms:

1. During the prophase of meiosis-I, crossing-over of genetic material (segments of DNA) between the homologous maternal and paternal chromosomes takes place. During the chromosomal crossing-over, the DNA double helix is broken in both a maternal chromatid and a homologous paternal chromatid and exchange of DNA fragments between the two chromatids takes place in a reciprocal fashion by a process called *genetic recombination*.
2. During the anaphase of the first meiotic division, there is a random distribution of maternal and paternal homologous chromosomes to the daughter cells which are produced as a result of this division.

MEIOSIS-I (Fig. 2.16 A)

In a primitive germ cell which is going to divide by meiosis, replication of nuclear DNA takes place before the first meiotic division, so that all the chromosomes of the cell become duplicated. Each duplicated chromosome is a double-stranded structure consisting of two identical strands. The meiosis-I also consists of four phases: prophase, metaphase, anaphase and telophase.

Prophase

During the prophase of meiosis-I, the process of genetic recombination takes place. Therefore, it is a long phase which is divided further into five stages: leptotene, zygotene, pachytene, diplotene and diakinesis.

The **leptotene stage** is also called ***leptonema*** (in Greek, leptonema means *thin threads*). In this stage, the duplicated chromosomes (each consisting of two sister chromatids) become visible in the nucleus of the cell as long and thin strands.

The **zygotene stage** is also known as ***zygonema*** (i.e., *paired threads*). In this stage, the double-stranded chromosomes line up with each other as homologous pairs; this process of pairing is called *synapsis* (or *syndesis*) and each of these pairs is known as a *tetrad* or *bivalent*. In each bivalent, the precisely paired homologous chromosomes are held together by means of numerous points of contact which are called *synaptonemal complexes*.

The **pachytene stage**, also called ***pachynema*** (i.e., *thick threads*). In this stage, the chromosomes become thicker and shorter due to coiling. The special feature of this stage is that small segments of non-sister chromatids break apart and become exchanged in a process called *crossing-over*. A point where such exchange takes place is called a ***chiasma***. The chiasmata are X-shaped, microscopically visible regions and constitutes a characteristic feature the pachytene stage of meiosis-I.

The **diplotene stage** is also termed ***diplonema*** (i.e., *two threads*). In this stage, the paired chromosomes of each bivalent begin to separate from each other. However, the chromatids remain connected to each other at the sites of chiasmata.

The **diakinesis** constitutes the last stage of prophase of meiosis-I. During this stage, separation of the chromosomes continues and the chiasmata become more obvious. The nucleoli disappear, the nuclear envelope disintegrates and the meiotic spindle begins to form by the end of the diakinesis.

Metaphase

During the metaphase of meiosis-I formation of the meiotic spindle is completed. The bivalent chromosome pairs gather in alignment on the center of the spindle and form the equatorial plate. It is to be noted that the bivalents are still attached to each other at the regions where chiasmata are located.

Anaphase

In the anaphase of the first meiotic division, the chromosomes of each homologous pair become completely detached from each other and move to the opposite poles of the spindle. The point to be particularly noted is that (unlike the anaphase of mitosis) no division of centromeres occurs during the anaphase of meiosis-I and the duplicated chromosomes (each consisting of two chromatids) move to the opposite poles of the dividing cell to make up the nucleus of each of the two daughter cells (which are obtained at the end of the telophase). It is also to be noted that the distribution of maternal and paternal chromosomes to the daughter cells is completely random and a matter of pure chance.

Telophase

In this stage the nuclei are reconstituted and cytokinesis divides the parent cell into two daughter cells. Each

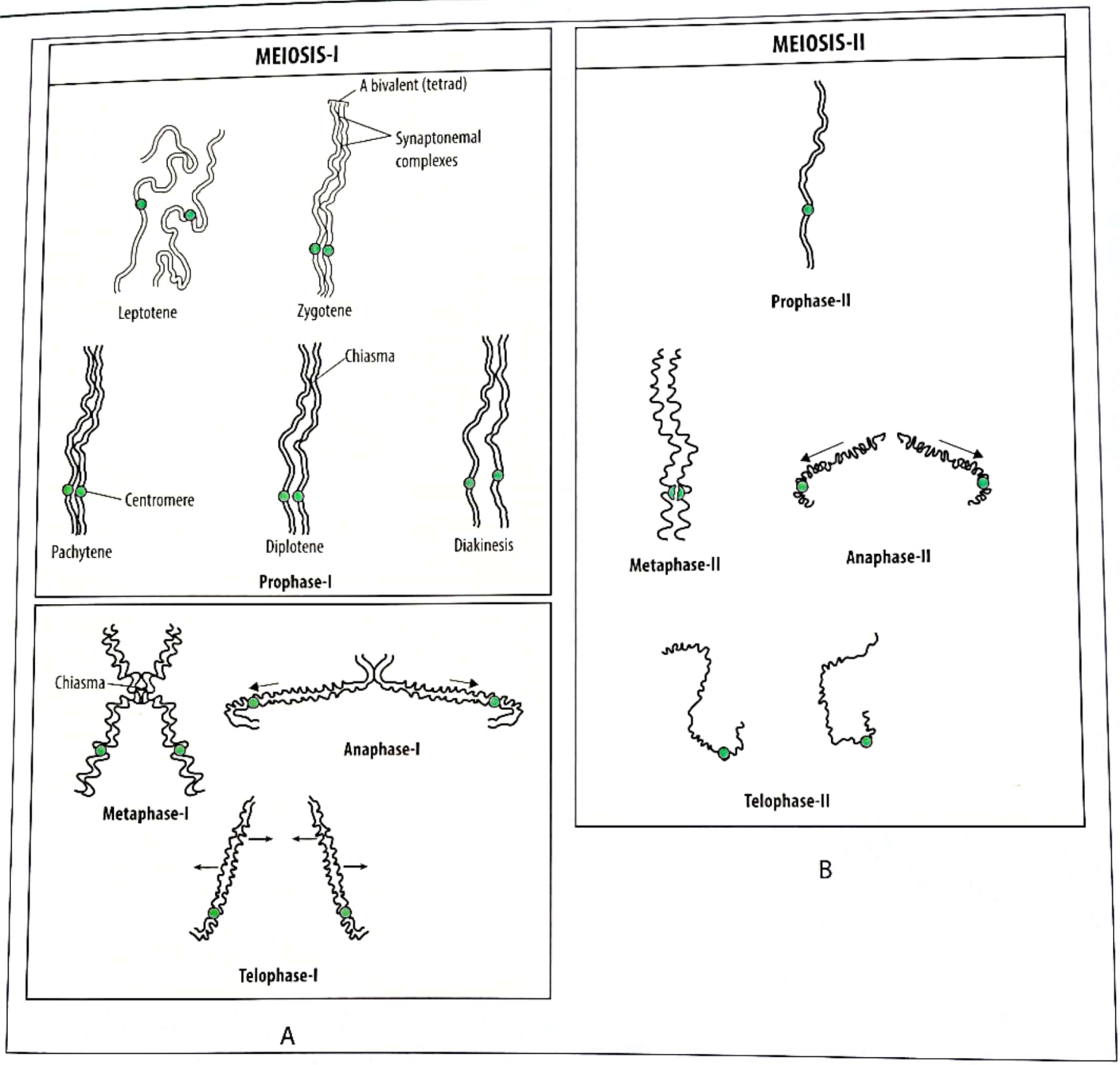

Fig. 2.16 A schematic diagram depicting behavior of chromosomes during various stages of meiosis-I (A), and meiosis-II (B).

daughter cell contains haploid (i.e., 23) chromosomes but each chromosome is a double-stranded chromosome consisting of two sister chromatids.

MEIOSIS-II (Fig. 2.16 B)

Each of the two daughter cells produced as a result of meiosis-I, soon enters meiosis-II which also consists of the usual four phases, i.e., prophase, metaphase, anaphase and telophase. The behavior of the chromosomes in meiosis-II is similar to that which is seen in mitosis. Division of the centromere in each doubled chromosome takes place, the sister chromatids separate and move as independent chromosomes to the opposite poles. Reconstitution of nuclei soon takes place, and then cytokinesis divides the cell into two daughter cells. Consequently, four cells are obtained at the conclusion of the second meiotic division. The nucleus of each of these cells contains a haploid number of chromosomes and 1n amount of DNA.

THE CELL CYCLE (Fig. 2.17 & 2.18)

Cells belonging to the renewing cell populations undergo a sequence of events that is repeated over and over again. This sequence of events is called *cell cycle*. This cycle is divided into two main parts: (1) **M phase** in which mitosis (cell division) occurs, and (2) **interphase,** which is the intervening period between consecutive cell divisions. During this period the cells make necessary preparations for cell division. Whereas mitosis takes only 30 to 60 minutes to complete, the interphase spans a much longer

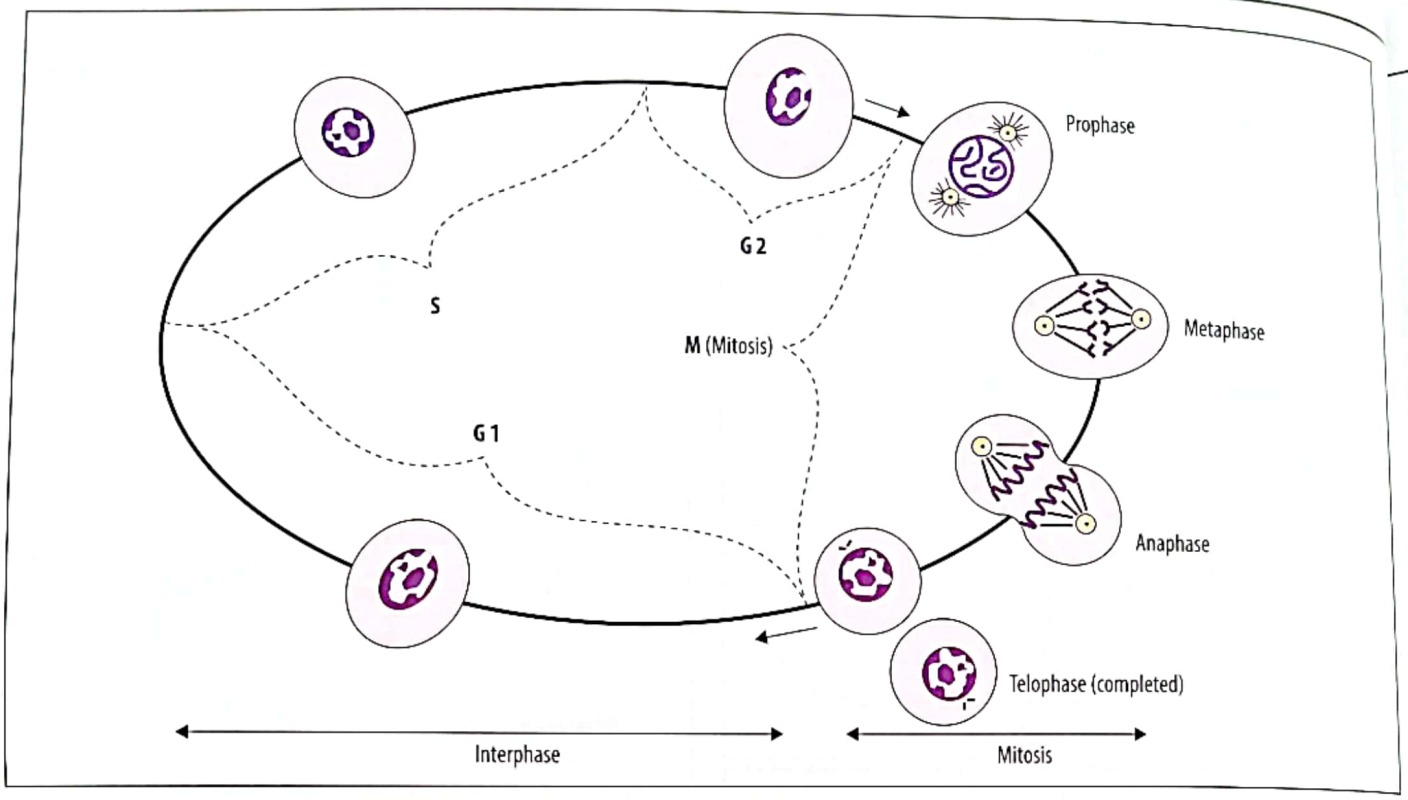

Fig. 2.17 Diagram showing different phases of the cell cycle.

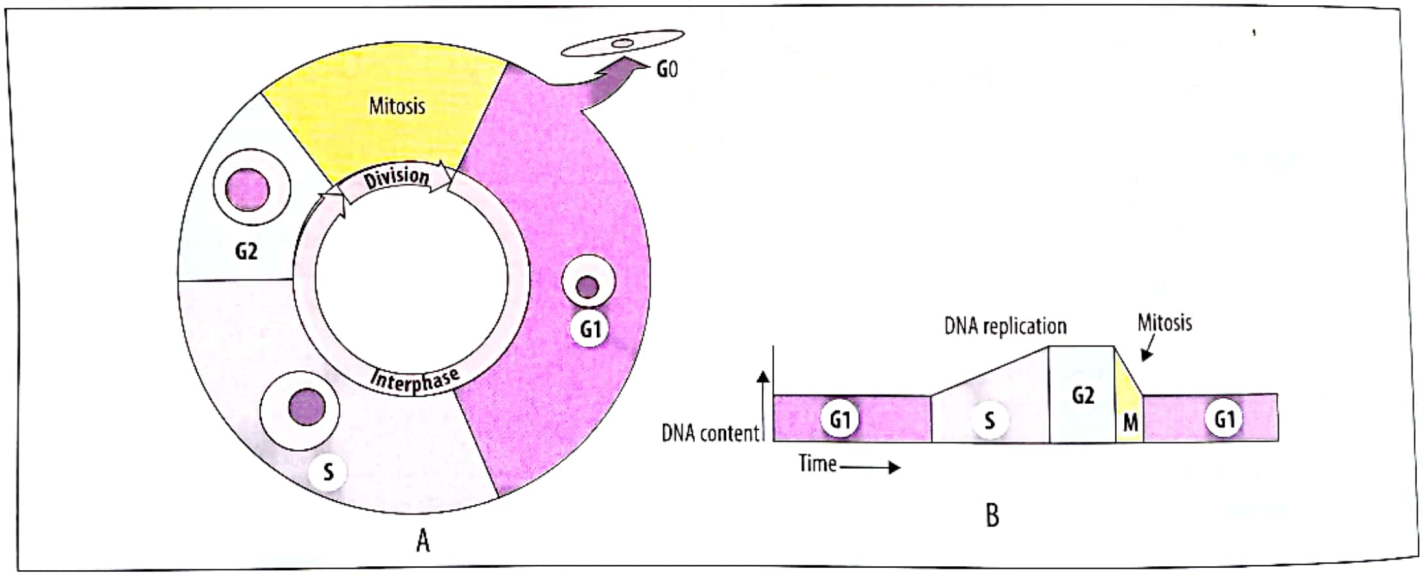

Fig. 2.18 A-Diagrammatic representation of the sequence of different phases in the cell cycle.
B-Diagram showing changes in the DNA content of a cell during various phases of the cell cycle.

period of time and is subdivided into three phases, which are described below:

1. **The G1 Phase** (*the first gap phase* or *first growth phase*) is the period that follows the mitosis. During this phase, the cell manufactures proteins (mainly histones) and ATP. The organelles increase in number (either by duplication of some organelles, e.g., mitochondria and centrioles, or by fresh formation of others, e.g., ribosomes and lysosomes). Consequent to these activities, the cell enlarges in size. In the regularly dividing cells, the duration of G1 is about 10 hours.
2. **The S Phase** (i.e., *synthesis phase*) is the period during which synthesis of DNA takes place. DNA synthesis results in preparation of an exact replica of the genetic material. Replication of centrioles also occurs during this phase. The s phase takes approximately 9 hours to complete.
3. **The G2 Phase** (the *second gap phase* or *second growth phase*) is the period between the end of the S phase and the beginning of mitosis. During this phase, the cell synthesizes ATP and the tubulin variety of proteins (which will be utilized to produce the microtubules

The **early endosomes** are located in the peripheral cytoplasm close to the plasmalemma of the cell. The pH of the interior of early endosomes remains around 6.0.

When an endocytotic vesicle fuses with an early endosome, the membrane components (membrane proteins, receptors, and clathrin) are from the endocytosed macromolecule (ligand) and returned to the cell membrane for re-use. As this recycling of the membrane components takes place, the early endosomes mature into late endosomes which move to the deeper part of the cytoplasm. The primary lysosomal vesicles emerging from the trans Golgi network fuse with the late endosomes to form the secondary lysosomes (i.e., when a primary lysosome fuses with a late endosome, a secondary lysosome is formed).

LYSOSOMES

The lysosomes are small, roughly spherical bodies, bounded by a single membrane, ranging from 0.05 to 0.2 µm in diameter. However, in some cells, e.g., neutrophilic leukocytes, the lysosomes are larger in size, reaching a diameter of 0.8 µm.

The lysosomes contain hydrolytic enzymes (acid hydrolases) which are capable of degrading almost all types of biological macromolecules (proteins, carbohydrates, lipids, and nucleic acids).

As already explained, the lysosomes are categorized into two varieties: (i) primary lysosomes, and (ii) secondary lysosomes. The primary lysosomes are the small, membrane-bound vesicles which bud off from the cisternae of the Golgi apparatus; they contain various acid hydrolases. The secondary lysosomes, also called heterolysosomes, are formed when the primary lysosomes fuse with the late endosomes. The heterolysosomes contain materials which are being digested by the degradative activity of the acid hydrolases.

Functions

The function of the lysosomes is to carry out intracellular digestion. They can be used either to degrade materials taken into the cell by endocytosis (*heterophagy*), or to degrade cell's own old and worn out organelles (*autophagy*).

Heterophagy is the chief function of the lysosomes. As mentioned earlier, the materials endocytosed by cell are delivered to the late endosomes. The primary lysosomes fuse with the late endosomes to form secondary lysosomes. In a secondary lysosome, the hydrolytic enzymes carry out the degradation of the macromolecules to small soluble products which cross the lysosomal membrane and enter the cytosol. These products are either reused by the cell or released from the cell into the extracellular space. The remaining indigestible compounds are retained within the used lysosomes, which are now called *residual bodies*. The residual bodies usually remain stored within the cytoplasm. These bodies frequently contain partially degraded lipidic material, some of which becomes converted to a brownish pigment called *lipofuscin* (described later).

Autophagy is also an important function of the lysosomes by which the old and worn out organelles are disposed of; such disposal is necessary to ensure normal turnover of the organelles. An organelle (e.g., a mitochondrion) which is needed to be disposed of becomes enclosed within a membranous covering derived from the smooth endoplasmic reticulum. The resulting vesicle (called an autophagosome) then fuses with a primary lysosome and its contents are digested. In certain pathological conditions or when cellular damage occurs, the lysosomes may rupture, release their enzymes and ultimately destroy the cell from within; this process is called *autolysis*.

PEROXISOMES

The peroxisomes (also called *microbodies*) are fairly similar to lysosomes in appearance but their contents, and therefore functions, are markedly different from lysosomes. Peroxisomes are small spherical bodies bounded by a single membrane, ranging from 0.2–1.0 µm in diameter. They contain oxidative enzymes that oxidize a variety of organic substrates, notably fatty acids and amino acids. These oxidation reactions produce hydrogen peroxide. Being a highly reactive oxygen intermediate, hydrogen peroxide can cause serious chemical damage to the cell and, therefore, is immediately decomposed into water and oxygen by the enzyme catalase which is an important content of the peroxisomes.

In addition to oxidizing the organic substrates normally found in the cell, the peroxisomes also oxidize and detoxify many harmful substances that are taken up by the cell, e.g., ethyl alcohol, formaldehyde, drugs, and poisons. This detoxification mainly occurs in the liver and kidneys and, therefore, the hepatocytes and the cells lining the kidney tubules are especially rich in peroxisomes.

PROTEASOMES

The proteasomes are small cylindrical structures composed of multisubunit protein complexes including proteases. The average diameter of a proteasome is about 13 nm and, like the ribosomes, the proteasomes are also not surrounded by a membrane. The proteasomes represent ATP-dependent protease complexes that destroy the denatured, malformed or nonfunctional proteins. This process of cytosolic proteolysis is very discreetly controlled by the cell. Any protein that is needed to be degraded by the proteasomes is first attached to a cytosolic protein called *ubiquitin*. Once a protein has been tagged to ubiquitin, it is readily degraded by the proteasomes into short peptides. These peptides pass into the cytosol and, at the completion of the degradation of a protein molecule, the ubiquitin molecule is also released into the cytosol for reuse.

CENTRIOLES AND CENTROSOME

Normally, each cell contains two centrioles which lie in a special area of the cytoplasm called **microtubule organizing center (MTOC)** or *centrosome*. The centrosome consists of: (i) the centrioles, and (ii) a dense *pericentriolar matrix* containing free tubulin subunits and

ii. Glycogen

The glycogen is a polysaccharide which is the most common form of energy storage in the cells. It is especially abundant in the liver cells and skeletal muscle cells. Whenever needed, the glycogen is converted into glucose by depolymerization.

In the cytoplasm, the glycogen exists as small, round masses called glycogen granules. These granules are not bounded by a membrane. During routine processing of tissues for H&E staining, the glycogen is dissolved out and, therefore, the areas occupied by the glycogen granules is represented by small, circular empty spaces in the cytoplasm of the cells; such spaces are a conspicuous feature of those cells which store large quantities of glycogen, e.g., liver cells and skeletal muscle cells. In alcohol-fixed tissues stained by PAS method*, the glycogen is preserved and appears as magenta (reddish-purple) granules. Under EM, the glycogen granules are seen as electron-dense, round masses measuring 15-30 nm in diameter.

2. Pigments

Certain cells of the body contain substances which naturally possess some kind of color. These substances, called pigments, can be visualized under the light microscope even in unstained tissues. Following is a description of the two most commonly found pigments in the human cells:

i. Lipofuscin

The lipofuscin pigment occurs in the cells as granules of a yellowish brown material. As already explained, this material is composed of lipid-containing residues of the lysosomal digestion. Lipofuscin pigment is an indication of the normal wear and tear of cells and its amount increases with age. Understandably, this pigment is especially abundant in long living cells like the cardiac muscle cells and nerve cells.

ii. Melanin

This pigment occurs in the form of dark brown granules. Melanin is chiefly found in special cells called *melanocytes*, which are mainly located in the basal layers of the epidermis of skin. After their formation in the melanocytes, the melanin granules are transferred to the keratinocytes of the epidermis. In the skin, the melanin serves to protect the epidermal cells against the harmful effects of the solar ultraviolet radiation.

Melanin pigment is also abundantly present in the eyeball where it is chiefly found in the pigment epithelia of the retina, ciliary body, and iris. In the eye, the melanin pigment performs the important function of absorption of light to prevent reflection and resultant glare.

Melanin granules are also found in the neurons of a large mass of gray matter, called substantia nigra, which is located in the midbrain.

* The PAS (periodic acid-Schiff) method is a special staining technique which is used to stain carbohydrates and carbohydrate-rich macromolecules.

iii. Hemosiderin

This iron-containing pigment and normally occurs as yellowish brown granules in the phagocytic cells of the spleen, liver and bone marrow, which phagocytose the old and worn out RBCs. The hemosiderin represents the indigestible residues of the hemoglobin and consists mainly of denatured ferritin bound to certain insoluble proteins. In some diseases, hemosiderin accumulates in large amounts in different body organs, especially in the liver, lungs and kidneys; such condition is called hemosiderosis.

CYTOSKELETON

The cytoplasm of all eukaryotic cells contains a filamentous array of cytosolic proteins, which are of three main categories: (1) **microfilaments** which average 5 nm in diameter and are composed of the protein *actin*, (2) **intermediate filaments** which have an average diameter of 10 nm and are composed of different types of proteins which vary in different cell types, and (3) **microtubules** which are very fine tubular structures composed of a protein called *tubulin*.

The cytoskeleton performs the following major functions:

1. As indicated by its name, the cytoskeleton forms an internal scaffolding in the cell and thus constitutes a structural framework that maintains the cell shape and gives the cell a mechanical resistance against deformation.
2. Through its association with the adjacent cells and with the extracellular connective tissue, the cytoskeleton helps to stabilize the entire tissue.
3. The cytoskeleton divides the cytosol into functionally separate areas and provides a scaffold that serves to keep various organelles and other components of the cytoplasm at their specific places in the cell.
4. The cytoskeleton facilitates cytoplasmic streaming, i.e., the movement of the cytosol within the cell, which results in the transport of nutrients, proteins, organelles, and cytoplasmic vesicles within the cell.
5. The cytoskeleton performs a very important function in the motile cells of the body (e.g., the leukocytes and macrophages). In such cells, the bundles of microfilaments undergo active contraction to produce pseudopodia which enable the cells to move from one location to another.
6. During the cell division, the microtubules form the mitotic spindle and the microfilaments facilitate cytokinesis.
7. The components of the cytoskeleton also contribute to the formation of the cores of specialized structures like microvilli, cilia, and flagella.

A description of the structure of various components of the cytoskeleton will now be given.

MICROFILAMENTS

The microfilaments, also called **actin filaments** or

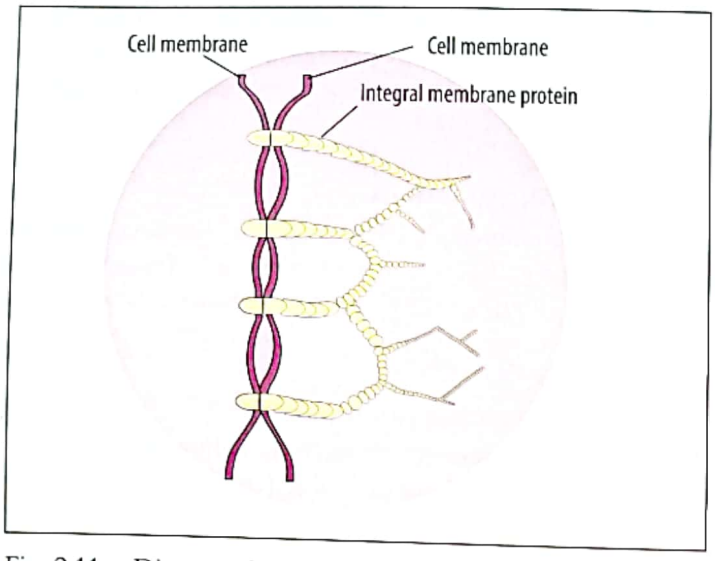

Fig. 2.11 Diagram showing the structure of zonula occludens type of cell junctions.

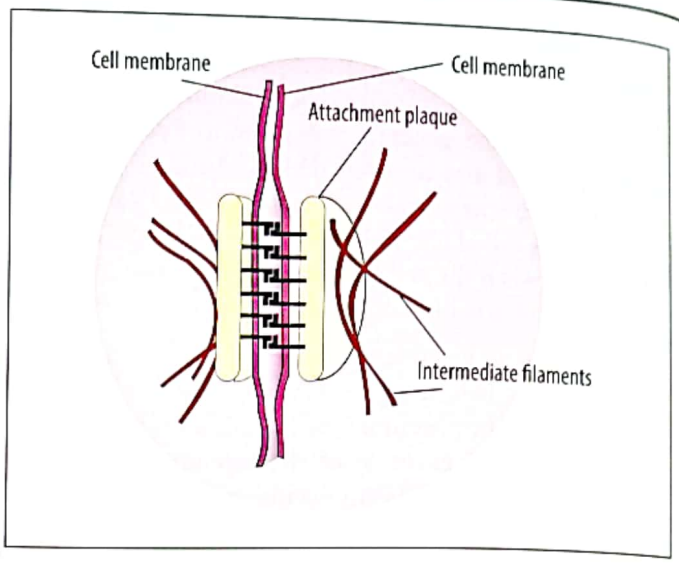

Fig. 2.12 Diagram showing the structure of a desmosome.

In addition, the actin filaments of the cytoskeleton attach to the zonula occludens through the ZO1 proteins.

ZONULA ADHERENS

The zonula adherens is also a belt-like junction that encircles the entire cell. However, there is no fusion of cell membranes. The intercellular gap (about 20 nm) is occupied by the extracellular portions of the molecules of an adhesive transmembrane glycoprotein *e-cadherin*. The extracellular portion of e-cadherin molecules of the opposing cell membranes are bound to each other in the intercellular space by Ca^{2+} ions. On the cytoplasmic side, the tails of the e-cadherin molecules are linked to cytoplasmic anchoring proteins *catenin* and *vinculin*, which in turn bind to actin filaments of the cytoskeleton.

The zonulae adherentes also serve to join the adjacent cells in the epithelial sheets and occur as one of three components of a junctional complex (described on page 26).

FASCIA ADHERENS

Structurally the fasciae adherentes are similar to zonulae adherentes but, as indicated by their name (in Latin, fascia means a band), these cell junctions are band-like in shape and have a limited extent. The fasciae adherentes are found among the cardiac muscle cells, smooth muscle cells, neurons, and neuroglial cells.

MACULA ADHERENS (DESMOSOME)

This variety of anchoring cell junctions occurs very frequently in the epithelial tissues. The maculae adherentes, more commonly known as *desmosomes,* are "spot-weld" like junctions which are randomly distributed along the lateral plasma membranes of the cells of the simple (i.e., single-layered) epithelia and throughout the plasma membranes of the cells of the stratified (i.e., multilayered) epithelia. However, desmosomes are not exclusive to the epithelial tissues but are also found between the cardiac muscle cells.

Electron microscopic studies show that the desmosomes have a complex structure. The cell membranes in the regions of the cell junctions are seen to be farther apart (about 30 nm) than the usual intercellular gap of 20 nm. This wide intercellular gap is occupied by the adhering proteins of the cadherin family. Disc-shaped, electron-dense **attachment plaques** are seen to be located opposite each other on the cytoplasmic aspects of the plasma membranes of the two cells taking part in the formation of a desmosome (Fig. 2.12). A very fine electron-dense line is seen to be extending along the midline of the comparatively wide intercellular gap. Intermediate filaments of the cytoskeleton are seen to be anchored to the attachments plaques. These filaments are either inserted into the attachment plaque or make hairpin turns and extend back into the cytoplasm. In the epithelial cells, keratin intermediate filaments are anchored to the attachment plaques, whereas in the c cardiac muscle ells the anchoring intermediate filaments are of desmin type. The attachment plaques have been found to be composed of several anchoring cytoplasmic proteins especially *desmoplakins* and *plakoglobins*.

Two types of the transmembrane proteins of the cadherin family provide adherence in the region of a desmosome; these cadherins are *desmocollins* and *desmogleins*. The cytoplasmic aspects of these transmembrane proteins bind to the proteins of the attachment plaque. The extracellular portions of desmocollins and desmogleins extend into the intercellular gap and bind to similar proteins extending out of the cell membrane of the adjoining cell. The electron-dense line in the middle of the intercellular gap represents the line of linkage between the transmembrane proteins of adjoining cells. This linkage is dependent upon the presence of calcium ions.

HEMIDESMOSOME

A hemidesmosome is also a spot-like adhering junction that gives the appearance of a half desmosome (Fig. 2.13). The hemidesmosomes serve to anchor the epithelial

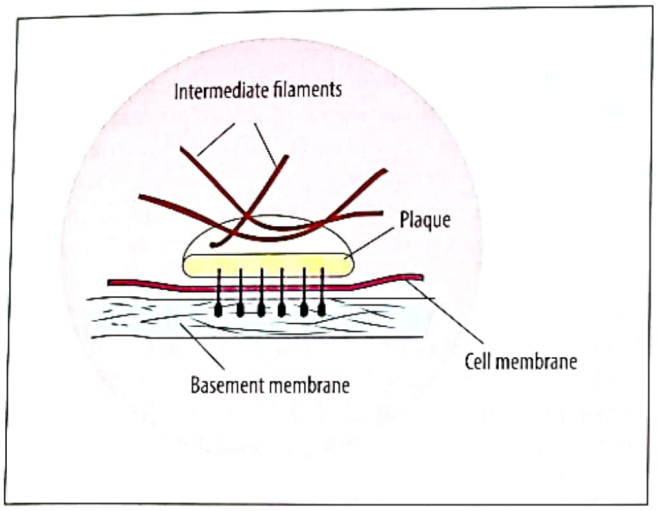

Fig. 2.13 Diagram showing the structure of a hemidesmosome.

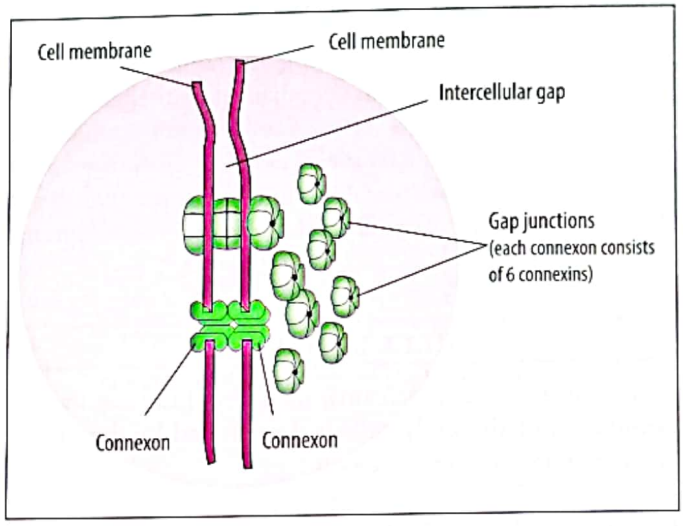

Fig. 2.14 Diagram showing the structure of macula communicans (gap junction) variety of cell junctions.

cells to their basal lamina. In hemidesmosomes the transmembrane linker proteins are *integrins* (and not cadherins). The extracellular portions of integrins bind to the laminin and type IV collagen present in the basal lamina. The intracellular parts of the integrins bind to the *desmoplakins* and *pectins* present in the attachment plaque of the hemidesmosome. The cytoplasmic intermediate filaments of keratin variety are seen to be inserted into the attachment plaque.

GAP JUNCTIONS

The gap junctions are communicating junctions which allow selective diffusion of molecules between adjoining cells. These junctions were named as gap junctions because, in early studies under the EM, they resembled occluding junctions but it was seen that the plasma membranes of the cells taking part in the junction were not fused but separated from each other by a gap of 3 nm. The name gap junction, although an unsatisfactory term, is still commonly used. However, alternative terms like nexus or macula communicans, which depict the function of these junctions, are replacing the old terminology.

The gap junctions are commonly found among the epithelial cells. They also occur between many other cells of the body including the cardiac muscle cells, smooth muscle cells, neurons, astrocytes, and osteocytes. No gap junctions are present between the skeletal muscle cells.

Each gap junction or nexus is a spot-like structure where the plasma membranes of the adjoining cells are closely apposed with an intercellular gap of only 3 nm. A gap junction contains a closely packed array of many **transmembrane channels** (Fig. 2.14). Each of these channels is composed of two hemichannels called ***connexons***; one hemichannel belongs to the plasmalemma of each of the two cells participating in the formation of the gap junction. The cylindrical wall of each connexon is formed by six transmembrane proteins called *connexins*. A connexon from one plasma membrane projects into the intercellular gap to fuse with a connexon from the adjoining cell membrane, thus a tubular channel is formed by the union of two hemichannels. This transmembrane channel, composed of 12 connexins, directly connects the two cells participating in the junction. The number of transmembrane channel in a macula communicans is not constant and varies widely depending on the location and functional activity of the junction.

The lumen of a tamsmembrane channel in the gap junction has an average diameter of 1.5 nm. These channels permit free passage of ions, cyclic AMP and GMP, amino acids, sugars, and other small molecules from one cell to the other. Different connexons specifically allow the passage of different ions and molecules. This specificity depends on the type of connexin proteins forming the connexon wall (more than 20 connexin proteins have been identified in the human body).

The permeability of the gap junctions is regulated, i.e., the connexons can be opened or closed as and when required. The regulating factors are the intracellular concentration of calcium ions and the cytosolic ph. Normally the cytosolic calcium level remains below the extracellular calcium level. As long as the calcium ion concentration in the cytosol remains low, the connexons remain open, but as soon as the intracellular calcium ion concentration rises above a critical level, the connexons become closed. Similarly, a decrease in the cytosolic pH also results in the closure of connexons.

In the cardiac and smooth muscle, the nexuses (gap junctions) function to provide electrical coupling of the adjacent cells, so that the waves of electrical excitation can spread unimpeded and synchronous contraction of the muscle can take place.

Nexuses are very frequently seen in the embryonic cells where they play an important role in the exchange of informational molecules between the developing cells.

during the mitosis). The G2 is a shorter phase that takes about 4 hours to complete.

It is to be noted that the cell cycle as described above occurs only in the renewing cell populations. The cells of the static and stable cell populations leave the cell cycle in G1 phase and enter a resting phase called **G0 phase** (G-Zero phase). In static cells the G0 phase is permanent; such cells leave the cell cycle permanently and lose the capability to undergo mitosis. The stable cells also enter G0 but they retain the potential to re-enter the cell cycle and undergo mitosis whenever needed.

CELL DEATH

The rates of cell proliferation and cell death determine the net cell population of the human body. Generally, two types of cell death are recognized: necrosis and apoptosis.

NECROSIS

Necrosis or ***accidental cell death*** occurs only in pathological conditions. When the cells are exposed to injury or disease, they undergo necrosis which is characterized by cell swelling followed by cell fragmentation resulting in release of the cellular contents into the extracellular space. The debris of the necrotic cells is engulfed by the phagocytic cells called macrophages. Fragments of the necrotic cells cause the release of cytokines from the macrophages, which trigger inflammation in the surrounding cells.

APOPTOSIS

Apoptosis, also called **programmed cell death,** is an essential process for removing cells that are stressed, damaged or worn out. It is estimated that, in a normal adult human, 50-70 billion cells die by apoptosis each day.

As the apoptosis begins, the cell loses its junctions and shrinks in size. The surface specializations (if any) are also lost. The mitochondria of the cell release cytochrome c (a protein of the electron transport chain) into the cytoplasm which causes breakdown and degradation of various intracellular structures in regulated manner. The nucleus becomes small and compact and then undergoes fragmentation. The cell organelles gradually disappear as they are broken down. Finally, the cell itself breaks up into several fragments that are called *apoptotic bodies*, which are engulfed by the macrophages.

Unlike necrosis, the engulfed fragments of apoptic cells do not trigger release of cytokines by the macrophages and, therefore, no inflammatory response is elicited and no damage to the neighboring cells occurs. It can be said that, as a result of apoptosis, *the cell dies by honor* without disturbing its neighbors.

SOME CLINICOPATHOLOGICAL TERMS IMPORTANT FOR THE MEDICAL STUDENTS

ATRESIA

In medical terminology, atresia is used to denote two conditions:

1. The congenital absence or closure of a normal body origin tubular passage such as the anus, vagina, or intestine.
2. The degeneration and resorption of one or more ovarian follicles before maturation.

HYPERTROPHY

Enlargement or overgrowth of an organ or part of the body due to an increase in the size of its constituent cells is known as hypertrophy.

ATROPHY

A diminution in the size of a cell, tissue, organ a part of the body called atrophy.

HYPERPLASIA

An abnormal multiplication or increase in the number of normal cells in normal arrangement in a tissue is called hyperplasia.

METAPLASIA

A change in the type of adult cells in a tissue to another type of adult cells, which are not normal for that tissue, is called metaplasia.

DIFFERENTIATION

Differentiation is the process by which a less specialized cell changes into a more specialized cell. Cellular differentiation is the chief process that occurs during the embryonic development and leads to the formation of a multicellular organism from the zygote (which is formed by the union of a male and a female gamete). However, the differentiation continues during the adult life as the adult stem cells divide and create fully differentiated daughter cells during tissue repair after injury, and in the ever-continuing process of normal turnover of the body cells.

ANAPLASIA

A loss of differentiation of cells and of their orientation to one another and to their axial framework and blood vessels is called anaplasia. It may simply be called as "undifferentiation" and is a characteristic feature of the tumor tissues.

NEOPLASIA AND NEOPLASMS

A new growth of a tissue/tissues, in which the growth is uncontrolled and progressive is called a neoplasm. The process of formation of a neoplasm is called neoplasia. The neoplasms could be benign or malignant. Histologically, the benign neoplasms exhibit little or no anaplasia, do not invade the neighboring tissues and do not metastasize to distant organs. The malignant neoplasms exhibit a significant degree of anaplasia, invade the surrounding tissues and metastasize through the lymph vessels and/or blood vessels to the distant regions/organs.

Metastasis. Transfer of a disease from one organ or body part to another part is called metastasis. It may occur by the transfer of pathogenic microorganisms (e.g. Tubercle bacilli) or by transfer of cells (as in malignant neoplasms).

Epithelium 3

THE TISSUES OF BODY

A *tissue* is defined as an organized aggregation of related or similar cells specialized to carry out some particular function (or functions). All tissues consist of cells and a variable amount of extracellular matrix. The extracellular matrix (also called *intercellular substance*) consists of a complex of macromolecules which are synthesized by the tissue cells and exported into the extracellular space.

The human tissues are generally classified into four main varieties, which are called the basic tissues of the body; these tissues are:

1. The epithelial tissue or *epithelium*.
2. The connective tissue.
3. The muscle tissue.
4. The nervous tissue.

Most of the organs of the body are comprised of two components: parenchyma and stroma. The **parenchyma** consists of those cells or tissues that perform the specific function (or functions) of a particular organ. The **stroma** constitutes the supporting tissue of the organ. Generally, the parenchyma is composed of epithelium and the stroma consists of connective tissue.

EPITHELIUM

The epithelium* is defined as a collection of closely apposed cells with very small amount of extracellular matrix. The epithelial tissue is avascular (i.e., it does not contain any blood vessels); therefore, it is always supported by a variable amount of loose connective tissue which contains blood capillaries. Between the epithelium and the associated connective tissue is present a layer of noncellular material called *basement membrane* or *basal lamina* which is composed of adhesive glycoproteins and collagen type IV fibrils (for detailed structure of the basement membrane see page 49). Oxygen and nutrients diffuse to the epithelial cells from the blood capillaries located in the connective tissue beneath the basement membrane. Conversely, the carbon dioxide and metabolic waste products diffuse from the epithelial cells to the blood capillaries.

The epithelia of the body are divided into two major groups: (i) glandular epithelia, and (ii) covering epithelia. The **glandular epithelia** are those which make the secretory portions of the glands of the body (and also line their ducts). The covering epithelia form continuous sheets, consisting of one or more layers of cells, that cover the external surface of the body and internal surface of the body cavities. The covering epithelia also line the luminal surface of the tubular structures of the body, e.g., the blood vessels and the gastrointestinal tract.

* The term epithelium derives from the fact that the early histologists studied the microscopic structure of the skin and noticed the cellular sheets usually showed nipple-like projections. Hence, the term epithelium was coined (in Greek, *epi* = on or over, and *thele* = nipple).

GLANDULAR EPITHELIA

Some epithelial cells of the body are specialized to produce substances that differ in composition from blood plasma or intercellular fluid. These substances, which are generally fluid in nature, are not used by the cell itself but are expelled to the extracellular compartment. These products are either secretions or excretions; the *secretions* are useful and are utilized by the body, whereas *excretions* are discarded and eliminated from the body.

The process of **secretion** involves intracellular synthesis of macromolecules of various types. The synthesized materials are temporarily stored in the cell as small, spherical, membrane-bound structures called *secretory granules*. The secretory granules, also called *secretory vesicles*, move toward the surface of the cell, fuse with the cell membrane and release contents by exocytosis.

The process of **excretion** does not involve intracellular synthesis of macromolecules but consists merely of a transfer of waste materials from the blood to the glandular cells which pass on these materials into the glandular lumen. After passing through the ducts of the ducts of the excretory glands, the excretions are finally eliminated from the body. The chief examples of the excretions are the urine and sweat.

COVERING EPITHELIA (Fig. 3.1)

The covering epithelia, also called *lining epithelia*, consist of one or more layers of cells, which cover the external body surface or line the tubular passages and cavities of the body. The most obvious example of the covering epithelia is the epidermis of skin, which is a multilaminar epithelium that covers the outer surface of the body. Those spaces and passages that open on to the exterior (e.g., the respiratory, digestive and urogenital passages) are all lined by covering epithelia of different types. The internal (luminal) surface of the heart, blood vessels, and lymph vessels is also lined by a special type of epithelium (called endothelium). The body cavities (i.e., the pericardial, plural and peritoneal cavities) are also lined by a special type of covering epithelium (called mesothelium).

CLASSIFICATION OF THE COVERING EPITHELIA

Depending on the number of cell layers present, the covering epithelia are divided into the following three main types:

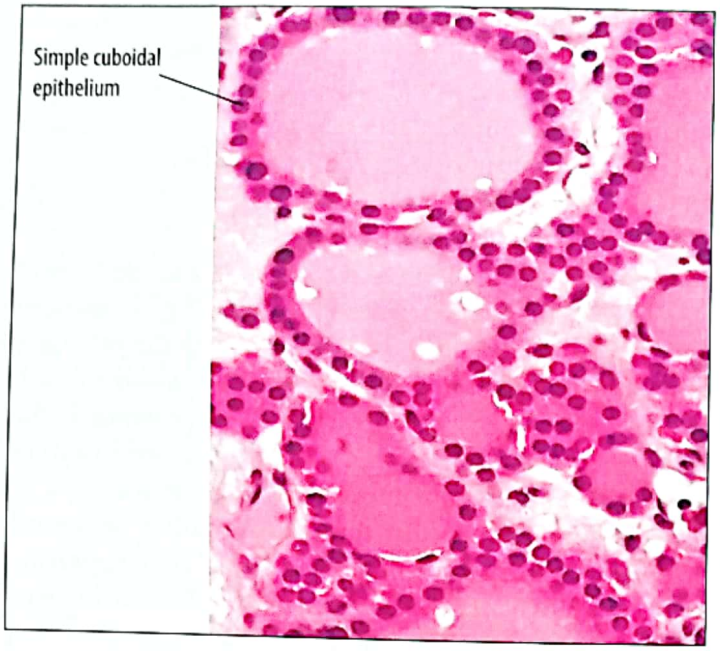

Fig. 3.2 A section of the thyroid gland showing many thyroid follicles. Note that each follicle is lined by a layer of simple cuboidal epithelium

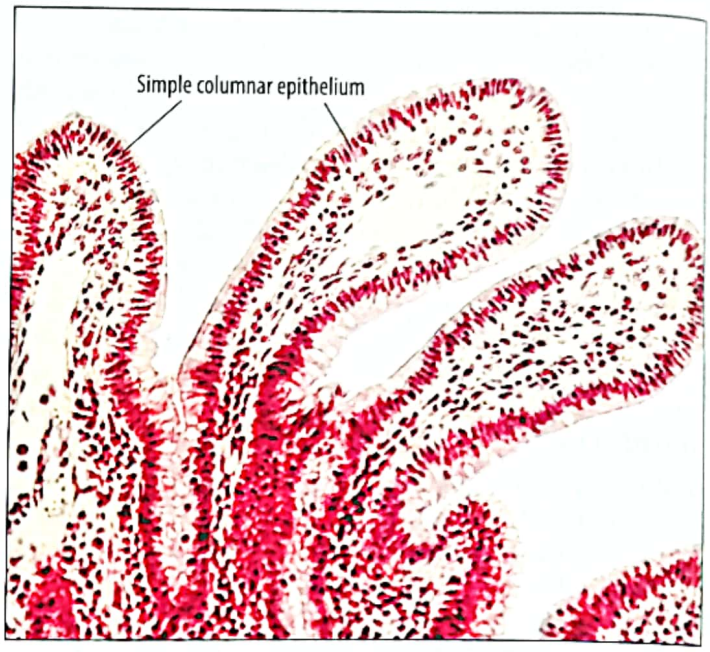

Fig. 3.3 A section of the small intestinal mucosa showing villi covered by simple columnar epithelium.

STRATIFIED SQUAMOUS EPITHELIUM

This variety of epithelium lines those surfaces which are subjected to wear and tear. The number of layers in this type of epithelium varies considerably in different locations but the shape and arrangement of the cells are quite characteristic. The deepest or basal layer, which rests on a basement membrane, is formed by low columnar or cuboidal cells which steadily divide mitotically to provide a constant supply of cells for the overlying layers. Next to the basal layer are present a few layers of larger polygonal cells. As the free surface is approached, the cells gradually become flattened and at the surface they assume a squamous (flat) shape.

The condition of the cells of the most superficial layers of the stratified squamous epithelium varies with the location and environment of the epithelium. Taking into account the state of the most superficial cells, two subvarieties of the stratified squamous epithelium are recognized: keratinized (also called cornified) and nonkeratinized (also called noncornified).

The stratified squamous keratinized epithelium covers those areas of the body which are subjected to abrasion and desiccation (drying). In this type of epithelium, the cytoplasm of the superficial cells accumulates large numbers of keratin filaments. Near the surface of the epithelia the cells lose their nuclei and organelles and become converted into dead, flattened plates (squamae) consisting of the protein *keratin*. The intercellular space between the squamous cells contains a water proofing glycolipid. The best example of the stratified squamous keratinized epithelium is the epidermis (Fig. 3.4) which covers the entire free surface of the body (for details on the structure of epidermis see Chapter 15).

The stratified squamous nonkeratinized epithelium lines those slippery surfaces in the body which are subjected to abrasion but remain wet. In this variety of the stratified squamous epithelium, the surface cells become very flat but mostly remain nucleated and their cytoplasm contains little or no keratin. The lining epithelia of most of the oral cavity, oropharynx, and esophagus (Fig. 3.5) belong to the stratified squamous nonkeratinized variety.

In both subtypes of the stratified squamous epithelium the most superficial cells constantly flake off from the surface, indicating the base to surface progress of the cells. As already noted, mitotic activity in the basal layer produces new cells which move into the overlying layers and become polygonal in shape. In the polygonal cell layers, the cells still keep on moving toward the surface until they reach the most superficial layers where they assume a squamous shape. The most superficial squamous cells lose their desmosomes and flake off from the free surface.

STRATIFIED CUBOIDAL EPITHELIUM

The stratified cuboidal epithelium consists of two or three layers of cuboidal cells. This type of epithelium has a very restricted distribution in the human body. It only occurs as the lining of the larger ducts of some exocrine glands like pancreas and salivary glands. The ducts of the sweat glands are also lined by this type epithelium. The stratified cuboidal epithelium has not been found to be involved in absorptive or secretory activity. Its probable function is to provide the ducts with a stronger lining than the simple cuboidal or simple columnar epithelium.

STRATIFIED COLUMNAR EPITHELIUM

This variety of stratified epithelium consists of columnar surface cells which rest on one or more layers of roughly cuboidal cells. This epithelium also has a restricted distribution in the body. The lining epithelium of the

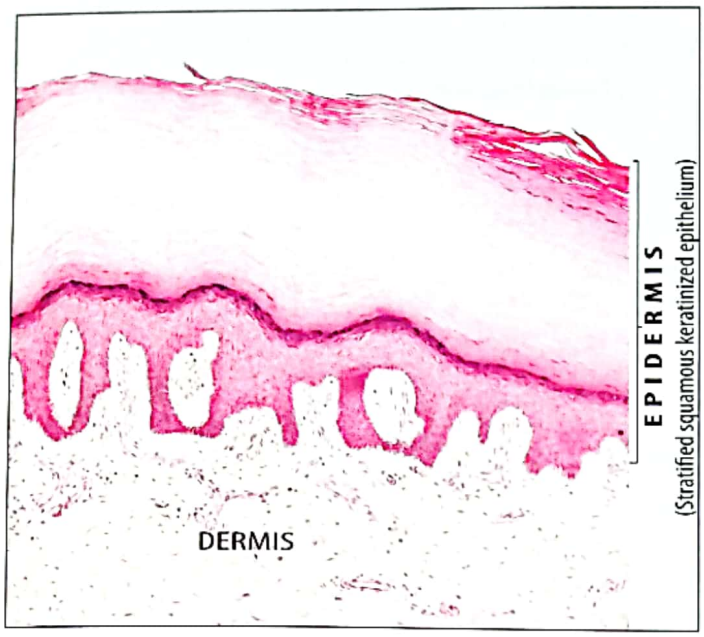

Fig. 3.4 A section of the skin showing epidermis and dermis. Note the stratified squamous keratinized epithelium constituting the epidermis.

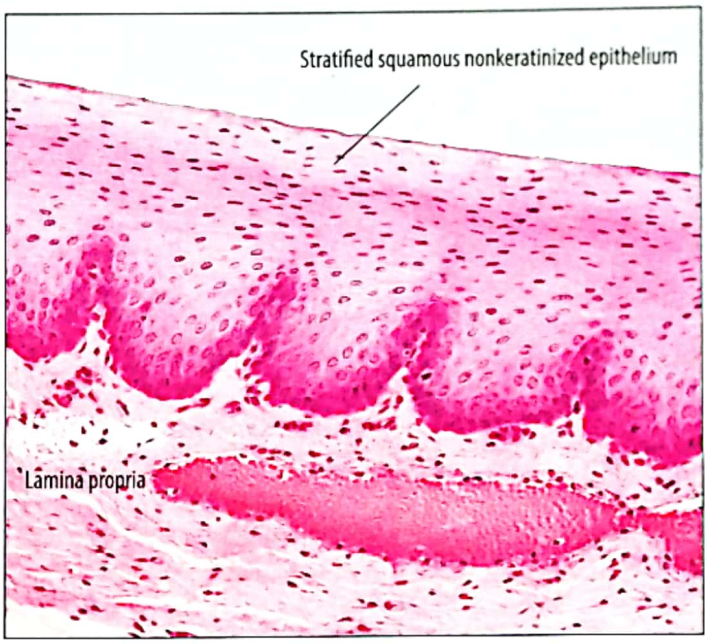

Fig. 3.5 A section of the esophagus showing stratified squamous nonkeratinized epithelium.

conjunctiva of the eye is a typical example of the stratified columnar epithelium. Some parts of the male urethra are also lined by the stratified columnar epithelium.

Note: Unlike the stratified squamous epithelium, the surface cells of the stratified cuboidal and stratified columnar epithelia are not continuously replaced by basal mitosis and there is no progress from the base to surface.

TRANSITIONAL EPITHELIUM

This is a special type of stratified epithelium that lines the lower urinary tract. This epithelium, also known as urothelium, is specially designed to withstand stretch produced by the distension of the urinary passages due to the collection and storage of the urine till is voided. The transitional epithelium is also specialized to prevent the diffusion of urine back into the tissues of the urinary tract and, thence, into the bloodstream. The urothelium lines the renal calyces, renal pelvis, ureters, urinary bladder, and proximal parts of the male as well as female urethra.

The structure of the transitional epithelium is best studied in sections of the wall of the urinary bladder. It is to be noted that the microscopic structure of this special type of multilaminar epithelium varies greatly according to the state of distension of the urinary bladder.

In the undistended (i.e., contracted) urinary bladder, the urothelium appears to consist of 6 or more layers of cells (Fig. 3.6). The basal layer of the epithelium consists of cuboidal cells. Over the basal layer are present several layers of polygonal cells. The most superficial layer of the epithelium appears to consist of very large dome-shaped cells, also called **umbrella cells**, whose highly convex apices bulge into the bladder cavity. Some of the umbrella cells contain two nuclei.

Distension of the urinary bladder stretches and flattens the epithelium. In the fully distended (i.e., stretched) state, the transitional epithelium is seen to consist of only 2 or 3 layers. There is a basal layer of low cuboidal cells over which are present one or two layers of large flat (squamous) cells.

The change in number of layers indicates the ability of the cells of the transitional epithelium to accommodate to stretching. The cells in the basal layer are not much affected by the distension but the cells in the next three or four layers become flattened and also re-arrange themselves, so that the number of layers becomes reduced. The large, highly convex surface cells not only flatten but also unfold their luminal plasma membranes to accommodate the increasing area. The folding and unfolding of plasma membranes covering the luminal surfaces of the most superficial cells of the urothelium occurs due to special structure of these membranes.

When a fully stretched transitional epithelium is studied under EM, the apical plasmalemma of the most superficial cells of this epithelium is seen to contain unusually thick and rigid areas, called **plaques,** which are about 12 nm thick. Between the plaques are present *interplaque regions* of normal, flexible cell membrane (about 8 nm in thickness). Microfilaments are seen to be extending from the inner surface of the plaques into the cytoplasm of the cells.

In the relaxed bladder the luminal plasma membranes of the superficial cells undergo folding and the plaques invaginate into the cytoplasm of these cells. The invaginated plaques remain temporarily stored in the cytoplasm of the surface cells as *fusiform vesicles.* However, lumina of these vesicles remain in continuity with the exterior of cells. As the urinary bladder undergoes distension, the stored plaques unfold and re-emerge on to the cell surface when the surface area

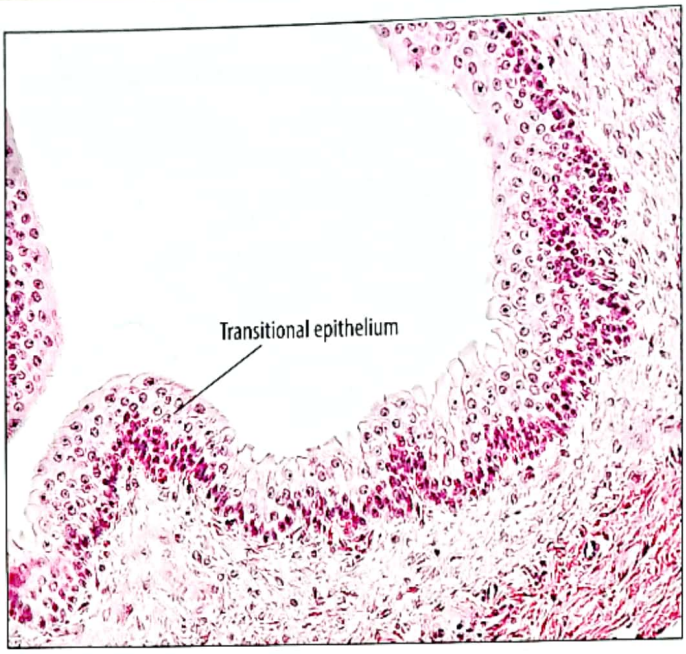

Fig. 3.6 A section of the urinary bladder (in contracted state) showing the bladder mucosa lined by the transitional epithelium.

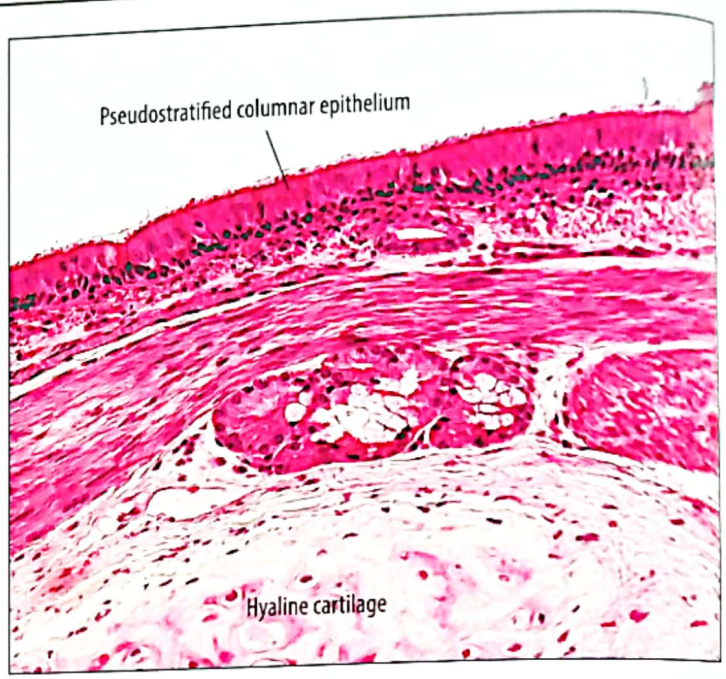

Fig. 3.7 A section of the trachea. The mucosa is seen to be lined by pseudostratified columnar (ciliated) epithelium.

of the epithelium increases as a result of stretching. The microfilaments attached to the plaques provide structural support during the folding and unfolding processes.

The transitional epithelial lining of the excretory urinary passages makes an impermeable barrier which prevents the urine from passing into the epithelium or beyond into the underlying tissues. This barrier function of the urothelium depends on two special features of cells of the most superficial layer of this epithelium: (1) the luminal plasmalemma of these cells is impermeable to salts and water, and (2) these cells are strongly bound together by zonulae occludentes and multiple desmosomes, which prevent the paracellular leakage of urine.

PSEUDOSTRATIFIED COLUMNAR EPITHELIUM

As explained earlier, this type of epithelium consists of tall cells and short cells. All cells of the psudostratified columnar epithelium rest on the basement membrane but all of them do not reach the free surface of the epithelium. Only the tall, columnar cells extend through the entire thickness of the epithelium. Each of these cells has a wider part which reaches the free surface of the epithelium and a narrow and slender basal part which passes downward to rest on the basement membrane. The short cells, also called basal cells, lie between the columnar cells. The basal cells are conical in shape having a broad base which rests on the basal lamina and a tapering upper part which terminates between the columnar cells and does not reach the free surface of the epithelium.

As the cell nuclei are located in the wider parts of both types of the cells of the pseudostratified columnar epithelium, they are present in the apical part of the tall cells and basal parts of the short cells. Consequently, the nuclei of all the component cells of the epithelial sheet do not lie at the same level and, in ordinary tissue sections examined under LM, the nuclei appear to be present in two or more rows. This arrangement creates a false appearance of cell stratification. Accordingly, the epithelium is titled as pseudostratified, i.e., falsely stratified (*pseudo* is a combining form meaning false).

The pseudostratified columnar epithelium (Fig. 3.7) lines the conducting part of the respiratory system (nose, nasopharynx, trachea, and bronchi). It also lines the major male genital ducts (duct of epididymis and ductus deferens). The tall cells of the pseudostratified epithelium usually exhibit special structural modifications on their apical surface (kinocilia in the in the conducting respiratory passages, and stereocilia in the male genital ducts).

Note. Because all cells of the pseudostratified columnar epithelium rest on the basement membrane, some authorities consider this epithelium to be a subtype of the simple (unilaminar) epithelia.

TWO SPECIAL VARIETIES OF EPITHELIAL CELLS

1. Neuroepithelial Cells

These are tall columnar cells bearing microvilli, cilia, or stereocilia on their free surface. Neuroepithelial cells are found in special sense organs like taste buds and vestibulocochlear receptor system of the internal ear. These cells are specialized as sensory receptors for the reception of external stimuli. Afferent nerve fibers terminate at neuroepithelial cells and form synapses with them.

2. Myoepithelial Cells

These cells are stellate (star-shaped) cells having many processes. Their cytoplasm contains contractile (actin and myosin) filaments. The myoepithelial cells contract when stimulated by neural or neurohormonal signals. They are

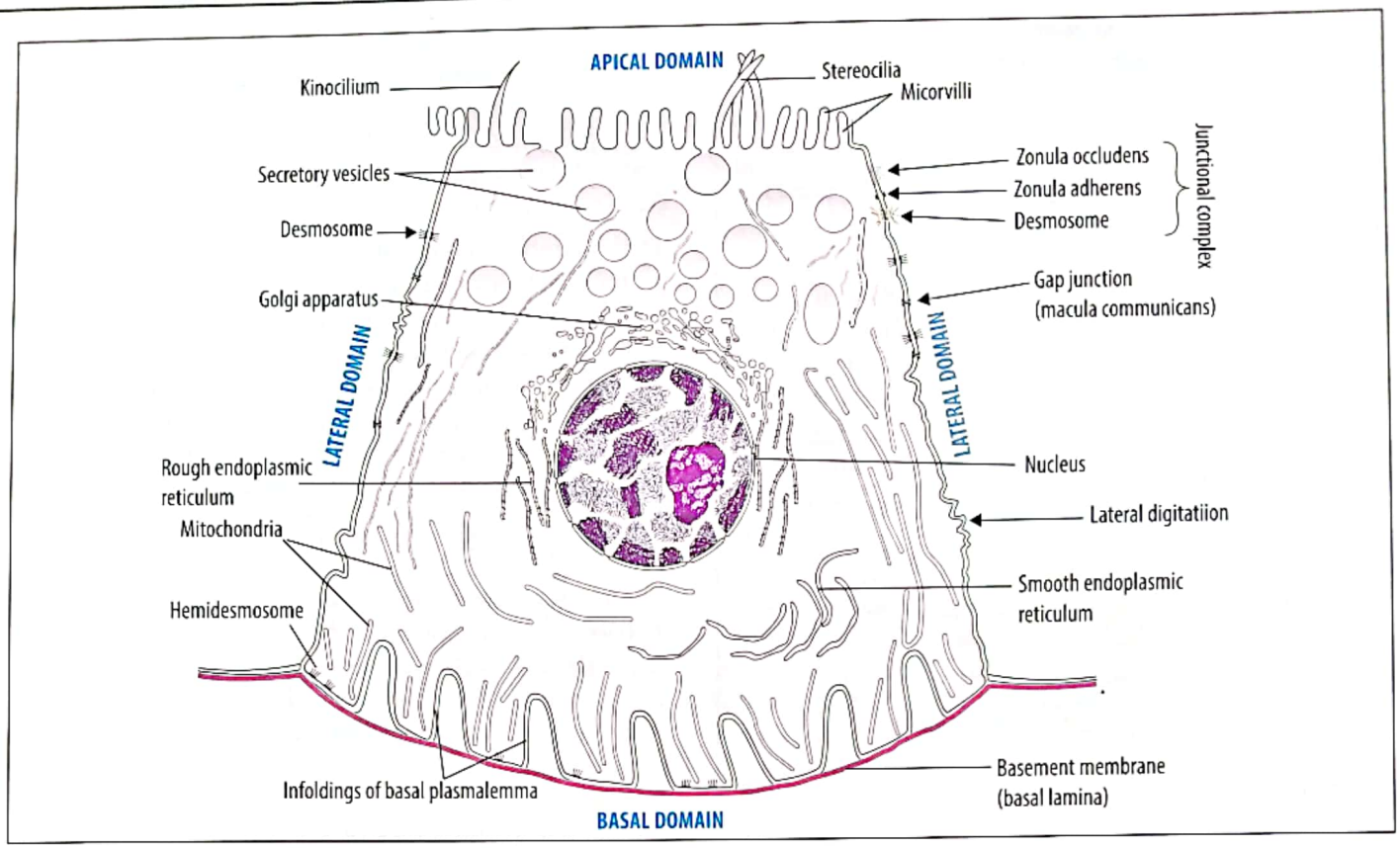

Fig. 3.8 Diagram showing major structural features and different domains of a hypothetical epithelial cell.

found around the secretory acini of the mammary, salivary, lacrimal and sweat glands. Their contraction assists in the flow of secretion into the larger channels.

POLARITY OF EPITHELIAL CELLS

Most of the epithelial cells are said to be polarized because distinct morphological and biochemical characteristics are associated with different surfaces of these cells. Generally, an epithelial cell has three domains which are related to different surfaces of the cell; these domains are: an **apical domain**, a **lateral domain** and a **basal domain** (Fig. 3.8). The structural and biochemical characteristics of different domains determine the functions of these domains.

APICAL DOMAIN

The apical domain is the region of the epithelial cell facing the lumen of an enclosed cavity or a tube. This contains ion channels, carrier proteins, hydrolytic enzymes, and aquaporins (channel-forming proteins that function in the regulation of water balance). In many epithelial cells, the apical domain shows special structural modifications which include microvilli, cilia and stereocilia.

MICROVILLI

These are small, slender, finger-like projections found on the free surface of epithelial cells in a variety of locations. Individually, the microvilli are too small to be seen with the light microscope. The electron microscope reveals that the microvilli are plasma membrane-covered cytoplasmic extensions, 1 to 2 µm in height and about 0.1 µm in diameter.

EM shows that each microvillus contains a central bundle of 20 to 30 microfilaments (actin filaments) which are cross-linked at regular intervals by the actin-binding proteins *fascin* and *fimbrin*. The microfilaments are anchored to the plasma membrane at the top and sides of the microvillus.

The microfilaments extend down into the apical cytoplasm, where they become embedded in the *terminal web*. Within the terminal web the actin filaments become associated with the molecules of spectrin and myosin-II.

The columnar epithelial cells lining the luminal surface of small intestine bear numerous microvilli on their free surface. In histological sections examined under LM, the intestinal mucosal surface appears vertically striated and is, therefore, referred to as *striated border*. Microvilli also occur in large numbers on the luminal surface of the cells lining the proximal convoluted tubules of the kidney. In this location they are longer and appear as a *brush border* on light microscopic examination.

Functions

Microvilli greatly increase the surface area of the cells in correlation with an absorptive function. Interaction between the actin filaments of the microvilli with the myosin-II molecules of the terminal web results in oscillatory contractile movement of the microvilli which facilitates the absorptive process.

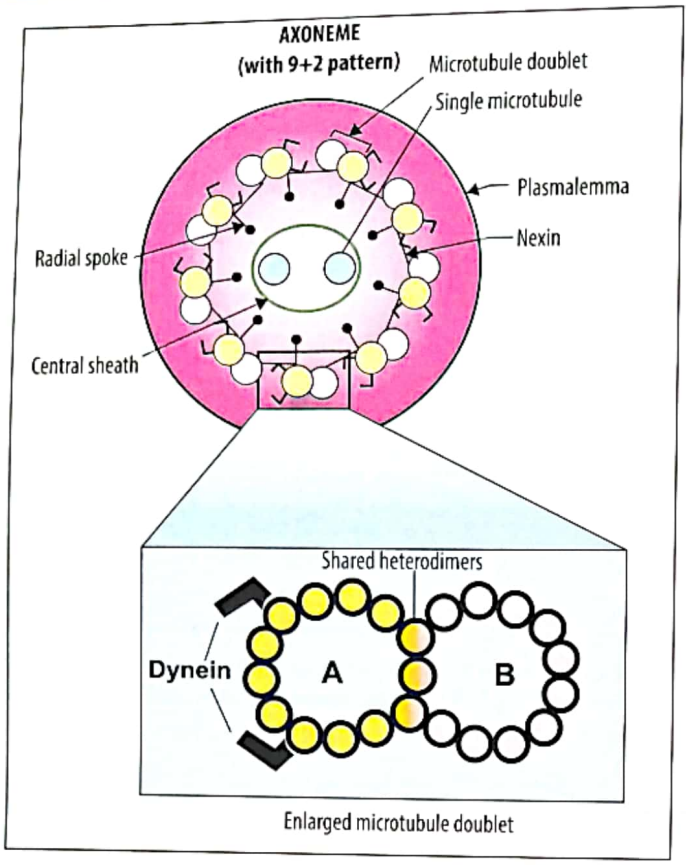

Fig. 3.9 Diagram showing internal structure of the axoneme of a kinocilium.

CILIA

Cilia are hair-like processes found chiefly on the free surface of those epithelial cells which are specialized for transport of fluid or a film of mucus over the epithelial surface. Due to their capability to make rapid to-and-fro oscillatory movements, cilia are also referred to as **kinocilia** to distinguish them from stereocilia which are nonmotile processes. Cilia are readily visible under LM, measuring 5-10 µm in length and about 0.2 µm in diameter.

In the human body, the kinocilia are principally found on the tall cells of the pseudostratified columnar epithelium of the conducting part of the respiratory tract, and the simple columnar epithelium lining the uterine tubes. In addition, kinocilia are also found on the neuroepithelial cells (hair cells) of the sensory regions (maculae and cristae) of the internal ear.

EM reveals that the shaft or free part of each cilium contains a core complex of microtubules which is called **axoneme**. This core complex is enclosed by the plasma membrane which, at the base of the cilium, becomes continuous with plasma membrane of the cell from which the cilium arises.

The axoneme consists of longitudinal microtubules arranged in a 9+2 pattern (Fig. 3.9). There are 9 doublet microtubules surrounding a central pair of single microtubules. The central microtubules are separate from each other; however, they are enclosed within a thin, tubular sheath called *central sheath*.

Each of the nine doublet microtubules consists of two members designated A and B. The microtubule A is complete and its wall consists of the usual 13 protofilaments. The wall of microtubule B has only 10 protofilaments and shares three protofilaments of the wall of the microtubule A.

The adjacent doublets are linked to each other by thin protein bridges called *nexins*. Each a microtubule of the nine doublets is connected to the central sheath by a *radial spoke* that is composed of an elastic protein. On the outer surface of the A microtubule are present tiny hook-like arms, which are made up of the motor protein dynein. When activated by ATP, these *dynein arms* make temporary cross-bridges with the microtubule B of the adjacent doublet. This results in ciliary movement by sliding of the doublets relative to each other.

The microtubules extend from the tip of the cilium to its base, which is known as *basal body* and is located just inside the free surface of the cell. As explained previously (in chapter 2), a basal body has the same structure as that of a centriole. It has a wall of circularly arranged 9 triplet microtubules, but there are no central microtubules, thus making a 9+0 pattern. As a cilium joins a basal body, the two central microtubules of the axoneme of the cilium, terminate at the base, while the peripheral nine doublets become continuous with the nine triplet microtubules of the basal body.

Functions

In the living epithelial cells, the kinocilia beat rapidly in a specific direction. Each kinocilium stiffens during the more rapid forward or *effective stroke*, whereas it bends during the slower *recovery stroke*. Successive cilia in each row begin their beat in sequence so that each is slightly more advanced in its movement than the cilium behind it in that row. This sequential activation of cilia results in the formation of waves that sweep slowly over the epithelial surface resulting in propulsion of a layer of mucus, fluid, particulate matter, or ovum over the epithelium.

The kinocilia present on the neuroepithelial cells of the vestibulocochlear apparatus of the internal ear have a quite different function. In these cells, the kinocilia perform the function of transduction of the mechanical stimuli into electrical impulses.

STEREOCILIA

Under the light microscope these structures appear as thin, hair-like structures which are seen to be in contact with each other to form small tufts. Under LM, they appear to resemble cilia but, because of their incapability to exhibit movements in the living cells, they were given the special name of *stereocilia* (stereo = solid, implying nonmotile). Electron microscopic studies reveal that the stereocilia are actually unusually long microvilli containing the central bundle of microfilaments. Because the stereocilia are very long, they are flexible and

the tip, which results in the tuft-like appearance seen under the light microscope. Average length of a stereocilium is 30 µm.

Cell bearing the stereocilia have a restricted distribution in the body. Chief location of the stereocilia is on the epithelial cells lining the duct of epididymis and vas deferens. In addition, the stereocilia are also found on the neuroepithelial cells of the internal ear.

Functions

In the duct of the epididymis and in the vas deferens, the function of the stereocilia is to increase the mucosal surface area to facilitate the reabsorption of the fluid that leaves the testes along with the spermatozoa.

Function of the stereocilia present on the neuroepithelial cells (hair cells) of the internal ear is quite different. Here, the stereocilia function in nerve signal generation. Deflection of stereocilia (by head movement or by the sound waves) causes depolarization of the hair cell plasma membrane and thus a nerve impulse is generated (which is transmitted to the CNS by the sensory nerve axons that terminate at the hair cells).

LATERAL DOMAIN

The epithelial tissues are characterized by close packing of the cells and, hence, the lateral domain of each epithelial cell lies in close contact with lateral domain of the neighboring cells. Two main functions of the lateral domain are: (1) cell adhesion, and (2) cell-to-cell communication. The function of **cell adhesion** is dependent on three factors: (1) presence of cadherins (adhesive glycoproteins) in the lateral plasma membranes of the adjacent cells, (2) presence of invaginations and evaginations in the lateral plasma membranes, due to which the cells interdigitate and thus interlock with each other, (3) presence of adhering and occluding junctions between the adjacent cells. The function of **cell-to-cell communication** is served by the nexuses (gap junctions).

BASAL DOMAIN

The basal surface of epithelial cells is characterized by the presence of three important features: (1) basement membrane, (2) hemidesmosomes, and (3) infoldings of plasmalemma.

The **basement membrane** (also called basal lamina) is a thin layer of extracellular material which lies between the epithelial cells and the underlying connective tissue.

The **hemidesmosomes** are junctions that anchor the epithelial cells to the basal lamina (the detailed structure of a hemidesmosome has been described in chapter 2).

The **infoldings of the basal plasmalemma** are a special feature of those epithelial cells which are involved in active transport of ions and molecules. These vertical infoldings of the basal plasmalemma increase its surface area, so that it can accommodate more transport proteins and channels. Numerous mitochondria are present between the infoldings of the basal plasmalemma, which provide energy for the active transport occurring through the cell membrane. The basal regions of those epithelial cells which have infoldings of the basal plasmalemma show eosinophilic vertical striations in the ordinary H&E stained sections examined under the LM. These eosinophilic ***basal striations*** are produced by the plasmalemmal infoldings and rows of mitochondria present between these infoldings. The basal striations are most prominent in the epithelial cells lining the proximal and distal convoluted tubules of kidney. In addition, the cells lining some segments of the ducts of the salivary glands also show basal striations, and therefore, these duct segments are called *striated ducts*.

BASEMENT MEMBRANE

The interface between the epithelium and the underlying (or surrounding) connective tissue is characterized by the presence of a thin layer of acellular material which is called basement membrane. This membrane is composed further of two layers: basal lamina and reticular lamina.

The **basal lamina** is finely fibrillar layer having a thickness of 80-100 nm. It lies directly beneath the basal surface of the epithelial cells. The major components of the basal lamina are fibrils of collagen type IV and a glycoprotein called laminin, which are held together by the adhesive glycoprotein entactin and a special type of heparan sulfate called perlecan. Under very high resolution electron microscopes, the basal lamina appears to consist further of two layers: (1) ***lamina lucida***, which is a nearly 40 nm thick electron-lucent zone lying just under the basal surface of epithelial cells; it is composed mainly of laminin and entactin, and (2) ***lamina densa***, which is a 50-60 nm thick electron-dense layer and contains collagen type IV fibrils, laminin, and perlecan.

The reticular lamina is a layer of variable thickness which contains reticular fibers and a variety of glycosaminoglycans (chondroitin sulfate, heparan sulfate, and hyaluronic acid, etc). The reticular lamina also contains fibrils of collagen type VII, which anchor the basement membrane to the underlying connective tissue.

It is important to note that in the past some histologists proposed that the reticular lamina should be considered to be a part of the connective tissue underlying the epithelium and, therefore, the term basal lamina should be used instead of basement membrane. This view received wide acceptance in the past years, but recently the trend has again shifted toward the use of the term basement membrane instead of basal lamina. However, the confusion continues and many histologists consider the terms basement membrane and basal lamina to be synonymous.

There are two locations in the body where a single basement membrane is found between two adjacent epithelial layers:

(1) alveoli of the lungs, and (2) glomeruli of the kidneys. In these locations, the basement membrane is unusually thick because it represents the fused basement membranes of the adjacent epithelial layers. Under EM, such a basement membrane is seen to consist of a central unusually thick lamina densa flanked on either side by a lamina lucida.

Functions of the Basement Membrane

1. It serves to bind the epithelial cells to the underlying or surrounding connective tissue.
2. It serves as a molecular sieve or ultrafilter. Generally, the basement membranes are freely permeable to small molecules but impede the passage of macromolecules, especially proteins.
3. The basement membrane serves as a scaffolding during the epithelial regeneration or wound healing.
4. The basement membrane also plays an important role in cell growth, proliferation, and differentiation by regulating interaction between the cell surface receptors and the molecules in the extracellular matrix.

FUNCTIONS OF THE EPITHELIUM

The most obvious function of the epithelial membranes is that of protecting the underlying connective tissue. In addition, the epithelial cells may be secretory or absorptive in function. In general, epithelia which secrete, absorb and filter are single-layered, their height (squamous to columnar) correlating with their efficiency. The pseudostratified epithelium is generally engaged in protection, secretion, and ciliary transport. Role of the stratified epithelia is mainly limited to protection.

A summary of different functions performed by the epithelium will now be given:

1. Protection

All covering epithelia provide protection to the underlying connective tissue. However, this ability is most marked in the stratified squamous epithelium. The stratified squamous epithelia of epidermis, oral cavity, esophagus and vagina furnish examples of protection against mechanical trauma. The stratified squamous keratinized epithelium of the epidermis also protects the body against drying, soaking, and bacterial invasion.

2. Secretion

Epithelial cells synthesize substances and pass them out on to a surface or into the blood. They may do this as isolated secretory cells (e.g., goblet cells and enteroendocrine cells), as secretory surfaces (e.g., gastric mucosal surface), or as organized multicellular structures called glands.

3. Absorption

Some epithelial cells are selectively permeable to substances in solution. For example, in the epithelial lining of the small intestine the absorptive epithelial cells (enterocytes) take up appropriate materials from the lumen of the gut but leave the unwanted substances behind. Similarly, the epithelial cells of the kidney tubules selectively resorb the useful constituents of the urinary filtrate (mainly water and sodium) and thus prevent the loss of these substances from the body via urine.

4. Excretion

Certain epithelial cells filter and eliminate those waste products which are carried in the blood. Urine and sweat are the examples of such filtrates, which are excreted through the kidneys and sweat glands, respectively.

5. Transport

Mucus and particulate matter are transported along the surface of the ciliated epithelium of the conducting respiratory passages. The ciliated epithelium of the uterine tubes helps in the transport of the ovum toward the cavity of the uterus.

6. Sensory Reception

In the special sensory areas of the internal ear and in the taste buds, the specialized epithelial cells (neuroepithelial cells) serve as intermediaries in neural transmission.

Glands 4

Some epithelial cells of the body are specialized to synthesize specific substances which are released from these cells onto an internal or external surface. Such cells are called *gland cells*. If the released substances are used elsewhere in the body, they are called *secretions*; if the products are discarded from the body, they are known as *excretions*. An aggregation of gland cells into a definite structure for the purpose of secretion or excretion is called a *gland*.

CLASSIFICATION OF GLANDS

The glands are generally classified into two major groups: (1) *exocrine glands,* which release their products onto an epithelial surface, either directly or through a duct, e.g., the salivary glands, and (2) *endocrine glands* which release their products into the bloodstream, e.g., the thyroid gland. It should, however, be noted that some glands possess both exocrine and endocrine functions, e.g., the pancreas.

An account of classification of exocrine glands will now be given; the endocrine glands will be dealt with separately in chapter 16.

EXOCRINE GLANDS

An exocrine gland may consist of a single cell constituting a *unicellular gland,* e.g., the goblet cell which is a mucus-secreting unicellular gland (Fig. 4.1). The goblet cells are abundantly found in the mucosa of the intestine and conducting respiratory passages. Most of the exocrine glands are, however, *multicellular glands* that are composed of many cells which line an epithelial invagination from a free surface. In some of these glands all the cells lining the lumen are secreting cells. But in most of the multicellular glands secretory activity is limited to the cells of the terminal portion (or portions) of the gland, which constitute the *secreting portion* of the gland. The gland passages serve as a nonsecretory *duct* or a system of ducts that carries the secretion to the surface.

The exocrine glands are classified into different types on the basis of:

1. Morphology of duct/ducts and secreting portion/ portions.
2. Nature of the secretory product.
3. Mode of secretion.

CLASSIFICATION OF EXOCRINE GLANDS ON THE BASIS OF MORPHOLOGY OF THE DUCTS AND SECRETORY PORTIONS

If an exocrine gland consists of a single secretory passage or a single system of secretory passages opening into an unbranched duct, it is called a *simple* gland. On the other hand, a gland containing a branched duct system is called a *compound* gland (Fig. 4.2).

The secreting portions of the glands also vary in shape. In some glands, the secretory portion/portions are tubular in shape; such glands are called ***tubular glands***. In other glands, the secretory portion/portions are dilated to form somewhat oval, sac-like structures; each of such a dilatation is known as an *acinus* and, accordingly, these glands are called ***acinar glands***. Older name for an acinus was alveolus and therefore such glands are sometimes also referred to as *alveolar glands*. In some glands, the secreting portions are neither typically tubular nor acinar, but combine certain features of both; such glands are known as ***tubuloacinar glands*** or tubuloalveolar glands (Fig. 4.2 & 4.3).

Upon the basis of the aforementioned two criteria (i.e., morphology of the duct and shape of the secreting portion), the exocrine glands are classified and subclassified as follows:

SIMPLE GLANDS

A simple exocrine gland is characterized by the presence of a single, unbranched duct. The simple glands are further classified into two subtypes: simple tubular glands and simple acinar glands.

SIMPLE TUBULAR GLANDS

In these glands the glandular (epithelial) cells make simple tubules which open onto the epithelial surface. All cells of the glandular tubule may be secretory in function. Alternatively, the secretory cells may be confined to the distal part of the tubular invagination. In such case the proximal part of the tube, lined by nonsecretory cells, acts as a *duct* that carries the secretions of the gland onto the epithelial surface.

In most of the simple tubular glands three parts can be distinguished: (i) a *mouth* which represents the opening on the epithelial surface, (ii) a *neck* which is a relatively narrow part of gland, and (iii) a *fundus*, which is the terminal, rather dilated secreting portion of the gland.

Taking into account the shape of the secretory portion, the simple tubular glands are further classified into three subtypes: straight, coiled and branched.

In the **straight simple tubular glands,** the secretory portion is in the form of a straight unbranched tubule, e.g., the intestinal glands (also called crypts of Lieberkuhn).

In the **coiled simple tubular glands,** the terminal secretory portion of the tubule is coiled or convoluted, e.g., the sweat glands.

In the **branched simple tubular glands,** the deeper

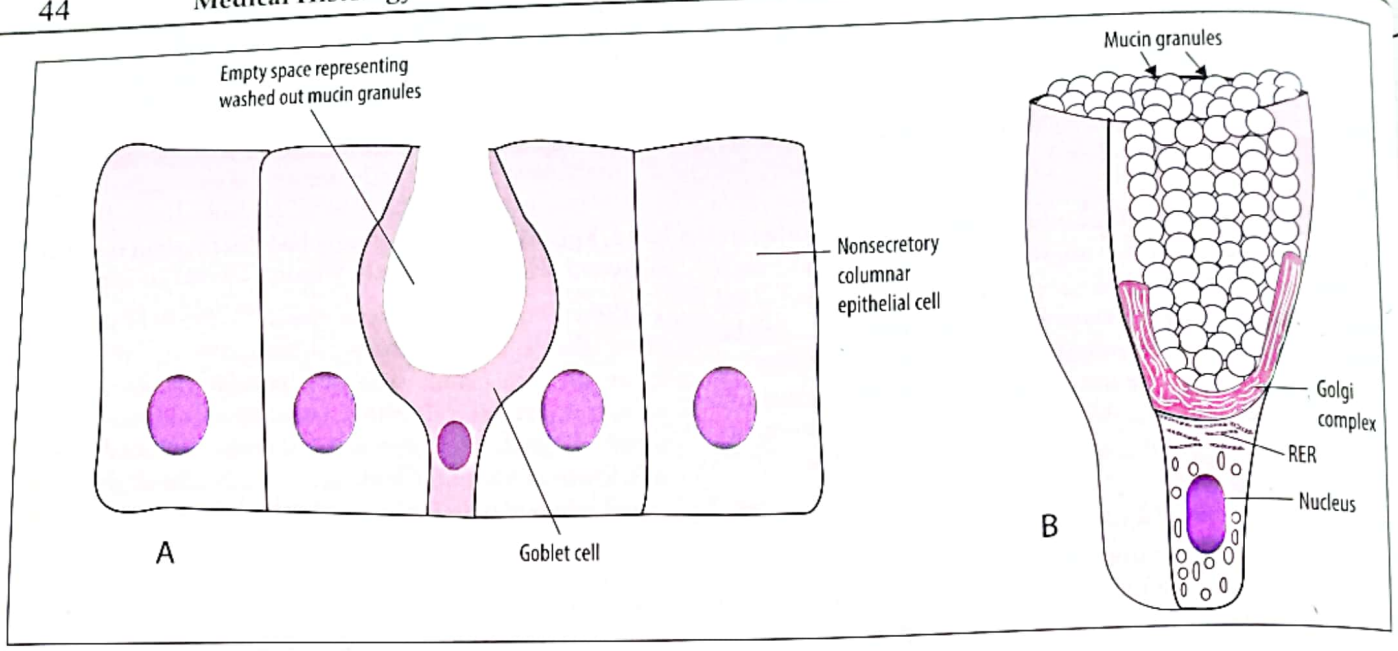

Fig. 4.1 A. A unicellular gland represented by a goblet cell present between nonsecretory columnar cells.
B. Diagrammatic representation of the structural features of a goblet cell.

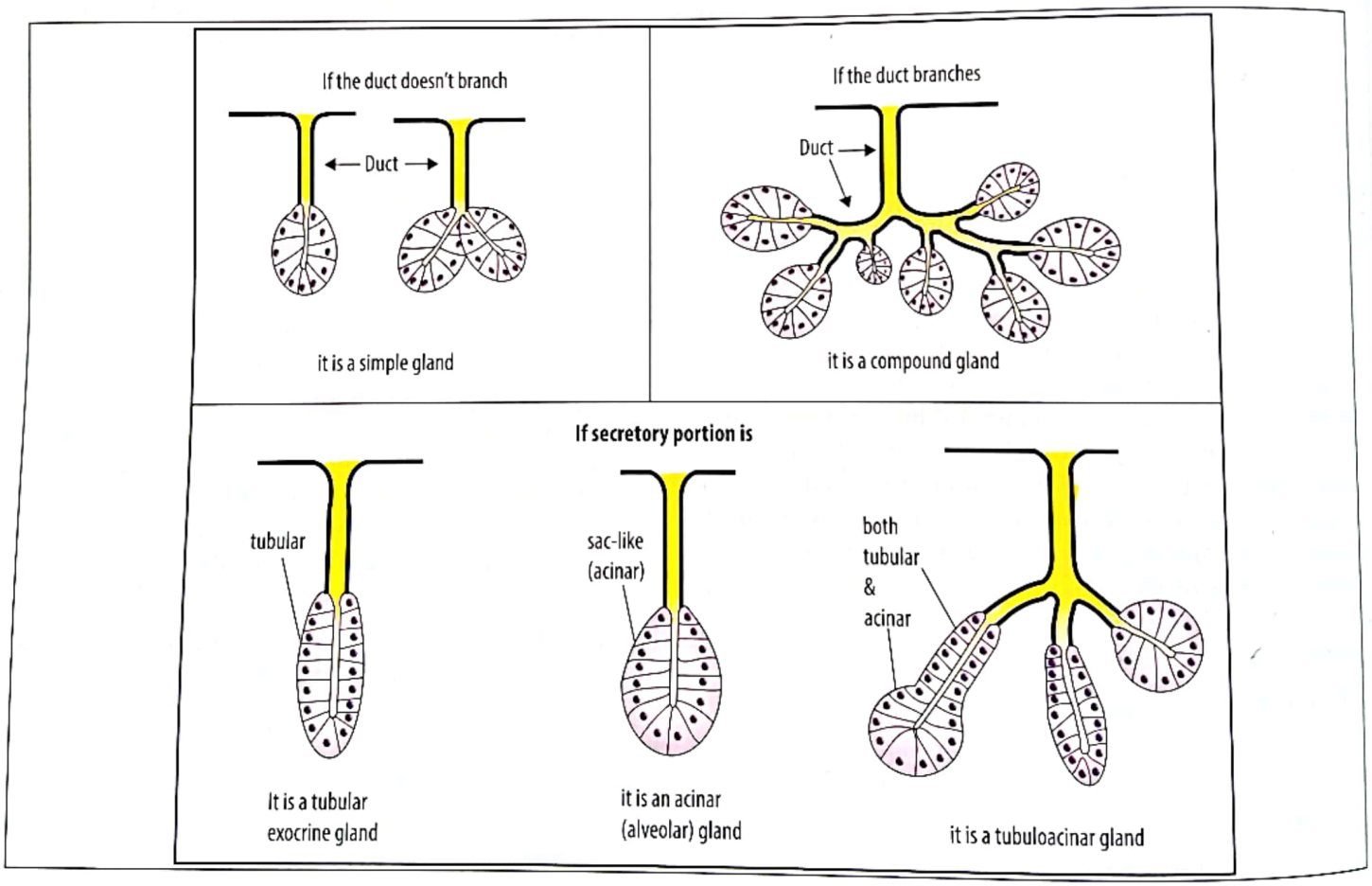

Fig. 4.2 Diagram showing the difference between the simple and compound glands and different types of secretory portions of the exocrine glands.

portion of the tubule is divided into two or more branches, e.g., the principal glands of stomach and endometrial glands of the uterus.

SIMPLE ACINAR GLANDS

A simple acinar gland consists of a single acinus (alveolus) which opens onto the surface by a very short duct. This variety of glands has a very restricted distribution in the human body. The urethral glands of the penile part of the male urethra constitute a typical example of simple acinar glands.

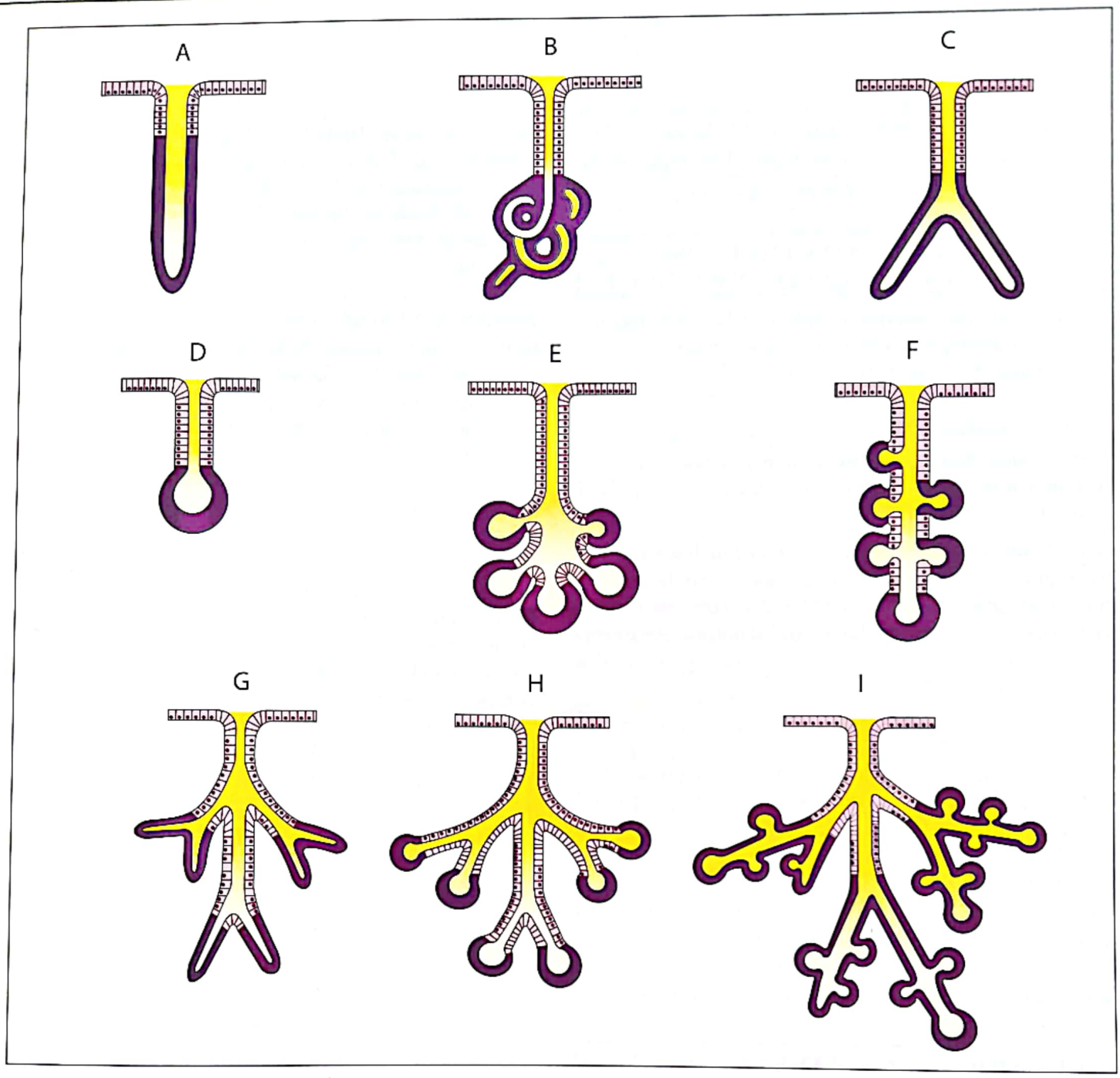

Fig. 4.3 A diagram illustrating morphology of different types of exocrine glands. A. Simple tubular, B. Simple tubular coiled, C. Simple tubular branched, D. Simple acinar (alveolar), E and F. Simple acinar branched, G. Compound tubular, H. Compound acinar, I. Compound tubuloacinar. Note: The terminal (secretory) portions of the glands are shown in solid purple color.

Simple branched acinar glands also occur in the human body. In this variety of glands, either the acinus is subdivided by partitions into several smaller compartments (e.g., the sebaceous glands of skin) or several acini are arranged along a duct (e.g., Meibomian glands of the eyelids).

COMPOUND GLANDS

The compound glands are characterized by the presence of a branched duct or duct system. Depending on the shape of the terminal (secretory) portions, the compound glands are further categorized into the following three varieties:

COMPOUND TUBULAR GLANDS

These glands consist of a number of distinct duct systems that open into a main excretory duct. The secretory portions are in the form of long branching tubules which are usually coiled. The brunner's glands of duodenum belong to the compound tubular variety of glands.

COMPOUND ACINAR GLANDS

In these glands, the duct system is branched and the secretory units are acinar in shape. The exocrine portion of pancreas is an example of compound acinar glands.

COMPOUND TUBULOACINAR GLANDS

In these glands the secretory portions are in the form of irregularly branched tubules with numerous acinar outgrowths on the walls of the tubules and at the blind end of each tubule. The duct system is highly branched. The submandibular salivary gland is a typical example of the compound tubuloacinar glands.

CLASSIFICATION ON THE BASIS OF NATURE OF THE SECRETORY PRODUCT

On this basis the exocrine glands are classified into the following three types: (i) mucous glands, (ii) serous glands, and (iii) mixed glands.

MUCOUS GLANDS

These glands produce a viscid, slimy, carbohydrate-rich secretion which is called *mucus*, e.g., the pyloric glands of stomach.

Actually the mucous cells secrete *mucin* which is a strongly hydrophilic glycoprotein. When released from the cell, the mucin becomes highly hydrated and is converted into a viscous, slimy substance which is called ***mucus***. The mucus spreads over the epithelial surface to serve as a protective lubricating gel.

SEROUS GLANDS

The serous glands produce a thin, watery, protein-rich secretion, often high in enzymatic activity, e.g., the parotid salivary gland.

MIXED (SEROMUCOUS) GLANDS

These glands produce both mucous and serous secretions and their secretory product is a mixture of these two types of secretions. The best examples of the seromucous glands are the sublingual and submandibular salivary glands.

CLASSIFICATION ON THE BASIS OF THE MODE OF SECRETION

Secretion released by most of the glands of body consists of the secretory product synthesized by the gland cells, and no part of the cell cytoplasm is lost in the secretion. However, in some of the exocrine glands the secretion contains a part or whole of the cell cytoplasm. Depending on their mode of secretion, i.e., the manner in which the secretory product is elaborated, the exocrine glands are classified into the following three varieties: merocrine glands, apocrine glands, and holocrine glands.

MEROCRINE GLANDS

These glands are also called *eccrine* glands. The secretory product of these glands is discharged from the gland cells without any loss of the cell cytoplasm. Most of the exocrine glands of the body belong are of merocrine verity, e.g., the salivary glands, lacrimal glands, and intestinal glands.

APOCRINE GLANDS

In these glands, some part of the apical cytoplasm of the secretory cell is lost along with the secretory product. Typical example of the apocrine glands is provided by the special sweat glands located in axillae (armpits) and perianal area. The ceruminous glands of the external auditory meatus (which secrete ear wax) also belong to this variety of glands. In the lactating mammary glands, the fat globules are also secreted by an apocrine mechanism of secretion.

HOLOCRINE GLANDS

In these glands entire cells laden with secretory material disintegrate and all of the cellular contents are discharged from the gland as secretion. The sebaceous glands of the skin constitute the only example of holocrine glands in the human body.

GENERAL STRUCTURE OF THE EXOCRINE GLANDS

Generally, the larger glands have the same structural pattern. Externally, a gland is surrounded by a dense layer of connective tissue which forms capsule of the gland. From the capsule connective tissue septa extend into the gland and divide the gland substance into a number of lobes. Thinner septa subdivide each lobe into smaller lobules. Blood vessels and nerves pass along the connective tissue septa to reach the secretory elements.

The *parenchyma* of a gland consists of its secretory units and ducts. The connective tissue capsule and septa constitute the *stroma* of the gland.

Connective Tissue 5

The connective tissue is actually a group of tissues which connect and support the other three varieties of the tissues of body, especially the epithelial and muscular tissues. The connective tissues are characterized by the presence of relatively fewer cells but a large amount of extracellular matrix (compare this with epithelial tissues, which consist of a large number of cells with relatively small amount of extracellular matrix). The extracellular matrix (ECM) of the connective tissue is composed of two components: (i) ground substance, and (ii) fibers. The ground substance consists of proteoglycans and glycoproteins. The thread-like fibers are composed of protein and are of three types: collagen fibers, reticular fibers and elastic fibers. Different types of connective tissue differ from each other in cell variety, in chemical composition of the ground substance, and in number and type of fibers present in the ECM.

Functions of the Connective Tissue

1. Provision of structural support

The support provided by the connective tissues is mainly of a mechanical nature. The connective tissue proper forms the internal supporting framework and protective coverings of various organs of the body. The cartilage and bone provide the supporting framework of the body as a whole and also make protective casings in some regions of the body, e.g., the thoracic cage which provides protection to the heart, lungs, and great vessels, etc.

2. Role as medium for exchange

The ground substance part of the connective tissue matrix serves as a medium through which oxygen, nutrients and metabolic wastes are exchanged between the body cells and blood.

3. Role in the defense and protection of the body

The connective tissue also plays an important role in the defense of the body against injurious agents; this is accomplished in three ways: (i) the intercellular substance (matrix) of connective tissue acts as a physical barrier to those bacteria which manage to penetrate the epithelial membranes, (ii) some connective tissue cells have the ability to engulf bacteria and other unwanted particulate matter, and (iii) some connective tissue cells produce antibodies which react with and inactivate the antigens.

4. Storage of fat

The connective tissue also performs the important function of storage of fat.

CONNECTIVE TISSUE CELLS

Many types of cells are found in different varieties of connective tissue. Cells found in the connective tissue proper will be discussed in this section, while the cells of the cartilage and bone will be taken up later. Blood cells will be discussed in chapter 9. Cells of the connective tissue proper can be classified into two main types: (i) resident cells, and (ii) migrant cells.

The **resident cells** (also called *fixed cells*) are those cells which develop and remain within the connective tissue. These cells include fibroblasts, myofibroblasts, adipocytes, and mesenchymal stem cells.

The **migrant cells** include those cells which enter the connective tissue from the blood stream. These cells include the macrophages, mast cells, plasma cells, and various types of the white blood cells.

Only the loose areolar variety of connective tissue proper contains all of the aforementioned cells; other varieties contain only a single cell type or a combination of two or more types of connective tissue cells. Morphology (and functions) of different types of connective tissue cells will now be described.

RESIDENT CELLS OF THE CONNECTIVE TISSUE

These cells originate in the connective tissue, remain in connective tissue and ultimately die there. This category of cells includes fibroblasts, myofibroblasts, adipocytes, and mesenchymal stem cells.

FIBROBLASTS

The fibroblasts constitute the most abundant variety of connective tissue cells. The structural features of fibroblast vary in relation to the function being performed by the cell. Generally, two varieties of fibroblasts are recognized: inactive fibroblasts and active fibroblasts.

The **inactive fibroblasts,** which are also known as *fibrocytes,* are usually small ovoid cells, each containing a small, condensed, rod-shaped nucleus. The cytoplasm of an inactive fibroblast is slightly acidophilic but takes little or no stain in the peripheral region of the cell. As the cell membrane is too thin to be resolved under LM, it is not possible to identify the cell boundaries of an inactive fibroblast in routinely prepared sections.

The **active fibroblasts** are larger, spindle-shaped cells that may show slender radiating processes (Fig. 5.1). Each active fibroblast has a large, ovoid nucleus which contains a prominent nucleolus. The cytoplasm stains

deeply basophilic. Electron microscopic studies reveal that in the cytoplasm of an active fibroblast there is marked increase in the amount of RER as well as free ribosomes. Other organelles also show an enlargement in size. Active fibroblasts usually reside in close proximity to collagen fibers.

Functions of the Fibroblasts

Principal function of the fibroblasts is to secrete proteins collagen and elastin, which polymerize to form various types of connective tissue fibers. In addition, the fibroblasts secrete various components of the ground substance of connective tissue (proteoglycans, and glycoproteins, etc).

Myofibroblasts

The myofibroblasts are not easily distinguished from the fibroblasts in ordinary H&E sections examined under the LM. However, electron microscopic examination shows that, in addition to prominent RER and a large Golgi apparatus, the cytoplasm of a myofibroblast contains bundles of actin filaments and dense bodies similar to those of smooth muscle cells. Myofibroblasts are abundantly found in areas of wound healing. The function of the myofibroblasts is **wound contraction** (which is a process that causes the closure of the wound).

ADIPOCYTES (Fig. 5.1)

The adipocytes are characterized by the presence of considerable amount of fat in their cytoplasm and, therefore, they are also called **fat cells**. The fat cells may occur singly in connective tissue but are often found in groups of varying sizes. In many locations in the body, the adipocytes are accumulated in such large numbers that they form a special type of connective tissue which is called *adipose tissue.*

An adipocyte is generally a large cell ranging 50-150 µm in diameter. Individual fat cells (as seen in the loose connective tissue) are usually spherical or ovoid in shape, but the closely-packed adipocytes (as in adipose tissue) appear to be polygonal in shape. Like the fibroblasts, the adipocytes are also derived from the mesenchymal cells.

An adipocyte may be unilocular (containing a single, large fat droplet) or multilocular (containing many small fat droplets). In the adults, the unilocular adipocytes are preponderant and their aggregations constitute *white adipose tissue*. The aggregates of multilocular fat cells constitute *brown adipose tissue.*

In the ordinary (unilocular) adipocytes, 80% of the cell is occupied by a single large fat droplet. Consequently, the cytoplasm is reduced to a thin rim around the fat droplet and the nucleus is found to be pressed against the cell membrane. In routine histological preparations, the fat is dissolved out of the adipocyte and the space occupied by it is represented by a large vacuole. The stained thin rim of cytoplasm of the unilocular adipocyte and its eccentric, flattened nucleus collectively give a characteristic ***signet-ring appearance*** (Fig. 5.1 & 6.3).

The chief function of the fat cells is to store the fat in the fed state and to release it into the blood in the fasted state (for further details see "Adipose Tissue" in chapter 6).

MESENCHYMAL STEM CELLS

These cells cannot be easily identified in the connective tissue. They are derived from the embryonal mesenchyme and retain the capability to give rise to fibroblasts and adipocytes throughout life. Some histologists maintain that these cells occur as *pericytes* on the capillaries of the connective tissue.

MIGRANT CELLS OF CONNECTIVE TISSUE

The migrant connective tissue cells are also called ***free cells*** or *transient cells*. These cells originate in the bone marrow and circulate in blood. Upon receiving appropriate stimuli, these cells leave the bloodstream and enter the connective tissue to carry out their specific function. The migrant connective tissue cells include macrophages, mast cells, plasma cells, and various types of white blood cells.

MACROPHAGES

Connective tissue macrophages, also called *histiocytes*, are motile, phagocytic cells found in the loose connective tissue throughout the body. When ordinary H&E stained tissue sections are examined under LM, it is difficult to distinguish the resting (inactive) macrophages from fibroblasts. The inactive macrophages are generally fusiform or stellate in shape and lie in close association with collagen fibers. However, the nucleus of a macrophage is smaller and darker staining than that of a fibroblast and its cytoplasm contains numerous granules which represents lysosomes.

The active macrophages (i.e., which are engaged in phagocytic activity) can be easily distinguished from the fibroblasts. An active macrophage is a large, spherical or oval cell measuring 10 to 35 µm in diameter. The nucleus is eccentric in position and kidney-shaped in appearance, showing a prominent indentation on one side (Fig. 5.1). The cytoplasm is basophilic and contains many small vacuoles and dense granules.

EM reveals that the surface of an active macrophage is highly irregular and shows numerous folds and cytoplasmic projections. The surface folds and projections enable the macrophage to engulf the material to be phagocytosed; in addition, they help in amoeboid movement of the cell. EM also shows that the cytoplasm of a macrophage contains a well-developed Golgi apparatus, prominent RER and a large number of lysosomes. The cytoplasm also contains residual bodies, phagolysosomes and numerous vesicles of both Golgi and pinocytotic origin.

Functions of the Macrophages

1. The major function of the macrophages is phagocytosis of unwanted particulate matter. This may occur as a defense activity, e.g., phagocytosis of bacteria, or as

a clean-up operation, e.g., phagocytosis of organic material (e.g., the debris of dead leukocytes) or phagocytosis of inorganic particulate matter (e.g., the carbon particles).

2. Some macrophages also act as **antigen-presenting cells (APCs)**. These macrophages phagocytose the antigenic proteins and degrade them into peptide fragments. These peptide fragments are transferred to the surface of the macrophage and presented, in combination with MHC II molecules, to the T lymphocytes, so that an immune response is initiated (for details regarding MHC molecules and APCs see chapter 14).
3. During the immune response against bacteria and other foreign antigens, the macrophages secrete a number of *cytokines* which belong to the interleukin 1 (IL-1) family. These cytokines stimulate the proliferation and maturation of lymphocytes, so that the immune response to the foreign antigen is amplified. The activated macrophages also have the capability to release the tumor necrosis factor-alpha (TNF-α) which can kill cancer cells if they are in small numbers.

Sometimes macrophages encounter a foreign body which is too large to be engulfed by a single cell, e.g., a piece of the material used in surgical sutures. In such case many macrophages fuse to form a huge multinucleated cell that engulfs the foreign body; such a cell is known as a ***multinuclear giant cell*** (also called *foreign body giant cell*).

At the sites of chronic infections that are caused by microorganisms which are resistant to phagocytosis, e.g., the causative organism of tuberculosis (Mycobacterium tuberculosis) and the causative organism of syphilis (Treponema pallidum), the macrophages enlarge in size and cluster together to form ***epithelioid cells*** (which are so named because the closely-packed macrophages give the appearance of epithelial tissue). The epithelioid cell clusters surround the phagocytosis-resistant microorganisms to wall them off and prevent them from damaging the healthy tissues. An organized collection of epithelioid macrophages is called a *granuloma*.

The macrophages are derived from monocytes of blood. The monocytes develop in the bone marrow and circulate in the blood. They leave the bloodstream by migrating through the endothelium of capillaries and enter the connective tissue compartment where they mature into macrophages. The transformation from a monocyte to a macrophage results in an increase in all of the cell organelles and cytoplasm. The macrophages can divide in the connective tissue to increase their number.

The monocytes, macrophages and other phagocytic cells of the body constitute the **mononuclear phagocyte system (MPS)**. The other phagocytic cells which belong to this system are: Kupffer cells of liver, alveolar macrophages of lungs, and Langerhans cells of skin. The microglia cells of the nervous tissue and osteoclasts of the bone tissue are also included in the mononuclear phagocyte system.

MAST CELLS

The mast cells are large, ovoid cells measuring 20 to 30 μm in diameter. The characteristic feature of a mast cell is the presence of large secretory granules in the cytoplasm (Fig. 5.1). These membrane-bound granules are so numerous that they frequently obscure the centrally placed, spherical nucleus. The cytoplasm also contains a few mitochondria, small amounts of RER, and a Golgi complex.

The cytoplasmic granules of the mast cells cannot be identified in routine histological sections because their contents are water soluble and are washed out when ordinary aqueous fixatives are employed to preserve the tissues. When special fixatives are used, the cytoplasmic granules of the mast cells stain intensely basophilic. When a basic aniline dye (e.g., toluidine blue) is used to stain the tissue, the granules of the mast cells stain purple (instead of blue). This property of changing the color of the stain is called *metachromasia*. The metachromasia of mast cell granules is due to the presence of heparin, which is a highly sulfated proteoglycan.

The mast cells secrete mediators of inflammation, which are classified into two categories: primary mediators and secondary mediators.

The ***primary mediators of inflammation***, also called *preformed mediators*, are stored in the basophilic granules of the mast cell. These include histamine, heparin, two proteolytic enzymes (tryptase and chymase), and two cytokines named neutrophil chemotactic factor (NCF) and eosinophil chemotactic factor (ECF).

The ***secondary mediators of inflammation*** are not stored in the cytoplasmic granules but are synthesized and released by the mast cells upon activation. These include leukotrienes C4, D4 and E4, tumor necrosis factor (TNF), prostaglandin D2, and several types of interleukins.

Mast cells originate from stem cells located in the bone marrow. Although the mast cells are functionally related to basophils of the blood, the two cells are derived from different stem cells in the bone marrow, and therefore, are unrelated to each other.

Functions of the Mast Cells

The mast cells play a key role in the development of allergic reactions that are called *immediate hypersensitivity reactions*. The cell membrane of the mast cells contains receptors for antibodies of the immunoglobulin E (IgE) type.

The IgE antibodies are produced by the plasma cells on the first exposure to an allergen which may be a protein antigen (e.g., plant pollen, bee venom, foreign serum) or a drug (e.g., penicillin). The IgE antibodies bind to the receptors located on the external surface of the plasmalemma of the mast cells. Upon a subsequent exposure to same allergen, an antigen-antibody reaction takes place on the surface of the mast cells; this reaction causes the discharge of primary and secondary mediators of inflammation from the mast cells. These mediators produce an *immediate hypersensitivity reaction* which may be local or systemic.

The *local immediate hypersensitivity reaction* may be manifested in the form of skin rashes (urticarial), asthma, or rhinitis (inflammation of the nasal mucosa manifested mainly by sneezing and runny nose).

The *systemic immediate hypersensitivity reaction*, is known as *anaphylactic shock*. It occurs in those *hyperallergic* persons who produce substantial amount of IgE upon first exposure to an antigen. Second exposure to the sensitizing antigen causes release of massive quantities of histamine and other mediators from the mast cells into the blood, resulting in the production of an anaphylactic shock, which is a potentially fatal condition as it can lead to cardiovascular collapse.

The primary and secondary mediators produced by the mast cells play various roles in the production of the inflammatory response and other manifestations of the immediate hypersensitivity reactions. Most important is the role of histamine which causes vasodilation and increases capillary permeability, thus causing edema (collection of fluid) in the surrounding tissue. It also produces bronchospasm (narrowing of pulmonary bronchioles due to contraction of their smooth muscle) and stimulates increased secretion of mucus in the respiratory tract.

The role of heparin (which is an anticoagulant proteoglycan) produced by the human mast cells is not clear. It is supposed that the small amount of heparin released by the degranulation of mast cells during the hypersensitivity reactions, binds to histamine and inactivates it, and thus serves to limit the inflammatory response.

PLASMA CELLS

The plasma cells, also called plasmacytes, are seen under the LM as large, ovoid cells averaging 20 µm in diameter. Their cytoplasm stains intensely basophilic except for a small pale-staining area near the nucleus (Fig. 5.1). The cytoplasmic basophilia is due to the presence of abundant rough endoplasmic reticulum. The pale-staining area near the nucleus contains an extensive Golgi complex and centrioles. Abundant RER and a large Golgi make the plasma cells well-suited for protein secretion.

The nucleus of a plasma cell is spherical in shape and occupies an eccentric position in the cell. It exhibits a characteristic clock face appearance (also called cartwheel appearance). This distinctive appearance of the nucleus is because of the fact that large clumps of darkly-staining heterochromatin alternate with pale-staining areas of euchromatin.

The **plasma cells secrete antibodies**, which are proteins belonging to the immunoglobulin (Ig) variety. A plasma cell has the capability to secrete several thousand antibody molecules per second.

The plasma cells are derived from the activated lymphocytes of the B type. When a B lymphocyte encounters an antigen, it engulfs the antigen and, after processing it in its lysosomal system, presents the antigenic determinant (epitope) of this antigen to the helper T cells, which are a specific variety of T lymphocytes. The helper T cells, in turn, release proteins that cause the B lymphocytes to differentiate into antibody-secreting plasma cells. The plasma cells formation occurs in the germinal centers of the lymphoid follicles of the secondary lymphoid organs, e.g., the lymph nodes and tonsils. The antibodies secreted by the plasma cells pass into the bloodstream and lymphatic system. Once released into the blood and lymph, the antibody molecules reach the target antigen and bind with this antigen to neutralize or destroy it.

Normally, the plasma cells reside in the lymphoid follicles of the secondary lymphoid organs, but some of them do enter the blood and then leave the bloodstream to enter the loose connective tissue, especially in those regions where the antigens tend to enter the body, e.g., the mucosa of the digestive and respiratory tracts. At these strategically important places, the plasma cells play an important role in defense against those antigens which manage to penetrate the mucosal linings (provided that these antigens are similar to those which originally caused the development of the plasma cells from the B lymphocytes). The plasma cells of the loose connective tissue release copious amounts of antibodies to neutralize the invading antigens as soon as these antigens cross the epithelial lining of the mucosa.

The plasma cells have a short life span of 10 to 20 days after which they die by apoptosis. However, the number of the plasma cells is maintained by the constant migration of these cells from the secondary lymphoid organs into the loose connective tissue via the bloodstream.

LEUKOCYTES

The leukocytes (white blood cells) normally migrate from the bloodstream into the connective tissue through the capillary walls. This migration increases during inflammation. Almost all types of leukocytes (neutrophils, eosinophils, lymphocytes, and monocytes) enter the connective tissue. The lymphocytes of B type develop into plasma cells and the monocytes give rise to macrophages. The role of neutrophils and eosinophils will be taken up in chapter 9.

EXTRACELLULAR MATRIX OF THE CONNECTIVE TISSUE

The extracellular matrix (ECM) of the connective tissue is composed of ground substance and fibers. The nature of its components determines the physical properties of various types of connective tissue like the ability to withstand shearing forces, stretch, and compression, etc.

GROUND SUBSTANCE OF THE CONNECTIVE TISSUE

The ground substance of connective tissue is an amorphous, gel-like material which has a high content of water. The water bound by the ground substance serves as the medium through which all nutrients and waste

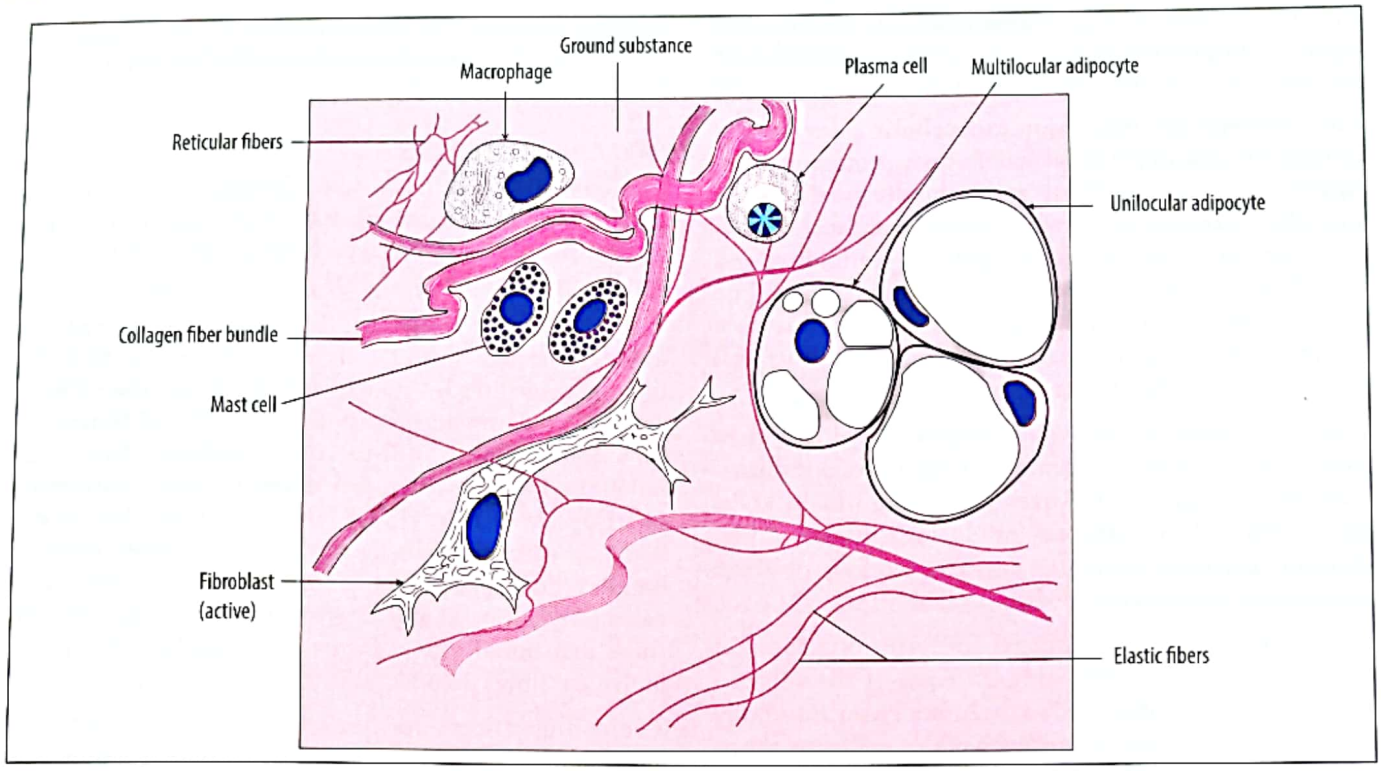

Fig. 5.1 Diagrammatic representation of the cells and fibers that may be seen in the loose connective tissue.

products must pass in transit between the blood and the parenchymal cells of the organs.

Because the ground substance is soluble in the reagents generally used to prepare the tissues for microscopy, in routine histological sections the ground substance cannot be seen and is represented by empty (unstained) areas.

The ground substance of connective tissue is composed of glycosaminoglycans and glycoproteins. These macromolecules interact with each other, with connective tissue cells and fibers, and with epithelial cells.

The **glycosaminoglycans (GAGs)** are long chain polysaccharides composed of repeating disaccharide units. All of the GAGs are sulfated, except hyaluronic acid. The sulfated glycosaminoglycans are covalently bound to small amounts of proteins to form *proteoglycans*. The ground substance of connective tissue contains the non-sulfated GAG hyaluronic acid and five sulfated GAGs which include: chondroitin sulfate, dermatan sulfate, keratan sulfate, heparan sulfate, and heparin.

The **glycoproteins** of the ground substance of connective tissues are adhesive glycoproteins which fasten various components of connective tissue with each other. These glycoproteins perform this function in the following two ways: (i) they have binding domains for various components of the extracellular matrix of connective tissue (collagen, elastin, and GAGs, etc.), and (ii) they bind to receptor proteins, called *integrins,* that are located in the plasmalemma of the cells. The major adhesive glycoproteins of the ground substance are *fibronectin* and *laminin*. The ground substance of the cartilage contains a special type of adhesive glycoprotein called *chondronectin*. Similarly, a special variety of adhesive glycoprotein, called *osteonectin*, is found in the ground substance of the bone.

CONNECTIVE TISSUE FIBERS

Connective tissue fibers are long, slender protein polymers which are found in differing proportions in various types of connective tissue. The constituent proteins of the connective tissue fibers are synthesized and secreted by the fibroblasts. Specific properties of any variety of connective tissue are largely dependent upon the type of fibers present in the extracellular matrix of that connective tissue.

Connective tissue fibers are of three major types: collagen fibers, reticular fibers, and elastic fibers. The first two types are composed of proteins belonging to the *collagen* family, whereas the elastic fibers are made up of a protein called *elastin*.

Before describing the features of various connective tissue fibers separately, it is essential to have some fundamental knowledge about the collagen family of proteins.

COLLAGENS

The collagens are a large family of proteins that are characterized by a triple helical molecular structure consisting of three polypeptide chains (called α-chains) which are wound around each other in a rope-like fashion to form a triple helix. Hydrogen bonding holds the α-chains together. Glycine, proline and hydroxyproline are the commonest amino acids that occur in the molecules of different types of collagen. Collagen synthesis occurs

EM shows that, despite their thinner diameter, the collagen type III fibrils exhibit the 65 nm banding pattern similar to that of collagen type I fibrils. The loosely packed collagen type III fibrils of the reticular fibers are held together by interfibrillar bridges composed of proteoglycans and glycoproteins.

Due to their thinness, the reticular fibers are not distinguishable in the ordinary H&E stained tissue sections examined under the LM. But, due to their association with carbohydrate-rich proteoglycans, the fibers can be visualized by employing special staining procedures like silver staining and Periodic-Acid Schiff (PAS) staining. When stained by silver salts (e.g., by Gomori's silver impregnation technique), the reticular fibers take a black color and their network becomes clearly identifiable (collagen fibers, on the other hand, stain yellow to brown). Because of their high affinity for silver salts, the reticular fibers are also called *argyrophilic fibers* (in Greek, *argyros* means silver). By the PAS stainingtechnique, the reticular fibers are seen as red strands.

The reticular fibers form the supporting framework in many organs which include lymph nodes, spleen, liver, endocrine glands, and bone marrow. Delicate sheaths of reticular fibers also surround the muscle fibers (as endomysium) and nerve fibers of the peripheral nerves (as endoneurium). In the loose connective tissue, networks of reticular fibers are present at the junction of connective tissue and epithelium. In this location they are seen to extend into the basement membrane of the epithelium.

In the bone marrow and lymphoid organs, the reticular fibers are produced by the reticular cells. In most other locations, the reticular fibers are produced by the ordinary fibroblasts. Two other sources of production of reticular fibers are worth noting. The reticular fibers of endoneurium are secreted by Schwann cells. In the muscularis of gastrointestinal tract and in the tunica media of arteries the reticular fibers are produced by the smooth muscle cells.

ELASTIC FIBERS

As indicated by their name, these fibers are highly elastic. They can be stretched easily even by weak traction forces but return to their original length when these forces are removed.

Elastic fibers are seen, in unstained preparations of loose connective tissue, as very thin highly refractile strands, 0.2 to 1.0 μm in diameter, which branch and re-join to form a loose network. In other locations, however, the elastic tissue may present different forms and arrangements. In elastic ligaments the elastic tissue is present as thicker parallel fibers.

In locations where elastic fibers are abundant, they impart a yellow color to the tissues. In the ordinary H&E stained tissue sections the elastic fibers cannot be identified easily because they are very faintly eosinophilic. However, a number of special staining procedures have been devised to selectively stain the elastic fibers, e.g., resorcin-fuchsin technique, by which the elastic fibers are colored bluish-black.

As in the case of the collagen and reticular fibers, the component proteins of the elastic fibers are also secreted by the fibroblasts, except in the walls of the blood vessels where they are produced by the smooth muscle cells.

Electron microscopic studies reveal that, during their formation, the elastic fibers pass through three successive stages. In some locations in the body, these fibers do not develop beyond the first or second stage of development, but in most of the locations they pass through all the three stages of development to achieve the final characteristics of elastic fibers. In the first stage, the fiber consists of a bundle of thin (11 nm diameter) microfibrils, which are composed of the glycoprotein *fibrillin*. At this stage of formation, the developing elastic fibers are also called **oxytalan fibers.** In the adult human body, the oxytalan fibers are found in the dermis of skin and in the periodontal ligaments of the teeth. The suspensory ligament of the lens of eye also consists of the oxytalan fibers. In the second stage, the protein elastin appears as an amorphous material within the bundles of the fibrillin microfibrils. The combination of fibrillin and elastin is called *elaunin* and at this stage the developing elastic fibers are called **elaunin fibers.** The elaunin fibers are also found in the periodontal ligaments and in the dermis of skin. In the third stage of development, the amorphous elastic component constitutes more than 90% of the fiber. The fibrillin microfibrils are then mostly seen to form a peripheral layer around the central core of elastin. Now these fibers are known as **elastin fibers** or *elastic fibers.* The oxytalan, elaunin and elastin fibers collectively constitute the *elastic fiber system*; the elastin fibers are the most numerous fibers of this system.

The *elastin* protein is rich in the amino acids glycine and proline. It also contains two special amino acids desmosine and isodesmosine which are responsible for the rubber-like properties of elastin. When a force is applied, the elastic fibers are capable of stretching one and a half time their original length. However, they return to their original length when the stretching force is removed.

In the walls of the blood vessels, elastin is secreted by the smooth muscle cells. In these locations elastin does not become associated with microfibrils of fibrillin. This nonfibrillar elastin occurs as fenestrated membranes, sheaths or lamellae, which are arranged concentrically in the wall of the blood vessels.

Elastic fibers are found in connective tissues throughout the body but they are particularly abundant in connective tissue framework of those organs that must yield to pressure or pulling forces and then return to their original size and shape, e.g., the lungs. Certain ligaments are also rich in elastic tissue and hence, are called *elastic ligaments.* In human beings, best example of elastic ligaments is constituted by the ligamenta flava of the vertebral column.

EMBRYONIC CONNECTIVE TISSUE

Mesenchymal Connective Tissue

The mesenchymal connective tissue or *mesenchyme* is found only in the developing embryo. It is composed of roughly star-shaped cells, called *mesenchymal cells* that lie in an abundant ground substance, which is viscous in nature. Scattered reticular (collagen type III) fibers are also present. The processes of the star-shaped mesenchymal cells extend in different directions and make contact with similar processes of neighboring cells. Gap junctions are present at the sites of such contacts. Mitotic figures are commonly seen in the mesenchymal cells. These cells are pluripotent in nature and give rise to different types of connective tissue cells. The mesenchymal cells, which are initially scattered throughout the embryo, gradually become depleted as they differentiate into various types of connective tissue cells. In the adult, representatives of the mesenchymal cells are found as mesenchymal stem cells of the loose connective tissue.

Mucoid Tissue

The mucoid tissue (also called mucous tissue) is an embryonic/fetal type of connective tissue which occurs as an intermediate stage in the development of adult type of connective tissue from mesenchyme. It consists of an abundant soft, jelly-like extracellular matrix composed chiefly of hyaluronic acid and a fine meshwork of type I and type III collagen fibrils. Stellate fibroblasts with branching processes are scattered within the extracellular matrix.

The mucoid tissue constitutes the principal component of the umbilical cord where it is known as Wharton's jelly. This mucoid tissue forms a compliant cushion around the blood vessels running in the umbilical cord.

CLASSIFICATION OF CONNECTIVE TISSUES

The connective tissues are classified into three main types:

1. Embryonic connective tissue
2. Connective tissue proper
3. Specialized connective tissue.

These three varieties of connective tissue are subclassified as under:

1. Embryonic connective tissue
- i. Mesenchymal connective tissue
- ii. Mucoid connective tissue

2. Connective tissue proper
- i. Loose connective tissue
- ii. Dense connective tissue
  - a. Dense irregular connective tissue
  - b. Dense regular connective tissue
    - Collagenous
    - Elastic
- iii. Reticular connective tissue
- iv. Adipose tissue

3. Specialized connective tissue
- i. Cartilage
- ii. Bone
- iii. Blood

chiefly in the fibroblasts, but some other cells also have the capability to produce collagens, e.g., the chondrocytes of the cartilage tissue and osteoblasts of the bone tissue.

The collagens are the major extracellular proteins and account for about 25% of the whole-body protein content. By self-association into fibrils and by binding to the GAGs and other extracellular matrix components, the collagens contribute to tissue integrity, act as tissue scaffolding, and provide tensile strength to the connective tissues. The collagen also interacts with the cells through cell surface receptors of integrin variety and, thus, mediates cell adhesion as well as cell migration.

Upon prolonged boiling, the collagen is hydrolyzed to form gelatin, which is commonly used in food industry. The name *collagen* is of Greek origin, in which 'kolla' means glue and 'gen' denotes 'producing' (in older times, glue was prepared from the skin and tendons obtained from horses and other animals).

By exploring the structure of collagens occurring in different locations in the body, 28 types of the collagen proteins have been identified so far. Although no universally accepted classification of various types of collagens exists, the collagens are loosely classified into three main groups: (i) fibrillar collagens, (ii) sheet-forming collagens, and (iii) anchoring collagens.

The ***fibrillar collagens*** (also called *fibril-forming collagens*) are the chief source of tensile strength in animal tissues. In this variety of collagens, the collagen molecules are linked to each other to form fibrils which are indeterminate in length and range in diameter from 12 to 500 nm, depending on their location in the body tissues. Collagen type I, II, III, V and XI belong to the fibrillar variety of collagens. Out of these, the type I is the commonest variety of collagen in the body. The diameter and length of the type I collagen fibrils are highly variable; their diameter ranges from 20 to 90 nm and they may be several micrometers in length. Generally, the type I collagen type I fibrils assemble to form the *collagen fibers* which are the commonest connective tissue fibers of the body. The type II collagen fibrils occur in the extracellular matrix of the cartilage. The collagen type III fibrils form the reticular fibers of the connective tissue. The collagen type V is co-expressed with collagen type I in skin, bone, cornea, and placenta, etc. Collagen type XI is co-expressed with collagen type II in the extracellular matrix of the cartilage.

The chief variety of the sheet-forming collagens is the collagen type IV, which provides the main structural support in the basement membranes of the epithelial tissues. The collagen IV also forms the external laminae of the adipocytes, Schwann cells, smooth muscle cells, and skeletal muscle cells.

The anchoring collagens (also called linking collagens) are those collagens that link the fibrils of the fibrillar collagens with each other. They also serve to anchor the fibrillar and sheet-forming collagens to the components of the surrounding or underlying connective tissue. The anchoring collagens include collagen type VII, IX, XII, and XIV.

COLLAGEN FIBERS

The collagen fibers (also called *collagenous fibers*) are the most commonly occurring connective tissue fibers. They are composed of collagen type I fibrils which are arranged parallel to each other.

When seen under LM in fresh unstained spreads of loose connective tissue, collagen fibers appear as white unbranched threads, which appear to be interwoven, running randomly in various directions. Their diameter is variable (ranging from 2 to 10 µm) and they have great indefinite lengths. When not under tension, collagenous fibers pursue a slightly wavy course. In many parts of the body the collagen fibers lie parallel to each other forming bundles (fascicles) of various sizes. Component fibers of a fascicle may leave it and interweave with another bundle (note that bundles of collagen fibers do branch but the individual fibers do not).

The collagen fibers are flexible but inelastic (i.e., non-extensible) and, thus, they provide a unique combination of flexibility and strength to the structures in which they are present.

In H&E preparations examined under LM, collagen fibers stain acidophilic, taking a pink color with eosin. When seen under high magnification, these fibers exhibit faint longitudinal striations indicating that they are composed of thinner fibrils.

EM makes it clear that each collagen fiber is composed of parallel fibrils, which vary from 50 to 90 nm in diameter. In electron micrographs of lead-stained sections, these fibrils appear to be cross-striated, with denser staining transverse bands repeating every 65 nm along their length. It has been found out that in a collagen fibril the collagen molecules are aligned side by side in a staggered fashion. As a molecule becomes incorporated into the parallel array, it extends beyond (i.e., overlaps) its neighbor by one quarter. The successive collagen molecules also have a 40 nm gap between their ends, which is called *lacunar zone*. The staggered arrangement of the collagen molecules and the presence of intermolecular gaps are the two factors which are responsible for the cross striations seen in the electron micrographs of the collagen fibrils.

RETICULAR FIBERS

These fibers are much thinner than the collagen fibers and range from 0.5 to 2 µm in diameter. Unlike collagen fibers, the reticular fibers do not form bundles but they branch extensively and form elaborate networks*. The reticular fibers are composed of type III collagen which exists in the form of fibrils ranging from 25 to 45 nm in diameter. The

* In Latin, *rete* means *net*, while *reticulum* is the diminutive of rete, meaning a "small net". *Reticular* means "resembling a network".

Connective Tissue Proper

6

The connective tissue proper is classified into four major types:

1. Loose connective tissue.
2. Dense connective tissue.
3. Reticular connective tissue.
4. Adipose tissue.

LOOSE CONNECTIVE TISSUE

The loose connective tissue is widely distributed in the body. It is the packing and anchoring material of the body and acts as embedding medium for many structures including blood vessels and nerves. It binds other tissues, organ components and organs together and allows, owing to its flexibility, a considerable degree of mobility between such parts. It also forms the supporting framework (stroma) of most of the organs. Chief examples of the loose connective tissue are: the subcutaneous tissue (superficial fascia and deep fascia), mesentery, and omentum.

All the three basic components of connective tissue (cells, fibers and ground substance) are best represented in the loose connective tissue. The two most common cell types include the fibroblasts and macrophages, but many mast cells and adipocytes are also present. In the ECM of the loose connective tissue, collagen fibers (composed of collagen type I) are most abundant, but elastic fibers are also present in a considerable number. Reticular fibers are scarce but they tend to increase in number at places where the loose connective tissue borders upon other structures. The ground substance of the ECM is dissolved out by the chemical agents (alcohol and xylol) used during the tissue fixing and staining procedures. Therefore, in stained sections, the ground substance of the loose connective tissue is represented by small circular or oval areas in which no structure can be seen. Such empty areas are called *areolae* and, therefore, the loose connective tissue is sometimes also referred to as ***loose areolar connective tissue.***

Small blood vessels (capillaries) run in the ground substance and supply the cells with oxygen and nutrients. Fine nerve fibers are also found in the ground substance of loose connective tissue.

DENSE CONNECTIVE TISSUE

This variety of connective tissue proper is characterized by close packing of its fibers. It contains fewer cells and much smaller amount of the ground substance as compared to the loose connective tissue. The dense connective tissue is less flexible but it is far more resistant to stress.

According to the arrangement of its component fibers, the dense connective tissue is subclassified into two categories: (1) dense irregular connective tissue in which the collagen fibers are arranged randomly, and (2) dense regular connective tissue in which the fiber bundles are arranged in an organized fashion, generally running parallel to each other.

DENSE IRREGULAR CONNECTIVE TISSUE

This variety of dense connective tissue usually occurs in the form of sheets. It consists mainly of bundles of collagen fibers which are interwoven into a meshwork. Such a meshwork resists stress from all directions. In addition to collagen fibers, the dense irregular connective tissue also contains fine networks of elastic fibers scattered among the collagen bundles.

Examples of the dense irregular connective tissue are deep fascia and capsules of organs like the thyroid gland, prostate gland, liver, kidneys, and testes, etc. The perichondrium of the cartilage and periosteum of the bone also consist of dense irregular connective tissue.

DENSE REGULAR CONNECTIVE TISSUE

In this variety of dense connective tissue, the fibers are densely packed and are arranged parallel to each other. Dense packing of component fibers provides maximum strength to this tissue. The dense regular connective tissue is further classified into two varieties: dense regular collagenous connective tissue and dense regular elastic connective tissue.

DENSE REGULAR COLLAGENOUS CONNECTIVE TISSUE

This tissue is composed of densely packed bundles of collagen fibers. The limited space between the fiber bundles is occupied by the ground substance and fibroblasts. The dense regular connective tissue occurs in the form of cord-like or band-like structures (tendons and ligaments) or broad sheet-like structures (aponeuroses).

Tendons and Ligaments

Tendons are cord-like structures which attach muscles to the bones, whereas ligaments are band-like structures that join bones to bones. They are composed almost entirely of collagen fibers, due to which white in color upon naked eye examination. The collagen fibers run parallel to each other and are closely packed in the form of bundles, which are separated from each other by a small quantity of ground substance. Fibroblasts are the only cell type present and even these cells are very small in number.

In a longitudinal section of a tendon, the fibroblasts are seen as elongated cells, aligned in rows between the bundles of

Brown adipose tissue is very abundant in hibernating animals. In humans, a large amount of brown adipose tissue is found in the new born. As the body grows, the amount of this tissue gradually decreases and become greatly reduced after the first decade of life. In the body of an adult human being, the brown adipose tissue is found to be located in the posterior triangle of neck and in the mediastinum. It is also found around the abdominal aorta and kidneys.

The principal function of the brown adipose tissue is production of body heat. The adipocytes of brown adipose tissue produce heat by *non-shivering thermogenesis*. Under the influence of norepinephrine released from sympathetic nerve endings, fat is mobilized and heat is generated by multilocular adipocytes. The mitochondria of these cells contain a unique uncoupling protein called *thermogenin,* which is located in the inner mitochondrial membrane. Thermogenin uncouples the mitochondrial metabolism from production of ATP, so that energy produced by the oxidation of fatty acids is not used to synthesize ATP but is dissipated as heat.

In the hibernating animals, the heat produced by the brown adipose tissue is used to warm the blood upon arousal from hibernation. In humans, the brown adipose tissue plays a vital role in the maintenance of normal body temperature in the new born and in young children. Physiological role of the brown adipose tissue in the adult humans is not well-understood.

Cartilage 7

The cartilage is a special type of connective tissue which is characterized by its resilient and pliant nature. It is found at those places in the body where structural support with flexibility is required. Like all connective tissues, the cartilage is also composed of cells and a large amount of extracellular matrix (ECM). The resilient nature of the cartilage is due to its specialized ECM, which is firm but rubbery and pliable.

The cells of the cartilage, known as *chondrocytes*, occupy small spaces within the ECM which are called *lacunae*. The ECM of the cartilage is secreted by the chondrocytes and consists of ground substance and connective tissue fibers/fibrils. The special feature of the ground substance of the cartilage is the presence of large quantities of sulfated glycosaminoglycans. The cartilage is generally surrounded by a layer of dense irregular connective tissue called *perichondrium*.

The cartilage is an avascular tissue and its substance is not traversed by any blood vessel or lymphatic vessel. The chondrocytes receive their nutrition by diffusion through the ECM from the blood vessels running in the surrounding connective tissue. In the synovial joints, the articular cartilages receive nourishment from the synovial fluid.

PERICHONDRIUM

It is a sheet of dense irregular connective tissue which surrounds the cartilage in most of the locations in the body (the fibrocartilage and articular variety of hyaline cartilage are not covered by perichondrium). Microscopically, the perichondrium consists of two layers: an outer fibrous layer and an inner cellular layer.

The outer **fibrous layer** of the perichondrium consists of type I collagen fibers and fibroblasts. The blood capillaries, from which oxygen and nutrients are supplied to the chondrocytes, run in the fibrous layer of perichondrium. In addition, lymph capillaries and nerves are also present in this layer.

The inner **cellular layer** of the perichondrium is also called *chondrogenic layer*. It lodges progenitor cells which have the ability to differentiate into chondroblasts. The chondroblasts divide and differentiate into chondrocytes. The chondrogenic layer ensures a continuous supply of chondroblasts in the growing cartilages in the children, but it can become active in the adults as well when repair of the cartilage is required after damage due to injury or disease.

CHONDROCYTES

All varieties of the cartilage contain only one type of cells called chondrocytes. They lie within small space (lacunae) in the ECM and are present either singly or in clusters known as *isogenous groups* or cell nests. Each isogenous group consists of 2-4 cells which represent the daughter cells formed as a result of mitotic division of one chondrocyte. The chondrocytes secrete and maintain the ECM of the cartilage. They not only produce collagenous components of the matrix but also the noncollagenous proteins and proteoglycans. In the elastic cartilage, the chondrocytes also produce the protein elastin. They also secrete special enzymes, called metalloproteinases that degrade the ECM to enlarge the lacunae, so that the growing chondrocytes can expand and the isogenous groups of these cells can reposition themselves in the enlarged lacunae.

The chondrocytes are roughly spherical cells with an average diameter of 20 µm. The cytoplasm of a chondrocyte contains a large amount of rough endoplasmic reticulum, a large Golgi apparatus, and numerous secretory granules. The RER synthesizes the proteins needed for the ECM of the cartilage and the Golgi apparatus packs these proteins into secretory granules. Due to the abundance of the RER, the cytoplasm of the chondrocytes stains basophilic.

EXTRACELLULAR MATRIX (ECM) OF THE CARTILAGE

The ECM of the cartilage consists of macromolecules which are secreted by the chondrocytes. These macromolecules are: (1) collagens, (2) noncollagenous proteins, (3) hyaluronan, and (4) proteoglycans. The collagens found in different varieties of the cartilage are collagen type I, type II, and type XI. The noncollagenous proteins of the cartilage are multiadhesive glycoproteins, mainly chondronectin and fibronectin. Hyaluronan (hyaluronic acid is a nonsulfated glycosaminoglycan. The major proteoglycan of the cartilage ECM is aggrecan, which consists of two sulfated glycosaminoglycans (chondroitin sulfate and keratan sulfate) which are attached to a multidomain protein core.

CLASSIFICATION OF THE CARTILAGE

The cartilage is conventionally classified into three types: (i) hyaline cartilage, (ii) fibrocartilage, and (iii) elastic cartilage. This classification is based on a number of characteristics which include naked-eye appearance of a freshly cut specimen of the cartilage, microscopic structure of the cartilage, and the mechanical properties of the cartilage, especially its degree of flexibility. The differences in the mechanical properties of different types of cartilage tissue are determined by the differences in the composition of the ECM.

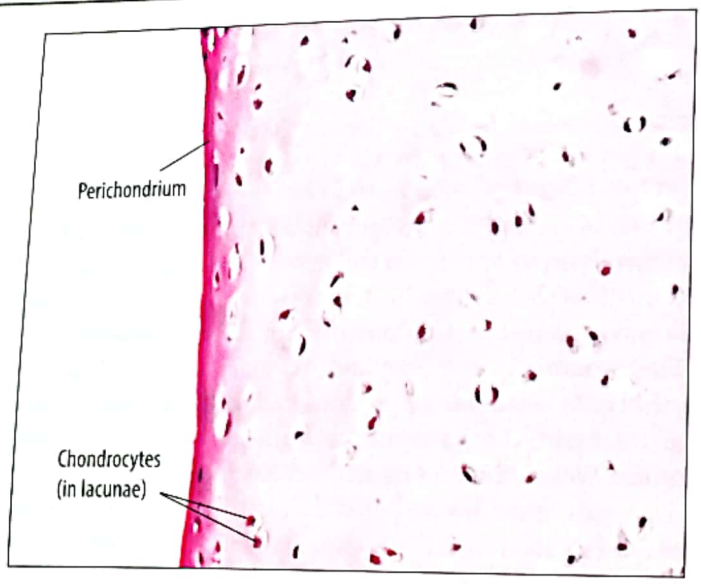

Fig. 7.1 A section through the hyaline cartilage (from a costal cartilage).

HYALINE CARTILAGE

Hyaline cartilage (Fig. 7.1) is the most commonly-occurring cartilage in the human body. When examined by the naked eye in the fresh state, this variety of cartilage gives a pearly-white, translucent, glassy appearance (hence the name hyaline, i.e., glass-like). The hyaline cartilage provides firm but resilient support in different parts and organs of the body. Due to its smooth and pliable surface, It also serves to reduce friction between articulating bony surfaces in movable joints.

All hyaline cartilages are covered by perichondrium except the articular cartilages.

Location of the Hyaline Cartilage in the Body

1. The initial skeleton of the fetus consists of hyaline cartilage.
2. In the children and young adults, the epiphyseal cartilages (growth plates) of the growing bones consist of hyaline cartilage.
3. The major laryngeal cartilages (cricoid cartilage, thyroid cartilage, and arytenoid cartilages) are of hyaline variety.
4. The cartilages present in the wall of the trachea and bronchi are of hyaline type.
5. The costal cartilages (present at the anterior ends of the ribs) are hyaline cartilages.
6. The articular cartilages (which cover the articular surfaces of bones involved in the formation of synovial joints) are also of hyaline variety.
7. The nasal cartilages, which form part of the skeleton of nose, are also of hyaline type.

ECM OF THE HYALINE CARTILAGE

The extracellular matrix of the hyaline cartilage contains all the four typical components of the cartilage ECM, i.e., collagen, adhesive glycoproteins (chondronectin and fibronectin), hyaluronan, and aggrecan. The collagen component of the hyaline cartilage consists chiefly of type II collagen fibrils. In addition, fibrils of type XI collagen are also present in a small quantity. However, the collagen type II and type XI fibrils cannot be seen in the ordinary stained sections because these fibrils are very thin and, therefore, are beyond the resolving power of the light microscope.

The ECM of the hyaline cartilage is highly hydrated and, therefore, 60-80% of the net weight of this variety of cartilage consists of water. This water id bound chiefly to the aggrecan-hyaluronan aggregates. The high water content gives the hyaline cartilage a high degree of resilience which is one of the chief properties of this type of cartilage.

Distribution of various components of the hyaline cartilage ECM is not uniform. Consequently, the ECM does not stain uniformly and, upon the basis of staining differences, different regions can be identified in the ECM of the hyaline cartilage; these regions are: (i) pericellualr matrix, (ii) territorial matrix, and (iii) interterritorial matrix (Fig. 7.2A).

The **pericellular matrix** (also called *capsular matrix*) lies immediately outer to the cell membrane of the chondrocytes. It stains intensely basophilic because it is devoid of collagen type II fibrils and consists chiefly of the sulfated proteoglycans (present as aggrecan molecules).

The **territorial matrix** surrounds the pericellular matrix. It contains a small number of collagen type II fibrils and large quantities of sulfated proteoglycans. The territorial matrix stains deeply basophilic (but less intensely than the pericellular matrix).

The **interterritorial matrix** lodges abundant collagen type II fibrils (and also collagen type XI fibrils) but contains relatively smaller quantity of sulfated proteoglycans. Therefore, the interterritorial matrix takes a light basophilic stain.

ARRANGEMENT OF CHONDROCYTES IN THE HYALINE CARTILAGE

In the central region of the hyaline cartilage, the chondrocytes are generally arranged in isogenous groups of 2-4 cells. The cells of an isogenous group are spherical or ovoid in shape and are flattened on adjacent sides. All the members of an isogenous group occupy a single lacuna.

Near the periphery of the cartilage, the chondrocytes are generally distributed singly and have an elliptical shape. In the subperichondrial region, the chondrocytes appear as rows of narrow elongated cells with their long axes parallel to the surface of the cartilage.

ARTICULAR CARTILAGE (Fig. 7.3)

It has already been mentioned that the articulating surfaces of the bones participating in the formation of the synovial joints of the body are covered by a layer of hyaline cartilage

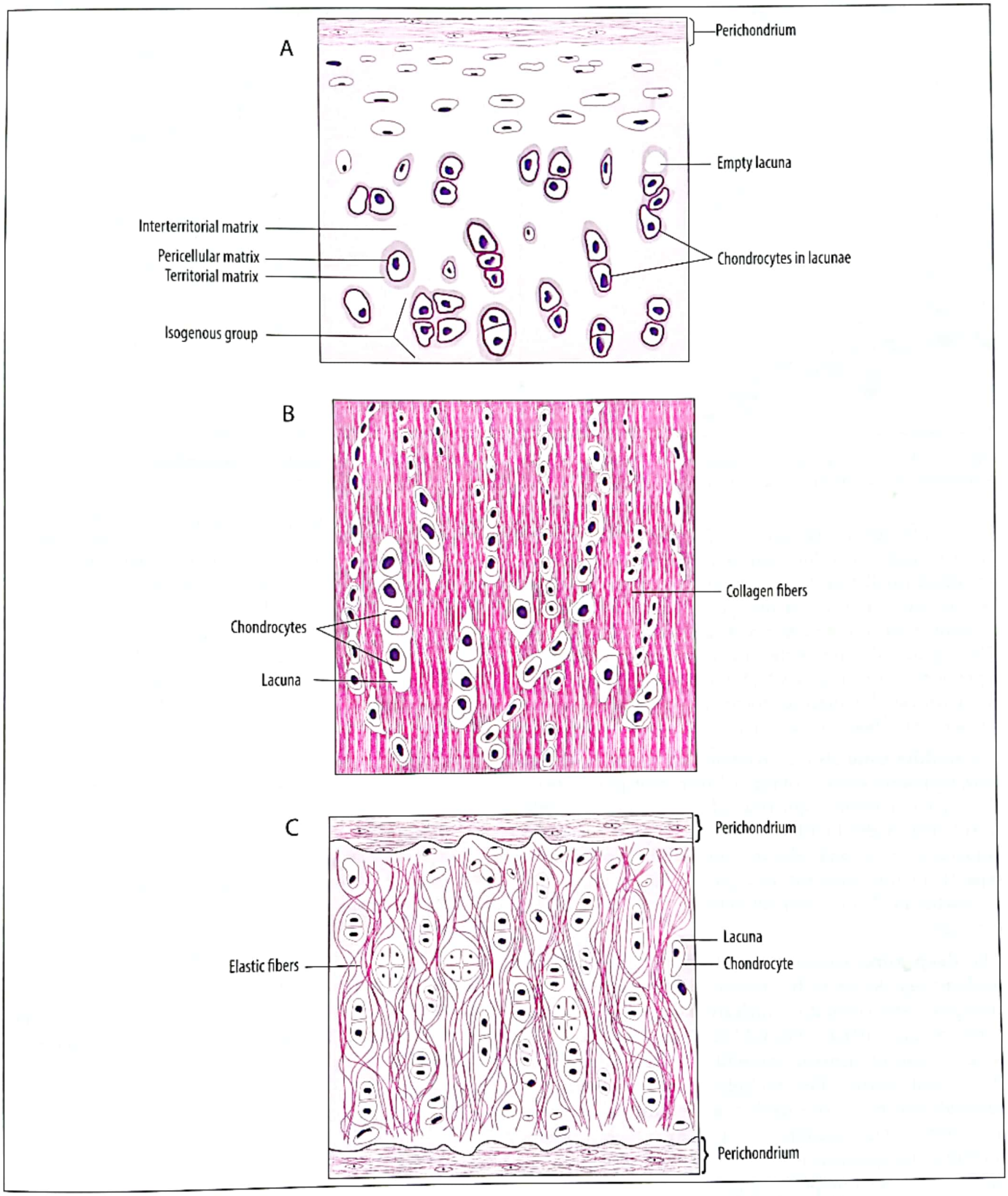

Fig. 7.2 Diagrammatic representation of histological features of the three varieties of cartilage A. Hyaline cartilage B. Fibrocartilage C. Elastic cartilage

called articular cartilage. This cartilage serves as a smooth, wear-resistant, almost frictionless, load-bearing surface. The thickness of the articular cartilage varies from 2 to 4 mm.

The articular cartilage can be divided into **four zones**: (i) the superficial zone, (ii) the middle zone, (iii) the deep zone, and (iv) the zone of calcified cartilage.

The superficial zone, also called *tagential zone*, makes up 10-20% of the articular cartilage thickness. It contains tightly-packed type II collagen fibrils arranged parallel to

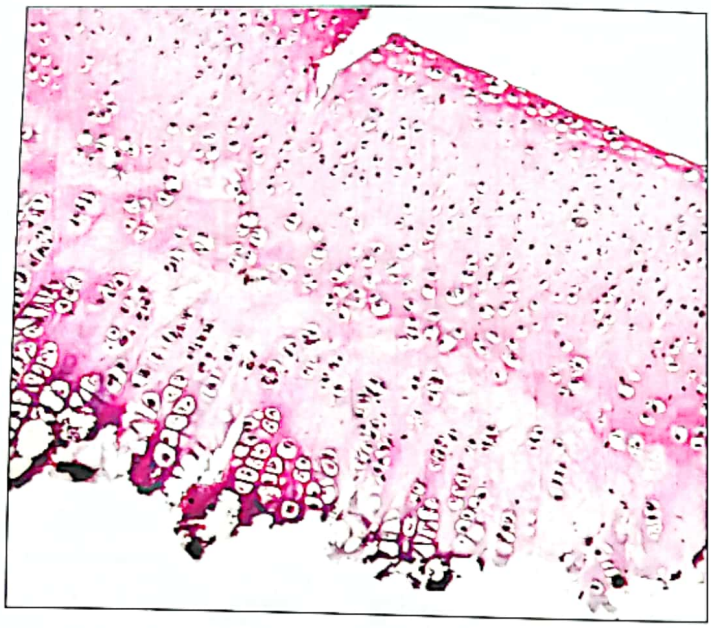

Fig. 7.3 A section through the articular cartilage. Note that no perichondrium covers this variety of hyaline cartilage.

the articular surface. Between the fascicles of the collagen fibrils are embedded elongated chondrocytes which are also organized parallel to the surface of the articular cartilage. Due to the abundance of the type II collagen fibrils, the concentration of proteoglycans in this zone is relatively low. The superficial zone of the articular cartilage constitutes a pressure-resistant layer which protects the deeper zones from damage by shearing forces to which the articular surfaces of the bones are subjected during joint movements.

The **middle zone**, also called *intermediate zone* or *transitional zone*, represents 40-60% of the articular cartilage thickness. This zone contains spherical chondrocytes randomly distributed in the ECM. The ECM of the middle zone contains aggrecan and relatively thicker fibrils of collagen type II. In this zone the collagen fibrils are aligned in somewhat an oblique orientation to the free surface of the cartilage.

The **deep zone** constitutes approximately 30% of the total cartilage thickness. It contains spherical chondrocytes arranged in short columns which are aligned perpendicular to the articular surface. The ECM of the deep zone contains large amount of aggrecan molecules and very thick type II collagen fibrils. The collagen fibrils are arranged perpendicular to the free surface of the cartilage between the columns of the chondrocytes. Due to its high aggrecan content and perpendicular alignment of the type II collagen fibrils, the deep zone provides greatest resistance against the compressive forces applied to the articular cartilage.

The **zone of calcified cartilage** constitutes 10-20% of the articular cartilage thickness. It consists of a calcified extracellular matrix in which are embedded small spherical chondrocytes. The calcified zone of the articular cartilage serves to bind the articular cartilage to the bone tissue by anchoring the collagen fibrils of the deep zone of the cartilage to the subchondral bone.

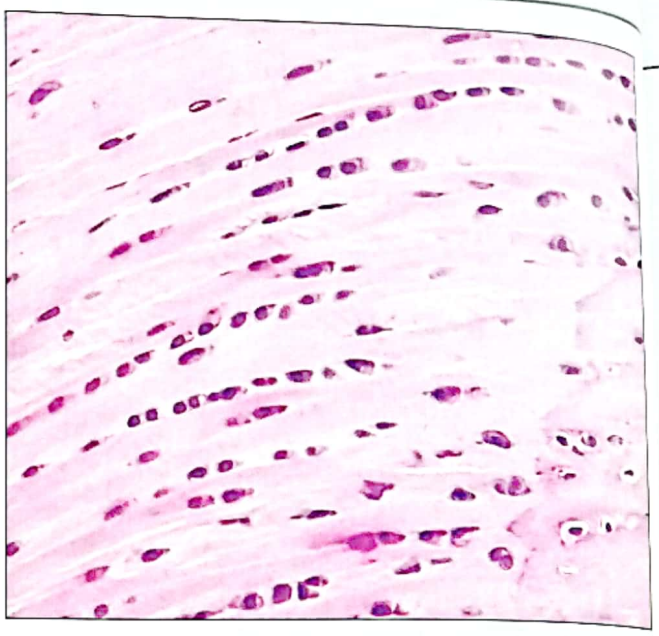

Fig. 7.4 A section through the fibrocartilage.

FIBROCARTILAGE (Fig. 7.4)

The fibrocartilage is a tough and strong but resilient tissue. Like other types of cartilage, it also consists of chondrocytes and ECM. The ECM of the fibrocartilage contains a very high number of type I collagen fibers (Fig. 7.2B). In addition, adhesive glycoproteins, hyaluronan, and aggrecan are also present. Some collagen type II fibrils are also found in the ECM. The fibrocartilage is not covered by perichondrium.

In stained histological sections the chondrocytes of the fibrocartilage are usually seen to be lying in rows between the bundles of type I collagen fibers. Many fibroblasts are present between the collagen fiber bundles. Due to the preponderance of type I collagen fibers, the ECM of the fibrocartilage stains generally acidophilic. However, the ECM immediately surrounding the rows of chondrocytes contains type II collagen fibrils and proteoglycans; therefore; it takes a light basophilic stain.

Location. The fibrocartilage is found in those places in the body where resistance against compression and shearing forces is required. The chief locations of the fibrocartilage are as under:

1. Intervertebral discs.
2. Disc of the pubic symphysis.
3. Intra-articular discs of the sternoclavicular and temporomandibular joints.
4. Menisci of the knee joint.
5. Labrum glenoidale (of the shoulder joint) and labrum acetabulare (of the hip joint).

ELASTIC CARTILAGE (Fig. 7.5)

The elastic cartilage is named after its mechanical property of flexibility and elasticity. This variety of cartilage tolerates

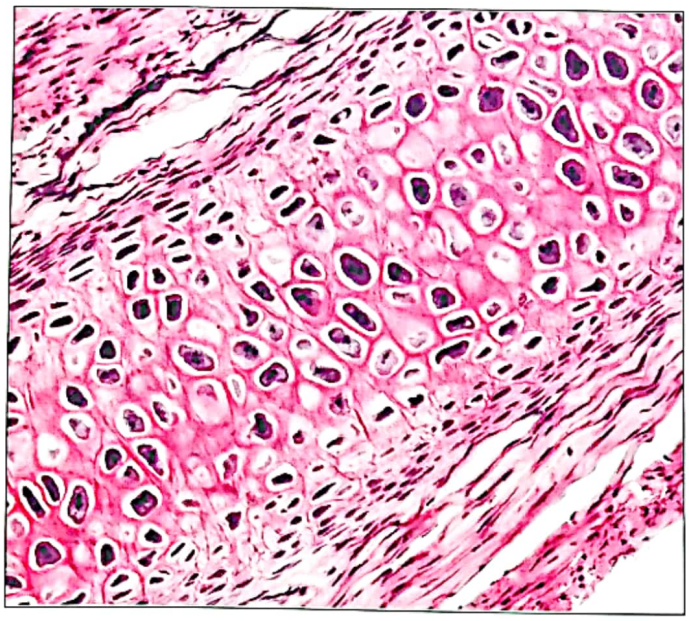

Fig. 7.5 A section through the elastic cartilage.

distortion without damage and returns to its original shape after being temporarily deformed.

Like the other varieties of cartilage, the elastic cartilage also consists of chondrocytes and extracellular matrix. The chondrocytes lie within lacunae and occur either singly or in isogenous groups of 2-4 cells. The distinguishing feature of the elastic cartilage is the presence of a dense network of branching and anastomosing elastic fibers (Fig. 7.2C). In addition to the elastic fibers, the ECM contains type II collagen fibers, aggrecan, hyaluronan, and the adhesive glycoproteins chondronectin and fibronectin.

The network of elastic fibers is especially dense in the regions immediately surrounding the chondrocytes. The elastic fibers can be easily seen under the LM in stained tissue sections.

The elastic cartilage is covered by a perichondrium which consists of the usual two layers: an outer fibrous layer and an inner cellular layer.

Location. The elastic cartilage has a limited distribution and is found in the following two major locations in the human body: (1) auricle of the external ear, and (2) epiglottis of the larynx.

In addition, the elastic cartilage is found in the wall of the external auditory meatus and auditory tube. The small cartilages of the larynx (corniculate cartilages and cuneiform cartilages) are also made up of elastic cartilage.

8 Bone

Like other types of connective tissue, the bone tissue (osseous tissue) also consists of cells and extracellular matrix. The matrix of bone has the special feature of being extremely rigid because it is impregnated with mineral salts, mainly calcium phosphate. Hence, the chief physical property of bone tissue is its toughness and hardness.

Functions of the Bone tissue

Due to their hard, unyielding nature, the bones form the main constituent of the adult skeleton. The bones give **support** to the fleshy structures of the body and provide **protection** to the vital organs (e.g., brain in the skull and heart and lungs in the thoracic cage). The bone tissue also acts as a storehouse of calcium, phosphate and some other ions. These ions especially calcium are released (or stored) in a controlled manner under the influence of specific hormones. Thus, the bone tissue plays an important role in the homeostatic *regulation of blood calcium level.*

BONE MATRIX

The extracellular matrix of the osseous tissue consists of organic and inorganic components.

ORGANIC COMPONENTS OF THE BONE MATRIX

The organic component of the bone matrix forms nearly 50% of the dry weight of the bone. It consists mostly of collagen but noncollagenous proteins of various types are also present in small quantity (see below).

The **collagen** accounts for more than 90% of the organic component of the bone matrix. It consists chiefly of type I collagen but a small amount of type v collagen is also present. This very high content of collagen makes the bone resistant to tensile stresses. The abundance of collagen imparts acidophilia to the bone matrix, due to which the matrix takes a pink color in the ordinary H&E stained sections.

The **noncollagenous proteins** constitute only 10% of the organic components of bone matrix, but are very important for the development, growth and repair of the bone tissue. The noncollagenous proteins of the bone matrix belong chiefly to the following three categories:

i. ***Proteoglycans***. As explained previously, the proteoglycans consist of a core protein which is covalently attached to side chains of glycosaminoglycans (GAGs). The GAGs of bone tissue are mainly hyaluronic acid, chondroitin sulfate, and keratan sulfate. The proteoglycans enable the bone to withstand pressure and compression.

ii. ***Multiadhesive glycoproteins***. These proteins ensure adhesion of the bone cells and collagen fibers to the mineralized bone matrix. The multiadhesive proteins of the bone tissue include osteonectin, osteopontin, and sialoproteins I and II.

iii. ***Osteocalcin***. This is a special calcium-binding protein found in the bone tissue. The osteocalcin captures calcium ions from the blood circulating in the capillaries running in the osseous tissue. In this way this protein plays an important role in the mineralization of the bone tissue.

INORGANIC COMPONENT OF THE BONE MATRIX

The inorganic (mineral) component of bone matrix is responsible for hardness of bone tissue and constitutes about 50% of the dry weight of the bone. Calcium and phosphorus are the chief bone minerals, but substantial quantities of sodium, potassium, magnesium, bicarbonate, and citrate ions are also found. Calcium and phosphorus exist mainly as rod-like crystals of calcium phosphate in a form nearly identical to the hydroxyapatite of the rock minerals [$Ca_{10}(PO_4)_6(OH)_2$]. However, substantial quantities of amorphous (non-crystalline) calcium phosphate are also present in the bone matrix.

The calcium phosphate crystals of the osseous tissue are about 40 nm in length and 1.5 to 3.0 nm in thickness. These crystals are deposited in a regular fashion in close association with the type I collagen fibers.

The association of minerals with the collagen imparts hardness to the bone and makes it capable of resisting tensile forces. Removal of the organic component of the bone (e.g., by burning) leaves the inorganic material as a white, brittle, and fragile mass which retains the original shape of the bone but breaks easily when handled. On the other hand, when the inorganic component is removed (e.g., by prolonged immersion in dilute mineral acids), the bone loses its rigidity and becomes as flexible as a tendon.

CELLS OF THE BONE TISSUE

Four varieties of cells are associated with the bone tissue: osteoprogenitor cells, osteoblasts, osteocytes, and osteoclasts.

OSTEOPROGENITOR CELLS

These cells are found in the inner layer of the periosteum and in the endosteum. They also line the Haversian canals and Volkmann's canals of the compact bone. Osteoprogenitor cells are flattened cells, each containing an oval nucleus in the central region of the cell. The scanty cytoplasm stains faintly basophilic. Under EM the cytoplasm is seen to contain a small Golgi apparatus, sparse rough endoplasmic reticulum, and numerous free ribosomes.

The osteoprogenitor cells are derived from embryonic mesenchyme and retain the potential to divide mitotically throughout life. On being stimulated (e.g., by injury), the osteoprogenitor cells differentiate into osteoblasts. Some histologists consider the osteoprogenitor cells to be the inactive form of osteoblasts.

OSTEOBLASTS

The osteoblasts are derived from the osteoprogenitor cells. Actually, the osteoprogenitor cells transform into osteoblasts, according to the need, in a bone which is undergoing growth or repair.

The osteoblasts are specialized, nondividing, bone-forming cells which synthesize and secrete the organic components of the bone matrix (type I collagen, proteoglycans and glycoproteins). The unmineralized bone matrix secreted by the osteoblasts is called ***osteoid***. The osteoblasts also secrete the enzyme alkaline phosphatase which brings about mineralization of the osteoid.

The active osteoblasts are cuboidal or low columnar cells that are found to be present in association to the growing or forming surfaces of bones (e.g., in a developing bone or in a bone undergoing repair after injury). The osteoblasts are aligned side by side and form a single layer that resembles a simple epithelium.

The cytoplasm of an active osteoblast stains markedly basophilic. The nucleus is eccentric in position and is located in that part of the cell which is present away from the forming surface of the bone. EM shows that the cytoplasm of the osteoblasts is characterized by the presence of abundant rough endoplasmic reticulum, many free ribosomes, and a large Golgi apparatus. The abundant RER and free ribosomes impart basophilia to the cytoplasm.

The chief protein produced by the osteoblast is the type I collagen. The collagen and other organic constituents of the bone matrix synthesized by the osteoblasts are packaged into secretory vesicles in the large Golgi apparatus and released by exocytosis from all parts of the cell surface. Those osteoblasts that become completely surrounded by the secreted bone matrix are transformed into osteocytes.

The osteoblasts exhibit thin cytoplasmic processes that extend into the newly formed osteoid. From these processes, the osteoblasts release membrane-bound vesicles, called matrix vesicles, which contain the enzyme alkaline phosphatase (ALP). In the osteoid, the ALP cleaves phosphate ions from other molecules and thus local concentration of these ions increases. At the same time, the calcium-binding glycoprotein osteocalcin raises the local concentration of the calcium ions. With the simultaneous increase in the concentration of the calcium and phosphate ions, the matrix vesicles become foci for the formation of calcium phosphate crystals.

The inactive osteoblasts become flattened and their cytoplasmic basophilia also diminishes (because of decrease in the amount of RER). In the adult bone, the inactive osteoblasts are found in the periosteum and endosteum.

The osteoblasts possess receptors for parathyroid hormone (PTH) on their cell membrane. When activated by PTH, the osteoblasts secrete a cytokine, called *osteoclast-stimulating factor,* which is an important stimulator of osteoclast activity (described later).

OSTEOCYTES

The osteocytes are actually mature osteoblasts that have become trapped within the bone matrix which they produced. The osteocytes comprise 95% of the living cells in the adult bone. Their number is enormous; almost 25000 osteocytes being present in every cubic millimeter of the healthy bone tissue. The osteocytes are not capable of mitosis but they are long living cells which may survive for decades within their lacunae. However, in the old age, the number of the osteocytes gradually decreases due to the death of these cells by apoptosis.

Osteocytes are ellipsoid cells containing an oval nucleus. Their cytoplasm surrounding the nucleus stains faintly basophilic and contains relatively few organelles. Each osteocyte lies in a small cavity in the bone matrix which is called a lacuna. Neighboring lacunae communicate with each other by narrow tubular channels called *canaliculi.* From the cell body of each osteocyte arise numerous fine dendritic processes which extend into the canaliculi and make contact with similar processes from the neighboring cells. Communicating junctions (nexuses) are present where the cytoplasmic processes of the neighboring osteocytes come in contact with each other.

The narrow space between the plasma membrane of osteocytes and the walls of the lacunae and canaliculi is called *periosteocytic space.* This space is occupied by extracellular fluid. This fluid serves as a medium for exchange of nutrients and metabolites between the osteocytes and nearest blood vessels (present in the periosteum, Haversian canals and perforating canals).

Functions of the Osteocytes

Maintenance of the bone matrix. The osteocytes are necessary for the maintenance of the bone matrix in a normal and healthy condition. Death of the osteocytes (due to trauma or aging) results in resorption of the bone matrix.

Maintenance of the blood calcium level. It has been proved that the osteocytes are capable of secreting special enzymes called matrix metalloproteinases (MMPs). These enzymes degrade the bone matrix immediately surrounding the osteocytes and their processes to release calcium ions from the matrix. The calcium ions are released into the subosteocytic space from where they pass into the bloodstream. This osteocytic osteolysis, which is controlled by the parathyroid hormone, plays an important role in maintaining the blood calcium level within the normal range.

OSTEOCLASTS

The osteoclasts are specially meant for the resorption of the bone matrix. They play a very important role in the

remodelling and renewal of the bone (which continues to occur throughout the life of the individual). These cells are found on those surfaces of the bones which are undergoing resorption. On such resorptive bone surfaces, the osteoclasts are seen to be located in shallow depressions called **resorption bays** (which are traditionally called *Howship's lacunae,* although they are not lacunae in true sense). The resorption bays are created by the erosive action of the osteoclasts on the underlying bone.

The osteoclasts are very large, multinucleated, motile cells. An inactive (resting) osteoclast is generally 40 µm in diameter and contains about 20 closely-packed, oval nuclei. However, an active osteoclast is a very large cell (up to 150 µm diameter) which may contain up to 50 nuclei. Due to the abundance of mitochondria and lysosomes, the cytoplasm of an osteoclast stains strongly acidophilic. The cytoplasm also contains many Golgi complexes. The nuclei of an osteoclast are clustered in that part of the cell which is farthest from the bone surface.

The border of the lower part of an osteoclast exhibits finger-like processes due to the presence of deep infoldings of the plasmalemma; this border is called ***ruffled border*** (Fig. 8.1). The ruffled border lies in contact with the bone surface within a resorption bay. The processes of the osteoclasts are different from the static microvilli found over the epithelial cells. The finger-like processes of the osteoclasts have been found to undergo frequent changes in shape as they are actively extended and retracted. The periphery of the ruffled border is surrounded by a ring-like zone of the cytoplasm which is devoid of the cell organelle but is rich in actin filaments; this zone is called ***clear zone*** or ***sealing zone***. The actin filaments enable the cell membrane surrounding the sealing zone to be anchored firmly to the bony wall of the Howship's lacuna. In this way a closed *subosteoclastic compartment* is created between the ruffled border and the bone that is undergoing resorption. The osteoclasts secrete hydrogen ions, collagenase, and hydrolytic enzymes into this compartment.

Resorption of the bone matrix by the osteoclasts involves two steps: (1) dissolution of the inorganic components (minerals), and (2) digestion of the organic component of the bone matrix.

The osteoclasts pump hydrogen ions into the subosteoclastic compartment and thus create an acidic microenvironment, which increases the solubility of bone mineral, resulting in the release and re-entry of bone minerals into the cytoplasm of the osteoclasts to be delivered to the nearby capillaries. After the removal of the minerals, lysosomal hydrolytic enzymes as well as non-lysosomal enzymes like collagenase and gelatinase are secreted into the subosteoclastic compartment. These enzymes digest and degrade the collagen and other organic components of the decalcified bone matrix. The degradation products are phagocytosed by the osteoclasts at the ruffled border. Transport vesicles containing the degraded bone material travel toward the basolateral region of the osteoclast and finally fuse with the cell membrane to release their contents to the cell exterior (from where they are absorbed by the blood capillaries

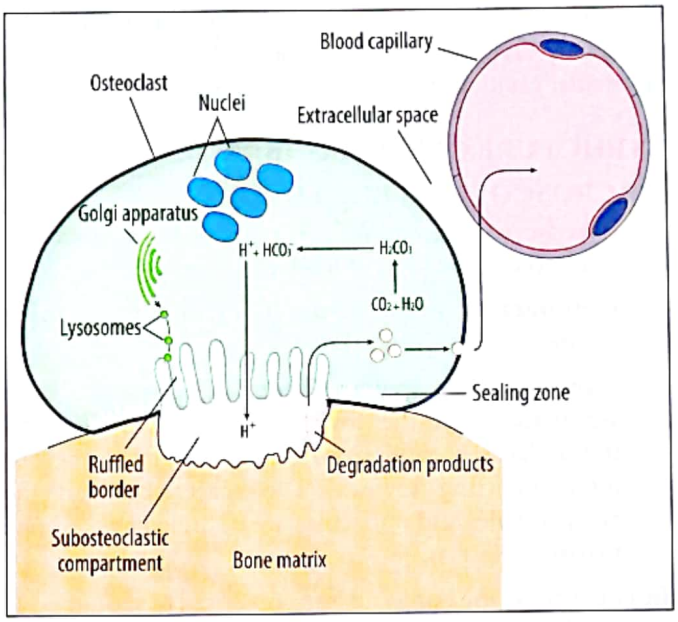

Fig. 8.1 A. Schematic drawing showing an osteoclast and its positional and functional relation to the bone matrix and a blood capillary. Note the ruffled border, subosteoclastic compartment, and sealing zone.

located in the vicinity of the osteoclast). Because of their phagocytic properties, osteoclasts are considered to be a component of the mononuclear phagocyte system (MPS).

The activity of the osteoclasts is controlled by hormones and cytokines. The osteoclasts possess receptors for calcitonin (a hormone produced by the parafollicular cells of the thyroid gland). This hormone suppresses the osteoclastic activity. The osteoclasts do not have receptors for parathyroid hormone (PTH). However, the PTH stimulates the osteoblasts to secrete the cytokine called *osteoclast-stimulating factor*, which is a potent stimulator of the osteoclastic activity. Along with osteocytes, the osteoclasts play an important role in maintaining the serum calcium to adequate levels.

Previously, the osteoclasts were supposed to be formed by the fusion of blood-borne monocytes. The recent evidence has indicated that the osteoclasts arise by the fusion of bone marrow-derived mononucleated cells.

PERIOSTEUM AND ENDOSTEUM

The external surface of the bones is covered by a layer of dense, irregular connective tissue called *periosteum.* This layer covers whole of the bone, except at the articular surfaces. The periosteum consists of two layers: an outer fibrous layer and an inner cellular layer. The outer **fibrous layer** consists of collagen fibers and fibroblasts. At the regions where ligaments and tendons are attached to the bone, some bundles of collagen fibers extend from the periosteum into the bone matrix. These fibers are called *perforating fibers* or *Sharpey fibers*. The inner **cellular layer** of the periosteum contains osteoprogenitor cells.

The cavities within the bone are lined by **endosteum,** which is a thin layer of vascular loose connective tissue

containing osteoprogenitor cells. The endosteum lines the medullary cavities of the shafts of the long bones and the trabecular cavities of the spongy parts of the bones.

STRUCTURE OF THE BONE TISSUE

MACROSCOPIC STRUCTURE

On naked-eye examination, two types of bone tissue can be seen in the cut section of a bone:

1. **Compact bone**, appearing as dense areas without cavities.
2. **Cancellous** or **spongy bone**, in which the bone substance is in the form of slender spicules and trabeculae separated from each other by numerous interconnecting cavities. The spaces between the bony spicules and trabeculae are occupied by the bone marrow (Fig. 8.4).

In a long bone, the bone ends are composed of spongy bone covered by a thin shell of compact bone. Conversely, the shaft consists of a thick coat of compact bone surrounding a large central cavity called medullary cavity. However, a thin layer of spongy bone is present on the inner aspect of the compact bone, so that the medullary cavity is lined by spongy bone. The short bones consist of a core of spongy bone completely covered by a layer of compact bone. The flat bones of the skull are composed of two layers (tables) of compact bone, separated by a layer of spongy bone, which is called *diploe*.

MICROSCOPIC STRUCTURE (Fig. 8.2)

Microscopically the bone tissue can be classified into two main types: primary bone and secondary bone.

Primary Bone

This type of bone tissue is also called **immature bone** or **woven bone**. This is the first bone tissue to appear during embryonic (and fetal) development and during bone repair. It is characterized by the presence of abundant osteocytes and irregularly arranged collagen type I fiber bundles in the matrix. The mineral content of the matrix of primary bone is much lesser in amount than that of the secondary bone. The primary bone becomes replaced by the secondary bone except in a few places, e.g., the alveolar sockets of the teeth and the places where tendons of muscles are inserted into the bones.

Secondary Bone

This variety of bone tissue is also called **mature bone** or **lamellar bone.** The adult skeleton normally consists of this type of bone tissue. The secondary bone is characterized by an organized arrangement of collagen fibers. It is composed of thin layers (plates) of bone matrix called ***lamellae***. The bone lamellae range from 3-7 µm in thickness and are arranged either parallel to each other or concentrically around a vascular canal. The osteocytes are found, lying within their lacunae between the lamellae. The neighboring lacunae are connected with each other by narrow **canaliculi** that contain processes of osteocytes. The canaliculi form a network of channels that allow the flow of nutrients, ions, hormones and waste products between the osteocytes and nearby blood vessels. Within the canaliculi, the processes of each osteocyte make communicating junctions with those of the neighboring osteocytes; these junctions allow the neighboring osteocytes to communicate with each other.

HAVERSIAN SYSTEMS (OSTEONS)

The bulk of compact bone in the adults is composed of cylindrical subunits called *Haversian systems* or *osteons* (Fig. 8.2 & 8.3). Each **osteon** consists of 5-15 lamellae of bone matrix that are concentrically arranged around a longitudinally running central canal called ***Haversian canal*** which has an average diameter of 50 µm. The lamellae constituting the osteons are known as ***Haversian lamellae***. In each osteon the collagen fibers of a lamella run parallel to each other, pursuing a helical or spiral course. The direction of fibers in adjacent lamellae is such that the fibers of one lamella make an angle of 90 degrees with those of the next. This alternating arrangement in fiber direction explains why lamellae appear to be so distinct from one another in histological sections. Each osteon is surrounded by a thin layer of mineralized bone matrix which is deficient in collagen fibers. This layer forms the boundary of each osteon is called *cement line*. The cement lines stain strongly basophilic because of their high content of proteoglycans and glycoproteins.

Each Haversian canal is lined by a layer of osteoprogenitor cells and contains one or two capillary blood vessels, a few nerve fibers and a small amount of loose connective tissue.

Since Haversian canals run along the long axis of the bone, in cross sections these appear as round openings surrounded by concentric Haversian lamellae. In longitudinal sections of the bone, the Haversian canals appear as long slits bordered by columns of lamellae. The longitudinal sections also show that the Haversian canals of the osteon are connected to the marrow cavity, the periosteum and to each other by transverse or oblique channels called *perforating canals* (also called *Volkmann canals)*. The Volkmann's canals perforate the lamellae but, themselves, they are not surrounded by any concentric lamellae. The perforating canals also contain blood vessels (capillaries) which connect the blood vessels of the Haversian canals with each other and with the blood vessels present in the periosteum and in the marrow cavity.

LAMELLAE OF THE COMPACT BONE

It has been stated above that each osteon consists of several *Haversian lamellae* surrounding a haversian canal. The irregular intervals between the neighboring osteons are occupied by parallel lamellae of variable length, which are called *interstitial lamellae* (also called intermediate lamellae). Under the periosteum of the shaft of a long bone are present lamellae that run parallel to the external surface of the bone. These lamellae are known as *outer circumferential*

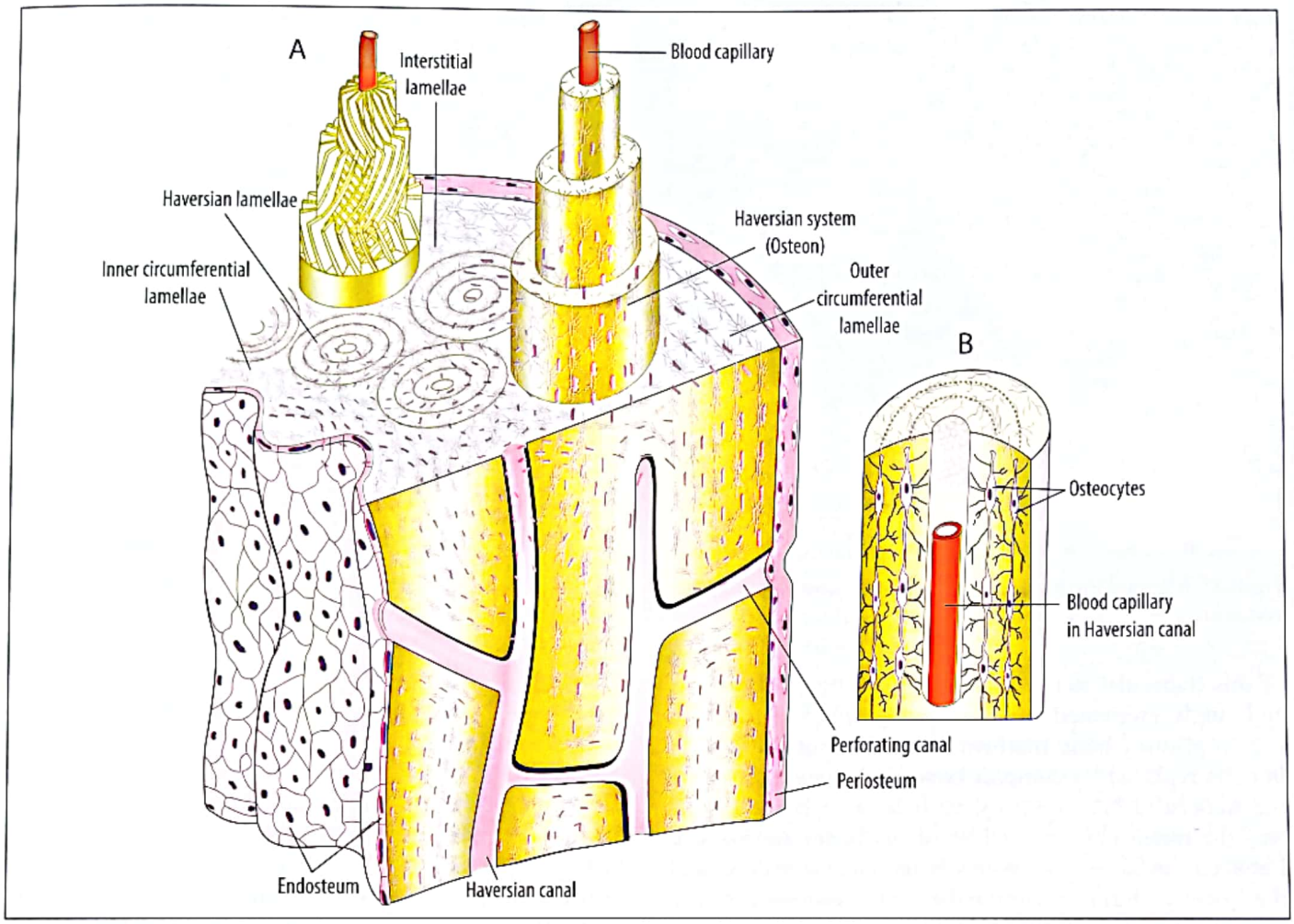

Fig. 8.2 A. Schematic drawing of the microscopic structure of the shaft of a long bone.
B. Diagram of an osteon showing a Haversian canal and Haversian lamellae. A blood capillary is present in the Haversian canal and several osteocytes are seen to be located in between the bone lamellae.

lamellae. Beneath the endosteum and encircling the marrow cavity are present *inner circumferential lamellae*, which are fewer in number than the outer circumferential lamellae.

BONE FORMATION

The process of bone formation is called **osteogenesis** or **ossification**. This process starts in the second month of embryonic life. Two different methods of bone formation are observed in the embryo: *intramembranous ossification* and *endochondral ossification*. In the intramembranous osteogenesis the bone is formed by replacing a membranous sheet of mesenchyme (embryonic connective tissue). In the endochondral osteogenesis, bone formation occurs by replacing a cartilaginous precursor of the bone. In both types of ossification, however, the manner of deposition of bone matrix is essentially the same. A description of the two methods of bone formation is given below.

INTRAMEMBRANOUS OSSIFICATION

The flat bones of skull, bones of the face, mandible, and clavicle develop by intramembranous ossification. This process begins in the 8th week of intrauterine life. In the areas where bones are needed to be formed by an intramembranous method, the mesenchyme becomes condensed in the form of sheets or membranes (hence the name *intramembranous* ossification). Each of such mesenchymal membrane becomes highly vascularized at several places by the ingrowth of capillaries which give rise to profuse networks. The mesenchymal cells in the center of vascularized region (called *primary center of ossification*) differentiate into osteoblasts. The osteoblasts in each primary center of ossification secrete osteoid (which is the immature, unmineralized bone matrix). The osteoid is deposited in the form of spicules and trabeculae whose surfaces are populated by osteoblasts. Soon after its formation, the osteoid becomes mineralized by the deposition of calcium phosphate crystals and, thus, mature bone matrix is formed. During this process, those osteoblasts which become trapped within the bone matrix become osteocytes. The osteoblasts on the surface of spicules and trabeculae secrete additional bone matrix, so that thicker bony trabeculae are formed.

As the bone formation progresses at several foci, a number of needle-like bony spicules are formed which progressively radiate from each of such foci. Consequently, the developing bone mass is initially spongy in nature and consists of trabeculae of bone tissue. The interstices

Fig. 8.3 A section through the compact bone showing Haversian systems.

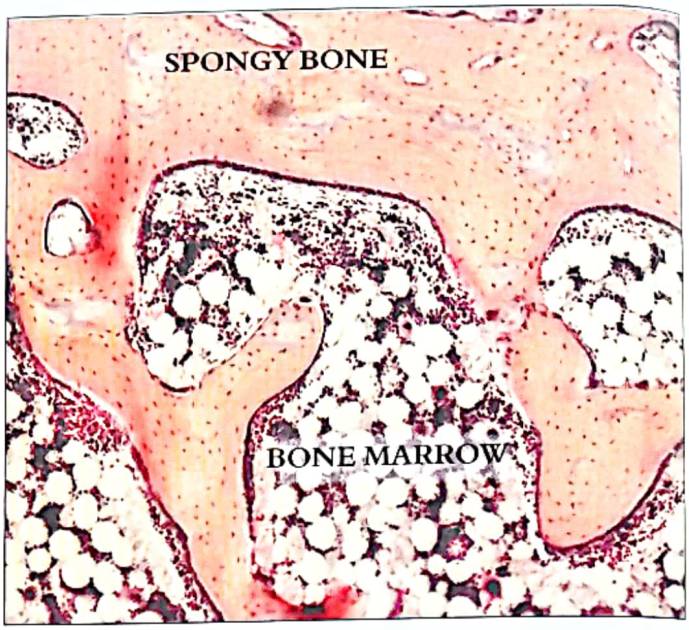

Fig. 8.4 A section through the spongy bone showing bony spicules. The space between the bony spicules is occupied by the bone marrow.

of this trabecular network are occupied by blood vessels and undifferentiated mesenchymal cells, which give rise to primary bone marrow. Later, some of the spongy bone is replaced by compact bone as the spaces between the trabeculae become filled with lamellar bone. In this way the inner and outer tables of flat bones are formed. Between the tables, the spongy bone remains as *diploe* and the spaces within it constitute the *primary marrow spaces*.

The entire primordium of the developing bone becomes surrounded by a thin layer of dense mesenchyme which gives rise to the periosteum.

ENDOCHONDRAL OSSIFICATION

Most of the bones of the body are formed by endo-chondral type of osteogenesis, which is also called **intracartilaginous ossification.** This process also begins in the 8th week of embryonic development. The endochondral type of bone formation involves three steps: (1) formation of miniature cartilaginous model of the bone, (2) continued growth of the cartilaginous model, which serves as a structural scaffold for bone growth, and (3) resorption and replacement of the cartilage by bone tissue.

Within each cartilaginous model of a developing bone, an orderly sequence of events occurs with the appearance of centers of ossification. These include a *primary center* which is located in the center of the shaft, and two or more *secondary centers*, which are located in the ends of the cartilaginous model. The primary centers usually appear in the third month of intrauterine life, while most of the secondary centers appear after birth (except the secondary center of ossification for the lower end of the femur which appears during the 9th month of the intrauterine life).

Course of Events in the Endochondral Ossification

Primary Center of Ossification

In the central region of the cartilaginous model of a bone, the chondrocytes undergo hypertrophy. As the osteocytes increase in size, their lacunae also become enlarged. The cartilage matrix forming the intervening walls of the lacunae becomes calcified. The cartilage cells now die and disappear leaving their enlarged lacunae vacant. Just at the time when these changes are occurring in the cartilage, the perichondrium around the primary center of ossification changes its character and becomes periosteum, i.e., the cells in its inner (cellular) layer transform into osteoblasts. These periosteal osteoblasts lay down a subperiosteal **bone collar** in the same way as described under intramembranous ossification (i.e., secretion of osteoid and its conversion into mature bone matrix by the deposition of mineral salts).

The bone collar surrounding the walls of empty and large lacunae of the calcified hyaline cartilage is invaded by vascular sprouts from the periosteum. These sprouts consist of blood capillaries, which are accompanied by mesenchymal cells. Some of these mesenchymal cells transform into osteoclasts. The osteoclasts excavate passages through the bony collar to allow the blood capillaries to pass into the underlying calcified cartilage in the primary center of ossification. The osteoclasts accompanying the blood capillaries erode the calcified cartilage matrix and produce an irregular system of intercommunicating spaces which are called *primary marrow spaces*. These spaces become filled with the embryonic bone marrow. The delicate walls of the primary marrow spaces, which are formed of calcified cartilage, become covered with a layer of osteoblasts (which are also derived from the mesenchymal cells). These osteoblasts lay down osteoid which is soon converted into mature bone matrix by the deposition of mineral salts.

In the developing long bones, deposition of subperiosteal bone continues, but formation of bone on the walls of the centrally placed primary marrow spaces stops and a

process of erosion begins. This results in the removal of early spicules of bone and enlargement and confluence of the primary marrow spaces until a primitive *medullary cavity* is produced in the center of the growing long bone.

The process of ossification gradually spreads from the primary center of ossification toward the peripheral parts of the developing bone and, progressively, the regions adjoining the primary center of ossification also undergo the above described sequence of changes. That part of a long bone which develops from the primary center of ossification (i.e., the shaft) is known as *diaphysis.*

Secondary Centers of Ossification

These centers appear in the cartilaginous peripheral parts of the developing bone and, here also, the cartilage is replaced by bone through the same sequence of events as described for the primary center of ossification. Those parts of a bone which develop from the secondary centers of ossification are called *epiphyses.*

GROWTH OF BONES

Growth in Length

The plate of hyaline cartilage intervening between the diaphysis and each epiphysis of a long bone is known as ***epiphyseal cartilage*** or **growth plate**. The growth plates are responsible for the longitudinal growth of the bone during childhood and early adult age. Starting from epiphyseal side, following five successive zones can be recognized in an epiphyseal cartilage:

1. Resting Zone

The zone is also known as the zone of reserve cartilage. In this zone, the chondrocytes are randomly distributed and show no cellular proliferation or active matrix secretion.

2. Proliferative Zone

In this zone the cartilage cells proliferate (i.e., divide mitotically) and become aligned in longitudinal columns. These cells produce cartilage matrix actively. It is the continuous mitotic division of cartilage cells in this zone which is responsible for the longitudinal growth of the bone.

3. Zone of Cartilage Hypertrophy

In this zone the cartilage cells undergo hypertrophy (i.e., increase in size) with corresponding enlargement of their lacunae. Enlargement of lacunae reduces the cartilage matrix to thin septa between the columns of enlarged chondrocytes.

4. Zone of Cartilage Calcification

In this zone the hypertropvhied chondrocytes die and disappear. Simultaneously, the cartilage matrix (present as septa and spicules) becomes calcified by the deposition of calcium phosphate crystals.

5. Zone of Ossification

This zone lies adjacent to the diaphysis. Blood capillaries and osteoprogenitor cells invade thin zone from the diaphyseal side. The osteoprogenitor cells differentiate into osteoblasts which become aligned on the spicules of the calcified cartilage matrix. The osteoblasts deposit bone matrix on the surface of the calcified cartilage. As more and more matrix is deposited, the calcified cartilage in the core of the spicules undergoes resorption and finally disappears.

When adequate size of the bone has been achieved and no more increase in length is required, the chondrocytes of the epiphyseal cartilages cease to divide mitotically and very soon the whole growth plate becomes replaced by bone, so that each bony epiphysis becomes fused with the bony diaphysis. Fusion of the epiphyses with the diaphysis generally occurs during the adolescence.

As a general rule, the epiphysis which is last to appear is the first to fuse with the diaphysis and, conversely, the epiphysis which appears first of all, joins the diaphysis last of all. When the epiphyses at the both ends of a long bone have fused with the diaphysis, the growth of the bone in length is complete and no increase in the length of the bone is possible in the later life.

Growth in Diameter

Growth of the long bones in diameter occurs by appositional growth. In a growing bone the osteoprogenitor cells in the inner layer of periosteum continuously divide and differentiate into osteoblasts that deposit a new layer of bone matrix on the pre-existing surface. This process of deposition of layers of compact bone under the periosteum occurs continuously throughout the growth of the bone.

Deposition of bone matrix on the subperiosteal surface of a long bone is accompanied by resorption of bone on the endosteal surface by the activity of the osteoclasts. This results in the enlargement of the marrow cavity. Well-balanced deposition of bone on the outer surface and resorption of the inner surface continue until the optimal diameter of the adult bone has been attained.

BONE REMODELING

The bones undergo continuous remodeling by deposition of osseous tissue at certain sites and resorption at others. This process, that allows a bone to maintain its shape, is called bone remodeling. This is a very active process in growing children. However, bone remodeling continues in the adult life, though at a much slower pace. In the adult the bones remodel themselves to meet the stresses to which they are subjected during the life of the person.

Bone Repair After Injury

A break or rupture in a bone is called a *fracture.* Such an injury causes destruction of the bone matrix and death of the osteocytes in the bone tissue adjoining the fracture site. The hemorrhage (bleeding) occurring from the damaged blood vessels produces a blood clot at the site of the injury. This blood clot is soon invaded by blood capillaries, fibroblasts and osteoprogenitor cells from the surrounding connective tissue (periosteum). New loose connective

collagenous fibers. In cross sections, the fibroblasts give a star-shaped (stellate) appearance with plate-like extensions between the bundles of collagen fibers. The substance of a tendon or ligament is generally subdivided into fascicles which are surrounded by loose connective tissue containing blood capillaries and fine nerves of the tendon. The loose connective tissue surrounding each fascicle of collagenous bundles is called *endotendineum*. Externally, the tendon is covered by a thin layer of dense irregular connective tissue, which is called *epitendineum*.

Aponeuroses

These structures are actually broad, flattened tendons that attach sheet-like muscles to the bones. In an aponeurosis the collagen fibers are usually arranged in multiple layers. Within an individual layer, the collagen fiber bundles run parallel to each other. However, direction of fibers changes in successive layers. Some collagen fibers cross over between the adjacent layers and thus prevent the separation of layers from one another.

DENSE REGULAR ELASTIC CONNECTIVE TISSUE

This variety of dense connective tissue occurs in the form of **elastic ligaments** which are composed of bundles of unusually thick elastic fibers arranged parallel to one another. Collagenous fibers and fibroblasts are also found between the elastic fibers. In human beings, the best examples of the elastic ligaments are: the true vocal cords, ligamenta flava of the vertebral column, and suspensory ligament of the penis.

RETICULAR CONNECTIVE TISSUE

This variety of connective tissue proper consists of *reticular fibers* and *reticular cells*. The **reticular fibers** are made up of collagen type III and are arranged in the form of a mesh-like network (Fig. 6.1). The **reticular cells** are considered to be a special variety of fibroblasts. These cells have a stellate shape and possess long processes which pass in different directions to make contact with those of the neighboring cells. Most of the protoplasmic processes of the reticular cells are wrapped around or extend along the reticular fibers. This specific arrangement creates a special trabecular system that forms the architectural framework of hemopoietic and lymphoid organs (bone marrow, spleen and lymph nodes). The spaces within the trabecular meshwork are occupied by scanty ground substance and cells, which are lymphocytes in the lymphoid organs, and precursors of blood cells in the bone marrow.

ADIPOSE TISSUE

Adipose tissue consists of adipocytes (fat cells) associated with abundant blood vessels (capillaries). As discussed earlier, individual adipocytes and small groups of adipocytes are found throughout the loose connective tissue. However, large aggregates of adipocytes are designated *adipose tissue*.

The fat within the adipocytes represents the excess nutritional calories which are kept in store and used whenever required. Thus, the adipose tissue constitutes the largest storehouse of energy in the body (the liver and skeletal muscles have a limited capacity to store energy as glycogen). In adipocytes the fat is stored as triglycerides.

There are two types of adipose tissue in the human body, which differ in color, vascularity, metabolic activity and distribution. These two types are: white adipose tissue and brown adipose tissue. The white adipose tissue is the predominant type in humans. The brown adipose tissue is present in considerable quantity in the fetus and newborn infants, but it diminishes during the first decade of life and is greatly reduced in amount in the adult humans. Both types of adipose tissue are richly supplied with capillaries and postganglionic fibers of the sympathetic nervous system. In the white adipose tissue, these fibers end on the walls of blood vessels, whereas in the brown adipose tissue the sympathetic nerve fibers end on the blood vessels as well as adipocytes.

WHITE ADIPOSE TISSUE (Fig. 6.2)

The color of this type of adipose tissue varies from white to light yellow. Histologically, the white adipose tissue is characterized by the presence of adipocytes which contain only a single fat droplet and are called ***unilocular adipocytes***. These cells have a large size and measure 50-150 µm in diameter.

Isolated adipocytes have a spherical shape but appear polyhedral in the adipose tissue because of close packing of cells. Most of the space within an adipocyte is occupied by the large fat droplet and, consequently, the remaining cytoplasm occupies the peripheral region of the cell and, in stained sections, appears as a thin rim around the large fat droplet. The nucleus of the adipocyte is also displaced to one side of the lipid droplet and is almost flat in shape. In routine H&E stained tissue sections the fat is dissolved out and the space previously occupied by the fat droplet appears as a colorless rounded or oval area. Consequently, the cytoplasm and the nucleus of the unilocular adipocyte are seen as a rim around the central empty space. The eosinophilic cytoplasm and the basophilic nucleus of the cell together exhibit a typical *signet-ring appearance* (Fig. 6.3A).

Each adipocyte is surrounded by a thin external lamina (which is analogous to the basal lamina of the epithelial cells). A fine network of reticular fibers supports each adipocyte and binds it to its neighbors. Thin connective tissue septa divide the white adipose tissue into incomplete lobules.

The white adipose tissue is widely distributed in the human body. It is present in the subcutaneous tissue as a layer called *panniculus adiposus*. This layer acts as an insulator under the skin and, by preventing heat loss from the body, plays an important role in the temperature regulation of body, especially in the newborn babies and infants. In the adults, large collections of white adipose tissue

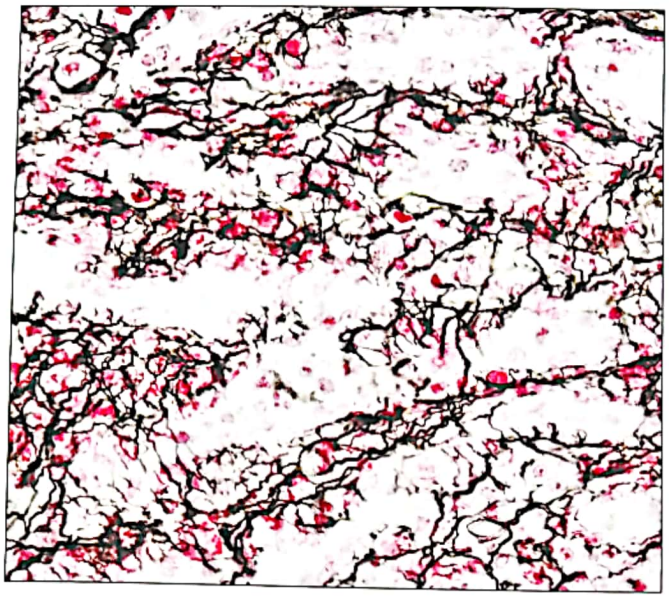

Fig. 6.1 Reticular connective tissue in the red pulp of spleen (silver-staining technique).

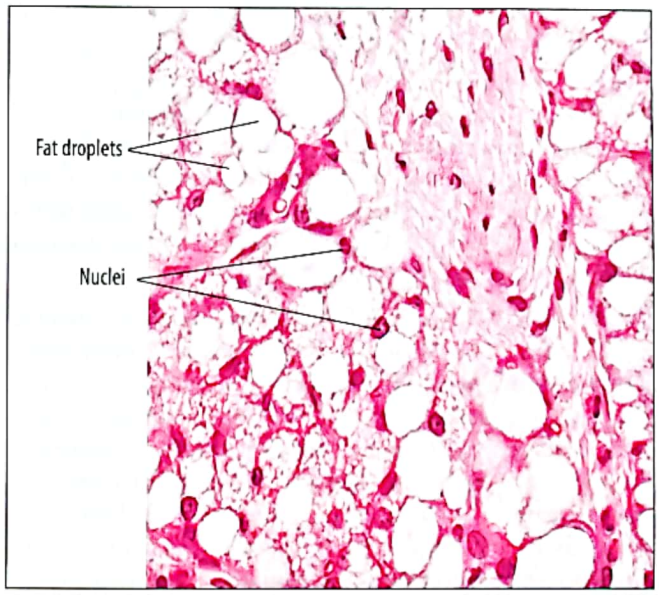

Fig. 6.2 A tissue section showing white adipose tissue. Note that a single fat droplet is present in each fat cell and nucleus of each cell is pushed to its periphery.

are found in the subcutaneous tissue of abdominal wall, axilla, buttocks and thighs. Internally, the fat is stored in both sexes in the greater omentum, mesentery, and in the retroperitoneal space, especially around the kidneys where it provides mechanical protection by forming 'padding' around these vital organs. In certain locations collections of white adipose tissue serve as shock absorbing cushions, e.g., in palms, soles and buttocks.

The white adipose tissue is the chief site of storage of excess energy in the body. When the body is in well-fed state, the fat cells store the excess energy as triglycerides. In the fasting or starving state, the triglycerides are split into the glycerol and fatty acids which are released into the blood. The fatty acids so released serve as a source of energy for the body cells.

BROWN ADIPOSE TISSUE

The brown adipose tissue consists of multilocular adipocytes, which store fat in their cytoplasm as multiple small fat droplets. Upon naked eye examination, this tissue ranges from tan to reddish brown in color because of two factors: (1) being richly vascular, the brown adipose tissue contains a large number of blood capillaries, (2) the adipocytes of brown adipose tissue possess exceptionally abundant mitochondria, which contain the colored enzymes cytochromes*.

The adipocytes of brown adipose tissue appear polygonal in sections and have a considerably smaller diameter than that of the adipocytes of white adipose tissue. The cytoplasm is also relatively abundant and contains numerous fat droplets of various sizes (Fig. 6.3 B). The nucleus is spherical

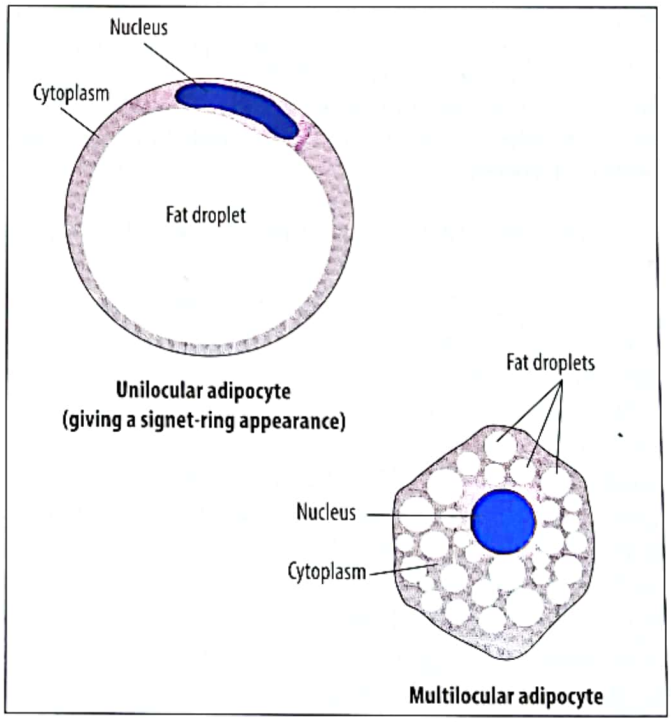

Fig. 6.3 Diagram showing the structural features of a unilocular adipocyte and a multilocular adipocyte.

in shape and eccentric in position (but not pushed to the periphery of the cell). Numerous mitochondria, a small Golgi apparatus and small amounts of SER and RER are also present.

Connective tissue septa divide the brown adipose tissue into well-defined lobules. These septa convey blood vessels and sympathetic nerve fibers to the tissue. A special feature of the multilocular adipocytes is that they receive direct sympathetic innervation.

* Cytochromes are electron-transfer hemoproteins that take part in oxidative phosphorylation. Because of their conjugation with iron, cytochromes impart a reddish tinge to the structures in which they are present in large quantities.

tissue called *granulation tissue* is formed at the site of the fracture. As fibroblasts continue to produce collagen fibers, this tissue becomes dense. Simultaneously, some of the periosteal cells differentiate into chondroblasts, which form hyaline cartilage in this connective tissue. This mass of connective tissue and cartilage, that fills the fracture site and surrounds the fractured ends of the bone, is called a **fibrocartilage callus.** The osteoprogenitor cells of the periosteum and endosteum undergo proliferation and differentiate into osteoblasts. These osteoblasts deposit bone in the peripheral part of the fibrocartilage callus. Later on, bone deposition starts in the central part of the callus and the fibrocartilage callus is gradually replaced by a **bony callus.** This callus consists of trabeculae of primary bone uniting the broken bone fragments. The spongy primary bone of the bony callus is gradually replaced by the secondary bone which is compact in nature. The excess bone formed at the fracture site is soon resorbed, so that the continuity of the marrow cavity is re-established and normal surface contours of the bone are once again restored.

JOINTS

A union between two or more bones is known as a joint. Depending on their structural features, the joints can be immovable, slightly movable, or freely movable. Structurally, the joints are classified into three types: fibrous joints, cartilaginous joints, and synovial joints.

FIBROUS JOINTS

In these joints the bones are held together by fibrous connective tissue. The fibrous joints are further classified into three varieties: suture, syndesmosis, and gomphosis.

The **sutures** (or *sutural joints*) are found between the flat bones of the skull. In these joints the margins of adjacent flat bones are joined to each other by thin bands of dense fibrous connective tissue. This connective tissue has osteogenic properties and, in the adult person, the fibrous tissue of the sutural joints becomes gradually replaced by osseous tissue, so that the suture disappears and the adjacent bones fuse with each other; this process is called synostosis.

The **syndesmosis** variety of the fibrous joints is characterized by the presence of a dense fibrous interosseous ligament between the articulating bones. The best example of a syndesmosis is the inferior tibiofibular joint.

The **gomphosis** joints are peg-and-socket joints found only between the roots of the teeth and their alveolar sockets. The roots of the teeth are covered by a special type of tissue called *cementum*. The cementum and the bone of the alveolar socket of each tooth are joined by a layer of fibrous connective tissue known as periodontal ligament (also called periodontal membrane). The joining medium is dense fibrous connective tissue (called in this location as *periodontal ligament*).

CARTILAGINOUS JOINTS

In these joints the articulating bones are joined to each other by cartilage. These joints are further classified into two subtypes: primary cartilaginous joints and secondary cartilaginous joints.

The **primary cartilaginous joints** (also called *synchondroses*) are formed between the epiphyses and diaphysis of the growing endochondral bones. In these joints, the bony masses are joined to each other by plates of the hyaline cartilage called growth plates. The synchondroses are temporary joints and become eliminated when the growth plates disappear and the epiphyses and diaphysis of a growing bone become fused with each other.

In the **secondary cartilaginous joints** (also called symphyses) the bones are joined to each other by fibrocartilage, e.g., the pubic symphysis. Unlike the synchondroses, the symphyses are permanent joints.

SYNOVIAL JOINTS

These are freely movable joints, in which the articulating bones are joined by a capsule of dense fibrous connective tissue. The articular surfaces of the bones forming the joint are covered by a layer of hyaline cartilage called *articular cartilage*. The inner surface of the joint capsule is lined by a membrane called *synovial membrane,* which secretes a lubricating fluid called *synovial fluid* which fills the joint cavity.

Synovial Membrane

The synovial membrane, also called synovium, is a layer of connective tissue that lines the inner surface of synovial joint capsules. In addition, it also lines the inner surface of the bursae and tendon sheaths.

Two types of cells are found in the **synovial membrane**: type A cells and type B cells. The **type A cells**, also called *macrophage-like synovial cells*, are phagocytic cells that perform the function of removal of unwanted particulate matter (like cartilage debris or bacteria) from the joint cavity. The **type B cells**, also called *fibroblastic synovial cells*, are much more numerous than the type A cells. These cells are involved in the secretion of some components of the synovial fluid.

Synovial Fluid

The synovial fluid, also called *synovia*, is a transparent, pale-yellow, highly viscid fluid found in the cavities of the synovial joints. It is an ultrafiltrate of the blood plasma to which are added secretions of the type B cells of the synovium which contain the nonsulfated glycosaminoglycan hyaluronan (hyaluronic acid).

The chief functions of the synovial fluid include lubrication of the articulating bone surfaces (to reduce friction between them), shock absorption, provision of oxygen and nutrients (from the blood capillaries underlying the synovium) to the cells of the articular cartilage, and transportation of the CO_2 and metabolic wastes in the reverse direction.

Blood 9

The blood is a special type of connective tissue that is composed of several types of cells and also cell-fragments suspended in a fluid matrix called ***plasma***. The blood cells and cell-fragments are known collectively as ***formed elements*** of blood. The formed elements are classified into three main categories: (1) erythrocytes, (2) leukocytes, and (3) platelets. The relative volume of the formed elements and plasma is 45% and 55%, respectively.

PLASMA

Centrifugation of the blood reveals that the plasma is a yellowish, homogeneous fluid. The major component of the blood plasma is water, which constitutes about 90% of its volume. Remaining 10% of the plasma volume consists of various solutes dissolved in the water component of the plasma. About 80% of these solutes consist of proteins (mainly albumin, globulins, and fibrinogen), while the remaining 20% consist of various nutrients (glucose, amino acids and lipids), inorganic salts, electrolytes, nitrogenous compounds (mainly urea and creatinine), hormones, enzymes, and dissolved gases (oxygen, carbon dioxide and nitrogen), etc.

FORMED ELEMENTS OF BLOOD

The formed elements of blood are: (1) erythrocytes or red blood cells **(RBC)**, (2) leukocytes or white blood cells **(WBC)**, and (3) **platelets** or *thrombocytes*, which are cytoplasmic fragments derived from large cells called megakaryocytes (which reside in the bone marrow).

Staining of Blood Cells

Blood smears are usually stained with a mixture of methylene blue (a basic dye), eosin (an acidic dye), and azures (which are also basic dyes). The dye mixtures commonly used for staining blood smears are Wright stain, Giemsa stain, Leishman stain, and Romanovsky stain; out of these, the Wright stain is most commonly employed in the laboratories.

After staining with any of the above mentioned stains, four staining characteristics can be distinguished, representing the affinity of various blood cell contents for the respective dyes of the mixture: (1) the affinity for methylene blue (a basic dye) is known as basophilia; the basophilic structures in the blood cells are stained purplish blue , (2) the affinity for eosin (an acidic dye) is known as acidophilia or eosinophilia; the eosinophilic structures in the blood cells are stained pink, reddish or orange, (3) affinity for the azures is known as azurophilia; the azurophilic structures take up a light blue color, and (4) affinity for a neutral dye complex, formed by the combination of the acidic eosin dye with the basic dyes (methylene blue and azures) is known as neutrophilia. The neutrophilic structures take up a pale pinkish- purple color (lilac color).

Morphological features (and functions) of various formed elements of blood (erythrocytes, leukocytes and platelets) will now be described. This will be followed by a description of the process of development of these elements which is called *hemopoiesis*.

ERYTHROCYTES

The erythrocytes or red blood cells (RBC) are the most numerous cells of the blood. A mature RBC is shaped like a circular, biconcave disc (Fig. 9.2). It does not contain a nucleus and is also devoid of typical cell organelles. Under normal conditions, the RBCs do not leave the circulatory system.

In the living state, an erythrocyte measures about 7.5 µm in diameter. Due to its biconcave form, it has a thickness of 2.6 µm at the periphery (rim), but is only 0.8 µm thick in the central region. In blood smears stained by the Wright stain, the erythrocytes are seen as circular bodies which stain pinkish in color (Fig. 9.1).

The red blood cells develop in the bone marrow as true cells. However, before entering the blood they extrude their nucleus and lose various cell organelles including the mitochondria. Subsequently, the erythrocytes generate energy by anaerobic glycolysis.

Main content of the cytoplasm of an erythrocyte is the iron-containing metalloprotein *hemoglobin,* which has the capability to combine with the oxygen molecules to form oxyhemoglobin and with carbon monoxide molecules to form carbaminohemoglobin. These combinations are, however, reversible due to which the which the hemoglobin acts as a gas-transporting medium. In the circulating blood, an RBC gives a reddish tinge because the hemoglobin in the cytoplasm of the erythrocyte turns red when it binds to oxygen. In dense masses, the erythrocytes give the blood its characteristic red color.

The biconcave shape of the erythrocytes facilitates the gaseous exchange by increasing the surface area of the cells and by bringing the plasma membrane of the cell close to the hemoglobin molecules present in the cytoplasm of the erythrocyte. The erythrocytes have a very pliable and elastic body due to which they are capable of considerable distortion in shape. This is evident from their capability to fold themselves to pass through the small capillaries which have lesser caliber than the diameter of the erythrocytes.

The RBCs are enveloped by a specialized cell membrane which performs several important functions peculiar to the

erythrocytes: (i) it imparts flexibility to these cells, (ii) it prevents adhesion of the erythrocytes to the endothelium of the blood vessels and to other blood cells, and (iii) it determines the antigenic properties of the erythrocytes (which form the basis of blood grouping).

Erythrocytes have an average life span of 120 days. The old and worn out erythrocytes are trapped, engulfed and degraded mainly by the phagocytic cells of the spleen and liver. To keep the RBC count in the circulating blood within the normal range, new erythrocytes are constantly produced in the red bone marrow (at the approximate rate of 2 million RBC per second).

Erythrocytes are the most numerous of the formed elements of blood. Average count of RBC in a normal adult person is about 5 million per microliter of blood. Women generally have a slightly lower total red cell count than men. The percent volume of blood occupied by the erythrocytes is called *hematocrit*. The normal value of hematocrit is 45%; a low hematocrit indicates anemia.

Reticulocytes

When stained with special dyes, e.g., cresyl blue, the cytoplasm of a few erythrocytes of the peripheral blood exhibits a bluish reticulum or a small number of bluish granules. Such cells, known as *reticulocytes*, represent the immature RBC in the peripheral blood. The reticulated appearance is produced by the basophilic staining of the residual ribosomes, which are still present in these immature erythrocytes. After entering the bloodstream, the newly produced erythrocytes undergo maturation and lose their basophilia within 24 hours. In a normal person the blood reticulocyte count is not more than 2 percent of the total number of circulating erythrocytes. Clinically, the reticulocyte count is used as a rough index of rate of erythrocyte production (a higher reticulocytes count in the blood indicates increased rate of production of RBC).

Anemia a medical condition that occurs due to a decrease in the number of erythrocytes in the circulating blood or due a reduction in the hemoglobin content of the erythrocytes, or both. Consequently, the oxygen-carrying capacity of the blood is reduced. The major clinical signs and symptoms of anemia include skin pallor, shortness of breath, and a feeling of being very tired or weak.

LEUKOCYTES

The leukocytes or white blood cells (WBC) are nucleated cells which are capable of ameboid movement. They are generally larger than the erythrocytes and their cytoplasm does not contain any hemoglobin. The primary function of the leukocytes is defense of the body against bacteria, viruses, parasites, and foreign proteins. They carry out their functions in the connective tissues. They arise, function, and die outside the bloodstream, using the blood circulation merely as means of transportation from their place of origin (i.e., bone marrow) to their destination (i.e., connective tissue).

In the adults, the normal **total leukocyte count (TLC)** ranges from 4,000 to 11,000 cells per microliter of blood; the count is higher in children. The number of leukocytes in the blood may increase or decrease under certain diseased conditions. A temporary increase in the number of leukocytes is called *leukocytosis*, whereas a reduction in the number of leukocytes below the lowest normal range is called *leukopenia*.

The leukocytes are classified into two main groups: (i) granulocytes, and (ii) agranulocytes.

1. **The granulocytes** are those WBC which contain specific granules in their cytoplasm.
2. **The agranulocytes** are those leukocytes which do not contain any specific granules in their cytoplasm.

However, it is important to note that both granulocytes and agranulocytes contain non-specific azurophilic granules in their cytoplasm, which represent the lysosomes of the cell.

GRANULOCYTES

The granulocytes are characterized by the presence of specific granules in their cytoplasm. They also have irregularly shaped nuclei.

According to staining reaction of the cytoplasmic granules, the granulocytes are classified into the following three types:

1. Neutrophils
2. Eosinophils
3. Basophils

NEUTROPHILS

The neutrophils constitute the most numerous variety of leukocytes, making 40-75% of the total WBC. They measure 9-12 μm in diameter in blood smears. The nucleus of a neutrophil shows a variety of forms, usually appearing as 3 to 5 irregularly ovoid masses (lobes) of chromatin connected to each other by narrow constrictions or fine strands of chromatin. The multi-lobed nucleus may be U-shaped, S-shaped, or irregular in appearance (Fig. 9.1). Because of the variable appearance of their nuclei, the neutrophils are also known as **polymorphonuclear leukocytes** (sometimes abbreviated as polymorphs or simply *polys*). In females, the inactive X-chromosome (Barr body) is present as a small drumstick-shaped appendage on one of the lobes of the nucleus of a neutrophil (Fig. 9.2). However, the appendage is not obvious in all the cells and can only be distinguished in about 3 percent of the neutrophils studied in a stained blood smear.

The major part of the cytoplasm of a neutrophil is occupied by three types of granules: ***azurophilic granules***, ***specific granules***, and ***tertiary granules***.

The **azurophilic granules** or ***primary granules*** are

actually lysosomes. They are larger in size but less numerous than the specific granules. Average diameter of azurophilic granules is about 0.5 µm. These granules stain more deeply than the specific granules and take a reddish purple color with Wright stain. The azurophilic granules (lysosomes) of the neutrophils contain myeloperoxidase and various acid hydrolases, which enable the neutrophil to digest the phagocytosed bacteria.

The **specific granules** or ***secondary granules*** are the most abundant of three types of granules. They are smaller in size than the azurophilic granules and measure about 0.2 µm in diameter. These granules take a faint pinkish-purple color with the Wright stain. The specific granules contain a number of enzymes and pharmacological agents, which aid the neutrophil in killing bacteria. The contents of the specific granules include: collagenase type IV, phospholipase, lactoferrin, lysozyme, and alkaline phosphatase. At the site of an injury or infection, the activated neutrophils also secrete cytokines and chemokines which attract other leukocytes and also direct the leukocytes and local tissue cells to begin the process of repair and restoration of the damaged tissue to its normal condition.

The **tertiary granules** of the neutrophils mainly contain various gelatinases and collagenases. These enzymes are released from the neutrophils to degrade the extracellular matrix of the connective tissue, so that the migration and movement of the neutrophils through the connective tissues is facilitated.

In addition to the three types of granules described above, the cytoplasm of a neutrophil contains a few mitochondria, a small Golgi apparatus, some vesicles of smooth endoplasmic reticulum, and a few ribosomes.

Functions of the Neutrophils

The neutrophils constitute the first line of defense against invading microorganisms especially bacteria. They are attracted to the site of infection by complement fragment C5a and the cytokine leukotriene B4, which are released at the site of tissue damage. Neutrophils usually reach the site of infection within 30 minutes. The histamine and heparin, released at the site of injury by the perivascular mast cells, increase the permeability of the capillaries by opening the intercellular junctions between the endothelial cells, and thus allow the neutrophils to migrate into the connective tissue. Upon reaching the site of infection, the neutrophils become actively phagocytic and begin to engulf bacteria.

The phagocytosed bacteria are first acted upon and killed by alkaline phosphatase, lysozyme, phospholipase and lactoferrin. Later on, the azurophilic granules contribute their myeloperoxidase and other hydrolytic enzymes which completely destroy the bacteria. In this process, a large number of neutrophils die and result in the formation of pus. The ***pus*** is a viscous, usually yellowish fluid that consists of dead leukocytes, dead bacteria, cellular debris and tissue fluid.

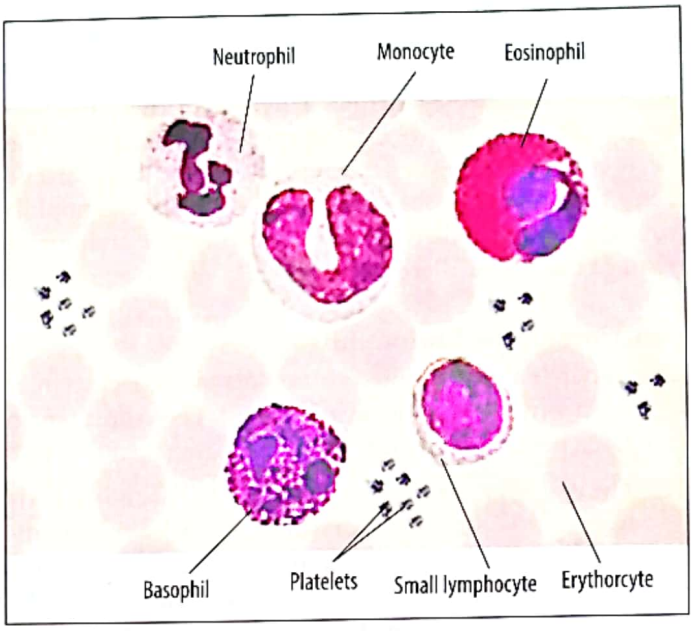

Fig. 9.1 Peripheral blood smear showing different types of blood cells. Clumps of platelets are also seen.

EOSINOPHILS

The eosinophils are slightly larger than the neutrophils and have a diameter of 10–14 µm. Under normal conditions, the eosinophils constitute only 1 to 3% of the total leukocytes. Although their number in the circulating blood is low, the eosinophils are found to be abundant in the loose connective tissue of the mucosa of the digestive, respiratory, and lower urinary tracts. They are also found in large numbers at the sites of chronic inflammation, e.g., in the lung tissues in patients suffering from asthma.

In stained blood smears, the eosinophils are characterized by two principal features: (1) their cytoplasm appears to be packed by large eosinophilic granules which exhibit a distinct red to orange color, and (2) each eosinophil has a characteristic bilobed nucleus, consisting of two large, globular lobes connected by a thin intermediate segment; in some cells the intermediate segment may be so thin that it appears strand-like (Fig. 9.1 & 9.2).

The large *eosinophilic granules* are the specific granules of these cells. Electron microscopic examination shows that these granules are roughly oval in shape and each has a flat, crystal-like core. Studies have revealed that these granules mainly contain four proteins that have a strong cytotoxic effect on multicellular parasites like helminths (worms). These proteins are: major basic protein (MBP), eosinophilic cationic protein (ECP), eosinophil-derived neurotoxin (EDN), and eosinophil peroxidase (EPO). In addition, the eosinophils also release several enzymes (like histaminase and arylsulfatase), many cytokines (like interleukins), and lipid mediators (like prostaglandins and leukoterienes) which serve to regulate and modulate the inflammatory response initiated by allergic or hypersensitivity reactions of different types.

The eosinophils also contain *azurophilic granules*, which are lysosomes containing various hydrolytic enzymes. These

enzymes carry out degradation of the antigen-antibody complexes phagocytosed by the eosinophil.

Whenever there is an allergic or hypersensitivity reaction to any antigen in the body, the mast cells present in the local connective tissue release eosinophil chemotactic factor (ECF). Under the influence of ECF, the eosinophils leave the blood capillaries and migrate into the connective tissue at the site of allergic reaction.

Functions of the Eosinophils

Like other leukocytes, the eosinophils also execute their functions in the connective tissues. The three main functions performed by the eosinophils are given below:

1. The eosinophils play a major role in defense of the body against multicellular parasites especially helminths (worms). At the site of a helminthic infection, the eosinophils degranulate and release MBP, ECP, EDN, and EPO, which are highly effective in killing the helminths.
2. The eosinophils control and modulate allergic reactions by releasing many cytokines and enzymes. Especially important in this regard is the enzyme histaminase which neutralizes and inactivates the histamine produced by the mast cells and basophils. The arylsulfatase breaks down leukotrienes released by the mast cells and by eosinophils themselves. Thus, the eosinophils serve to regulate the allergic reactions, so that the effects of the mediators of inflammation do not reach dangerous limits.
3. The eosinophils phagocytose the antigen-antibody complexes that are formed as a result of the allergic reactions. These complexes are degraded by the lysosomal enzymes of the eosinophil.

The count of eosinophils is unusually high in the blood of those individuals who are having helminthic infections (worm infestations). The eosinophil count is also high in persons suffering from allergy due to exposure to some allergen, e.g., pollen. The condition of having abnormally increased eosinophils in the peripheral blood is called *eosinophilia*.

BASOPHILS

The basophils are the least numerous of the white blood cells, constituting only 0.5-1% of the total leukocytes. They measure 8-10 μm in diameter in blood smears.

The nucleus of a basophil is relatively large in size and divided into irregular lobes. However, it cannot be easily seen in stained smears because it is masked by the large specific granules present in the cytoplasm (Fig. 9.1 & 9.2).

Like other granulocytes, the basophils also contain ***specific granules*** and ***azurophilic granules.*** The latter are lysosomes that contain various acid hydrolases similar to those of neutrophils.

The specific granules of the basophils are large in size, stain strongly basophilic, and are so numerous that they nearly fill the cytoplasm. These granules contain a variety of substances which include histamine, heparin, heparan sulfate, interleukin-4, interleukin-13, and some leukotrienes. These granules also contain eosinophil chemotactic factor, platelet activating factor, and phospholipase. The strong basophilia of the specific granules of the basophils is due to the presence of sulfated glycosaminoglycans heparin and heparin sulfate.

Functions of the Basophils

The basophils are functionally related to the mast cells of the connective tissue. The plasma membrane of the basophils contains Fc receptors which have affinity for IgE antibodies. When these antibodies bind with the Fc receptors, the contents of the specific granules of the basophils are released into the extracellular space. Along with the mast cells, the basophils take part in the production of the hypersensitivity reactions.

Although functionally related to each other, the basophils and mast cells are not the same. Not only the basophils differ in structure from the mast cells, but also the two cells originate from different stem cells in the bone marrow.

AGRANULOCYTES

The agranulocytes are comparatively undifferentiated cells and can reproduce by mitosis in connective tissues or in blood forming organs. The agranulocytes do not exhibit any specific granules in their cytoplasm. Each of them contains a large spherical or kidney-shaped nucleus. The agranulocytes are of two types: (1) lymphocytes, and (2) monocytes.

LYMPHOCYTES

The lymphocytes are basically the cells of the immune system of the body. Besides circulating in the peripheral blood, the lymphocytes enter the tissues and also form the major cell component of the lymph (hence the name *lymphocytes*). In addition to the circulating lymphocytes of the blood and lymph, vast numbers of lymphocytes are found in the parenchyma of the thymus, spleen, lymph nodes, and other lymphoid organs.

The lymphocytes constitute 20-40% of the total leukocytes in the adult. However, the lymphocytes count in the children is higher, being 30-70% of the circulating leukocytes in the peripheral blood. The higher number of lymphocytes in children is related to the development of immunity against various infections.

The lymphocytes in the circulating blood are loosely classified into two types: small lymphocytes and large lymphocytes. The *small lymphocytes* have approximately the same diameter as that of the erythrocytes (7.5 μm) and constitute about 90% of the circulating lymphocytes. The remaining 10% of the circulating lymphocytes are *large lymphocytes*, which generally range from 9 to 20 μm in diameter. The large lymphocytes either represent the activated lymphocytes (i.e., those lymphocytes which

have reacted with a specific antigen), or belong to a special variety of lymphocytes called *natural killer cells* (described later).

The lymphocytes are generally spherical cells containing a large, round nucleus. The large nucleus occupies major portion of the cell volume and the cytoplasm is seen just as a thin rim around the nucleus (Fig. 9.1 & 9.2).

The **nucleus** of a lymphocyte is generally spherical in shape but may have a slight indentation on one side. It is densely packed with coarse clumps of heterochromatin and, therefore, takes a deep purplish blue color in stained blood smears or tissue sections. The **cytoplasm** of a lymphocyte appears as a thin, light blue rim surrounding the nucleus. It lacks any specific granules, but some azurophilic granules, representing the lysosomes, can be seen. The cytoplasm also contains a small Golgi complex, a few mitochondria, some profiles of RER, and abundant free polyribosomes. Because most of the cell volume of a lymphocyte is occupied by the nucleus, the lymphocytes just appear as purplish blue dots in the stained smears and tissue sections examined under the low power of the light microscope.

Functional Varieties of Lymphocytes

On the basis of their functions, the lymphocytes are classified into three varieties:

1. T lymphocytes
2. B lymphocytes
3. NK cells

Although these three types of lymphocytes are morphologically indistinguishable from each other, they can be identified by immunocytochemical techniques. These techniques reveal that the three varieties of lymphocytes differ from each other in possessing distinctive protein molecules on the external surface of their plasmalemma. These molecules are called **CD markers** (cluster of differentiation markers). In the circulating blood, approximately 70% of the lymphocytes are T cells, 25% are B cells, and 5% are NK cells.

Although all the three varieties of the lymphocytes develop in the bone marrow from a common precursor cell, they differ from each other in their life history and function.

T LYMPHOCYTES

The T lymphocytes originate in the bone marrow but soon after their production they enter the blood circulation and reach the thymus. In the thymus these cells undergo proliferation and differentiation. As a result of differentiation their plasmalemma acquires specific CD markers and special receptors called T cell receptors (TCRs). Because the cells acquire their final differentiation and maturation in the thymus, they are titled T lymphocytes, i.e., *thymus-dependent lymphocytes*. After maturation in the thymus, the T lymphocytes enter the blood to join the continuously recirculating population of lymphocytes. Through the blood the T cells are also distributed to other lymphoid organs like spleen and lymph nodes. The primary function of the T lymphocytes is to launch the *cell-mediated immune response*. The T lymphocytes are further classified into several subtypes on the basis of CD markers present on their surface (for further details see chapter 14).

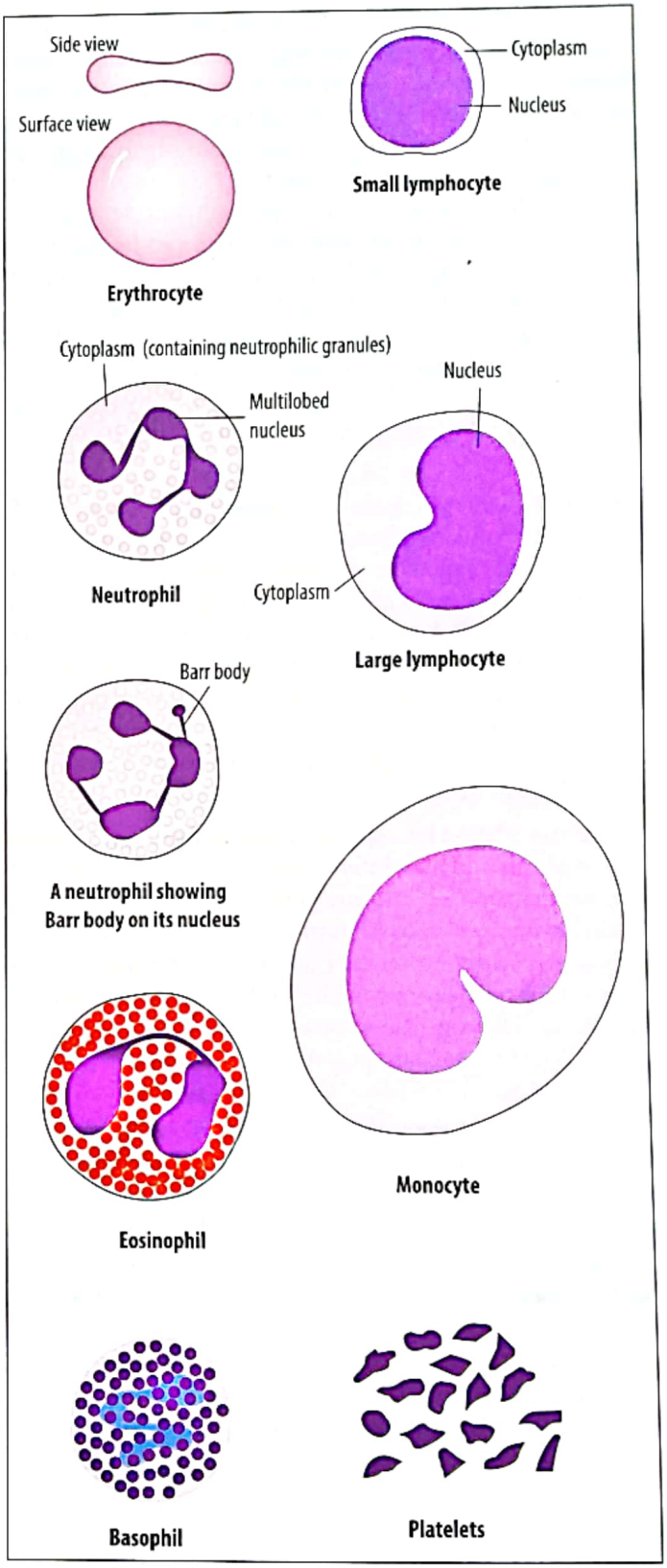

Fig. 9.2 Diagrammatic representation of the morphology of various types of blood cells and platelets.

B LYMPHOCYTES

This variety of lymphocytes was first identified in birds. Investigations revealed that, after originating in the bone marrow, some of the lymphocyte precursors migrate to bursa of Fabricius, which is a lymphoid organ located in the cloaca of birds. In the bursa of Fabricius these cells undergo differentiation and maturation and develop into a distinctive type of lymphocytes which can be identified by their specific CD markers. These cells were named B lymphocytes (implying 'Bursa-dependent Lymphocytes'). The bursa of Fabricius does not exist in mammals, but it has been found out that in mammals, including humans, the development of B lymphocytes in the prenatal life takes place in the fetal liver, whereas after birth it occurs in the bone marrow.

As pointed out earlier, the B lymphocytes constitute only 25% of the circulating lymphocytes, but it is to be noted that a large number of these cells resides in the bone marrow, spleen, lymph nodes and other lymphoid organs (except thymus). The B lymphocytes express **B-cell receptors (BCRs)** on their surface. These receptors enable a B lymphocyte to bind a specific antigen against which it will initiate an antibody response. The B lymphocytes also possess specific CD markers on the external surface of their plasmalemma.

Upon stimulation by a specific antigen, the B lymphocytes become activated and differentiate into plasma cells, which produce antibodies (immunoglobulins) against the antigen which caused the activation of the B lymphocytes. In this way the B lymphocytes play a very important role in the *humoral immune response* of the body against antigens (for details, see chapter 14).

NK CELLS

The blood also contains a small number of lymphocytes that lack the characteristic markers of the B and T lymphocytes but possess their own specific CD markers. These cells are programmed during their development to attack certain cellular targets directly and, therefore, are called **natural killer cells** (abbreviated as *NK cells*). These cells possess the capability to kill certain types of cell especially those host cells which either have been infected by a virus or have become cancerous.

The NK cells are larger than the B and T lymphocytes and contain cytoplasmic granules which can be seen under the light microscope and, therefore, these cells are also known as *large granular lymphocytes.*

MONOCYTES

The monocytes constitute about 3-8% of the total leukocytes of normal blood. They are the largest cells in a blood smear and range from 15 to 20 µm in diameter.

The nucleus of a monocyte is deeply indented and generally exhibits a U-shaped appearance (Fig. 9.1 & 9.2). The location of the nucleus is usually away from the cell center. Its chromatin is less condensed than that of a lymphocyte and, therefore, it stains lighter than the nucleus of a lymphocyte.

The cytoplasm of a monocyte stains basophilic and takes a bluish gray color with Wright stain. The cytoplasm contains a small Golgi complex, many mitochondria, some profiles of RER, a few free ribosomes, and numerous lysosomes (which are seen as azurophilic granules in the stained blood smears).

The monocytes are the principal cells of the mononuclear phagocyte system (MPS). They originate in the bone marrow, circulate in the blood for only a few days and then migrate into other tissues to give rise to other cells belonging to the MPS, e.g., in the loose connective tissue the monocytes develop into macrophages, in the bone tissue they give rise to osteoclasts, and in the nervous tissue they differentiate into microglial cells.

PLATELETS

The platelets, also called ***thrombocytes***, are small disc-like, cytoplasmic fragments, which have a round or oval shape and measure 2-4 µm in diameter. They are enveloped by a plasma membrane but do not contain a nucleus. The plasma membrane of the platelets has a thick (15-20 nm) glycocalyx coat, which plays an important role in platelet adhesion. Average life span of a platelet is about 10 days. Normal count of platelets ranges from 200,000 to 400,000 per microliter of blood. In blood smears, platelets are mostly seen as clumps of various sizes (Fig. 9.1).

In stained smears, a platelet shows two regions: a darkly staining central region called *granulomere* and a lighter staining, homogeneous, peripheral zone knows as *hyalomere.*

Electron microscopic examination of the **hyalomere** reveals that around the periphery of the platelet is present a *marginal bundle* of 10-15 microtubules arranged parallel to each other. The ring-like marginal bundle assists the platelet in maintaining its oval shape. The microtubules of the marginal bundle are associated with actin and myosin molecules that can rapidly assemble to form a contractile apparatus, which functions in platelet movement and aggregation. The hyalomere also contains two important tubular systems: a dense tubular system and an open canalicular system. The *dense tubular system* consists of a number of electron-dense irregular tubules which serve as storage site for calcium ions. The *open canalicular system* consists of membrane-bound vesicles connected to invaginations of the plasmalemma of the platelet. The open canalicular system performs two functions for the platelet: (1) it facilitates the uptake of chemical mediators and clotting factors, e.g., serotonin and fibrinogen from the plasma into the granules of the platelet, and (2) the degranulation of the platelets, i.e., release of the contents of the cytoplasmic granules into the blood, occurs through the open canalicular system.

Electron microscopic studies of the centrally-located **granulomere region of the platelet** reveal that the

cytoplasm in this region contains three types of granules which are named alpha granules, delta granules, and lambda granules. The alpha granules are the largest of all and measure 300-500 nm in diameter. These granules contain clotting factors, fibrinogen, thromboplastin, plasminogen, and plate-derived growth factor, etc. The contents of alpha granules perform their role in the initial phase of the blood vessel repair, blood clotting, and platelet aggregation. The delta granules measure 250-300 nm in diameter and contain platelet-activating mediators like serotonin, histamine, ADP, ATP, and calcium, etc. When released from the platelets, the contents of these granules facilitate platelet adhesion and vasoconstriction in the area of the injured blood vessel. The lambda granules measure about 200 nm in diameter and represent the lysosomes of the platelet. The hydrolytic enzymes contained in these granules function in clot resorption during the final stages of blood vessel repair.

Functions

The platelets play a very important role in hemostasis i.e., stoppage of the blood leakage from an injured blood vessel. Injury disrupts the endothelial lining of the blood vessels and exposes the subendothelial collagen. When platelets come in contact with this collagen, they become activated. Activation of platelets leads to the following three events: (1) **platelet adhesion**, i.e., adherence of platelets to the damaged region of the vessel wall, (2) **platelet activation** which results in the release of the chemicals stored in the platelet granules, and (3) **platelet aggregation,** i.e., adherence of more platelets to those already attached to the injury site. Platelet aggregation temporarily seals off the bleeding vessel. Ultimate result of interaction between the tissue factors, platelet-derived factors, and plasma borne factors is the formation of a blood clot or **thrombus**. The thrombus, which consists mainly of platelets and fibrin, serves as an effective seal at the site of blood vessel injury.

Under abnormal conditions, the number of platelets in the circulating blood may fall below or rise above the normal range. The deficiency of platelets is called **thrombocytopenia**. It may be the result of decreased production or increased destruction of platelets. For example, decreased production of thrombocytes occurs in aplastic anemia and dengue fever, whereas increased destruction of platelets occurs in idiopathic thrombocytopenic purpura. Excessive number of platelets in the blood is called **thrombocytosis**. This condition may result from reactive overproduction of platelets, e.g., after an acute blood loss, or may be due to a serious pathological condition called *myeloproliferative disorder*, in which there is an uncontrolled proliferation of the blood-forming cell colonies in the bone marrow.

HEMOPOIESIS

The blood cells have a limited life span and are dying or being destroyed constantly. The number of blood cells is kept fairly constant by continuous replacement from sources outside the circulation. The process of formation of formed elements of blood is known as hemopoiesis (also called hematopoiesis).

During the intrauterine life, the primitive blood vessels and blood cells begin to develop in the mesoderm during the 3rd week of development. The developing liver begins to form blood cells by the 6th week. Hemopoiesis also starts in the spleen during the middle of second trimester. Both liver and spleen continue to form blood cells until the end of intrauterine life. As the skeletal system develops, the hemopoiesis begins to occur in the bone marrow by the end of the second trimester of gestation. After birth hemopoiesis occurs only in the bone marrow. However, the liver and spleen retain the potential to form blood cells and can start the hemopoietic activity in special circumstances when there is an urgent need to form blood cells.

BONE MARROW

The bone marrow is a gelatinous vascular connective tissue, which is found in the marrow cavity of long bones and in the interstices between the trabeculae of spongy bones. The bone marrow is of two types: red marrow and yellow marrow. The *red bone marrow* is actively engaged in hemopoiesis and appears red because of the enormous number of RBCs being produced in it. In a new born, all of the bone marrow is of red variety, but after the age of 6 years the red bone marrow is gradually replaced by the yellow marrow. The *yellow bone marrow* contains large quantities of fat and hence gives a yellowish appearance when examined by the naked eye. By the adult age, red marrow remains only in the vertebrae, sternum, ribs, hip bones, and in the proximal ends of the humerus, femur and tibia. The red bone marrow actively forms blood cells throughout the life of the individual, but the yellow bone marrow does not take part in hemopoiesis in normal circumstances. However, the yellow bone marrow retains its hemopoietic potential and can transform into red marrow whenever there is an urgent need for hemopoiesis, e.g., after severe loss of blood.

RED BONE MARROW

The **red bone marrow** consists of three components: (1) blood sinusoids, (2) a stroma of reticular cells and reticular fibers, and (3) hemopoietic cords.

The sinusoids of red bone marrow receive blood from the branches of the nutrient arteries of the bone. Lined by endothelial cells and supported by a basal lamina, these sinusoids are surrounded by a network of reticular cells and fibers. The interstices of this network are occupied by hemopoietic cords.

The **hemopoietic cords** consist of self-renewing stem cells, differentiating precursors of the platelets and different types of blood cells, and the mature platelets and blood cells. The mature RBC, WBC and platelets are released into the sinusoids and taken away by the veins

draining the sinusoids. The hemopoietic cords also contain macrophages, which perform a variety of important functions. These macrophages phagocytose the malformed cells, the discarded cytoplasmic fragments of the developing blood cells and platelets, and the extruded nuclei of the developing erythrocytes. In addition, the macrophages and reticular cells release cytokines that promote proliferation and differentiation of the blood cell precursors.

STEM CELLS

The red bone marrow contains **pluripotent hemopoietic stem cells (PHSCs)** which give rise to all the different types of blood cells. The PHSCs do not divide regularly but undergo periodic bursts of mitotic division. These divisions are of two types: (1) *self-renewing divisions*, that produce more PHSCs to maintain the pool of these stem cells, and (2) *differentiating divisions* that give rise to two types of **multipotential hemopoietic stem cells (MHSCs).** One variety of MHSCs is called colony forming unit-lymphocyte (CFU-L), whereas the other type is known as colony forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte (CFU-GEMM). The CFU-L cells give rise to **lymphoid cell lines** that migrate from the bone marrow to the thymus, lymph nodes, spleen and other lymphoid organs/structures, where they undergo development. The CFU-GEMM gives rise to various cell lines that undergo further development in the bone marrow, and therefore, are called **myeloid cell lines.**

It is pertinent to mention here that the term colony forming units (CFU) was adopted upon the basis of *in vivo* and *in vitro* experiments, which indicated that these cells can produce colonies (groups) of cells with various pontentialities. An alternative term for these cells is CFC, i.e., colony forming cells.

PROGENITOR AND PRECURSOR CELLS

The MHSCs give rise to daughter cells called **progenitor cells**, which are either unipotential (committed to forming a single cell line) or bipotential (committed to form two different cell lines). The progenitor cells have a limited capacity for self-renewal and their mitotic activity is controlled by specific hemopoietic growth factors. The progenitor cells give rise to **precursor cells** that have specific morphological features that allow them to be recognized as the first cells of a specific cell line. The precursor cells undergo cell division and differentiation and, finally, give rise to a clone of mature blood cells.

HEMOPOIETIC GROWTH FACTORS

Hemopoiesis is regulated by a variety of growth factors produced by various cells. Each growth factor acts on specific stem cells, progenitor cells or precursor cells to induce mitosis or differentiation, or both. A brief description of the most well-known hemopoietic growth factors is given below:

Colony-stimulating Factors. These factors stimulate the cell division and differentiation of the unipotential or bipotential progenitor cells of the granulocytic and monocytic series. The colony stimulating factors are produced by macrophages, endothelial cells and fibroblasts.

Interleukins. Some cytokines belonging to the interleukin (IL) family stimulate the proliferation of stem cells as well as differentiation of progenitor and precursor cells of various cell lines. Most of such interkeukins are produced by the T lymphocytes. Examples of interleukins acting as hemopoietic growth factors are IL-3, IL-5, IL-7, IL-11, and IL-18.

Erythropoietin. This is a glycoprotein hormone produced by the kidneys. It specifically stimulates the division and differentiation of the progenitor and precursor cells of the erythrocytic series. A potent stimulant for the increased production of the erythropoietin is tissue hypoxia (reduction of oxygen supply to the tissues).

DEVELOPMENT OF ERYTHROCYTES (ERYTHROPOIESIS)

The erythrocytes are derived from the unipotential progenitor cells called colony forming unit-erythrocyte (CFU-E), which themselves arise from CFU-GEMM.

From CFU-E to mature RBC, the erythropoiesis comprises the following stages: proerythroblast, basophilic erythroblast, polychromatophilic erythroblast, orthochromatophilic erythroblast, reticulocyte, and mature erythrocyte.

1. Proerythroblast

The CFU-E differentiates into a proerythroblast which is the earliest recognizable cell of the erythrocyte series. A proerythroblast is a very large cell (15-20 µm in diameter) having a deeply basophilic cytoplasm. The nucleus, which is spherical and centrally located, shows a uniform chromatin pattern and one or two large nucleoli. Some hemoglobin is present in the cytoplasm but its amount is too small to be detected by ordinary staining techniques. After undergoing a number of mitotic divisions, the proerythroblasts give rise to basophilic erythroblasts.

2. Basophilic Erythroblast

This cell is smaller than the proerythroblast and averages 10 µm in diameter. The nucleus shows a coarse network of dense heterochromatin. The cytoplasm exhibits intense basophilia which indicates a further increase in the number of ribosomes. Hemoglobin increases in amount but is still masked by the basophilia of the cytoplasm.

3. Polychromatophilic Erythroblast

Basophilic erythroblasts undergo a number of mitotic divisions and produce cells in which hemoglobin is present in sufficient quantities to be easily identified in stained smears. With each mitotic division, there is a decrease in the basophilia of cytoplasm and an increase in the quantity of hemoglobin (which is acidophilic). Thus, the cytoplasm

of these cells takes varying amounts of acid and basic components of the Wright stain and, therefore, shows mixed colors varying from purplish-blue to a light pink or gray. Therefore, these cells are named polychromatophilic erythroblasts.

The nucleus of a polychromatophilic erythroblast shows a dense chromatin network with the coarse chromatin masses giving a characteristic *"checker-board appearance"*.

4. Orthochromatophilic Erythroblast

Each polychromatophilic erythroblast undergoes a number of mitotic divisions with a gradual decrease in the basophilia and an increase in the amount of hemoglobin. When the cytoplasm becomes as acidophilic as that of a mature erythrocyte, the cells are known as orthochromatophilic erythroblasts or *normoblasts*. An orthochromatophilic erythroblast is only slightly larger than an erythrocyte. Its nucleus is small and condensed and stains deeply basophilic. EM studies reveal that the ribosomes have almost disappeared from the cytoplasm of a normoblast. The Golgi apparatus and mitochondria are also seen to be undergoing degeneration.

The early orthochromatophilic erythroblasts undergo mitosis actively and eventually reach a stage at which their nuclei become very much shrunken (pyknotic) and no further division is possible. Finally, the nucleus is extruded from each cell.

5. Reticulocyte

The reticulocytes are immature erythrocytes. Their cytoplasm shows a delicate bluish network (reticulum) when the blood smears are stained by special dyes such as cresyl blue. The reticulocytes contain remnants of the Golgi apparatus, some mitochondria and a few polyribosomes. The RNA of the ribosomes is responsible for the basophilic reticulum seen in the cytoplasm of these cells. The reticulocytes are released from the red bone marrow into the circulating blood where they undergo maturation to become red blood cells. The maturation period ranges from 24 to 48 hours. During this period remnants of Golgi apparatus, ribosomes, centrioles, and mitochondria are lost to make the cell a mature erythrocyte. Under normal conditions, reticulocytes constitute 0.5 to 2 percent of the circulating erythrocytes.

DEVELOPMENT OF GRANULOCYTES (GRANULOCYTOPOIESIS)

The progenitor cells of the granulocytic series are derived from the multipotential hemopoietic stem cells CFU-GEMM. The eosinophils and basophils are derived from unipotential progenitor cells titled CFU-Eo and CFU-Ba, respectively. However, neutrophils are derived from bipotential progenitor cell CFU-GM. The CFU-GM undergoes mitosis to produce two unipotential progenitor cells: CFU-G of the neutrophil lineage and CFU-M of the monocyte lineage. The CFU-G, CFU-Eo and CFU-Ba divide to give rise to precursor cells of the granulocytic series called *myeloblasts*. From myeloblast to mature granulocytes, the granulocytopoiesis consists of the following stages: myeloblast, promyelocyte, myelocyte, metamyelocyte, and mature granulocyte.

1. Myeloblast

A myeloblast measures 12-15 µm in diameter and contains a large spherical nucleus that shows a delicate chromatin network. The scanty cytoplasm is markedly basophilic and contains numerous free ribosomes but a very sparse rough endoplasmic reticulum. The myeloblasts divide to produce daughter cells called promyelocytes.

2. Promyelocyte

The promyelocytes are larger than myeloblasts and average 20 µm in diameter. A promyelocyte has a big, round nucleus containing coarse chromatin. The cytoplasm is very basophilic and characterized by the presence of numerous azurophilic granules, which represent lysosomes. However, no specific granules are present in the cytoplasm of a promyelocyte. The Golgi apparatus and rough endoplasmic reticulum are also well-developed. The promyelocytes divide and differentiate into myelocytes.

3. Myelocyte

The myelocytes are much smaller than the promyelocytes, averaging about 10 µm in diameter. The nucleus is roughly oval in shape and stains more intensely than that of the promyelocyte. Distinguishing feature of a myelocyte is the appearance of specific cytoplasmic granules. These granules permit the recognition of myelocytes as neutrophilic, eosinophilic or basophilic. The myelocytes divide to give rise to metamyelocytes.

4. Metamyelocyte

The metamyelocytes are nearly of the same size as myelocytes. In the cytoplasm of a metamyelocyte the specific granules become more prominent. The nucleus becomes deeply indented and assumes a kidney-shaped appearance. The nuclear chromatin becomes more compact.

The metamyelocytes do not undergo mitosis but develop into mature granulocytes of their own lineage i.e., **neutrophils**, **eosinophils** or **basophils**. The specific granules increase in number and the nucleus assures a lobated appearance.

Before assuming a typical lobate form, the nucleus of an immature neutrophilic granulocyte passes through a stage in which it appears as a curved rod. Because of this band-shaped appearance of the nucleus, the juvenile neutrophil at this stage is known as a ***band cell***. The band cells may be found in the peripheral blood but their normal count is less than 5% of the total leukocytes. An increase percentage of band cells in the peripheral blood is an indication of very active hemopoiesis.

Muscle Tissue 10

The muscle tissue, also called *muscular tissue*, is made up of excitable cells which are capable of contraction (which results in a decrease in their length). This tissue is the most abundant tissue of the body and is responsible for the movement of different parts of the body, or the body as a whole. It also brings about changes in the size and shape of the internal organs of the body. The cells of the muscle tissue (*myocytes*) are generally narrow but very long and, therefore, are usually referred to as *muscle fibers* (instead of muscle cells or myocytes). The muscle fibers are aggregated in parallel arrays to facilitate the contraction of the muscle mass. The contractile ability of the muscle cells is derived from the presence of *myofibrils* in their cytoplasm, which are thread-like, contractile structures.

The plasmalemma of the muscle cells is known as ***sarcolemma*** and their cytoplasm called ***sarcoplasm*** (*sarkos* is a Greek word meaning flesh). Generally, an *external lamina* lies outer to the sarcolemma. The external lamina is secreted by the muscle cells and its structure of resembles that of the basement membrane of the epithelial cells, consisting chiefly of collagen type IV, the glycoprotein laminin and the proteoglycan perlecan. However, it is to be noted that no definite external lamina can be distinguished around the cardiac muscle cells.

Classification of the Muscular Tissue

Upon the basis of the light microscopic structure, the muscular tissue is classified into two main varieties: (1) striated muscle, and (2) nonstriated muscle.

1. **Striated Muscle**. The cells (fibers) of this variety of the muscle tissue exhibit cross-striations in the form of alternating light and dark bands. The striated muscle exists chiefly as the **skeletal muscle** (which is attached to the bones or cartilages of the body), but the heart wall is also formed by a special type of striated muscle called **cardiac muscle**. Functionally, the skeletal muscles are generally voluntary (i.e., their contraction is under the control of the will of the person), but the cardiac muscle is involuntary and its contraction is not under the control of the will. The skeletal muscles receive nerve supply from the somatic nervous system, whereas the nerve supply of the cardiac muscle is obtained from the autonomic nervous system.
2. **Nonstriated Muscle**. The cells (fibers) of this type of the muscular tissue do not show any cross-striations, and therefore, it is more commonly known as ***smooth muscle***. Functionally, the smooth muscle is also involuntary and receives its nerve supply from the autonomic nervous system.

Being simpler in structure, the smooth muscle is described before the two varieties of the striated muscle.

SMOOTH MUSCLE

This variety of the muscle tissue is titled "smooth" because its cells do not show any cross-striations in sections examined under the microscope. Generally, the smooth muscle occurs in the form of bands, bundles, or sheets. It is found mainly in the walls of the hollow viscera, e.g., stomach, intestine, urinary bladder, and uterus, etc., and, therefore, is also known as ***visceral muscle***. However, in addition to viscera, the smooth muscle is found in several other locations in the body. It forms an important component of the walls of the blood vessels and lymphatic vessels. It is also found in the eye as the ciliary muscle and muscle of the iris. The smooth muscle is also found in the skin as arrector pili muscles of the hair follicles and dartos muscle of the scrotal skin. The smooth muscle is also present in considerable quantity in the stoma of the prostate gland.

SMOOTH MUSCLE FIBERS

The smooth muscle fibers (i.e., smooth muscle cell) are spindle-shaped (fusiform); each of them is thick in the central part but tapers towards both ends (Fig. 10.1). The fusiform shape of the smooth muscle fibers allows tight packing of these fibers into bands, bundles or sheets because the narrow tapering portions of the spindle-shaped cells fit well with the wider parts of the adjoining cells.

The average greatest diameter of a smooth muscle fiber (in its central region) is about 6 µm. However, the length of the smooth muscle fibers is highly variable in different locations, being about 20 µm in the walls of small blood vessels and approximately 200 µm in the wall of the intestine. Longest smooth muscle fibers are found in the wall of the pregnant uterus where they may reach a length of 500 µm.

The adjacent smooth muscle fibers are connected to each other by maculae communicantes (gap junctions). These junctions provide intercellular communication needed to regulate the integrated contraction of an entire bundle or sheet of the smooth muscle.

Due to their tightly-packed arrangement, the smooth muscle cells give a mosaic-like appearance in longitudinal sections. In cross sections, circular outlines of various diameters are seen, in which nuclei are visible in only the largest profiles which represent the widest parts of the smooth muscle cell. The smaller profiles without nuclei represent the tapering ends of the cells.

Sarcolemma and External Lamina

The cell membrane (sarcolemma) of each smooth muscle fiber is an ordinary trilaminar cell membrane. As already

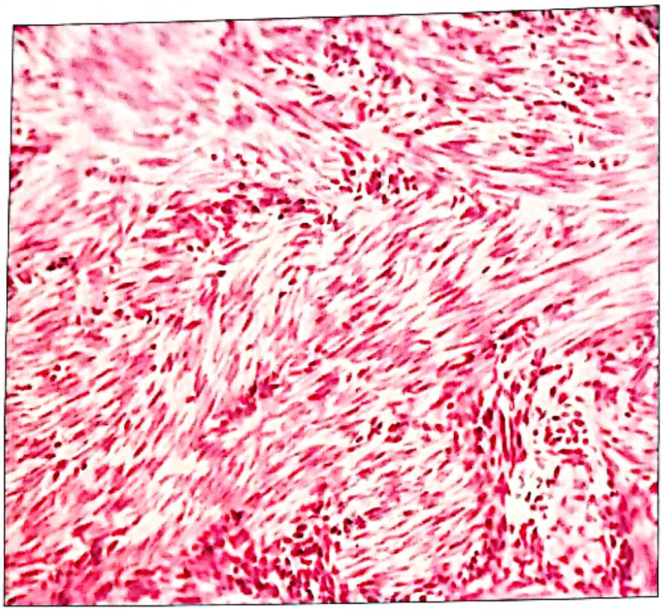

Fig. 10.1 A section through the muscle layer (myometrium) of the uterus showing bundles of smooth muscle fibers.

described, the sarcolemma is surrounded by an external lamina which is equivalent to the basement membrane.

Nucleus

Each smooth muscle cell contains a single, rod-shaped nucleus, located in the central thick portion of the fusiform cell. In longitudinal sections the nucleus often exhibits a corkscrew appearance, which is the result of contraction of the cell during fixation of the tissue.

Cytoplasm

EM shows that the cytoplasm (sarcoplasm) of a smooth muscle cell contains a contractile apparatus of thin filaments and thick filaments, and a cytoskeleton of intermediate filaments.

The sarcoplasm also contains mitochondria, SER, RER, polysomes, a small Golgi apparatus, and some glycogen granules. Most of these organelles and inclusions are concentrated in the region around the nucleus especially at its poles.

Electron microscopic studies also reveal that the plasmalemma of the smooth muscle cells shows numerous invaginations called **caveolae**. The caveolae contain receptors, pumps, and ion channels, and are usually found to be located close to the mitochondria and SER. The caveolae function to organize signaling molecules in the sarcolemma. The caveolae also serve to regulate the entry of the calcium ions into the smooth muscle cells, or their ejection from these cells, in accordance with the contraction or relaxation of the smooth muscle.

The **contractile apparatus** enables the smooth muscle to undergo shortening as and when required. The **thin filaments** are composed of the protein actin and measure 5-7 nm in diameter. The thin filaments form bundles that are arranged longitudinally and obliquely in the cytoplasm of the smooth muscle cell. The **thick filaments** are composed of *myosin II*. These filaments average 15 nm in diameter and generally run parallel to the thin filaments (detailed structure of the myosin II is described later in this chapter).

Generally, the **cytoskeleton** of smooth muscle cell consists of a network of ***desmin intermediate filaments***. However, in the smooth muscle of blood vessels, vimentin filaments are also present.

Examination under the EM reveals that the actin filaments are inserted into electron-dense structures called **dense bodies**, which contain actin-binding proteins (mainly α-actinin). The dense bodies are distributed in an irregular manner within the cytoplasm and some of them may also be associated with the sarcolemma. Serving as the anchoring sites for the actin filaments, the dense bodies are functionally similar to the Z discs of the skeletal muscle fibers (described later). The intermediate filaments of the cytoskeleton are also inserted into the dense bodies.

Nerve Supply of the Smooth Muscle

The smooth muscle is supplied by sympathetic and parasympathetic divisions of the autonomic nervous system, all nerve fibers being postganglionic and unmyelinated. On the basis of innervation, two types of smooth muscle are recognized. The first type called, *multiunit smooth muscle*, has a rich nerve supply and nearly all muscle cells receive nerve terminals, e.g., smooth muscle of the iris and ductus deferens. Because of its rich innervation the multiunit type of smooth muscle is capable of producing precise and graded contractions. The second type called *unitary smooth muscle*, has a relatively poor nerve supply, only a few muscle cells having nerve terminals. The nerve impulse passes from one cell to another through the gap junctions and the muscle functions in a syncytial fashion (because individual muscle fibers cannot contract separately). The unitary smooth muscle occurs in the walls of most of the hollow viscera. The smooth muscle in the walls of the blood vessels also belongs to the unitary variety.

Functions of the Smooth Muscle

As a result of its contractile properties, the smooth muscle carries out different functions in various locations in the body. The major functional role of the smooth muscle in various locations in the body is given below.

The smooth muscle in the walls of the blood vessels (especially arteries) contracts and relaxes to regulate the blood pressure and flow of blood. The smooth muscle in the wall of the digestive tract undergoes slow, rhythmic, involuntary contractions (called *peristalsis*) which propel the food through the digestive tube. In the urinary bladder, the smooth muscle helps to push the urine out. The smooth muscle in the wall of the uterus contracts during the childbirth to push the baby to the exterior. In the lungs, the smooth muscle in the wall of the bronchi and bronchioles

contracts and relaxes to control the air flow through the lungs. In the male reproductive tract, the contraction of the smooth muscle leads to onward movement of the spermatozoa and semen. In the eye, the pupillary smooth muscle controls the size of the pupil (to regulate the amount of light entering the eye), and contraction and relaxation of the ciliary smooth muscle regulates the curvature of the lens (to allow accommodation for the near and far visions). In the skin, the arrector pili muscles contract to make the hair stand erect. The smooth muscle in the walls of the seminal vesicles and in the stroma of the prostate gland contracts at the time of ejaculation to push the stored secretions of these glands into the urethra.

SKELETAL MUSCLE

The skeletal muscle is a type of striated muscle which is under the voluntary control. The skeletal muscles are titled as 'skeletal' because of the fact that they are attached to the skeleton of the body. Each skeletal muscle consists of numerous muscle cells (myocytes) held together by connective tissue. As explained earlier, the length of the muscle cells is enormous and, therefore, they are generally referred to as ***muscle fibers*** instead of muscle cells.

Depending on their relationship with the muscle fibers, the connective tissue investments of the skeletal muscle are named as epimysium, perimysium and endomysium (Fig. 10.3).

The **epimysium** is a sheath of dense irregular connective tissue that surrounds the entire skeletal muscle. The major blood vessels and nerves of the muscle penetrate the epimysium to gain entry into the muscle.

The **perimysium** surrounds bundles (fascicles) of muscle fibers within a skeletal muscle. It consists of less dense connective tissue. The blood vessels and nerves, that have penetrated the epimysium, run in the perimysium to be distributed to the muscle fascicles.

The **endomysium** is a thin layer of loose connective tissue that surrounds each skeletal muscle fiber individually. This layer lies immediately outer to the external lamina and consists of fine collagenous fibers embedded in proteoglycan matrix. Blood capillaries and nerve fibers run in the endomysium to reach the muscle fibers.

SKELETAL MUSCLE FIBERS

When the stained sections of the muscle tissue are examined under the microscope, the longitudinally sectioned skeletal muscle fibers give a striated appearance because they exhibit alternating light and dark bands that run across the entire width of the muscle fiber (Fig. 10.9).

The skeletal muscle fibers are cylindrical in shape. Their length is very variable and ranges from a few millimeters in very small muscles (e.g., stapedius muscle of the middle ear) to several centimeters in strap-like muscles (e.g., sartorius muscle of the thigh). The average diameter of the skeletal muscle cells is also variable, but averages and ranges 50-60 µm. An important feature of the skeletal muscle fibers is that they do not branch. **The cell membrane (sarcolemma) of** the skeletal muscle fibers is covered by the external lamina which binds each muscle fiber to its endomysium.

Each skeletal muscle fiber contains several nuclei (about 35 nuclei per mm of length). The nuclei are ovoid in shape and are situated peripherally, just beneath the sarcolemma. The fact that a single skeletal muscle cell contains multiple nuclei has a developmental basis. Studies on developing embryos have revealed that each skeletal muscle cell develops by the fusion of several small mononucleated cells called *myoblasts* and, therefore, each mature skeletal muscle cell (myocyte) comes to have multiple nuclei.

CYTOPLASM (SARCOPLASM)

The cytoplasm of a skeletal muscle fiber is almost filled by thin, cylindrical structures called *myofibrils,* which are composed of long proteins (described later). The myofibrils have an average diameter of about 1 µm but are very long and their total length depends on the length of the muscle fascicle in which the muscle fiber is present. The myofibrils run parallel to the long axis of the muscle fiber and extend its entire length, from one end to the other.

Relatively clear sarcoplasm is found around the nuclei beneath the sarcolemma. It contains a small Golgi complex, abundant mitochondria, a small amount of rough endoplasmic reticulum, and a large number of glycogen granules. The abundant mitochondria and glycogen granules are needed to provide energy to the chemical reactions involved in in the contraction of the muscle fiber. The smooth endoplasmic reticulum of the skeletal muscle fiber, called *sarcoplasmic reticulum*, is very extensive and has a special form and arrangement. The sarcoplasmic performs a very important function in the process of contraction of the muscle fiber (described in detail later).

It has already been mentioned that, under the light microscope, the longitudinally sectioned skeletal muscle fibers show *cross-striations* in the form of alternating dark and light bands. The **dark bands** are also called ***A bands*** because they are found to be *anisotropic* under the polarizing microscope. The **light bands** are also referred to as ***I bands*** because they are found to be *isotropic* under the polarizing microscope.

Myofibrils

The myofibrils are cylindrical, thread-like structures composed of long proteins filaments (described below). They are 1 to 2 µm in diameter and have a great length, extending from one end of the muscle fiber to the other. The myofibrils are very closely packed and occupy most of the space in the interior of the muscle fiber, so that very scanty cytoplasm is present between the myofibrils.

The dark and light bands observed on a muscle fiber under the light microscope are actually present on the myofibrils

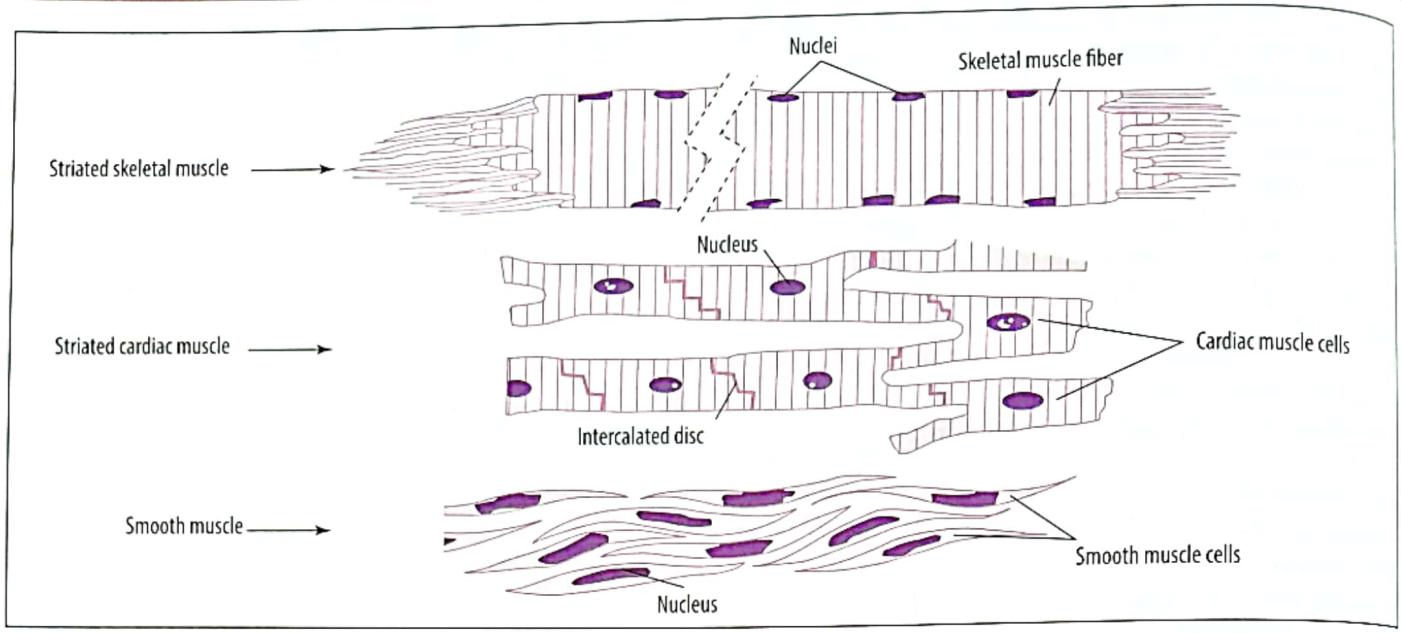

Fig. 10.2 Diagrammatic representation of structural feature of the three varieties of muscular tissue.

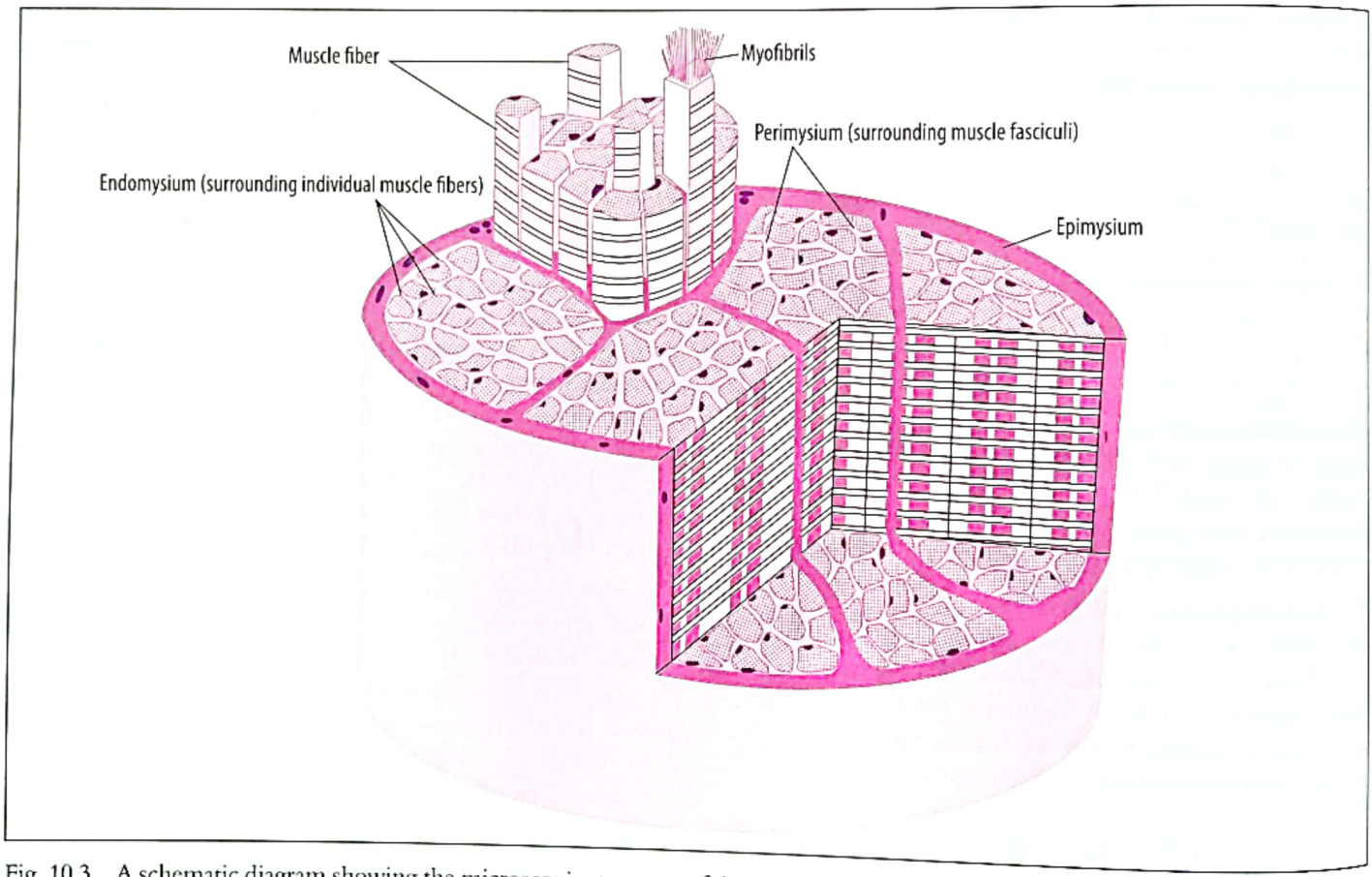

Fig. 10.3 A schematic diagram showing the microscopic structure of the skeletal muscle.

and are not present in the scanty interfibrillar cytoplasm, but because the myofibrils are very closely packed and run parallel to each other in perfect alignment, the dark and light bands appear to cross the entire width of the muscle fiber.

Electron microscopic studies on the myofibrils reveal that each dark band (A band) has a relatively brighter central region; this region is named **H* zone** (also called H band). The light band (I band) is seen to be bisected by a dark transverse line which is titled **Z* disc** (also called *Z line*). Very high magnifications show that the H zone of each A band is also bisected by a thin, dark line which is known as **M* line** (Fig. 10.4 & 10.5).

* These abbreviations represent three German words: **H** stands for *helle* (meaning *bright*), **Z** represents zwischen (meaning *between*), and **M** symbolizes *mittel* (meaning *middle*).

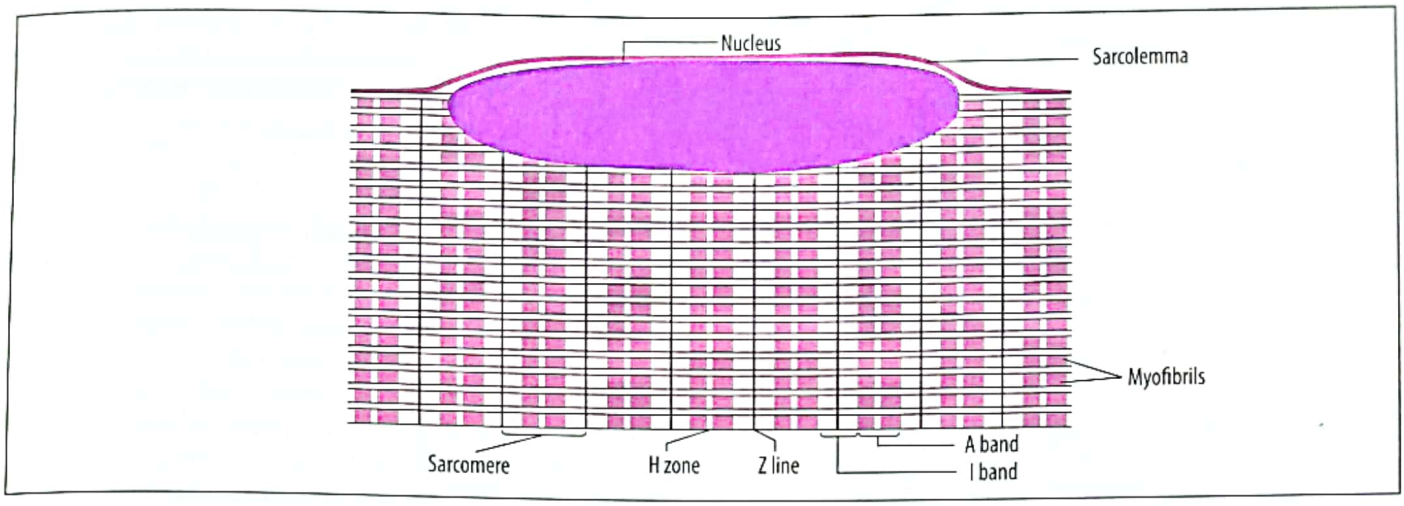

Fig. 10.4 Diagrammatic representation of electron microscopic structure of a skeletal muscle fiber.

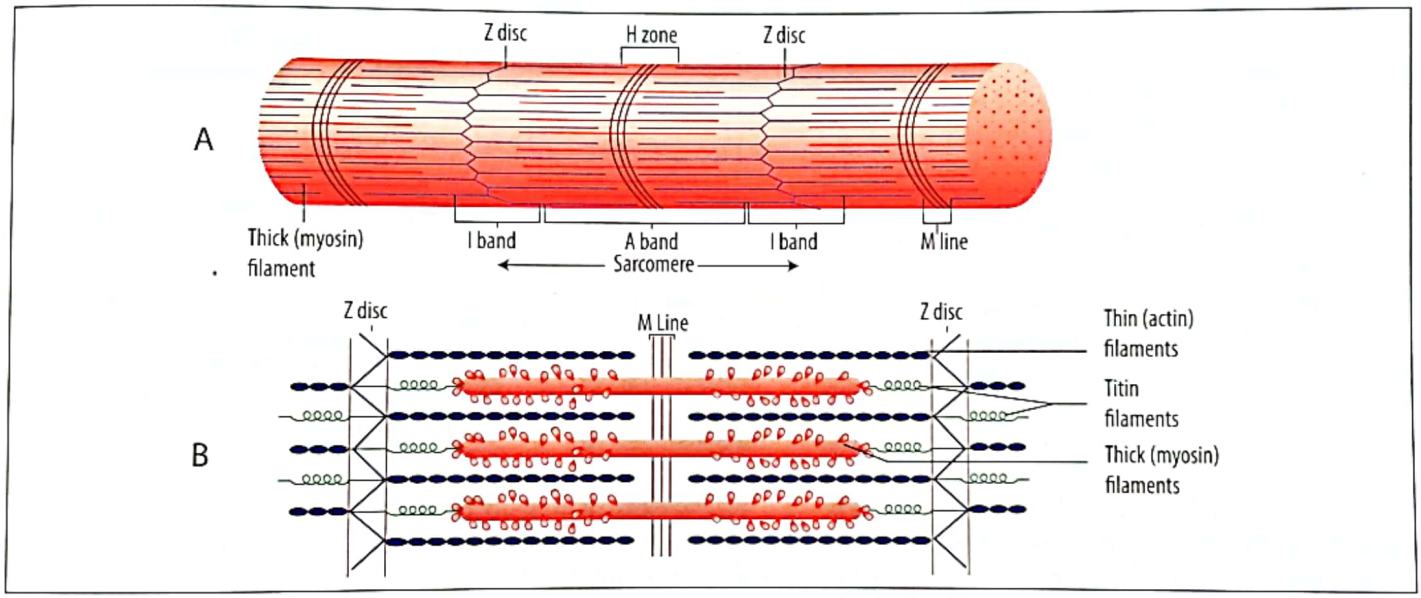

Fig. 10.5 A. Diagrammatic representation of the fine structure of a myofibril. B. Diagrammatic representation of the molecular structure of a sarcomere.

That segment of a muscle cell which lies between two successive Z discs is known as a ***sarcomere***. The sarcomere constitutes the basic contractile unit of the muscle fiber and measures about 2.5 µm in length in a resting muscle. It is obvious that each myofibril consists of an end-to-end repetitive arrangement of sarcomeres.

ULTRASTRUCTURE OF THE MYOFIBRILS

Studies with high resolution electron microscopes reveal that myofibrils are composed of bundles of **myofilaments** that lie parallel to the long axis of the myofibril. Each myofibril consists of two types of myofilaments:

1. **Thick myofilaments,** each of which measures about 15 nm in diameter and 1.5 µm in length.
2. **Thin myofilaments,** each measuring about 7 nm in diameter and are about 1.0 µm in length.

The thick and thin myofilaments are arranged parallel to long axis of the myofibril in a symmetric pattern.

The Thick Myofilaments

Each thick myofilament is composed of 200-300 molecules of myosin II (which is an actin-binding protein). The myosin II molecule is a complex molecule, being composed of one pair of heavy chains and two pairs of light chains (i.e., each myosin II molecule consists of two heavy chains and four light chains). Each *myosin heavy chain* is shaped like a golf club, having a long, thin tail (shaft) and a large, globular head (Fig. 10.6). The tails of the two heavy chains of a myosin molecule are twisted around each other in a rope-like fashion. Their globular heads project almost at a right angle at one end of the shaft. Each of the two globular heads of a myosin molecule is associated with a pair of *myosin light chains*.

The globular heads of the myosin II molecules have binding sites for actin and ATP. The heads also possess ATPase activity to release energy by the hydrolysis of ATP.

The thick myofilaments span the entire A band of the

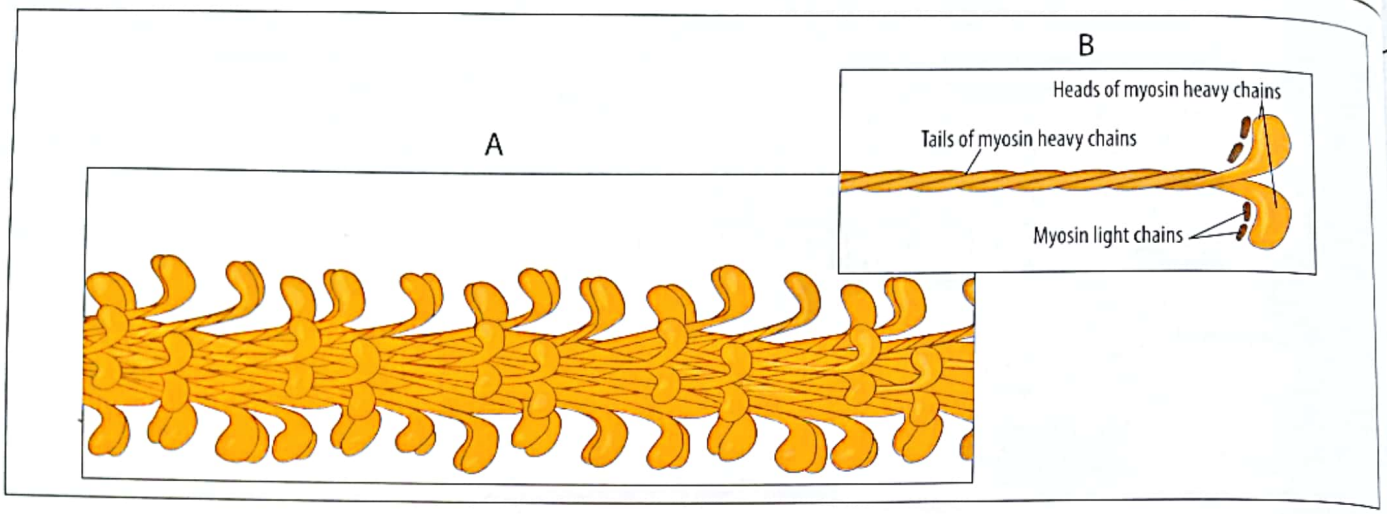

Fig. 10.6 A. Molecular structure of a thick myofilament which is seen to be composed of multiple myosin II molecules. B. Diagrammatic representation of the structure of a myosin II molecule; each of these molecules consists of two myosin heavy chains and two myosin light chains.

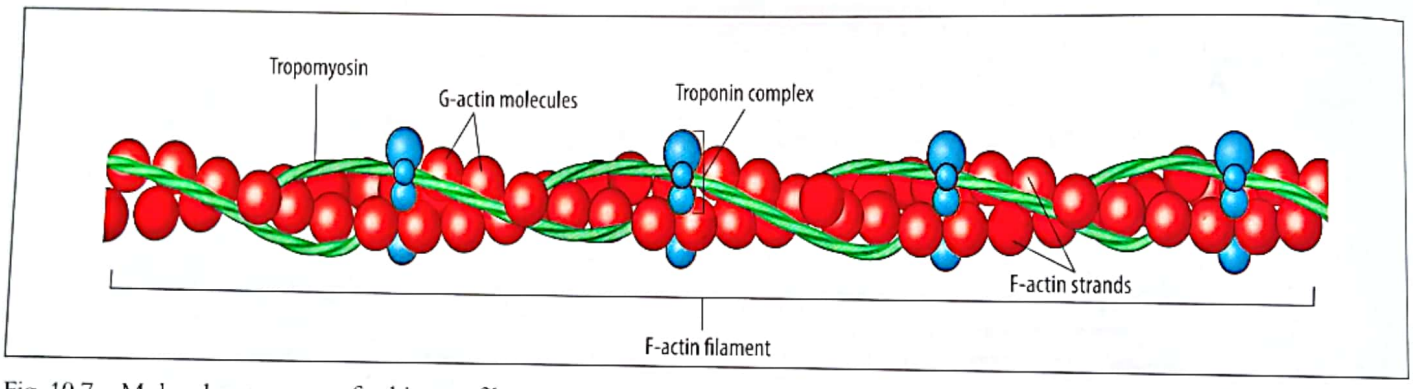

Fig. 10.7 Molecular structure of a thin myofilament.

myofibril. One end of each thick myofilament is bound to the proteins of the M line, while the other end is linked to the z disc by a giant protein called titin (which is more than 1 μm in length). The titin serves as a molecular spring which is responsible for the passive elasticity of the skeletal muscle.

The Thin Myofilaments

The thin myofilaments are composed mainly of the protein *actin*. The thin filaments are anchored to the Z disc by α-actinin (which is an actin-binding protein). In addition to actin, the thin myofilaments contain two other proteins named *tropomyosin* and *troponin*.

The actin exists as long filamentous polymers called *F-actin polymers*. Each filament of F-actin is composed of two strands, each of which is composed further of globular protein subunits of *G-actin* (Fig. 10.7). The two F-actin strands are twisted around each other in a helical manner. The *tropomyosin* molecules are present in the form of 40 nm long, slender filaments which occupy the shallow grooves of the F-actin double helix. A *troponin molecule* is attached at one specific site on each tropomyosin molecule.

Each troponin molecule actually consists of a complex of three globular subunits: troponin-T (TnT), troponin-I (TnI), and troponin-C (TnC). The TnT subunit binds the troponin molecule to the tropomyosin. The TnI subunit regulates the actin-myosin interaction. The TnC is the smallest subunit of the troponin molecule. It has special affinity for calcium ions. Binding of Ca^{2+} by TnC is an essential step in the initiation of contraction of the muscle fiber.

In the resting muscle, tropomyosin and troponin mask the myosin-binding sites on the actin filament. Binding of Ca^{2+} by TnC exposes the previously blocked active sites on the actin filament. This allows the globular heads of the myosin II molecules to flex and form cross-bridges with the actin molecules.

During muscle contraction, the globular heads of the myosin II molecules bind to the actin of the thin filaments. In a sequence of binding and release movements, the globular heads move along the actin filaments and, consequently, the actin filaments slide along the myosin filaments, resulting in shortening of the sarcomere. The repetitive binding and release of the myosin heads is powered by the energy released by the hydrolysis of ATP by the ATPase located in the globular head.

STRUCTURAL ORGANIZATION OF THE SKELETAL MUSCLE FIBERS

The cross bandings seen upon the inspection of a skeletal muscle fiber (under LM) or a myofibril (under EM) are simply a reflection of the distribution of myofilaments in the sarcomere. The thick filaments (myosin filaments) occupy the A band, i.e., the central portion of the sarcomere. The thin filaments (actin filaments) extend from either side of the Z line through the adjacenat I band and partway into A bands, interdigitating there with the thick filaments as far as the H zone. It is due to this arrangement that, in a cross section of the A band, each thick filament is seen to be surrounded by six thin filaments in a hexagonal array. The H zone is simply the central area of the A band which is free of the thin filaments. The width of the H zone is not constant and depends on the state of contraction of the muscle fiber. As the muscle fiber undergoes contraction, the actin filaments slide along the myosin filaments and the width of the H zone gradually decreases. In a fully contracted muscle, the H zone becomes almost completely extinguished.

It is clear from the above given description that the cross bands seen in the longitudinal section of a myofibril are due to the presence or absence of overlap between the two types of myofilaments. The light band (I band) contains only thin filaments, while the extremities of the dark band (A band) contain both thick and thin filaments. Only thick filaments are present in the H zone. The thin filaments terminate at the Z disc where they are anchored and kept in register. The thick filaments are anchored in the M line region of the H zone.

In the longitudinal section of a myofibril, the **Z disc** appears as a zigzag structure, which is bisected by an amorphous material called *Z-matrix*. The Z-matrix contains a large number of proteins especially α-actinin which serves to anchor the actin filaments from the adjacent sarcomeres to the Z disc. The Z-matrix also contains a protein called *nebulin* which helps α–actinin in anchoring the thin myofilaments to the Z disc.

The **M line,** which bisects the H zone of the dark band, contains a myosin-binding protein called *myomesin*. In addition, the M line region contains the enzymes creatine kinase that catalyzes the transfer of phosphate groups to ADP, so that ATP is formed which is required to supply energy for muscle contraction.

THE SARCOPLASMIC RETICULUM AND TRANSVERSE TUBULAR SYSTEM

The sarcoplasmic reticulum is a special type of smooth endoplasmic reticulum which is found only in the striated muscle. Electron microscopic studies reveal that the sarcoplasmic reticulum exists in the form of a network of cisternae or membranous tubules which course between and around the myofibrils. The tubules course chiefly in a longitudinal direction. Lateral anastomoses between longitudinally disposed tubules form a perforated collar

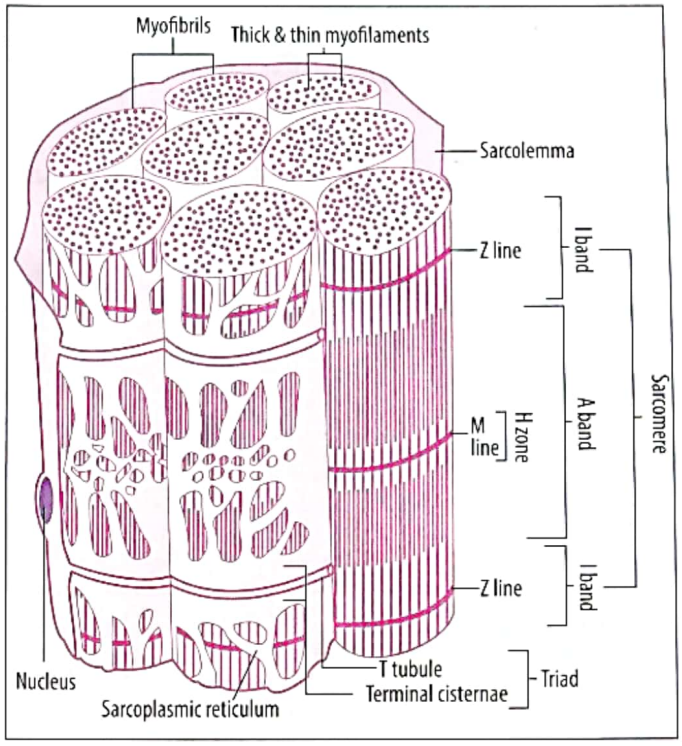

Fig. 10.8 Three-dimensional drawing of small part of a skeletal muscle fiber. The sarcoplasmic reticulum has been removed from one myofibril to show the bands and lines.

around the myofibrils at the level of each H zone. At the level of A-I junctions, the longitudinal tubules join terminal cisternae which are present in the form of rings around the myofibrils. Two such transversely disposed terminal cisternae are found at each A-I junction but are separated from one another by a more slender, transversely running tubule known as T-tubule. The T-tubules are not a component of the sarcoplasmic reticulum but are invaginations of the sarcolemma.

The special arrangement of two terminal cisternae of sarcoplasmic reticulum with a central T-tubule is called a *triad* (Fig. 10.8). As there are two A-I junctions in a sarcomere, two triads are present in relation to each sarcomere.

The **T-tubules (i.e., transverse tubules)** are actually invaginations of the sarcolemma of the muscle fiber. Their lumens open on to the extracellular space around the fiber. These tubules pass from the sarcolemma at regular intervals into the interior of the muscle fiber, undergo branching and lie between terminal cisternae of the sarcoplasmic reticulum to form triads at A-I junctions. At places, the wall of a T-tubule forms junctions (couplings) with the membranes of the terminal cisternae.

The sarcoplasmic reticulum and transverse tubular system play a very important role in the contraction and relaxation of the muscle fiber. The terminal cisternae of the sarcoplasmic reticulum serve as reservoirs for calcium ions. The membrane of these cisternae contains numerous gated **calcium release channels**. The T-tubules possess **voltage sensor proteins.** These proteins are activated

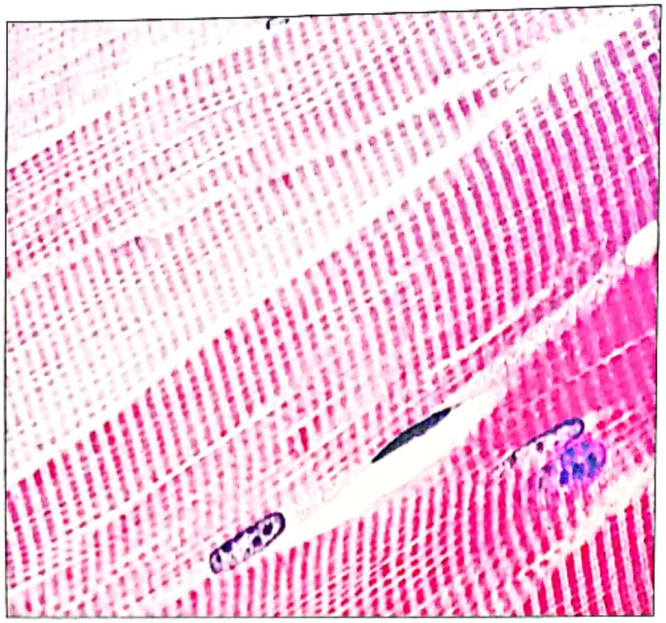

Fig. 10.9 A longitudinal section through the skeletal muscle fibers showing cross-striations and peripheral nuclei.

when the sarcolemma depolarizes. Activation of the voltage sensor proteins directly affects the calcium release channels located on the adjacent terminal cisternae of the sarcoplasmic reticulum and results in the release of calcium ions from the terminal cisternae into the sarcoplasm. The increased concentration of Ca^{2+} ions in the sarcoplasm triggers contraction of the muscle fiber by binding of calcium ions to TnC subunits of the troponin molecules. As soon as the contraction cycle is over, the calcium ions are transported back into the terminal cisternae by the activity of the **calcium membrane pumps** located in the membrane of the sarcoplasmic reticulum. Thus, the resting concentration of calcium ions in the sarcoplasm is once again restored. These calcium ions remain stored in the cisternae of the sarcoplasmic reticulum until a new muscle action potential arrives along the T-tubules.

Innervation of the Skeletal Muscles

Each skeletal muscle receives two types of nerve fibers: motor (efferent) and sensory (afferent).

Motor Innervation

The motor nerve fibers supplying the skeletal muscles originate from large motor neurons (called alpha motor neurons), which are located in the anterior gray horn of the spinal cord (or in the brainstem). As the nerve containing the motor nerve fibers reaches a muscle, it divides into multiple branches that penetrate the epimysium of the muscle and then run and branch further in the perimysial connective tissue. Finally, individual axons branch in the endomysium and give rise to terminal branches that end on individual muscle fibers at motor nerve endings called *neuromuscular junctions (motor endplates)*. The motor nerve fibers convey impulses that cause contraction of the muscle fibers.

A motor nerve and the muscle fibers it innervated by it constitute a *motor unit*. In the muscles involved in delicate movements (e.g., extraocular muscles), a motor neuron may be responsible for the innervation of 3 to 10 muscle fibers. On the other hand, in postural muscles of the back, a motor neuron may innervate several thousand muscle fibers.

Sensory Innervation

The sensory (afferent) fibers supplying the skeletal muscles pass to a special type variety of sensory nerve endings called *muscle spindles*, which are located on groups of muscle fibers that are distributed throughout the muscle. The sensory nerve fibers convey (to the CNS) the information about the degree of tension (stretch) in a muscle.

CARDIAC MUSCLE

The cardiac muscle is involuntary but striated. It is found only in the myocardium (muscle layer of the heart). The cardiac muscle cells, also called *cardiomyocytes*, are aligned in the form of chains. Cells within a chain often bifurcate or branch and make junctions with cells in the adjacent chains. It is to be understood that, while each skeletal muscle fiber represents a single cell, the cardiac muscle fibers are formed by the linear cell-to-cell attachment of several cardiac muscle cells by means of junctional complexes located in specialized regions which are called *intercalated discs*.

A delicate network of collagenous and reticular fibers lies between the cardiac muscle cells and represents the endomysium of these cells. Large bundles of cardiac muscle fibers are surrounded by coarse connective tissue which corresponds to the perimysium of the skeletal muscle.

CARDIAC MUSCLE CELLS (Fig. 10.10)

The cardiac muscle cells (cardiomyocytes) are elongated, branching cells with irregular contours at their junctions. They show a cross-striated banding pattern of alternating dark and light bands (identical to those of the skeletal muscle cells). The average diameter of a cardiomyocyte is about 20 µm. The length of the cardiac muscle cells ranges from 90 to 120 µm.

NUCLEUS

Generally, a single, large, pale-staining, oval nucleus is present in the central region of each cardiac muscle cell, but some cardiomyocytes are binucleated.

SARCOPLASM

Major part of the sarcoplasm of a cardiac muscle is occupied by myofibrils. These myofibrils consist of thin and thick myofilaments and show dark and light bands similar in appearance to those of the skeletal muscle. Consequently, longitudinal section of a cardiac muscle fiber also exhibits the typical dark and light bands, H zones, M lines, and Z discs. The sarcoplasm also contains a very large number

of mitochondria. In addition, an elaborate sarcoplasmic reticulum and many Golgi complexes are also present. Abundant glycogen granules and fat droplets, representing storehouses of energy, are also found in the cytoplasm of cardiac muscle cells. Considerable amounts of lipofuscin pigment are usually present in cardiac muscle cells of the old people.

As stated above, each cardiac muscle cell contains abundant mitochondria which occupy almost 50% of the cytoplasmic volume. Mitochondria of ordinary size are clustered beneath the sarcolemma and in the perinuclear zone, but especially large mitochondria with numerous cristae are found to be densely packed between the myofibrils. Abundance of mitochondria correlates with the need for continuous aerobic metabolism in the cardiac muscle .

The sarcoplasm of the cardiac muscle cells of the right and left atria of the heart contains membrane-bound granules, called *atrial granules*, which measure 0.2 to 0.4 μm in diameter. These granules contain a polypeptide hormone called *atrial natriuretic factor (ANF)*. The ANF is secreted when the atria are distended because of plasma volume expansion and induces diuresis (increased excretion of urine) by inhibiting the reabsorption of sodium by the kidney tubules, particularly the collecting tubules.

Intercalated Discs

When stained sections of the cardiac muscle are examined under the light microscope, it is seen that a special feature of this variety of the striated muscle is the presence of dark-staining transverse bands that cross the chains of the cardiac muscle fibers. These dark-staining cross-bands, called *intercalated discs*, represent highly specialized attachment sites between adjacent cardiac muscle cells. The intercalated discs are located at the level of Z lines and cross the muscle fiber like a stairway (showing multiple steps).

Studies by EM reveal that the intercalated discs represent special junctional complexes consisting of three varieties of intercellular junctions: desmosomes, fasciae adherents, and gap junctions.

The desmosomes and fasciae adherentes provide strong mechanical attachment between the adjoining cardiomyocytes, so that they do not become separated from each other during muscle contraction. The gap junctions (maculae communicantes) provide electrical coupling between the adjacent cardiac muscle cells.

The electrical coupling provided by the gap junctions is of great functional importance because, by permitting transfer of ions between the adjoining cells, it allows the spread of electrical impulse from one cardiomyocyte to the other. This rapid transmission of the electrical impulses enables the network of cardiac muscle fibers to function as a syncytium, so that a coordinated contraction of the myocardium can take place.

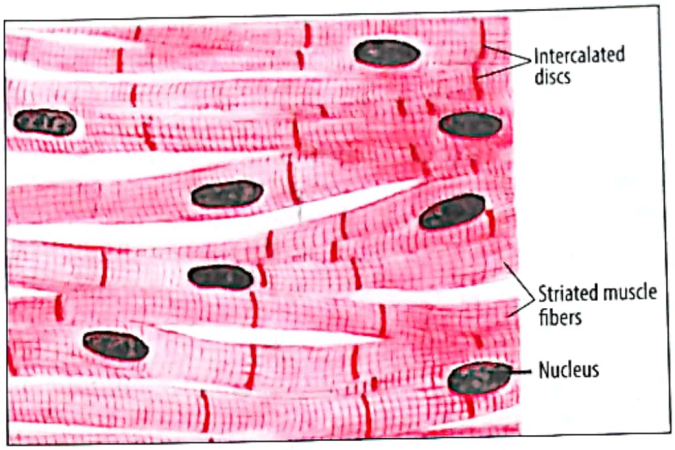

Fig. 10.10 A longitudinal section through the cardiac muscle showing striations and intercalated discs. Note that a single, oval nucleus is present in each cardiac muscle cell.

T-tubules and Sarcoplasmic Reticulum

The T-tubules of cardiac muscle resemble those of the skeletal muscle in that they are invaginations of the sarcolemma, but differ in that they are of greater diameter and lie at the level of Z discs (and not at A-I junctions). In cardiac muscle cells the sarcoplasmic reticulum consists of longitudinally running tubules which interconnect and anastomose with each other so frequently that an overall plexiform pattern is formed. No large terminal cisternae are present around the Z discs or at any other level and, therefore, no triads are formed. However, each T-tubule of cardiac muscle cell is generally associated with one small terminal cisternae of the sarcoplasmic reticulum; the association of these two structures is known as a *diad*.

Nerve Supply of the Cardiac Muscle

Branches of sympathetic and parasympathetic nerves follow the connective tissue and terminate in fine endings scattered among the cardiac muscle fibers.

MUSCLE TISSUE REPAIR AND REGENERATION

All the three varieties of the muscular tissue have different potentials for repair and regeneration after injury due to trauma or disease.

The **smooth muscle** has remarkable regenerative capability. When an injury leads to damage and death of smooth muscle cells in a hollow viscous or blood vessel, the surviving smooth muscle cells undergo mitotic division and replace the damaged cells. In blood vessel injury, the pericytes derived from the walls of small blood vessels also take part in the repair process. Serving as reserve cells or stem cells, the pericytes differentiate into smooth muscle whenever needed.

The **skeletal muscle** has a limited regenerative capacity. The skeletal muscle cells themselves are incapable of mitotic division. However, the skeletal muscle fibers are always associated with a sparse population of reserve cells called *satellite cells*, which are small, mononucleated

cells that lie between the skeletal muscle fibers and their external lamina. The satellite cells are dormant myoblasts which normally remain quiescent. In the event of injury to the muscle, these cells become activated and re-enter the cell cycle. They proliferate and fuse to form new skeletal muscle fibers. However, the regenerative capability of satellite cells is limited. Therefore, after major muscle injuries, the injured site is repaired by the fibroblasts. The activated fibroblasts produce collagenous scar tissue which fills the space created by the massive muscle damage.

The cardiac muscle has almost no regenerative capability in the adults. Therefore, the damaged cardiac muscle cells are replaced by scar tissue consisting of collagen fibers produced by the fibroblasts.

Nervous Tissue 11

The nervous system of body is composed mainly of nervous tissue. However, connective tissue also makes some contribution to this system by forming capsules of ganglia, tubular investments of nerves, and meninges of brain.

The nervous tissue, also called ***nerve tissue*** or ***neural tissue***, consists of the following two main components:

1. **Nerve cells** or **neurons** which form the structural and functional units of nervous system. These cells possess the special physiologic properties of excitability and conductivity.
2. **Supporting cells** which protect, nourish and maintain the nerve cells and their processes, but are themselves nonexcitable and nonconducting. In the central nervous system, the supporting cells are known collectively as ***neuroglia;*** the neuroglia comprises three categories of cells: astrocytes, oligodendrocytes, and microglial cells. In the peripheral nervous system, the supporting cells occur in relation to nerve fibers as ***Schwann cells*** and within the ganglia as *satellite cells*.

THE NEURON (Fig. 11.1)

The neurons differ from other body cells in having the following two special properties:

i. **Excitability**, i.e., the ability to respond to physical or chemical stimuli by generating an electrical impulse.

ii. **Conductivity**, i.e., the ability to conduct the electrical impulse to other parts of nervous system (via the processes of the neuron).

A neuron consists of cell body and processes. The cell body (*soma or perikaryon**) contains the nucleus and most of the cell organelles. The processes, also called, *neurites*, form the most characteristic feature of the neurons. The neurites are of two types: (i) *axons*, and (ii) *dendrites*.

Each neuron bears only one **axon**, which is usually a long process that carries the nerve impulse away from the nerve cell body; the axon may or may not be branched. The axon and its branches end in specialized, dilated terminals which make synaptic junctions with another neuron or with an effector cell (e.g., a muscle cell or a glandular epithelial cell). All other processes of the neuron are known as dendrites. The **dendrites** are generally short processes which usually branch close to the cell body like the branches of a tree (in Greek, *dendron* means 'a tree' and *dendrite* implies 'tree-like'). The dendrites receive impulses (from other neurons) and convey them to the cell body.

The neurons belong to the static variety of cell populations and do not divide mitotically. Postnatal increase in the size of the brain and spinal cord occurs by the mitotic division of the neuroglial cells.

NERVE CELL BODY

The nerve cell body, also called *soma* or perikaryon, contains the nucleus and cytoplasm. It primarily serves as a nourishing center for the neuron and supplies organelles and macromolecules to the cell processes. The nerve cell soma is also receptive to excitatory or inhibitory stimuli, which are generated by other neurons and conveyed to it by the axons terminals of those cells. The perikarya vary considerably in size and may be as small as 4 µm in diameter, (e.g., those of the granule cells of the cerebellar cortex) or as large as 135 µm (e.g., those of the anterior horn cells of the spinal cord). The shape of a perikaryon depends on the number and orientation of the cell processes. The perikarya of the unipolar neurons are usually globular and those of the bipolar neurons are generally elongated or fusiform, while the cell bodies of the multipolar neurons may be stellate, pyramidal, or spherical in shape.

NUCLEUS

Most nerve cells contain a single large, pale-staining, vesicular nucleus which is spherical in shape and central in position. The characteristic vesicular appearance of the nucleus indicates that it contains a large amount of euchromatin. Presence of abundant euchromatin in the nuclei of the neurons is indicative of very active protein synthesis in the cytoplasm of these cells. The nucleus of a neuron contains a relatively large, deeply basophilic nucleolus which appears particularly prominent in the pale-staining nucleus.

CYTOPLASM

The cytoplasm of the perikaryon contains organelles, inclusions and elements of the cytoskeleton. The most conspicuous organelle of the cytoplasm of a neuron is the rough endoplasmic reticulum (RER). Free polyribosomes are also present in large numbers. Abundance of the RER and free ribosomes correlates with the active protein synthesis occurring in the perikaryon. The synthesized proteins are meant for local use as well as for transport across the axon. In stained sections examined under the LM, the abundant RER and free polysomes appear as clumps of basophilic material called **Nissl substance** (also called ***chromatophillic substance*** or *Nissl bodies*). The amount of Nissl substance in a neuron depends on its type and functional state; it is most abundant in the large-sized motor neurons.

The nerve cell soma also contains smooth endoplasmic

* In Greek, *peri*=around , and *karyon*=nucleus.

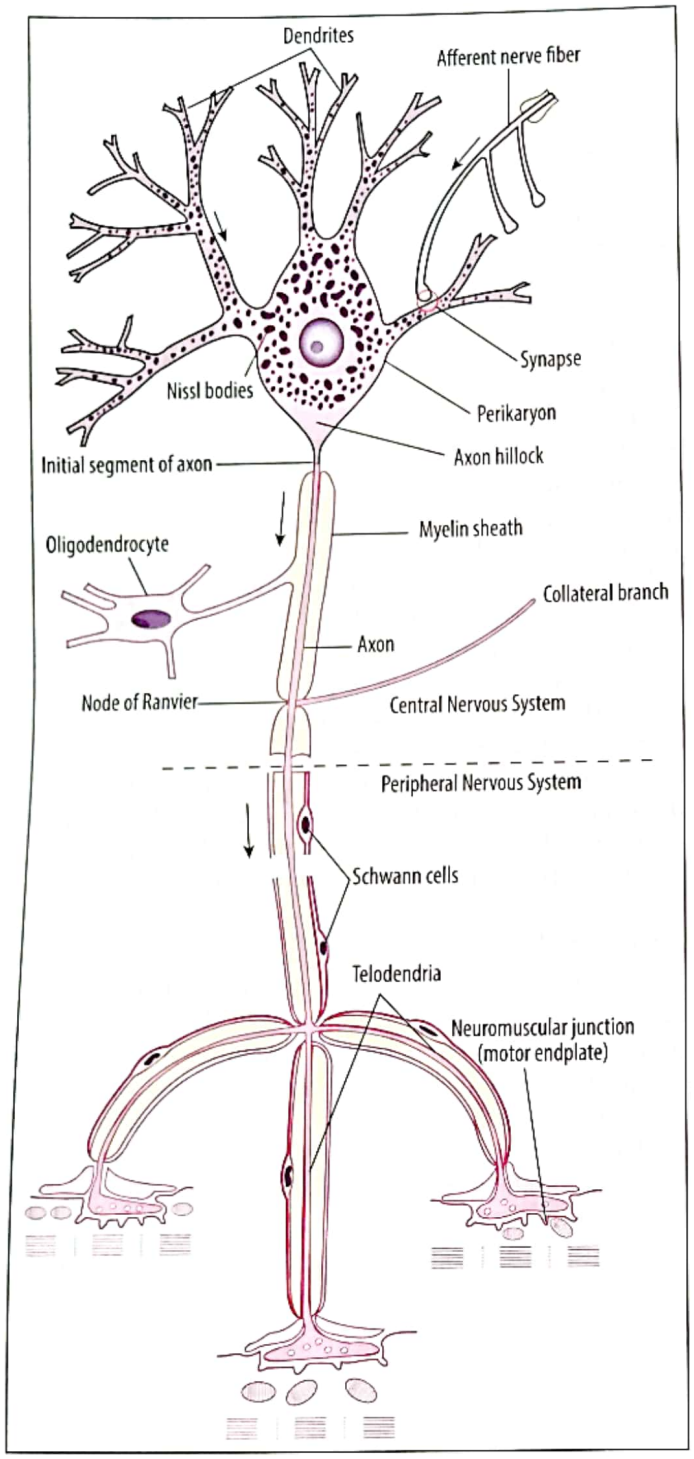

Fig. 11.1 Schematic drawing of a motor neuron. The arrows indicate the direction of nerve impulse.

reticulum, numerous mitochondria, abundant lysosomes, and a large Golgi apparatus. The well-developed Golgi complex correlates with the requirement of packaging of the huge amounts of proteins being synthesized by the RER. The large number of lysosomes correlates with the high turnover of cell membrane and other cell components. The most common inclusions of the neuronal cytoplasm are granules of the ***lipofuscin*** pigment, which occurs in the form of yellowish brown granules. This pigment represents the remnants of lysosomal activity and its amount increases with age.

The cytoskeleton of nerve cells consists of neurofilaments and microtubules which are randomly scattered in the cytoplasm. The **neurofilaments** are intermediate filaments having an average diameter of 10 nm. As such, the neurofilaments are not visible under LM, but under the effect of chemicals used for tissue fixation, these filaments become aggregated to form bundles; these neurofilament bundles, called *neurofibrils*, can be seen under LM when appropriate staining techniques (e.g., silver impregnation) are used. The **microtubules** form an important component of the cytoskeleton of the neuronal cytoplasm. They also enter into the axon and run along its whole length. The microtubules play an important role in the transport of proteins and other materials from one region of the neuron to the other.

NERVE CELL PROCESSES

DENDRITES

The dendrites are afferent processes of the neuron. They branch repeatedly like the branches of a tree. Most nerve cells have numerous dendrites which serve to increase the receptive area of the cell and enable one neuron to synapse with a large number of axonal terminals from other neurons. The dendrites contain nearly all the components of the neuronal cytoplasm except the Golgi apparatus. The outer surface of a dendrite shows numerous small, knob-like projections called *dendritic spines*. The dendritic spines are the sites where synapses occurring on a dendrites are located.

AXON

Each neuron has a single axon, which is the efferent process of the nerve cell and serves to transmit stimuli to other neurons or to the effector cells. The axon originates from the perikaryon at a short pyramid-shaped region known as the *axon hillock*. Unlike the dendrites, the axons usually have a smooth contour and a uniform diameter. Also, the axons are generally longer, straighter and thinner than the dendrites. Axons vary from less than a micrometer to several micrometers in diameter and from the fraction of a millimeter to more than a meter in length.

The cytoplasm of an axon is referred to as *axoplasm* and its plasmalemma as *axolemma*. The axoplasm contains mitochondria, SER, microtubules, and neurofilaments. No Nissl substance is seen in the axons indicating that no RER or free ribosomes are present in the axoplasm.

In contrast to dendrites, the axons generally have a uniform diameter and do not branch profusely. Along its course an axon often gives side-branchings, known as collateral branches (or, simply, *collaterals)*, which leave the axon nearly at right angles. Axons usually end by dividing into a number of terminal branches called *telodendria*. The axon collaterals and telodendria generally terminate as small knob-like expansions which are called *boutons*. The boutons are usually applied to the perikarya or dendrites of adjoining neurons to form synaptic junctions with them.

In addition to conduction of impulses, an important function of the axons is **axonal transport**, which occurs along the microtubules. As a result of this transport, movement of materials occurs between the perikaryon and the axon. The transport from the perikaryon to the axon terminals is called ***anterograde transport***, whereas the transport in the reverse direction is termed ***retrograde transport***. As a result of the anterograde transport, proteins, lipids, mitochondria, and synaptic vesicles are transported from the cell soma into the axon. A continuous supply of the lipids and proteins is essential for the maintenance of the normal structure of the axolemma. The retrograde transport conveys damaged membranes components and worn out mitochondria back to the perikaryon for final disposal. The macromolecular materials endocytosed by the axon by (e.g., viruses and toxins) are also conveyed to the nerve cell soma by means of the retrograde transport.

MORPHOLOGICAL CLASSIFICATION OF NEURONS (Fig. 11.2)

The neurons are classified according to the number of processes into the following three categories: unipolar neurons, bipolar neurons, and multipolar neurons.

1. **Unipolar Neurons.** These cells, also known as **pseudounipolar neurons**, possess a single process which divides into two branches in a T-shaped manner a short distance away from the perikaryon. One of these branches passes toward the periphery and functions as a dendrite, while the other branch passes toward the CNS as an axon. Unipolar neurons are found in the dorsal root ganglia of the spinal nerves and sensory ganglia of the cranial nerves.
2. **Bipolar Neurons.** These cells bear a single axon and a single dendrite which emerge from the opposite poles of a spindle-shaped cell body. Neurons of this variety are found in cochlear and vestibular ganglia, retina, and olfactory epithelium.
3. **Multipolar Neurons.** These cells have a single axon and a number of dendrites arising directly from the cell body. Most neurons of the human body belong to the multipolar variety. Some of the examples are: pyramidal cells of the cerebral cortex, Purkinje cells of the cerebellar cortex, and anterior horn cells of the spinal cord.

FUNCTIONAL CLASSIFICATION OF NEURONS

1. **Sensory Neurons.** These cells receive sensory stimuli from the environment or from the tissues and organs of the body and pass them on to the CNS.
2. **Motor Neurons.** These neurons convey impulses from the CNS or ganglia to the effector cells (i.e., muscle or gland cells) or to other neurons.
3. **Interneurons.** These neurons are located completely in the CNS. They form communicating and integrating circuits between sensory and motor neurons and other interneurons: Almost 99.9% of the neurons in the CNS are interneurons.

NERVE FIBERS

A nerve fiber consists of an axon and its coverings (if any). Two types of nerve fibers are found in the neural tissue: (i) myelinated nerve fibers, and (ii) unmyelinated nerve fibers.

MYELINATED NERVE FIBERS

In a myelinated nerve fiber, the axon is surrounded by a covering of a lipoprotein material; this material is called *myelin* and the covering is called myelin sheath. This sheath forms an interrupted, insulator covering that facilitates salutatory conduction in the myelinated nerve fibers which increases the conduction velocity up to 10 times.. The myelin is highly refractile in nature, due to which the myelinated nerve fibers, when seen in a mass, appear white on gross inspection (hence, the alternative name *white nerve fibers*). As mentioned earlier, the myelin sheath is not a continuous layer but shows interruptions (gaps) at regular intervals; these gaps are called *nodes of Ranvier, also termed nodal gaps*. Each segment of the nerve fiber lying between two consecutive nodes is called an *internode* (Fig. 11.3 & 11.4).

The myelin sheath is actually a greatly extended and modified plasmalemma wrapped around the axons in a spiral manner and, therefore, it shows concentric lamellae in transversely sectioned nerve fibers. The myelin lamellae are formed by the Schwann cells in the PNS and by the oligodendrocytes in the CNS. The process of formation of myelin sheath around the axons is called myelination or myelinogenesis. Myelination of axons in the neural tissue begins in the last three months of the intrauterine life, but occurs mainly during the period of infancy (first 12 months after birth). Myelination continues during the childhood and is completed in the adolescent period of life.

The **Schwann cells**, also called *neurolemmocytes*, are the supporting cells of the PNS which also perform the important function of forming myelin sheath around the axons. To make a myelin covering around an axon, multiple Schwann cells are involved. Each internode is myelinated by one Schwann cell and, therefore, longer the axon, greater the number of Schwann cells employed. Each Schwann cell extends its plasma membrane that spirals around the axon and the lipids and proteins of the plasmalemma give rise to the myelin sheath. Microscopic examination of a myelinated nerve fiber reveals that in each internode, outer to the myelin sheath, the residual cytoplasm of the Schwann cell forms a thin covering which is called *neurilemma* or *sheath of Schwann*. The flattened nucleus of the Schwann cell is also seen to be present under the neurilemma in the middle part of each internode.

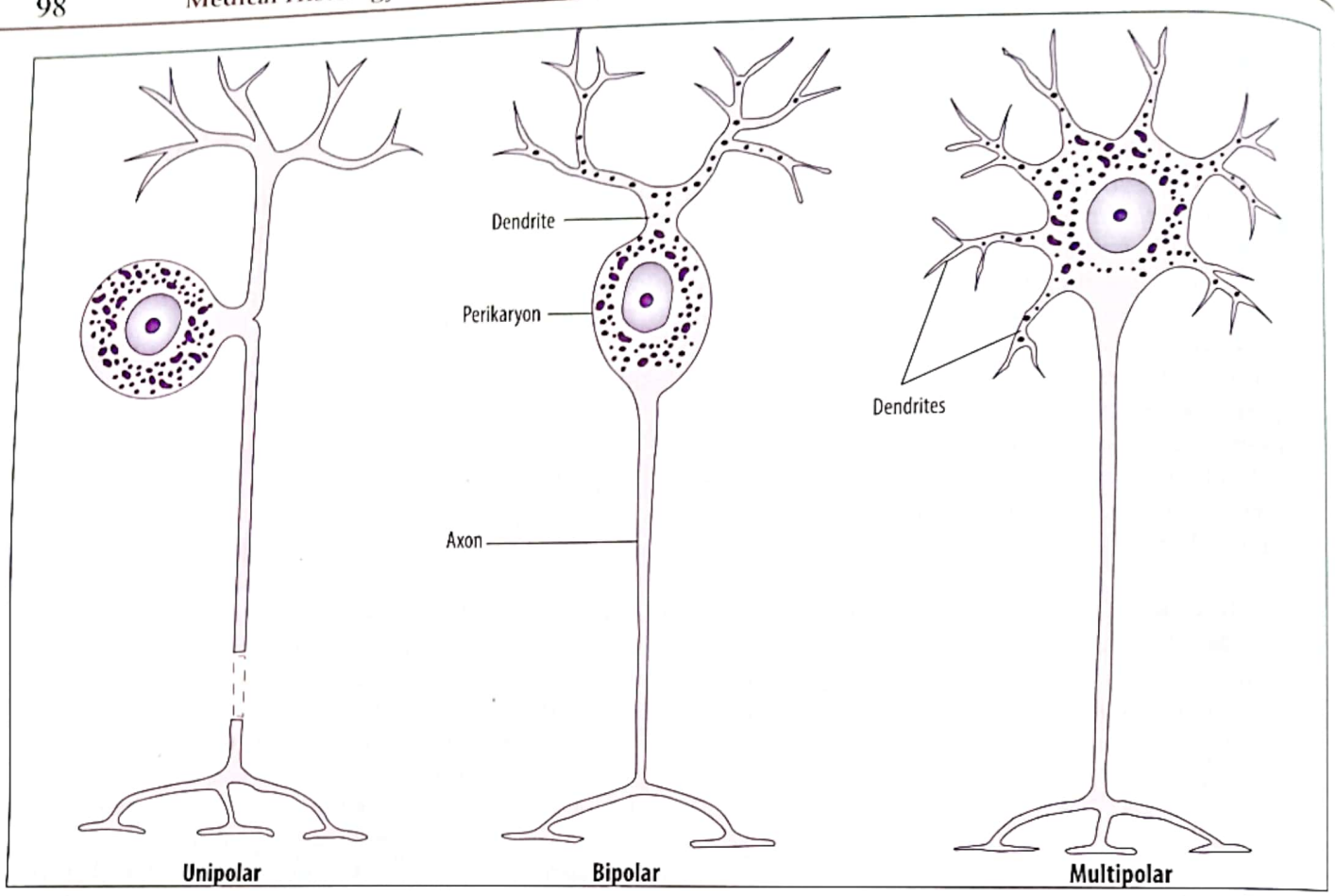

Fig. 11.2 Diagram showing different types of neurons according to their morphological features.

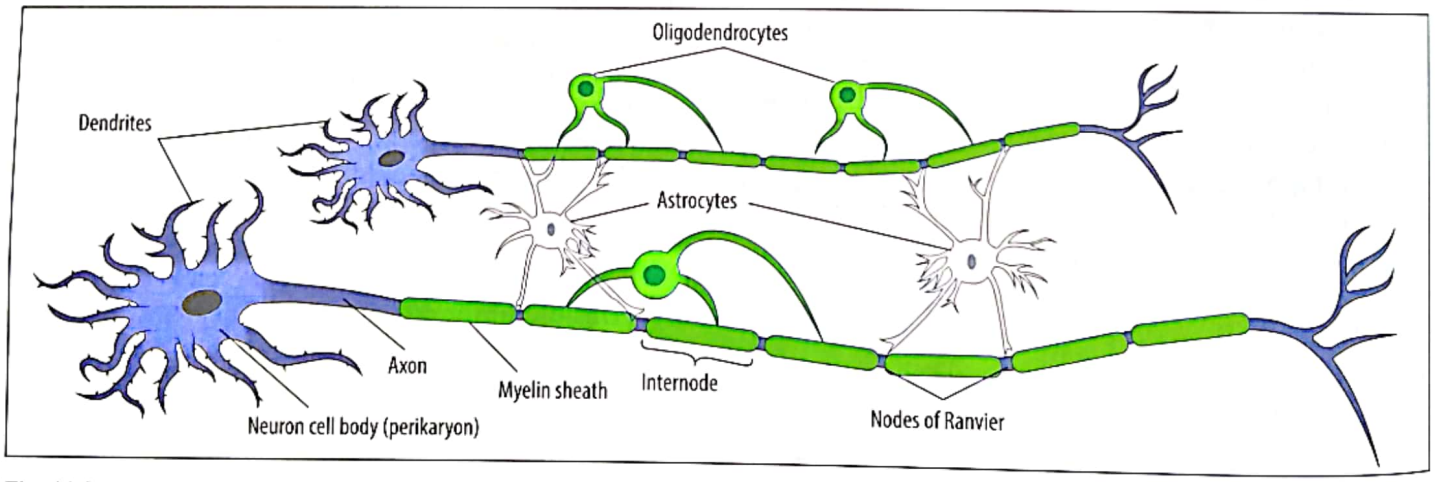

Fig. 11.3 Diagram showing two multipolar neurons in the CNS with their myelinated axons. Relationship of the myelinated nerve fibers to oligodendrocytes and astrocytes is also shown.

In the CNS, the oligodendrocytes form myelin around the axons. Each oligodendrocyte has several processes which form myelin sheath around many adjacent axons simultaneously (Fig. 11.4). The plasma membrane of each oligodendrocytic process wraps around a segment (prospective internode) of an axon and forms the myelin sheath in that internode. Several oligodendrocytes are employed to form myelin in different intermodal segments of an axon in the CNS. The cell body of the oligodendrocytes remain in between the nerve fibers and these cells continue to maintain the myelin sheath and provide trophic support to the axons.

UNMYELINATED NERVE FIBERS

The axons of smaller calibre (less than 1 μm in diameter) are not surrounded by a myelin sheath or any other covering. However, the unmyelinated nerve fibers receive trophic support from Schwann cells in the PNS and oligodendrocytes in the CNS. Therefore, in the peripheral nervous system the unmyelinated fibers run in grooves in successive Schwann cells (Fig. 11.5). In the CNS the unmyelinated axons run in grooves in the oligodendrocytes.

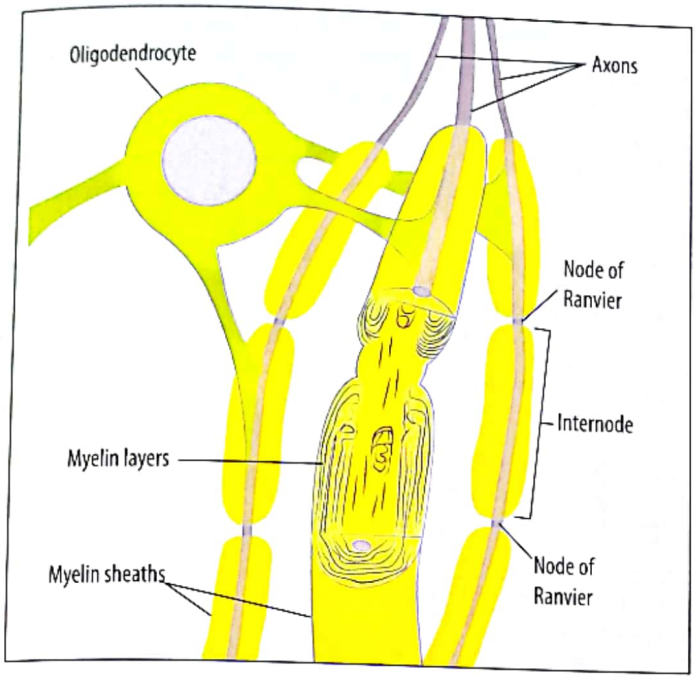

Fig. 11.4 Diagram showing an oligodendrocyte forming myelin sheaths around many axons in the CNS.

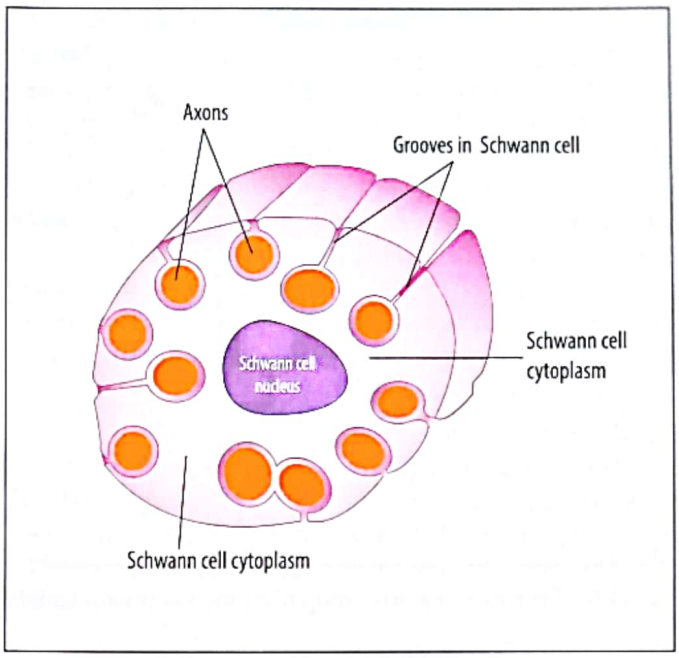

Fig. 11.5 Diagram showing the relationship of unmye-linated nerve fibers (axons) to a Schwann cell in the peripheral nervous system.

NERVE TERMINATIONS

The nerve fibers travelling in the peripheral nerves terminate in peripheral structures to which or from which they convey nerve impulses. Accordingly, the nerve terminations are classified into the following two major groups: (1) motor nerve endings, and (2) sensory nerve endings.

MOTOR NERVE ENDINGS

The motor (efferent) nerve fibers terminate in tissues in which they excite activity by releasing a neurotransmitter substance. At the termination of the somatic efferent fibers (which supply the skeletal muscle), the transmitter released is acetylcholine. On the other hand, at the termination of the visceral efferent fibers (which supply the smooth muscle and glandular epithelium) two different transmitters are released: (i) norepinephrine (noradrenaline) which is released at the sympathetic nerve endings, and (ii) acetylcholine, which is released at the parasympathetic nerve endings.

TERMINATIONS OF SOMATIC EFFERENT NERVE FIBERS

Neuromuscular Junctions (Fig. 11.6)

The motor nerve fibers supplying the skeletal muscle cells are myelinated axons that run in the perimysial connective tissue of the muscle. Each axon loses its myelin sheath and then branches to give rise to several terminal twigs. At the site of contact between each terminal twig and the muscle cell, a motor nerve ending is formed which is known as a *neuromuscular junction (also called motor endplate or myoneural junction).*

At a neuromuscular junction each terminal axonal twig ends in a bulbous expansion, which lies in a shallow depression in the sarcolemma of the muscle cell. This trough-like depression is called **synaptic cleft.** Within each bulbous axonal termination are present a large number of mitochondria and numerous small, round, clear **synaptic vesicles** containing acetylcholine. The space between the axon and the muscle fiber is filled with an amorphous material. In the base of the synaptic cleft the sarcolemma is thrown into folds which are called *junctional folds.* Beneath the junctional folds the sarcoplasm exhibits an abundance of muscle nuclei, glycogen granules, ribosomes, and mitochondria.

Acetylcholine receptors are present in the sarcolemma of the junctional folds. When a nerve impulse, travelling along the axon, reaches the axonal terminal, it causes release of acetylcholine from the synaptic vesicles into the synaptic cleft, where it binds to the acetylcholine receptors present on the sarcolemma of the junctional folds. This binding opens gated cation channels located in the sarcolemma in close vicinity of the acetylcholine receptors. This results in an influx of sodium ions which causes *depolarization* of the sarcolemma and, thus, an action potential is generated.

TERMINATIONS OF VISCERAL EFFERENT NERVE FIBERS

The postganglionic unmyelinated nerve fibers from the autonomic ganglia terminate in the following effectors: heart muscle (*cardiomotor*); smooth muscle of blood vessels (*vasomotor*); smooth muscle of viscera (*visceromotor*); smooth muscle (arrectores pilorum) of hairs (*pilomotor*); and glandular epithelium (*secretomotor*).

Near their termination, the visceral efferent fibers branch repeatedly and form complicated networks. Very fine

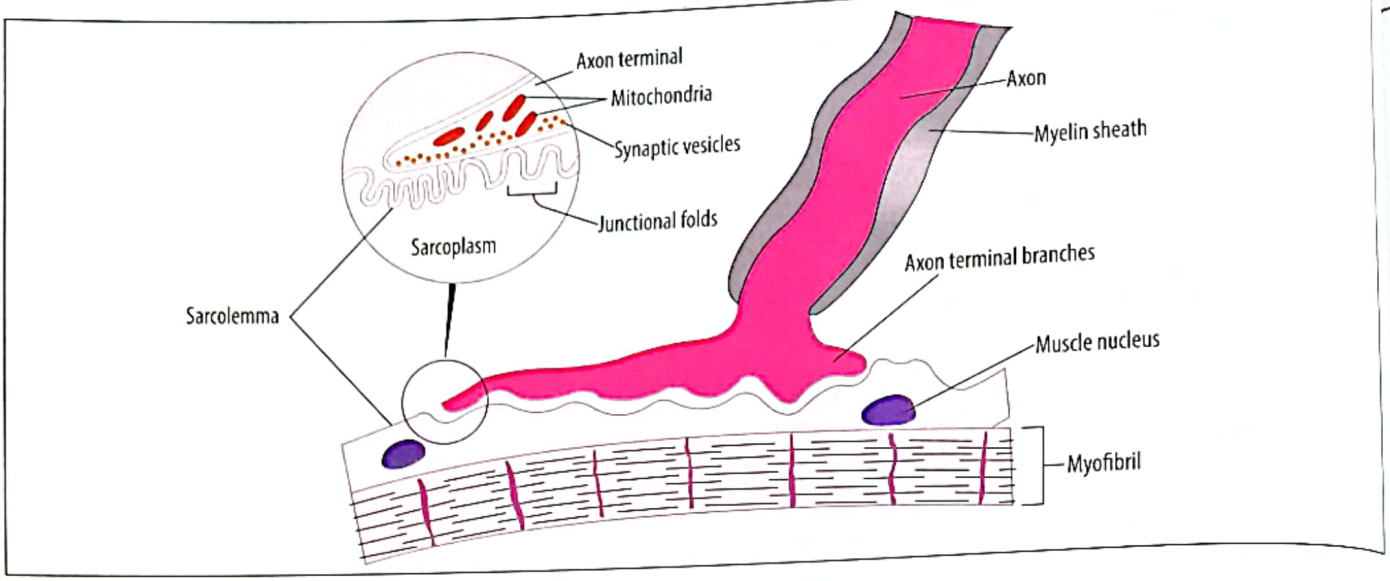

Fig. 11.6 Structure of a neuromuscular junction (motor endplate).

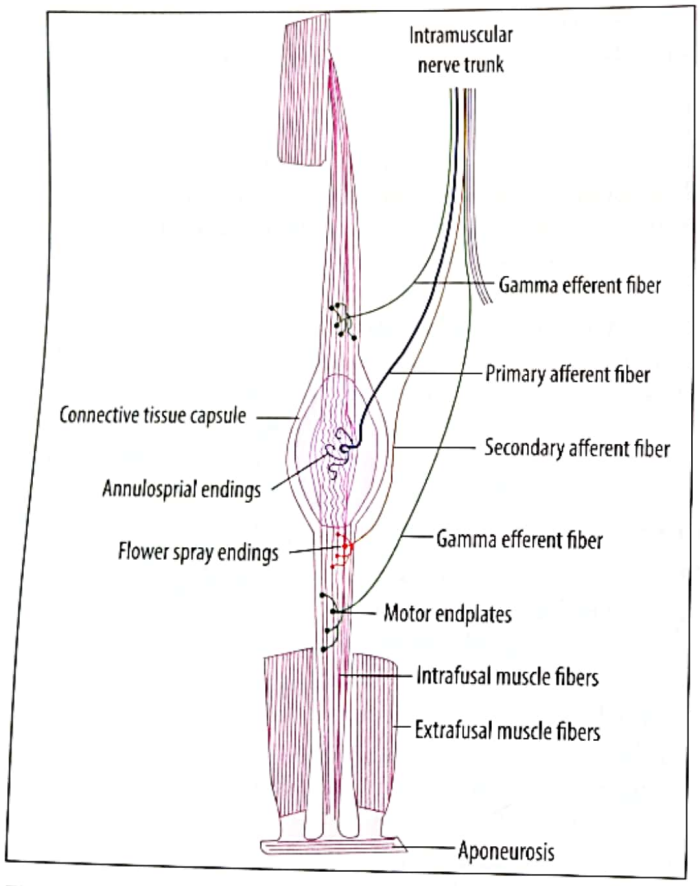

Fig. 11.7 Diagram showing the structure of a muscle spindle.

fibers arise from these networks and terminate in bulbous enlargements in the vicinity of the cells to be supplied. These enlargements contain vesicles of neurotransmitter substance (acetylcholine in case of postganglionic parasympathetic nerve fibers and norepinephrine in most of the postganglionic sympathetic nerve fibers). No special neuromuscular (or neuroglandular) junctions are formed at the terminations of visceral efferent nerve fibers. The neurotransmitter released at these terminations directly influences the responsive cells.

SENSORY NERVE ENDINGS (RECEPTORS)

The sensory nerve endings respond to stimuli in the periphery and send nerve impulses to the central nervous system (hence they may be called beginnings). Morphologically the sensory nerve endings can be classified into two main types:

A. Nonencapsulated (free) nerve endings.

B. Encapsulated nerve endings.

NONENCAPSULATED OR FREE NERVE ENDINGS

Free nerve endings constitute the most common variety of sensory nerve endings in the body. These endings are free (uncovered) terminal branches of the nerve fibers which represent the peripheral processes of the pseudounipolar neurons located in the dorsal root ganglia of the spinal nerves or sensory ganglia of the cranial nerves. These nerve fibers are either unmyelinated or finely myelinated in type. Before terminating, these nerve fibers lose their coverings like neurolemma and myelin sheath and each naked axon divides into several branches which end blindly between the epithelial cells (Fig. 11.8A). Similar free nerve endings are also found among the connective tissue fibers and cells, and around the muscle fibers. Free nerve endings do not show any structural specializations but it is believed that different fibers are functionally specialized to respond to the sensation of pain, temperature, and fine touch.

In the skin of the highly sensitive areas, like the fingertips and lips, a number of free nerve terminals form expanded, disc-like endings, each of which becomes associated with a special epithelial cell, called Markel cell. The Merkel cells are located in the deepest layers of the epidermis of skin. A Merkel cell and the associated free nerve ending are collectively referred to as *Merkel cell-neurite complex* (Fig. 11.8B). These specialized nerve endings are slowly-adapting mechanoreceptors concerned with the perception of light touch sensation.

ENCAPSULATED NERVE ENDINGS

In this group of sensory nerve endings, the nerve fiber terminals are enveloped by specialized connective tissue capsules. Encapsulated sensory nerve endings vary greatly in shape and size. They are classified into the following six varieties:

1. Meissner Corpuscles (Fig. 11.8C)

These sensory nerve endings, also called **tactile corpusles,** are located in the dermal papillae and are found chiefly in the nonhairy skin especially that of the finger tips, palms and soles. They are also found in the eyelids, lips and nipples.

The tactile corpuscles are oval or elliptical bodies measuring about 150 µm in length and 75 µm in diameter. Each corpuscle is covered by a connective tissue capsule, which is continuous with the epineurium of the nerve supplying the corpuscle. Within the capsule are present multilayered stacks of transversely disposed and flattened Schwann cells. Two or more myelinated nerve fibers supply each corpuscle, which lose their myelin sheaths before entering the corpuscle. The naked axons then pass upward through the stacked cells, pursuing a spiral or helical course.

The tactile corpuscles of Meissner are rapidly-adapting mechanoreceptors concerned with the perception of light touch.

2. Pacinian Corpuscles (Fig. 11.8D)

These nerve endings, also called **lamellated corpuscles** or *lamellar corpuscles*, are large, ovoid structures, about 1-2 mm in length and 0.5-1 mm in diameter. Each Pacinian corpuscle contains one nerve fiber, which loses its myelin sheath before entering the corpuscle.

In the lamellated corpuscle, the naked axon divides into several branches. The terminal part of the nerve fiber and its branches are surrounded by a capsule consisting of an inner core and an outer core. The *inner core* of the capsule immediately surrounds the axon along its entire length and consists of many layers of tightly-packed, flattened Schwann cells. The *outer core* forms the bulk of the capsule. It consists of several (15 to 50) concentric connective tissue lamellae. Each of these lamellae consists of fibroblasts and collagen type II and type IV fibrils. The adjacent lamellae are separated from each other by narrow fluid-filled spaces. Due to this specific lamellar arrangement, the cross section of a Pacinian corpuscle gives the appearance of a sliced onion in histological sections.

The function of the Pacinian corpuscles is to perceive the sensation of coarse touch, pressure and vibration. These nerve endings are mainly found in the deeper dermis and hypodermis of the palms, soles and digits. However, the Pacinian corpuscles are also found in the connective tissue in deeper parts of the body, e.g., in the interosseous membranes and in the connective surrounding the intestines and urinary bladder.

3. Ruffini Corpuscles (Fig. 11.8E)

These encapsulated nerve endings, also called Ruffini endings or bulbous corpuscles, are elongated, fusiform structures, each measuring about 1-2 mm in length and 0.2 mm in diameter. A Ruffini corpuscle consists of a thin connective tissue capsule enclosing a fluid-filled space. This space is traversed by bundles of collagen fibers that frequently pass through the capsule and anchor it to the surrounding connective tissue. A single myelinated nerve fiber enters the capsular space, loses its myelin sheath, and breaks up into a large number of unmyelinated branches that intertwine with the bundles of collagen fibers.

The Ruffini corpuscles are slowly-adapting mechanoreceptors and are chiefly located in the dermis, especially in the skin of the finger tips. These receptors are sensitive to skin stretch and sustained pressure on the skin. They are also thought to be responsible for the perception of heat.

4. Krause End Bulbs (Fig. 11.8F)

These nerve endings are oval bodies consisting of a thin connective tissue capsule that surrounds a central cavity. A myelinated nerve fiber enters the cavity, loses its myelin sheath, and divides into a number of branches which terminate in club-like endings. The Krause end bulbs are found in the skin, oral mucosa, clitoris and penis. These nerve endings are concerned with the perception of touch and pressure, but they are also thought to be thermoreceptors sensitive to cold.

5. Muscle Spindles (Fig. 11.7)

These proprioceptive nerve endings are also called ***neuromuscular spindles***. These encapsulated sensory receptors are located among and in parallel with the muscle fibers and are numerous toward the tendinous attachment of the muscle. A muscle spindle is generally less than a millimeter wide and up to 5 mm long.

Each muscle spindle consists of 6 to 14 modified striated muscle fibers enclosed in a connective tissue capsule. These muscle fibers, called *intrafusal fibers*, are much smaller, in both diameter and length, than the ordinary muscle fibers (which are called *extrafusal* muscle fibers).

Two varieties of intrafusal muscle fibers can be distinguished in a neuromuscular spindle: nuclear bag fibers and nuclear chain fibers. The **nuclear bag fibers** show a central bag-like dilatation in which are clustered numerous myonuclei. The central dilated portion is devoid of myofibrils and, hence, does not exhibit cross striations. The **nuclear chain fibers** are thinner than the nuclear bag fibers and exhibit only a single row of myonuclei in their central part which is devoid of myofibrils and cross striations. It should, however, be noted that the peripheral parts of the intrafusal muscle fibers contain myofibrils, show cross striations and possess contractile capability.

A muscle spindle is supplied both by afferent (sensory) and efferent (motor) nerve fibers that enter the spindle by

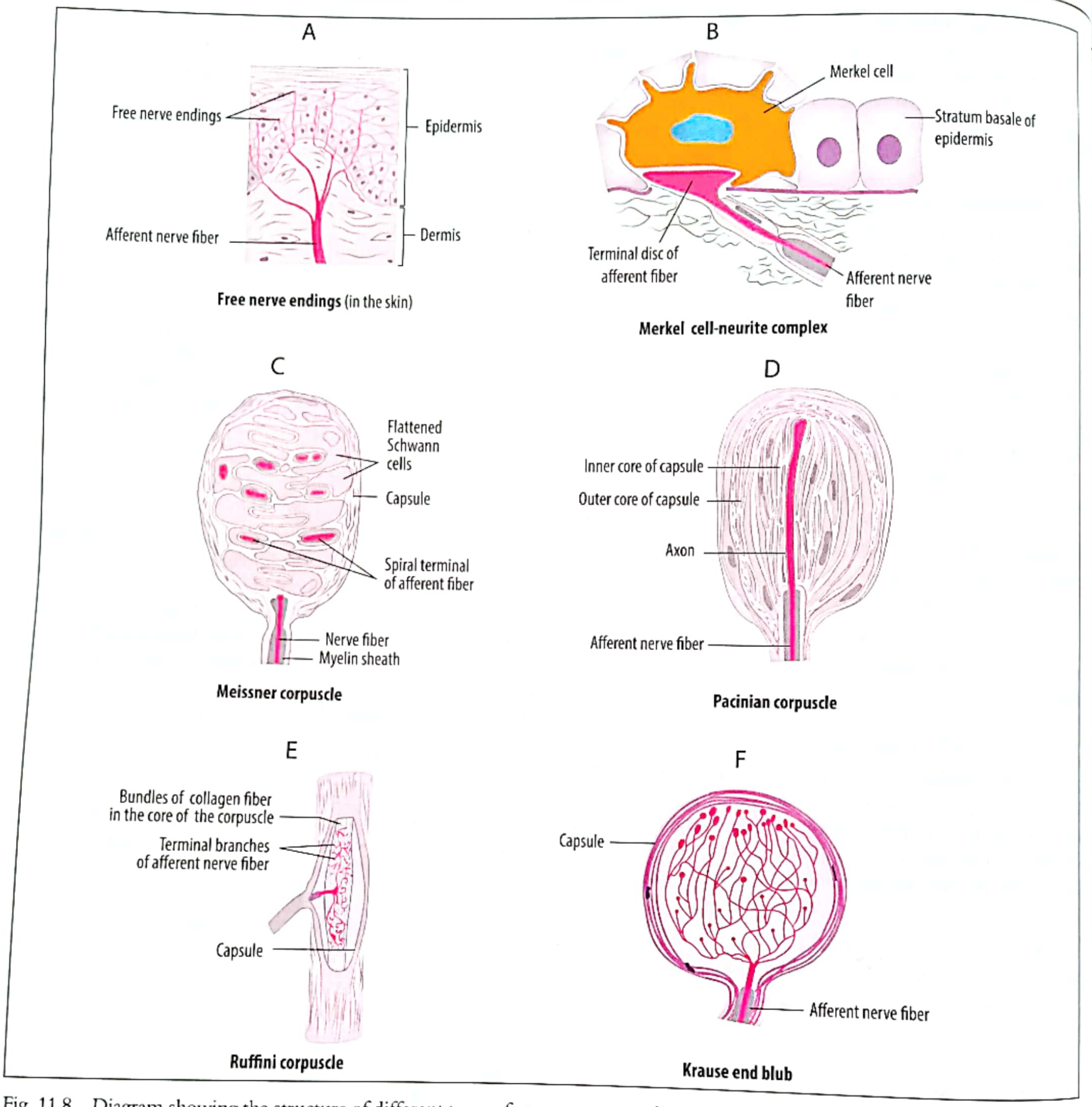

Fig. 11.8 Diagram showing the structure of different types of sensory nerve endings.

piercing the capsule. Each spindle receives a large and a small myelinated afferent nerve fiber. The large myelinated afferent nerve fiber loses its myelin sheath inside the capsule and divides into several branches which wind around the central parts of the nuclear bag and nuclear chain fibers in a spiral manner. These endings are called *annulospiral nerve endings* or *primary sensory nerve endings*. The smaller afferent nerve fiber loses its myelin sheath and divides into branches which terminate as small dilated nerve endings on the striated portions of the intrafusal muscle fibers, some distance away from the equatorial region. These nerve endings are known as *flower spray nerve endings* or *secondary sensory nerve endings*.

Each muscle spindle also receives fine gamma motor fibers (which originate from gamma motor neurons located in the spinal cord). These efferent nerve fibers terminate at small motor endplates on the peripheral striated portions of the intrafusal muscle fibers.

Function of the Muscle Spindle

The muscle spindles keep the CNS informed about the length of a muscle and the rate of change in its length and, thus, enable the CNS to control the degree of stretch in the voluntary muscles.

When a muscle is stretched (either actively or passively),

the intrafusal muscle fibers of the muscle spindles are also stretched. Lengthening of the intrafusal muscle fibers stimulates the primary and secondary sensory nerve endings of the muscle spindles. Stimulation of these nerve endings generates nerve impulses that are conducted to the spinal cord where the terminal branches of the axons synapse with the alpha motor neurons. The latter send impulses via efferent motor nerves to cause the contraction of the extrafusal muscle fibers; this constitutes the *stretch reflex*. Stimulation of the muscle spindles stops when the muscle contracts because the intrafusal fibers now return to their original length.

The gamma efferent nerve fibers modulate the sensitivity of the muscle spindles by regulating the length of the intrafusal muscle fibers.

6. Golgi Tendon Organs

These sensory nerve endings are found in tendons and are located near the muscle-tendon junctions. The Golgi tendon organs, also called *neurotendinous organs*, are cylindrical structures about 0.1 mm in diameter and 1 mm in length. They are positioned in series with the muscle fibers. Each Golgi tendon organ consists of a fibrous capsule which contains a bundle of loosely arranged, wavy collagen fibers, which are called *intrafusal collagen fibers*. A large myelinated nerve fiber enters the organ, loses its myelin sheath and divides into numerous smaller unmyelinated branches which end on the intrafusal collagen fibers as club-shaped free endings.

The Golgi tendon organs are stimulated by an increase of tension on the tendon. When a muscle contracts forcefully, tension on the intrafusal collagen fibers of the Golgi tendon organs is increased. To protect the muscle, bone and tendon, Golgi neurotendinous organs provide an inhibitory feedback to the alpha motor neurons of the same muscle. Nerve impulses from the Golgi tendon organs reach the interneurons in the spinal cord, which in turn exert an inhibitory influence on the alpha motor neurons located in the anterior gray horns of the spinal cord, causing relaxation of the muscle attached to tendon.

SYNAPSES

Synapses are the special surface contact sites where impulses are transmitted from a presynaptic cell (a neuron) to a postsynaptic cell (which may be a neuron or an effector cell, e.g., a muscle cell or a gland cell). The synapses permit neurons to contact with each other or with the effector cells.

Synapses between the neurons are commonly classified into the following three types:

1. **Axosomatic synapses**. An axosomatic synapse is a synaptic contact between an axon of a neuron and cell body of another neuron.
2. **Axodendritic synapses**. An axodendritic synapse consists of a synaptic contact between the axon of a neuron and the dendrite of another neuron.
3. **Axoaxonic synapses**. An axoaxonic synapse implies the synaptic contact of the axon of a neuron with the axon of another neuron.

Impulse transmission at synapses can occur electrically or chemically and, therefore, synapses are classified as electrical synapses and chemical synapses.

Electrical Synapses

The electrical synapses contain gap junctions that permit free movement of ions from one neuron to another. Movement of ions causes a flow of electrical current from one neuron to the other. Impulse transmission is much faster across the electrical synapses than across the chemical synapses. Although the electrical synapses are not very common in the human body, they have been found to be present in the retina, brainstem and cerebral cortex.

Chemical Synapses

In this variety of synapses, conduction of impulses occurs by the release of chemical substances (neurotransmitters). Chemical synapses constitute the most common variety of synapses in the nervous system.

A typical chemical synapse consists of three components:

1. A ***presynaptic knob*** (also called *bouton terminal*) which contains membrane-bound synaptic vesicles 40-60 nm in diameter) and a large number of mitochondria. The synaptic vesicles contain neurotransmitter substances like acetylcholine, norepinephrine, dopamine, serotonin or glycine, etc. That part of the plasma membrane of the bouton terminal that faces the synaptic cleft is called *presynaptic membrane*. A layer of dense material is present under the presynaptic membrane; this layer is called *presynaptic density*.
2. The ***synaptic cleft*** is the intercellular gap (20-30 nm wide) that separates the membrane of the presynaptic knob from the postsynaptic membrane belonging to the postsynaptic neuron.
3. The ***postsynaptic membrane*** is a portion of the cell membrane of the postsynaptic neuron. It contains the receptors with which the neurotransmitter released from the presynaptic knob interacts. The postsynaptic membrane also shows a *postsynaptic density* due to the presence of a layer of dense material under the cell membrane.

Mechanism of Chemical Synaptic Transmission

When a nerve impulse reaches the bouton terminal, it causes the opening of voltage-gated calcium ion channels located in the plasma membrane of the bouton. This allows influx of calcium ions from the extracellular space into the bouton. This Ca^{2+} influx causes the synaptic vesicles to fuse with the presynaptic membrane and release their contents (neurotransmitters) into the synaptic cleft. The specific receptors on the postsynaptic membrane bind the released neurotransmitter. This leads to the opening of sodium ion channels in the postsynaptic membrane,

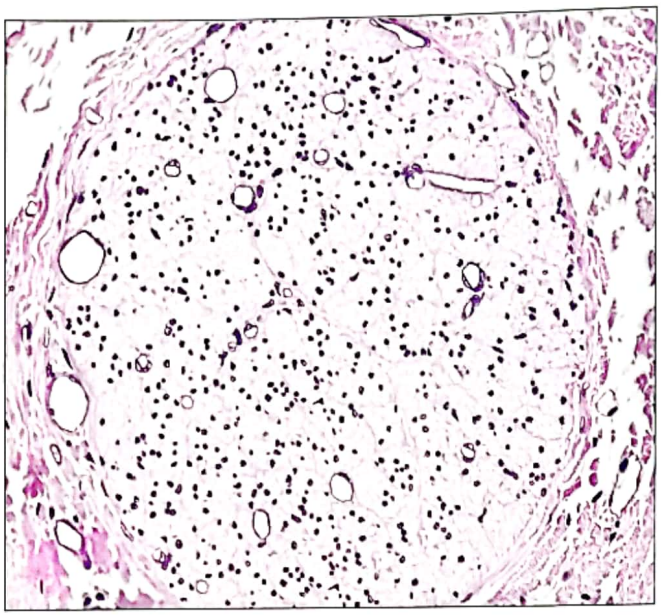

Fig. 11.9 Transverse section of a peripheral nerve.

resulting in depolarization of this membrane and thereby generating a nerve impulse.

PERIPHERAL NERVES (Fig. 11.9 & 11.10)

The peripheral nerves are composed of bundles of nerve fibers (axons and their supporting Schwann cells). The nerve fibers are held together by connective tissue, which is organized into distinct components: epineurium, perineurium, and endoneurium.

EPINEURIUM

This is a sheath of dense irregular connective tissue surrounding a peripheral nerve. The epineurium contains blood vessels and lymphatics of the nerve.

PERINEURIUM

Within the epineurium, the nerve fibers are arranged into bundles or fascicles. Each fascicle is surrounded by a connective tissue sheath called perineurium. This sheath generally consists of several layers of flattened epithelioid cells called *perineurial cells*. The number of these layers depends on the diameter of the nerve; large nerves may have up to 6 concentric layers but only a single layer is present in the nerves of very small diameter. Each layer is one cell thick and basal laminae of the perineurial cells intervene between the successive layers. The cells of each layer of the perineurium are joined at their edges by zonulae occludentes, so that each sleeve is a complete cylinder around the nerve fascicle. The perineurium functions as a diffusion barrier (called *blood-nerve barrier*) that maintains the ionic milieu of the nerve fibers within the perineurial sheath.

ENDONEURIUM

Within a fascicle, each nerve fiber (along with its supporting Schwann cells) is surrounded by a thin layer of connective tissue, known as endoneurium. The endoneurium contains delicate collagen fibers, reticular fibers, scattered fibroblasts, macrophages, capillaries, and perivascular mast cells.

GANGLIA

A collection of nerve cell bodies outside the central nervous system is called a *ganglion*. The ganglia form subsidiary nerve centers which receive and send out nerve fibers. They usually occur as ovoid structures associated with peripheral nerves.

Microscopically (and functionally), the ganglia can be grouped into the following two types:

1. Sensory ganglia.
2. Autonomic ganglia.

Both types of ganglia have some common features: (1) a connective tissue *capsule* surrounds each ganglion, (2) the capsule is continuous with a fine connective tissue network found throughout the substance of the ganglion, and (3) the perikaryon of each neuron in the ganglion is enveloped by a layer of small, flat or low cuboidal supporting cells called *satellite cells*. These cells control the microenvironment around the perikarya of the neurons and thus regulate their nourishment. In addition, the satellite cells provide electrical insulation to the nerve cell bodies.

SENSORY GANGLIA

The sensory ganglia receive afferent impulses from the periphery and convey them to the CNS. These ganglia are also called *craniospinal ganglia* because some of them are associated with the sensory roots of some cranial nerves (CN V, VII, IX, and X), while others are associated with the dorsal roots of all the 31 pairs of spinal nerves.

Each sensory ganglion is surrounded by a connective tissue capsule which is continuous with the epineurium of the nerve on which the ganglion is situated. The ganglion cells are unipolar neurons (also called pseudo-unipolar neurons). Each of these neurons gives rise to a single process which makes several irregular turns around the cell body and then divides into two branches in a T-shaped manner. One of these branches functions as a dendrite and passes in a spinal (or cranial) nerve to periphery where it terminates (or, actually, originates) at a receptor organ. The other branch, which is thinner of the two, becomes a functional axon and passes to the vcentral nervous system.

Within a sensory ganglion the nerve cell bodies may be small (only 15-30 μm in diameter) or large (about 120 μm in diameter). Those neurons which have small cell bodies give rise to unmyelinated processes, whereas large perikarya give origin to myelinated processes.

The nerve cell bodies are arranged as groups in the peripheral (cortical) zone of the ganglion. Each perikaryon is surrounded by a single layer of the supporting satellite cells (Fig. 11.11). The central (medullary) zone of the ganglion shows a great predominance of nerve fibers but is

devoid of nerve cell bodies.

The nerve impulse passes directly from the periphery to the CNS, bypassing the perikaryon of the unipolar neuron. Due to this reason, the perikarya of these neurons exhibit no synapses and perform an exclusively trophic function, providing nourishment for the cell's main process and its both branches.

AUTONOMIC GANGLIA

This group comprises the ganglia associated with the sympathetic and parasympathetic divisions of the autonomic nervous system.

The autonomic ganglia are also generally surrounded by a fibrous connective tissue capsule. However, the *intramural ganglia,* which are located in the walls of some viscera, are devoid of a proper connective capsule. The autonomic ganglia contain multipolar neurons having many branched dendrites and a single myelinated axon. Dendrites of these neurons make synaptic junctions with the incoming preganglionic nerve fibers, while their axons pass out as the postganglionic nerve fibers.

The perikarya of the neurons of the autonomic ganglia range from 20 to 60 µm in diameter. They are evenly distributed throughout the substance of ganglion and do not show the peripheral localization seen in the sensory ganglia. Generally, each neuron contains a single large, eccentrically-placed nucleus, but some cells may be binucleate. Because the neurons of these ganglia are multipolar, the perikarya of these neurons usually have an irregular or star-shaped appearance. Each multipolar neuron of an autonomic ganglion is associated with small satellite cells. In stained sections, the flat or low cuboidal satellite cells are seen to be located between the dendrites closely opposed to the plasmalemma of each perikaryon.

NEUROGLIA

The supporting tissue of the central nervous system is called neuroglia (also known simply as *glia*). The neuroglial cells cannot generate action potentials and their main function is to provide mechanical and metabolic support to the neurons. The neuroglia is considered to be essential for the maintenance and viability of neurons. The number of glial cells is about 10 times more than that of the neurons.

The neuroglia cannot be studied properly in sections stained by the routine H&E technique which only stains their nuclei (the nuclei of the neuroglial cells are generally smaller than those of the neurons). The neuroglia is best studied by special staining techniques like silver or gold impregnation which demonstrate the glial cells along with their processes.

The neuroglia is divided into the following two main types:

1. **Neuroglia proper** which consists of various types of supporting cells that occur between the neurons and their processes in the CNS. The neuroglia proper is further classified into three subtypes: astroglia, oligodendroglia, and microglia. The cells included in the ***astroglia*** are called astrocytes. The ***oligodendroglia*** consists of cells called oligodendrocytes. The ***microglia*** consists of cells referred to as microglial cells.
2. **Ependymal cells** (or ependyma) which line the cavities in the brain and spinal cord.

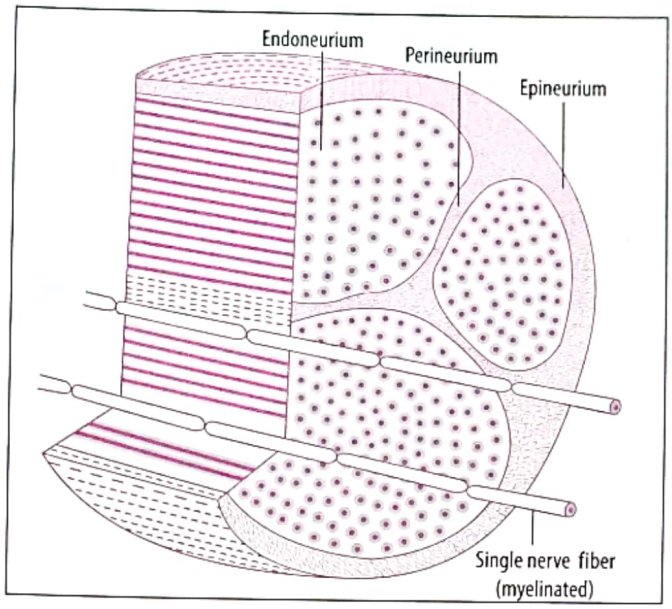

Fig. 11.10 A three-dimensional diagram showing the structure of a peripheral nerve.

Developmentally, the astrocytes, oligodendrocytes and ependymal cells are derived from the ectoderm of the neural crest, whereas the microglial cells are derived from precursor cells that originate in the bone marrow.

NEUROGLIA PROPER

ASTROGLIA

The astroglia consists of cells called **astrocytes**. The astrocytes are star-shaped cells with many branching processes. (in Greek, *aster* = star). The astrocytes are the largest and most numerous of the glial cells and are collectively also known as *astroglia*. They have large, centrally-located, spherical nuclei that stain lightly due to their vesicular nature. The cytoplasm of an astrocyte contains Golgi apparatus, lysosomes, a few ribosomes, and a small amount of rough endoplasmic reticulum. A special feature of the cytoplasm is the presence of bundles of intermediate filaments which extend into the processes. These intermediate filaments are composed of *glial fibrillary acid protein,* which is unique to the astrocytes.

Some of the cytoplasmic processes of the astrocytes end in small expansions called *perivascular endfeet*, which are applied to the walls of blood capillaries. Processes of astrocytes also extend to the surface of the brain and spinal cord forming a layer beneath the pia mater. Astrocytes are further classified into two subtypes: protoplasmic astrocytes and fibrous astrocytes.

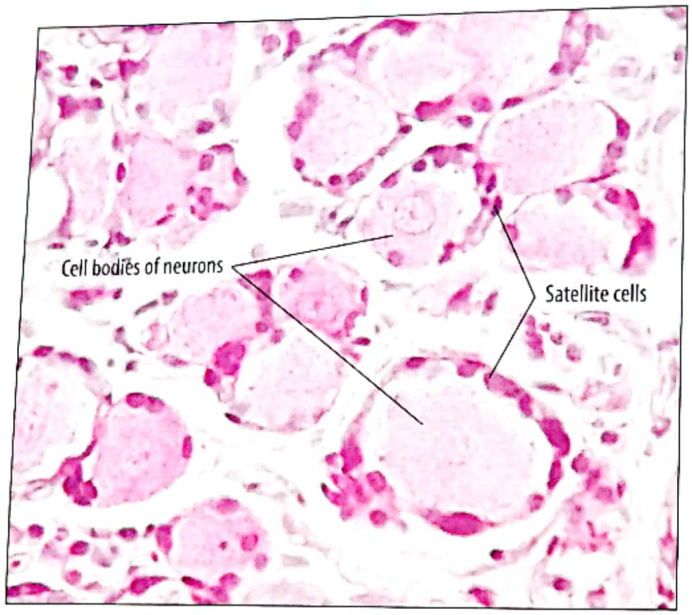

Fig. 11.11 A section through a sensory ganglion.

The **protoplasmic astrocytes** are found in the gray matter of brain and spinal cord. Their processes are generally short and thick but branch extensively. Most of these processes expand at their distal ends to envelop the neuronal surfaces and synaptic areas, while others terminate on blood capillaries as dilated perivascular endfeet (Fig. 11.12A). The protoplasmic astrocytes lying on the surface of the brain and spinal cord send some of their processes toward the pia mater. These processes reach the pia mater and terminate in expansions called *subpial feet*. The subpial feet of the adjacent protoplasmic astrocytes join with each other to form a membrane called *glia limiting membrane* or ***glia limitans***. The pia mater and the underlying glia limitans together form a relatively impermeable barrier that separates the neurons of the CNS from the CSF in the subarachnoid space.

The **fibrous astrocytes** are found in the white matter of the brain and spinal cord. As compared to the protoplasmic astrocytes, the fibrous astrocytes have fewer processes which branch infrequently and are thinner, straighter and longer (Fig. 11.12B). Some processes of the fibrous astrocytes terminate on blood capillaries as perivascular endfeet. As compared to the protoplasmic astrocytes, the cytoplasm of the fibrous astrocytes contains a much higher number of intermediate filaments.

Functions of the Astrocytes

1. The astrocytes regulate the passage of nutrients and metabolites between the blood and neurons. The perivascular endfeet of the astrocytes cover the walls of blood capillaries almost completely and, therefore, the oxygen and nutrients are transferred to the neurons through the astrocytes. Similarly, the CO_2 and metabolites pass from the neurons to the blood capillaries through the astrocytes.
2. The astrocytes modulate neuronal activities by regulating the ionic concentration in the extracellular space, especially the concentration of potassium ions which are released into the extracellular space as a result of the neuronal activity. The plasma membrane of the astrocytes contains abundant K^+ channels through which the K^+ ions pass from the extracellular space surrounding the neurons into the cytoplasm of the astrocytes. Thus buffering of the potassium ions occurs and the microenvironment around the neurons is kept in an optimal condition which allows the neurons to function normally.
3. The astrocytes and their perivascular feet play an important role in maintaining the blood-brain barrier.
4. The astrocytes, (especially the fibrous astrocytes) perform the function of formation of the scar tissue in the damaged areas of the CNS; such scars are known as *glial scars*.

OLIGODENDROGLIA

The oligodenroglia consists of cells called **oligodendrocytes** (Fig. 11.12D). As compared to astrocytes, the oligodendrocytes have smaller perikarya, smaller and denser nuclei, and fewer and shorter processes (the term oligodendrocyte implies "a cell with a few branches"). The oligodendrocytes are found in both gray and white matter of the CNS. They are very abundant in the white matter where they are often located as rows of cells between the neuronal processes; these oligodendrocytes are called interfascicular oligodendroglia. The interfascicular oligodendrocytes are involved in the formation and maintenance of myelin around the axons. In the gray matter, the oligodendrocytes are found in the vicinity of neurons; these oligodendrocytes are termed perineuronal satellite cells. The cell bodies of the oligodendrocytes are usually located near the blood capillaries but they do not have perivascular feet.

Functions of the Oligodendrocytes

In the infants and children, the interfascicular oligodendrocytes form myelin sheath around those axons in the CNS which are to be myelinated. In the adults, these cells serve to maintain the myelin sheaths around the myelinated nerve fibers of the CNS. In addition, these cells provide trophic support to the unmyelinated nerve fibers (naked axons) of the CNS as these axons run in cleft and grooves formed by the invaginations of plasmalemma of the oligodendrocytes.

Research has revealed that the oligodendrocytes (mostly perineuronal satellite cells) secrete a number of neurotrophins which provide local trophic support for the neurons. These neurotrophins include nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and neurotrophin-3.

MICROGLIA (Fig. 11.12C)

This variety of neuroglia consists of small, elongated cells,

each having a darkly-staining rod-shaped nucleus. The microglial cells have delicate tortuous processes which bear small spines. These cells are found both in the gray matter and white matter, and are often seen to be present in relation to blood vessels.

Functions of the Microglia

The microglial cells are resident macrophages of the brain and spinal cord. Like macrophages elsewhere, their chief function is phagocytosis of the unwanted particulate matter and they are regarded to be the representatives of the mononuclear phagocyte system (MPS) in the CNS. Whenever the nervous tissue of the CNS is invaded by bacteria (or other microorganisms), the microglial cells quickly move to the site of infection and phagocytose the invading microorganisms. They also engulf the debris of the damaged neurons and neuroglial cells. Being phagocytic in function, the microglial cells also act as antigen-presenting cells to activate the T lymphocytes. The microglial cells also secrete many types of immunoregulatory cytokines.

EPENDYMAL CELLS

The ependymal cells are cuboidal or low columnar cells that form an epithelium-like lining of the ventricles of brain and the central canal of the spinal cord; this lining is called *ependyma*. The ependyma lacks a basal lamina and the basal ends of the ependymal cells send branching processes into the adjacent white matter.

The ependymal cells are tightly bound together by junctional complexes. The apical surface of these cells bears kinocilia and microvilli (Fig. 11.12E). Rhythmic beating of the kinocilia ensures directional flow of the CSF. Presence of microvilli on the apical surface increases the surface area and facilitates the absorption of the CSF by the ependymal cells, so that the CSF contents (especially the sodium, potassium and chloride ions) can be transferred to the neural tissue to ensure optimum activity of the neurons.

GRAY MATTER AND WHITE MATTER

The brain and spinal cord are composed of gray matter and white matter. The **gray matter** consists mainly of nerve cell bodies. In addition, it contains unmyelinated and myelinated nerve fibers (mostly unmyelinated), protoplasmic astrocytes, oligodendrocytes, and microglial cells. The tangled meshwork of axons, dendrites, and neuroglial cell processes present within the gray matter is called *neuropil*.

The **white matter** does not contain nerve cell bodies and consists mainly of myelinated and unmyelinated nerve fibers. It also contains oligodendrocytes, fibrous astrocytes and microglial cells. The characteristic color of the white matter is due to the predominance of myelinated nerve fibers.

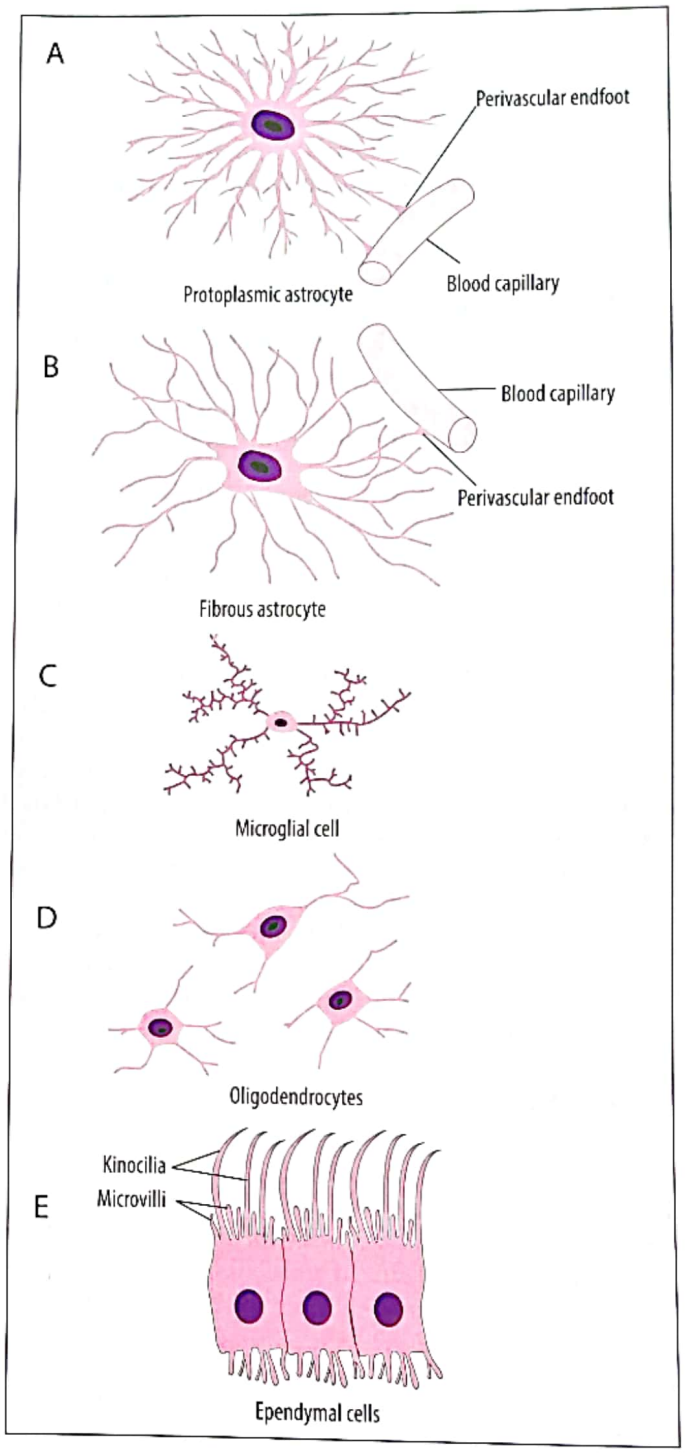

Fig. 11.12 Diagrammatic representation of morphological features of different types of neuroglia.

BLOOD-BRAIN BARRIER

The blood-brain barrier (BBB) is a highly selective permeability barrier that separates the circulating blood from the extracellular fluid in the CNS. The BBB does not permit any bacteria, bacterial toxins or harmful drugs and chemicals to pass from the blood circulation into the neural tissue.

The blood-brain barrier depends mainly on the following three structural factors:(i) occluding junctions are present

MONOCYTOPOIESIS

It has already been described that the bipotential CFU-GM stem cell is derived from the multipotential CFU-GEMM cells in the bone marrow. The CFU-GM divides and gives rise to CFU-G cell (progenitor of granulocytes) and CFU-M, which is the progenitor cell for the monocytes. The CFU-M cell is titled as monoblast.

The **monoblast** is quite similar to a myeloblast (precursor cell of the granulocytic series). The monoblasts differentiate into promonocytes. A **promonocyte** is a large cell (16-18 µm in diameter) containing a large kidney-shaped nucleus. The cytoplasm of a promonocyte stains basophilic (bluish) due to the abundance of RER and free ribosomes. There are no specific granules, but many azurophilic granules (representing the lysosomes) can be seen.

The promonocytes divide two or three times and then differentiate into **monocytes** which enter the bloodstream. The monocytes remain in the blood for a short period and then leave the bloodstream to give rise to different types of cells belonging to the mononuclear-phagocyte system like macrophages, osteoclasts, and microglial cells, etc.

DEVELOPMENT OF PLATELETS (THROMBOPOIESIS)

In the red bone marrow, the multipotential stem cells CFU-GEMM give rise to unipotential platelet progenitor cells titled CFU-Meg, which are committed to differentiate into **megakaryoblasts.** The megakaryoblasts differentiate into **megakaryocytes.** The platelets are formed by detachment of small, membrane-bound fragments from the cytoplasm of the megakaryocytes.

MEGAKARYOBLAST

The megakaryoblast is a large cell (25-50 µm in diameter) that contains a large lobated nucleus with multiple nucleoli. Its cytoplasm is basophilic and does not show any granules. Megakaryoblasts differentiate into megakaryocytes by a special type of nuclear division called **endomitosis**, whereby the chromosomes replicate and divide repeatedly, but neither karyokinesis (division of nucleus) nor cytokinesis (division of cytoplasm) occurs. The cell becomes large and the nucleus becomes highly polyploid (as much as 64n); this cell is now known as a *megakaryocyte*. Differentiation of megakaryoblast into a megakaryocyte occurs under the influence of a hormone called *thrombopoietin,* which is produced chiefly by the liver.

MEGAKARYOCYTE

The megakaryocyte is a giant cell (50-100 µm or more in diameter), with a large lobated nucleus. The cytoplasm of a megakaryocyte is basophilic and exhibits many azurophilic granules. Electron micrographs reveal that the cytoplasm of a megakaryocyte contains an extensive Golgi apparatus, numerous mitochondria, abundant RER, SER, many lysosomes, and multiple centrioles. Most of these organelles are seen to be concentrated in the perinuclear zone. The nucleus of a megakaryocyte is lobated in a complex manner. Individual lobes may be closely packed or connected by fine strands of chromatin. No nucleoli are visible.

The megakaryocytes are irregular cells having many cytoplasmic processes which extend into the sinusoids of the red bone marrow. These cytoplasmic processes, called proplatelets, become gradually subdivided into small compartments (1-3 µm in diameter), by invaginations of the plasma membrane, which are called *demarcation membranes*. Fragmentation of the cytoplasmic processes of the megakaryocyte along the demarcation membranes results in the formation of platelets which are released into the blood.

Megakaryocytes have a limited life span of about 10 days. After the peripheral cytoplasm is shed off as platelets, the remaining cell shows apoptotic changes and is finally phagocytosed by the macrophages. A single megakaryocyte can produce several thousand platelets before it dies by apoptosis.

DEVELOPMENT OF LYMPHOCYTES (LYMPHOPOIESIS)

The lymphocytes originate in the bone marrow from the multipotential hemopoietic stem cells CFU-L. The stem cells divide to produce **lymphoblasts** which are the progenitor cells of the lymphocytic lineage. After further division and differentiation, the lymphoblasts give rise to smaller cells called **prolymphocytes.** The prolymphocytes do not possess any surface marker and, hence, cannot be distinguished into T cells or B cells. A pro-lymphocyte has a deeply staining nucleus containing condensed chromatin. The nucleus is surrounded by a thin rim of basophilic cytoplasm containing very few organelles.

Some prolymphocytes remain in the bone marrow throughout life and keep on dividing there to produce daughter cells that differentiate into B lymphocytes. Other prolymphocytes enter the blood and reach the gut-associated lymphoid tissue (GALT), spleen and lymph nodes where they differentiate into B lymphocytes.

During the fetal and early postnatal life, the lymphoblasts migrate from the bone marrow to the thymus via the bloodstream. In the thymus, the lymphoblasts settle down in the peripheral part of the thymic cortex and proliferate there to give rise to daughter cells that are called *thymocytes*. The thymocytes move through the cortex to reach the thymic medulla. During their journey through the thymic cortex, the thymocytes differentiate into T lymphocytes (by obtaining surface markers that are characteristic of T lymphocytes). From the medulla of the thymus, the T lymphocytes are released into the blood. Most of these T lymphocytes keep on circulating in the blood, but some of them settle down in the peripheral lymphoid organs like spleen, lymph nodes, and tonsils, etc.

It is to be noted that B lymphocytes differentiate in the bone marrow as well as in lymph nodes, spleen and GALT, but the T lymphocytes differentiate only in the thymus.

LEUKEMIA

Leukemia is a progressive malignant disease characterized by distorted proliferation of the leukocytes and their precursors in the bone marrow. This results in: (i) a tremendous increase in the total number of leukocytes in the blood, and (ii) presence of abnormal leukocytes in the peripheral blood; these abnormal cells are actually the WBC precursors which spill over from the bone marrow into the blood.

The leukemia is further classified into two major types: myeloid leukemia and lymphocytic leukemia.

The **myeloid leukemia**, also called *myelogenous leukemia*, is characterized by a malignant change in those bone marrow cells which give rise to the polymorphonuclear leukocytes.

The **lymphatic leukemia**, also known as *lymphogenous leukemia*, is characterized by a malignant change in those bone marrow cells which ultimately produce lymphocytes.

Both types of leukemia described above are further categorized into acute and chronic types. The acute leukemia is characterized by a very high percentage of undifferentiated leukocyte precursors in the peripheral blood. In the chronic leukemia, there is a high percentage of relatively mature, but still not completely differentiated, leukocytes in the blood.

between the endothelial cells lining the blood capillaries of the neural tissue, (ii) abundant pericytes surround the endothelial cells of these capillaries, and (iii) the expanded perivascular endfeet of the astrocytes form an almost complete sheath around the basal lamina of the endothelial cells of the capillaries running in the neural tissue.

MENINGES

The brain and spinal cord are invested by three connective tissue coverings, which are known collectively as meninges*. These coverings are: (1) pia mater, which is the innermost layer, (2) arachnoid mater, which lies external to the pia mater, and (3) dura mater, which is the outermost layer.

PIA MATER

The pia mater, also known simply as *pia,* is the innermost layer of the meninges. It lies directly on the surface of the brain and spinal cord and closely follows their contours. As indicated by its name (in Latin, *pia* = tender; *mater* = mother), the pia mater is a delicate layer of highly vascular connective tissue. It is composed mainly of a thin layer of flat, modified fibroblasts. In addition, fine collagen fibers, elastic fibers, macrophages, mast cells, and lymphocytes are also present in the pial layer. Blood vessels (capillaries) are abundant in this layer. From the pia mater the blood capillaries are conveyed to the neural tissue. The outer surface of pia mater (which forms the inner boundary of the subarachnoid space) is covered by a single layer of squamous epithelial cells. The inner surface of the pia mater is in contact with the glia limitans.

ARACHNOID MATER

The arachnoid mater consists of fibroblasts, collagen fibers and a few elastic fibers. This meningeal layer is composed of two regions: (1) a sheet of connective tissue which lies in contact with the dura mater, and (2) a system of loosely arranged trabeculae that connect the flat, outer layer of arachnoid mater with the pia mater. The *arachnoid trabeculae* traverse the *subarachnoid space* (which is the space that lies between the pia mater and sheet-like portion of the arachnoid mater, and is filled with cerebrospinal fluid). Due to the presence of the trabeculae, the arachnoid mater gives the appearance of a spider's web (in Greek *arachnoiedes* means cobweb-like). The arachnoid trabeculae are composed of *arachnoid trabecular cells* (which are modified fibroblasts) along with a few collagen Fibers. The arachnoid trabecular cells possess long processes that attach to each other by desmosomes and communicate with one another by gap junctions.

The inner and outer surfaces of the sheet-like layer of arachnoid mater as well as the arachnoid trabeculae are covered by a single layer of flat epithelial cells, which are similar to those covering the outer surface of the pia.

* The word *meninges* is the plural of the Greek term *meninx* that means *membrane*.

The arachnoid is itself avascular, although blood vessels pierce it to pass from the dura to the pia mater. During embryonic life, the pia mater and arachnoid mater develop from a single sheet of mesenchyme surrounding the developing brain and spinal cord. In the adult the interface between the two layers is often difficult to distinguish. Therefore, the two layers are often collectively referred to as *pia-arachnoid*. The pia and arachnoid are also called *leptomeninges* (in Greek, *lepto* = thin).

DURA MATER

The dura mater (literally meaning *tough mother*) is a thick and tough layer of dense fibroelastic connective tissue. The **cranial dura mater**, which covers the brain, is fused with the periosteal lining of the inner surface of the bone of the neurocranium. The inner surface of the cranial dura is lined by a simple squamous epithelium. The narrow space between the dura mater and arachnoid mater is known as the *subdural space*. The dura mater covering the spinal cord, called **spinal dura mater**, is not fused with the periosteum of the vertebrae and, therefore, a narrow but definite *epidural space* exists between the outer surface of the spinal dura and periosteum of the wall of the vertebral canal. This space contains loose connective tissue and a network of thin-walled veins. Both the inner and outer surfaces of the spinal dura mater are covered by simple squamous epithelium.

Choroid Plexus

Each ventricle of the brain contains tufted projections on its roof or wall which consist of folds of highly vascularized pia mater covered by a layer of modified ependymal cells.

The function of the choroid plexus is to secrete the cerebrospinal fluid (CSF). The specialized ependymal cells remove mainly water and ions (Na^+, K^+, and Cl^-) from the blood capillaries of the choroid plexus and secrete these into the brain ventricles as the CSF. Some glucose and a very small amount of proteins are also secreted. The CSF is produced continuously and fills the brain ventricles, central canal of the spinal cord, and the subarachnoid space. The CSF is finally conveyed to the venous circulation through the arachnoid villi which drain this fluid into the superior sagittal venous sinus. The major **functions of the CSF** are: (1) it provides the Na^+, K^+, and Cl^- ions to the neural tissue which are required for optimal neuronal activity, and (2) by filling the subarachnoid space, it serves as a shock absorber to protect the neural tissue from injury during sudden and severe movements of the head or vertebral column.

Cerebrum, Cerebellum and Spinal Cord

12

CEREBRUM

The cerebrum consists of two cerebral hemispheres. Each of these hemispheres is covered by a layer of gray matter called *cerebral cortex* or *pallium*. The central region underneath the cortex consists of white matter. However, small masses of gray matter also occur in the white matter, which are called *nuclei*.

CEREBRAL CORTEX

As stated above, the layer of gray matter covering the cerebral hemispheres is called cerebral cortex or pallium. It is to be noted that the structure of the pallium is not same in all parts of the cerebrum. Most (90%) of the cerebral cortex belongs to a type called isocortex, neocortex or neopallium (because it represents the new cortex that developed during the course of evolution). The remaining (10%) cortex represents phylogenically old cortex and is called allocortex. The allocortex is structurally much simpler than the neocortex and comprises the olfactory cortex and the cortex covering the hippocampal formation. A description of the structure of the neocortex is given below. It should also be noted that, unless otherwise specified, the term cerebral cortex generally implies the neocortex.

Before discussing the structure of various layers of the cerebral cortex, a description of various types of neurons found in the cerebral cortex is given below.

Cells of The Cerebral Cortex (Fig. 12.1)

Before discussing the structure of different layers of the neocortex, it is necessary to have the knowledge of the various types of nerve cells found in the cerebral cortex. These neurons can be classified into the following two major groups:

i. Pyramidal cells.

ii. Nonpyramidal cells.

PYRAMIDAL CELLS

The pyramidal cells are multipolar neurons which have pyramid-shaped perikarya. Each cell has one main dendrite which arises from the apex of the cell soma and passes outward to end in the most superficial layer of the cortex. In addition, four or more branching dendrites pass outward from the base of the perikaryon. The axon emerges from the center of the base and runs into the underlying white matter to become a projection fiber or an association fiber.

The pyramidal cells are further classified into small, medium and large varieties on the basis of the height (length) of their cell bodies, which generally ranges from 10 to 50 µm. However, in the internal pyramidal layer of the primary motor area, giant pyramidal cells, called *Betz cells*, are found that have cell bodies that measure up to 100 µm in length.

NONPYRAMIDAL CELLS

This group includes the neurons of the following types: granule cells, horizontal cells, Martinotti cells, and fusiform cells. Most of these cells are interneurons.

1. **Granule Cells**. These cells have small, polygonal perikarya. The granule cells are also known as *stellate cells* because each of these cells gives off a number of small dendrites that pass in various directions, giving a star-shaped appearance to the cell. Each granule cell gives off a short axon which ramifies close to the cell body. Most of the extrinsic inputs to the cerebral cortex (e.g., those from the related thalamic nuclei) terminate on the dendrites of the granule cells.
2. **Horizontal Cells**. These are small, fusiform, horizontally oriented neurons found in the most superficial cortical layer. A horizontal cell has many short dendrites and a long axon which runs parallel to the surface of the cortex, making contact with the dendrites of the pyramidal cells.
3. **Martinotti Cells**. These are small, multipolar neurons found in the deeper layers of the cerebral cortex. A Martinotti cell has short dendrites, but the long axon ascends toward the cortical surface to end in a more superficial layer, commonly reaching the outermost layer of the cortex.
4. **Fusiform Cells.** These cells have spindle-shaped cell bodies that are oriented at right angles to the surface of the cerebral cortex. The axon arises from the side of the cell body (near its middle part) and descends into the white matter. Each end of the fusiform cell body gives rise to several branching dendrites; these dendrites extend outwards into the superficial layers and inwards into the deeper layers of the cortex.

LAYERS OF THE CEREBRAL CORTEX

The cerebral cortex (neocortex) consists of six layers of cells and fibers which are superimposed one uon another. These layers can easily be identified in a stained tissue section which has been cut perpendicular to the cortical surface. The six cortical layers (from superficial to deep) are as described below:

1. **Molecular Layer**. This layer, also called *plexiform layer*, is composed chiefly of cell processes (dendrites and axons). In sections stained for nerve fibers, the transversely cut cell processes give a punctate or

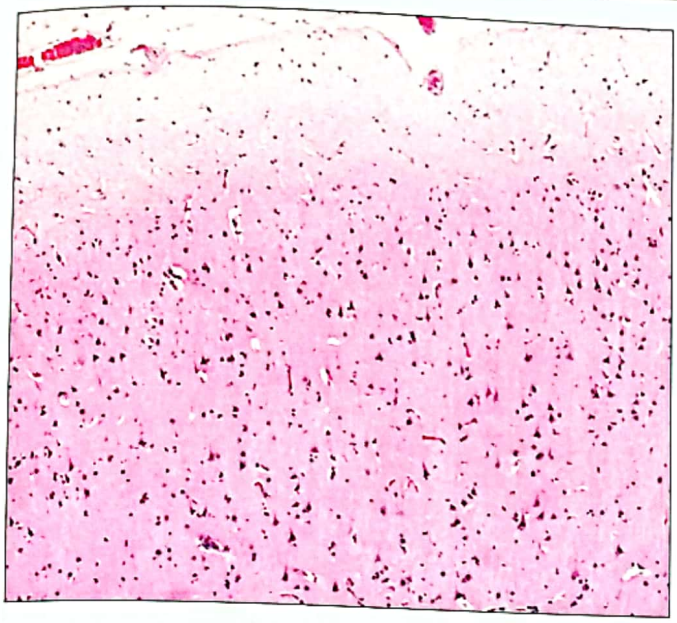

Fig. 12.2 A section through the cerebral cortex (neocortex).

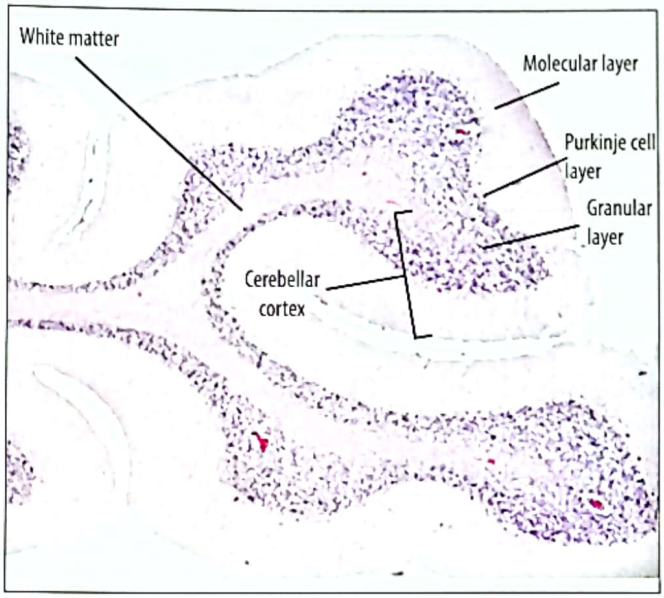

Fig. 12.3 A section through a lobule of the cerebellum.

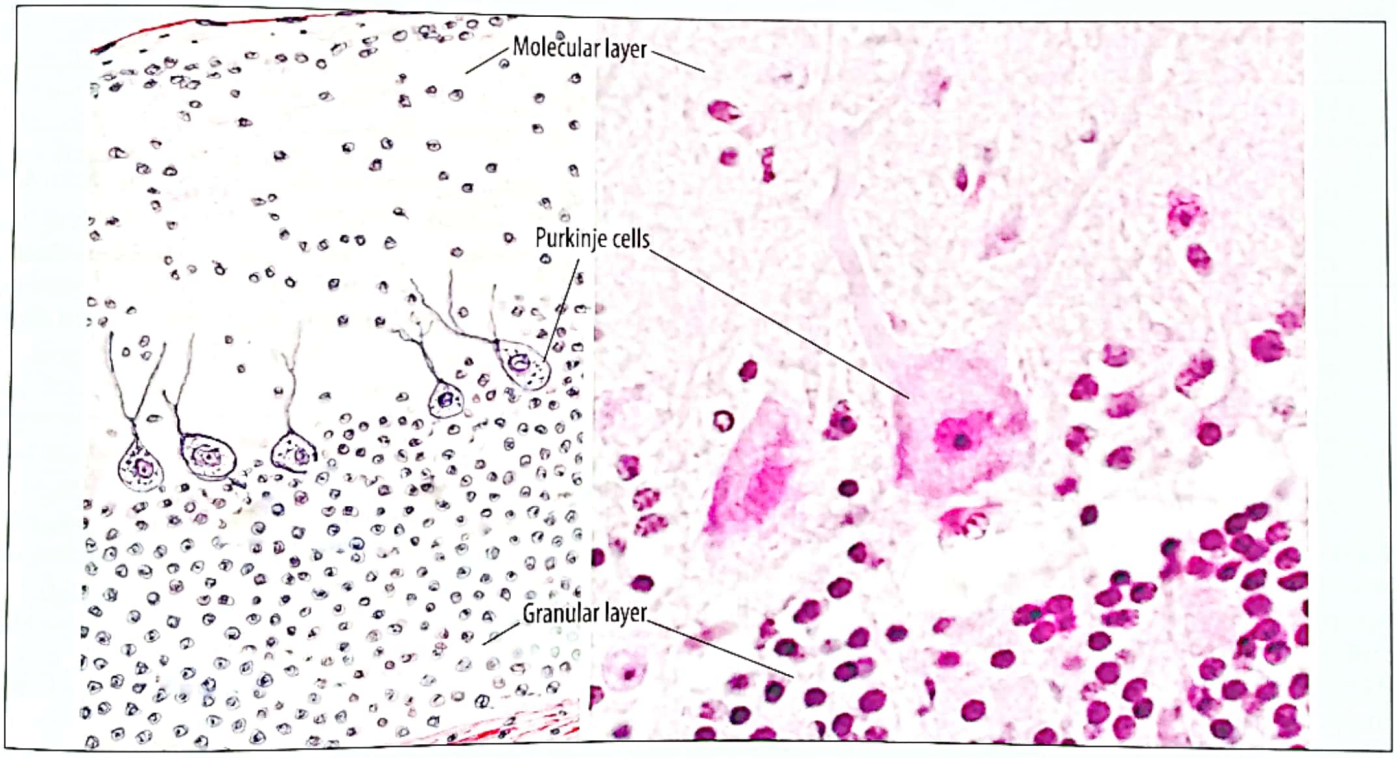

Fig. 12.4 Structure of the cerebellar cortex. The box on the right shows a section through the cerebellar cortex under high power of the light microscope. A diagrammatic representation of the structure of the cerebellar cortex is given in the box on the left.

The ***basket cells*** also have small perikarya which give rise to numerous branching dendrites that ascend toward the surface. The unmyelinated axon of a basket cell runs horizontally. Along its course, this axon gives off many descending branches which form basket-like terminal arborizations around the bodies of the Purkinje cells.

2. **Purkinje Cell Layer.** This layer lodges the cell bodies of large multipolar neurons called ***Purkinje cells***. The massive flask-shaped cell bodies of these cells are arranged as a single row along the outer margin of the granular layer.

Each Purkinje cell contains a vesicular nucleus with a prominent nucleolus. The cytoplasm contains a large number of Nissl granules which are arranged concentrically around the nucleus.

The dendritic tree arises from the apex of the perikaryon of each Purkinje cell as two or three large primary dendrites which enter the molecular layer and branch repeatedly to form a fan-shaped arborization. The axon arises from the base of the cell, acquires a myelin sheath, and passes through the granular layer to enter the underlying white matter. Most of the axons

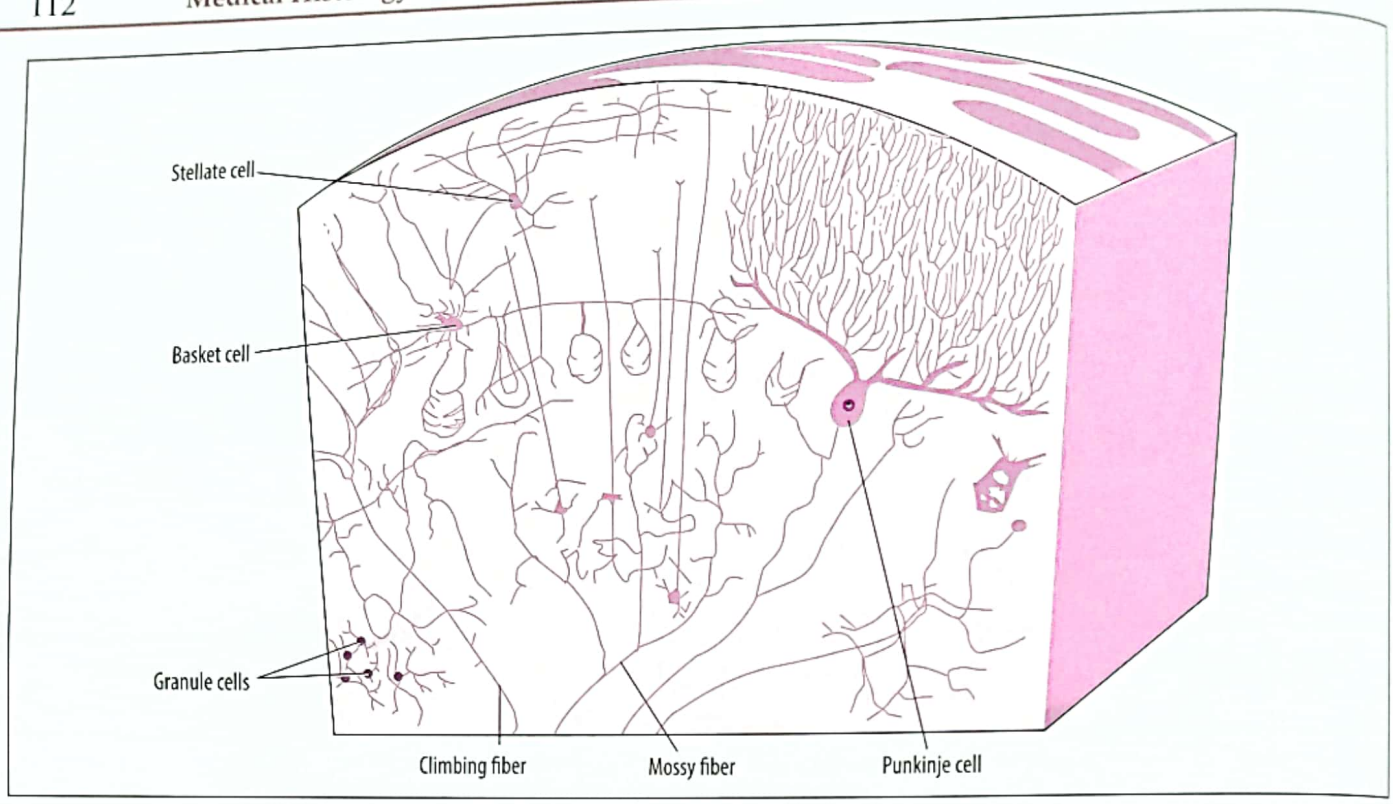

Fig. 12.5 A schematic diagram showing various cells and fibers in the cerebellar cortex.

of the Purkinje cells terminate in the deep cerebellar nuclei.

3. **Granular Layer.** This layer contains a dense population of small neurons called *granule cells*. In routine H&E stained sections, the cytoplasm of the granule cells does not stain well and the granular layer appears to be composed of closely packed nuclei which stain deeply basophilic. This layer also shows irregularly scattered lighter staining areas called *glomeruli* or *cerebellar islands*.

Each granule cell gives rise to four or five short dendrites which branch within the cerebellar islands. In these islands synapses occur between the dendrites of the granule cells and terminations of the mossy fibers (described below). The unmyelinated axon of a granule cell ascends to the molecular layer, where it bifurcates into two branches. These branches run parallel to the surface of the cortex and are known as *parallel fibers*. These fibers make synapses with dendrites of the Purkinje cells.

Afferent Fibers of the Cerebellum

1. **Climbing Fibers**. These nerve fibers originate in the inferior olivary nucleus of the medulla oblongata. Upon their entry into the cerebellum, these fibers run in the white matter to reach the cerebellar cortex, where they synapse with the dendrites of the Purkinje cells in the molecular layer.
2. **Mossy Fibers**. These fibers originate in the spinal cord (spinocerebellar tracts) or in the brain stem (e.g., vestibulocerebellar and pontocerebellar fibers). They terminate in the glomeruli present in the granular layer of the cerebellar cortex. At their termination these fibers branch repeatedly to give a moss-like appearance (hence the name *mossy* fibers). The terminal branches of mossy fibers make synapses with the dendrites of granule cells in the glomeruli of the cerebellar cortex.

SPINAL CORD (Fig. 12.6 & 12.7)

In transverse sections, the spinal cord is seen to be roughly oval in shape and is more flattened ventrally than dorsally.

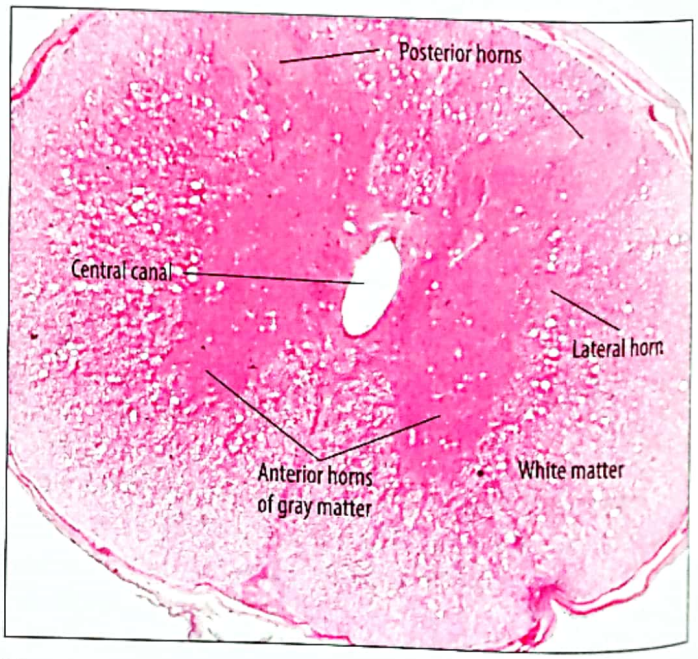

Fig. 12.6 A section through the thoracic part of the spinal cord.

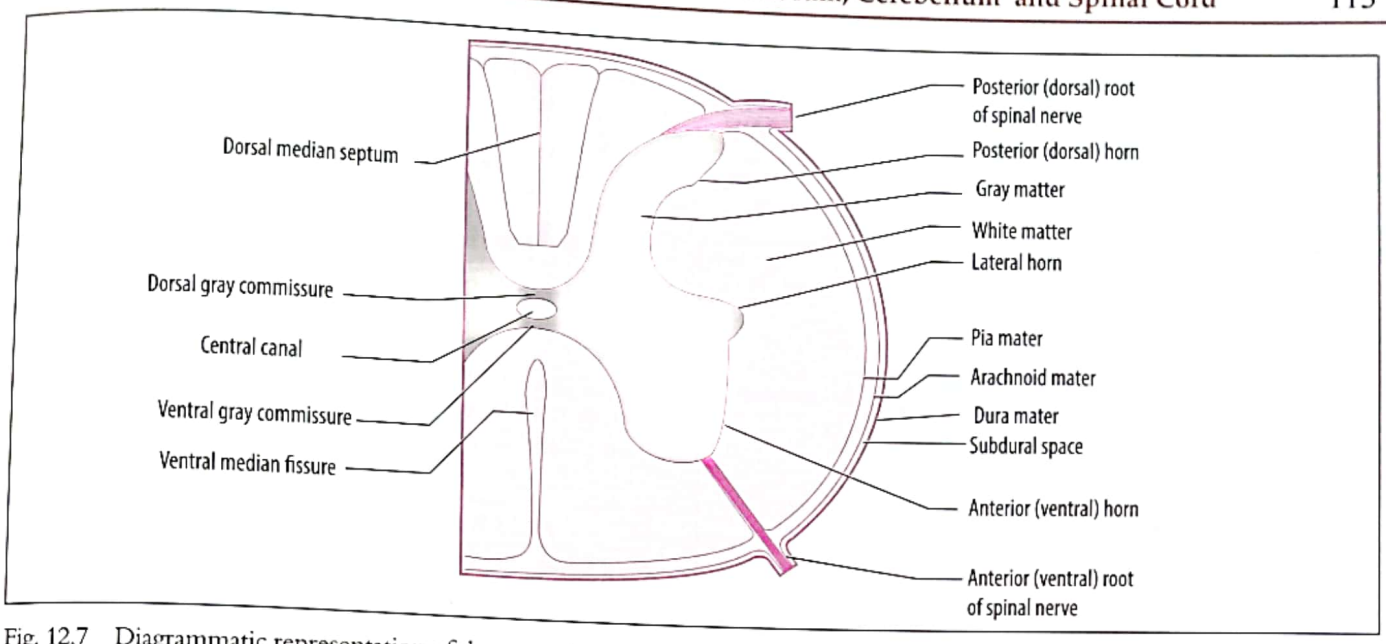

Fig. 12.7 Diagrammatic representation of the general structural plan of spinal cord

In the center there is a circular cavity called **central canal** which is lined by ependymal cells and contains CSF. Dorsal to the central canal is a septum which is composed of neuroglia and is called **dorsal median septum**. Opposite the dorsal median septum is a groove on the dorsal surface of the cord which is known as **dorsal median sulcus**. Ventrally, there is a deep cleft or fissure called **ventral median fissure**. In the dorsolateral part there are shallow grooves on either side which are called **dorsal lateral sulci**. Although the shape and structure of the cord vary at different levels (cervical, thoracic, lumbar and sacral), the basic structural pattern is similar at all levels.

Gray Matter of the Spinal Cord

The gray matter of the spinal cord is so arranged as to give the appearance of an H. The two dorsal limbs of the H are known as **posterior horns**. Similarly, the ventral limbs of the H are named as **anterior horns**. The posterior horns contain sensory neurons, while the anterior horns contain motor neurons concerned with the innervation of skeletal muscles. The central canal is situated in the horizontal bar of the H. The gray matter present dorsal to the central canal connects the two posterior horns and is known as the **dorsal gray commissure**. Similarly, ventral to the central canal is the **ventral gray commissure**. In all the thoracic and upper one or two lumbar segments, the gray matter of spinal cord shows an additional horn, called *lateral horn*, which is situated between the anterior and posterior horns. The lateral horns contain motor neurons which give rise to preganglionic sympathetic fibers.

White Matter of the Spinal Cord

The gray matter of the spinal cord lies outer to the gray matter and, on each side, it be divided into two major parts: dorsal white column and ventrolateral white column.

1. The **dorsal white column** or *dorsal funiculus* lies between the dorsal median septum and the posterior gray horns.
2. The **ventrolateral white column** lies between the posterior horn and the ventral median fissure. This column is further divided by the anterior horn of the gray matter into a *ventral white column* and a *lateral white column*.

Circulatory System 13

The circulatory system circulates blood and lymph through the body. Accordingly it consists of two divisions:

1. Blood vascular system.
2. Lymph vascular system.

The entire vascular system is internally lined by a special type of simple squamous epithelium known as *endothelium*.

THE BLOOD VASCULAR SYSTEM

The blood vascular system, also known as *cardiovascular system (CVS), circulates blood through all parts of the body. The blood* carries oxygen, nutrients, and hormones, etc., to body's organs and tissues and removes waste products produced as a result of metabolic activities of the cells. The CVS consists of heart (a muscular organ that rhythmically pumps blood) and a complex network of blood vessels extending to every part of the body. The blood vessels include capillaries, arteries and veins.

CAPILLARIES

Capillaries are delicate endothelial tubes that connect the arterial and venous sides of the circulation. Generally, they are arranged in the form of networks called *capillary beds*. In these networks oxygen and nutrients pass from the blood into the tissues, whereas CO_2 and other products of cellular metabolism pass from the cells into the blood. From within outward, the capillary wall consists of a layer of simple squamous epithelium called endothelium, basement membrane of the endothelium, and irregularly scattered contractile cells known as *pericytes* (Fig. 13.1). The diameter of capillaries is variable and depends on the type of capillary (described later).

The flat cells of the endothelium are arranged as a mosaic with their long axes parallel to the long axis of the capillary. The cell borders are usually serrated or wavy. When cut transversely, the wall of a capillary is seen to consist of portions of 2 or 3 endothelial cells. Central part of each endothelial cell shows a bulge because it lodges the nucleus. The cytoplasm also contains a small Golgi complex, some mitochondria, a small amount of RER, and a few free polyribosomes.

Pericytes

The pericytes, also known as *mural cells*, are contractile cells that wrap around the endothelial cells of the capillaries and postcapillary venules. Each pericyte is an elongated cell having long cytoplasmic processes, which partially surround the capillary wall. The pericytes lie adjacent to the outer surface of the endothelial cells and, therefore, share a common basement membrane with these cells (Fig. 13.1). In addition to ordinary organelles, the cytoplasm of the pericytes contains myosin, actin, and tropomyosin. Therefore, these cells have contractile property and play an important role in the regulation of blood flow through the capillaries and postcapillary venules. The pericytes also serve as reserve cells because they have the potential to differentiate into endothelial cells, smooth muscle cells or fibroblasts, as and when needed (e.g., after an injury).

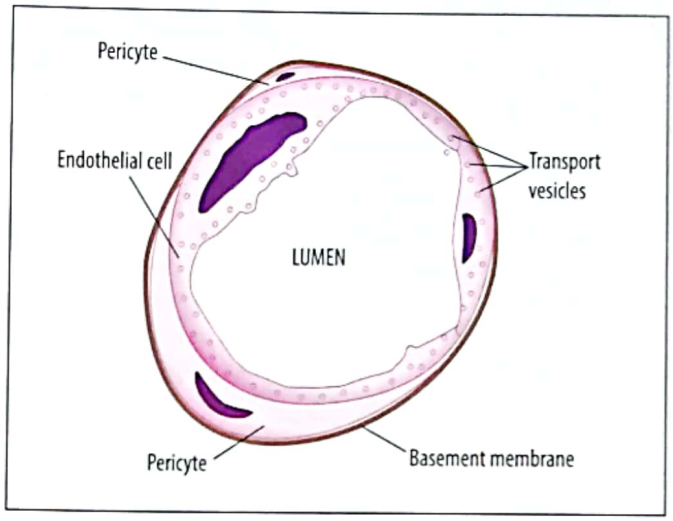

Fig. 13.1 Diagram showing the cells in the wall of a continuous capillary (endothelial cells and pericytes).

CLASSIFICATION OF CAPILLARIES

On the basis of their structure, the capillaries are classified into the following three types:

1. Continuous capillaries.
2. Fenestrated capillaries.
3. Discontinuous capillaries.

CONTINUOUS CAPILLARIES (Fig. 13.2A)

The continuous capillaries are endothelial tubes having a diameter of 7-9 µm. They constitute the most common variety of capillaries in the body. These capillaries do not have intercellular gaps between the lining endothelial cells and, therefore, there are no discontinuities in their wall. The basement membrane of the endothelial cells is complete and dose not show any interruptions. Also, the lining endothelial cells of the continuous capillaries do not have any fenestrations (pores).

The adjacent endothelial cells of the continuous capillaries are joined to each other by occluding junctions and, therefore, transport of materials between the blood and tissues occurs by *transcytosis* only. It is for this reason that numerous small vesicles can be seen in the cytoplasm of

arteries, the outermost boundary of the tunica intima consists of fenestrated layer made up of the elastic material (elastin) called *internal elastic lamina*, which separates tunica intima from the tunica media.

2. The **tunica media**, or *media*, is the middle layer. Generally, it is composed of many concentric layers of smooth muscle fibers. Variable amounts of collagen fibers, elastic fibers, reticular fibers, and proteoglycans are found between the smooth muscle fibers. An *external elastic lamina* may also be present at the junction of the tunica media and tunica adventitia in some arteries.
3. The **tunica adventitia**, or *adventitia*, is the outermost coat. It is composed of collagenous and elastic fibers coursing mainly in a longitudinal direction. The adventitia gradually merges with the connective tissue through which the vessel runs. The tunica adventitia of large arteries and veins also contains *vasa vasorum*, which are arterioles, capillaries and venules that supply blood to the walls of the large blood vessels. In addition, the tunica adventitia contains *nervi vascularis*, i.e., nerves of the vessel. The nervi vascularis (also called nervi vasorum) form a network of unmyelinated sympathetic nerve fibers that supply the smooth muscle of the tunica media.

ARTERIES

Arteries are the vessels that take the blood away from the heart. Depending on their size, the arteries are classified into the following three main types:

1. Arterioles.
2. Medium-sized arteries.
3. Large arteries.

The structure and relative thickness of the three component tunics vary according to the type of the artery.

ARTERIOLES

The arterioles, also called small arteries, begin as branches arising from the medium-sized arteries. They measure 0.3 mm or less in diameter and end by giving off several capillaries.

In the wall of an arteriole, all the three tunics of a typical blood vessel are distinguishable (Fig. 13.5). The **tunica intima** consists of endothelium and a very thin layer of subendothelial connective tissue consisting of a small number of reticular and elastic fibers. An internal elastic lamina may be seen in the larger arterioles. In the arterioles of smaller caliber, the **tunica media** consists only of one or two layers of circularly arranged muscle fibers; however, in the larger arterioles, up to five layers of smooth muscle may be present in the tunica media. The tunica **adventitia** of arterioles is scanty and consists of a thin layer of longitudinally oriented collagenous and elastic fibers.

Due to their narrow lumen and relatively thick muscular walls, the arterioles function to regulate the distribution of blood to different capillary networks by constriction or dilatation. Most arterioles can dilate 60 to 100% from their resting diameter and, therefore, these vessels are considered to be the chief controllers of the systemic blood pressure.

MEDIUM-SIZED ARTERIES

These arteries are also known as *muscular arteries* because their thick walls contain a large amount of smooth muscle in the tunica media (Fig. 13.3). The medium sized arteries perform the function of distribution of blood to various regions and organs of the body by contracting or relaxing the smooth muscle of their wall according to the functional demands of the region concerned. Therefore, these arteries are also referred to as *distributing arteries*. Most of the named arteries of the body belong to this group, e.g., the axillary, brachial, femoral, and popliteal arteries.

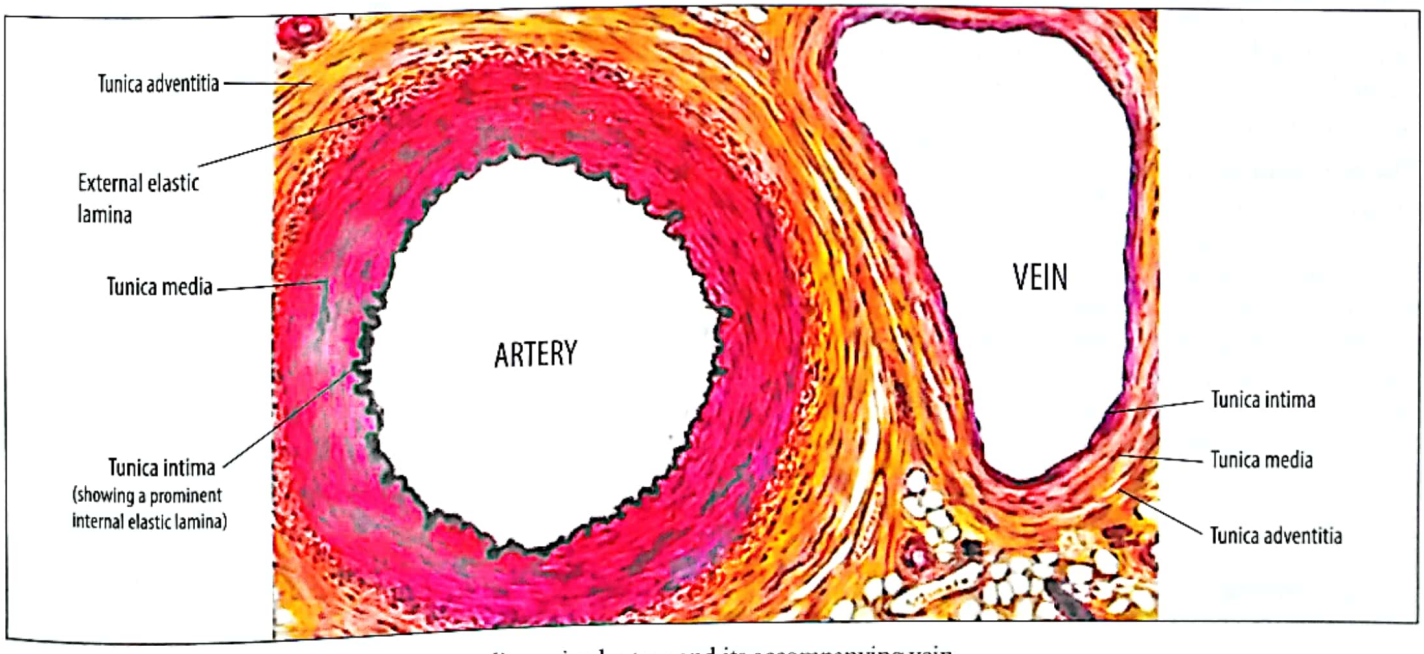

Fig. 13.3 A transverse section through a medium-sized artery and its accompanying vein.

The **tunica intima** of a muscular artery shows all the three typical layers, i.e., the endothelium, subendothelial connective tissue and internal elastic lamina. The subendothelial connective tissue consists of delicate collagenous and elastic fibers. The **internal elastic lamina** is very prominent and exists in the form of a thick, fenestrated sheet of elastin. In histological sections this lamina appears corrugated because post-mortem contraction of smooth muscle of the arterial wall causes folding of the elastic lamina.

The **tunica media** of a medium-sized artery is very prominent and may consist of as many as 40 layers of circularly disposed smooth muscle fibers. However, the muscle layers decrease in number as the diameter of the artery diminishes. Between muscle layers are present elastic fibers and reticular fibers in variable numbers. In the large muscular arteries an *external elastic lamina* may be seen as a network of elastic fibers at the junction of media and adventitia.

The **tunica adventitia** of a muscular artery is usually considerably thick and in many medium-sized arteries the media and adventitia are nearly equal in thickness. The tunica adventitia is composed of longitudinally coursing collagenous and elastic fibers.

LARGE ARTERIES (Fig. 13.4).

This group consists of arteries having a very large diameter. Structurally, the large arteries are characterized by the presence of a large amount of elastic tissue in their wall and, therefore, are also called ***elastic arteries***. The wall of a large artery is relatively thin in proportion to its diameter. The aorta and its main branches belong to this variety of arteries. The large arteries are also referred to as *conducting arteries* because they conduct blood from the heart to the medium-sized distributing arteries.

The **intima** of an elastic artery is relatively thick. Under the lining endothelium is present the subendothelial connective tissue consisting of collagenous and elastic fibers running in a longitudinal direction. No separate internal elastic lamina can be identified due to the abundance of elastic tissue in the media.

The **media** of an elastic artery consists of elastin, smooth muscle, collagen fibers, and a ground substance consisting of proteoglycans and glycoproteins. The elastin (which is a highly elastic protein) occurs as several fenestrated lamellae which are arranged in the form of concentric layers. The number of elastic lamellae increases with age. The aorta of the newborn has approximately 40 elastic lamellae, whereas about 70 lamellae are present in the adult. The spaces between the fenestrated elastic lamellae are occupied by circularly arranged bundles of smooth muscle fibers. No external elastic lamina can be identified in the wall of the large arteries.

The **adventitia** of an elastic artery is quite thin and consists mainly of collagenous and elastic fibers arranged in longitudinal spirals. Many fibroblasts and macrophages are also present in this layer.

The abundance of elastic tissue in the wall of large arteries enables them to perform the special function of maintaining the arterial blood pressure during diastole. During ventricular contraction (systole) of the heart, the elastic lamellae of the large arteries expand, and thus, store some force of the heartbeat. During ventricular relaxation (diastole), the walls of the large arteries undergo elastic recoil, which helps to maintain the blood pressure during diastole. This mechanism ensures continuous flow of blood through the capillary beds, in spite of the intermittent contraction of the heart.

Arteriosclerosis

With increasing age, the arteries undergo progressive degenerative changes, which result in thickening and loss of elasticity of their walls; this pathological alteration in the arterial walls is called *arteriosclerosis*. Major effect of arteriosclerosis is a decrease in the caliber (i.e., narrowing of the vessel lumen) which

results in *ischemia* (i.e., a decrease in blood supply) of the tissues being supplied by the diseased artery. The progressive ischemia may ultimately result in tissue death (necrosis) that may manifest as infarction or gangrene. The *infarction* leads to the formation of a localized area of dead tissue which is referred to as an *infarct*. The *gangrene* implies death of a considerable amount of tissue followed by bacterial invasion and putrefaction.

Arteriosclerosis occurs in three different forms: (1) *arteriolosclerosis*, which is characterized by the thickening of wall of the small arteries, (2) *Monckeberg's calcific sclerosis*, in which deposits of calcium occur in the tunica media of the arteries in persons older than 50 years, and (3) *atherosclerosis*, which is clinically very important and is described below.

Atherosclerosis. This condition is characterized by the deposition of fatty material in the tunica intima of arteries, which leads to the formation of localized thickenings in the vessel wall which are called *atheromas*. An atheroma consists of lipids (cholesterol and fatty acids), macrophages, calcium, and a variable amount of fibrous connective tissue. The atheromas result in partial or complete occlusion of the vascular lumen. An excessive intake of fatty diet by a person produces elevated blood lipid levels which result in the formation of atheromas. Some arteries of the body are especially prone to atherosclerosis; these include the coronary arteries, cerebral arteries, and internal carotid arteries.

VEINS

The veins carry blood away from the tissues and organs and return it to the heart. The basic structural pattern of the veins resembles that of the arteries but there are marked differences as well. The main difference is that the vein contains a greater amount of collagenous connective

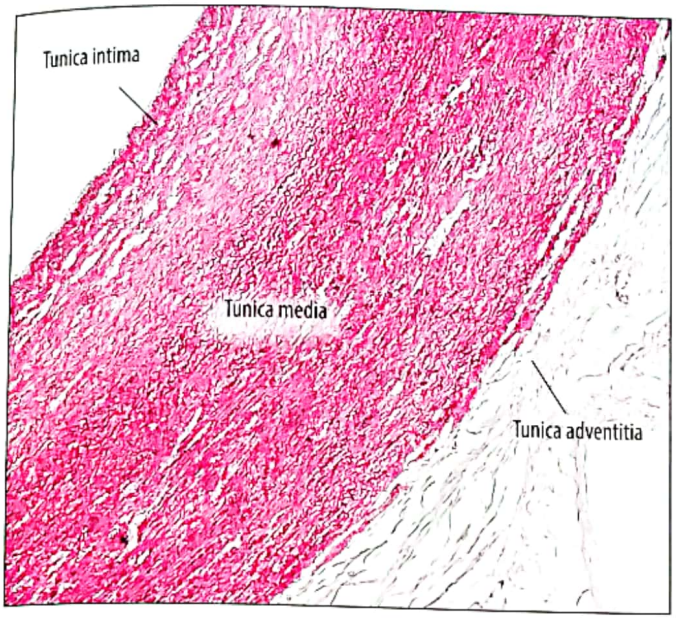

Fig. 13.4 A section showing a part of the wall of the aorta.

tissue but a lesser quantity of smooth muscle and elastic connective tissue in its wall. In addition, the three tunics of the vessel wall are not as clearly distinguishable in the wall of a vein as they are in the wall of an artery. In the tissue section examined under the microscope, it is also seen that the veins have thinner walls but larger lumen as compared to their accompanying arteries.

The veins are also classified into three major categories:

1. Venules.
2. Medium-sized veins.
3. Large veins.

VENULES

The capillary networks are drained into venules, which are further classified into postcapillary venules and muscular venules.

The **postcapillary venules** have a wider caliber than that of the capillaries and their diameter ranges from 15 to 20 μm. Their wall consists of endothelium which is extensively covered by pericytes and, therefore, these venules are also called *pericytic venules*. Intercelluar gaps are present between the endothelial cells of the postcapillary venules which make these vessels are very permeable. The postcapillary venules are the principal vessels through which leukocytes emigrate and fluid escapes from the blood into the tissues in case of inflammation and during allergic reactions. The pericytes of the postcapillary venules share a common basement membrane with the endothelial cells and also contribute to the synthesis of this membrane. The pericytes and the endothelial cells are joined to each other by tight junctions and gap junctions.

The **muscular venules** are located distal to the postcapillary venules. They have a larger diameter (up to 1 mm) and are characterized by the presence of one or two layers of smooth muscle outer to the endothelium (Fig. 13.5). These smooth muscle layers constitute tunica media of the vessel. A thin tunica adventitia consisting of fine collagen fibers may also be distinguishable. The muscular venules are also known as *small veins*.

MEDIUM-SIZED VEINS

This group includes most of the named veins of the body and their principal tributaries. Their diameter ranges from 1 to 10 mm. The tunica ***intima*** of a medium-sized vein consists of endothelium and a thin subendothelial layer of delicate collagenous and elastic fibers. A thin internal elastic lamina is usually distinguishable in medium-sized veins. The ***media*** is composed of circularly arranged smooth muscle cells and collagen fibers. The smooth muscle cells are loosely arranged and interwoven with collagen fibers. Many fibroblasts and elastic fibers are also present. The ***adventitia*** of medium-sized veins is thicker than the tunica media. It is composed chiefly of bundles of collagen fibers running in a longitudinal direction. Some elastic fibers are also present.

The ***great saphenous vein*** of the lower limb is a medium-sized vein of the lower limb having a special structure. It is often titled as a *muscular vein* because it contains smooth muscle in all of its three tunics. The thick tunica media of this vein consists of circularly-arranged smooth muscle fibers. In addition, this vein contains longitudinally running smooth muscle fibers in the tunica intima and tunica adventitia. Due to its special structure and easy accessibility, segments of the great saphenous vein are used as grafts in coronary artery bypass surgery.

LARGE VEINS

Veins having a diameter larger than 10 mm are classified as large veins. This group includes the superior and inferior venae cavae and the pulmonary, portal, internal jugular, azygos, common iliac, and renal veins. The ***intima*** of a large vein consists of endothelium and a well-developed subendothelial connective tissue containing collagen fibers, elastic fibers, fibroblasts, and a few smooth muscle cells. A thin internal elastic lamina is usually distinguishable in the large veins. The ***media*** of a large vein is thin and poorly defined. It consists of circularly-arranged smooth muscle fibers, elastic fibers, collagen fibers, and fibroblasts. The ***adventitia*** of a large vein is the thickest of the three coats and consists of longitudinally running bundles of collagen fibers and smooth muscle fibers; some elastic fibers are also seen to be intermingled with the collagen fibers.

Valves of the Veins

The medium-sized and large veins of the upper and lower limbs, which carry blood against the gravity, contain valves which prevent back flow of blood. A venous valve consists of two semilunar flaps of tunica intima. Each flap has a central core of collagenous and elastic fibers, which are continuous with those of the tunica media of the vein. Both surfaces of each valvular flap are covered by endothelium.

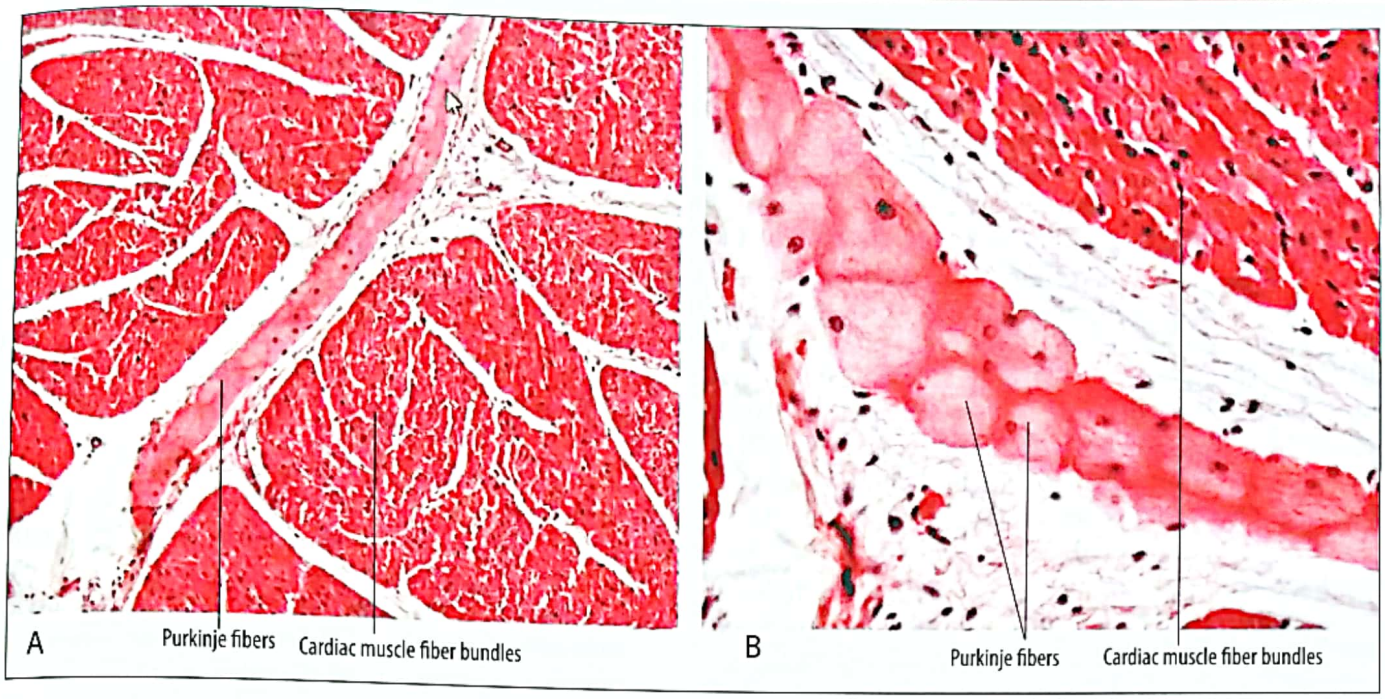

Fig. 13.6 A section through the left ventricle of the heart showing transversely cut Purkinje fibers and bundles of cardiac muscle fibers. A. Low power magnification of light microscope. B. High power magnification of light microscope.

from the impulses within the heart itself, although the autonomic system can control the rate of the heartbeat. The rhythmic stimulus for contraction of the myocardium is generated in the **sinoatrial (SA) node** which is located in the wall of the right atrium just lateral to the junction of the superior vena cava to this atrium. The SA node acts as a pacemaker and stimulates spontaneous rhythmic contraction of the atrial myocardium. Traveling through the atrial wall, the electrical impulse reaches the **atrioventricular (AV) node** which is located beneath the endocardium of the medial wall of the right atrium at the base of the interventricular septum. The AV node then initiates the contraction of the ventricular myocardium. For this purpose, the impulse from the AV node passes along a bundle of specialized muscle fibers which is called **atrioventricular (AV) bundle** (also called *bundle of His*). The AV bundle passes through the dense collagenous tissue of the cardiac skeleton (that separates the atria from the ventricles) and then splits into two branches in the interventricular septum; one of these branches is called right bundle branch while the other is referred to as left bundle branch. The right and left bundle branches are meant for the activation of the myocardium of the right and left ventricles, respectively, and therefore they run in the subendocardial tissue on the right and left sides of the interventricular septum. Both branches of the AV bundle taper out to produce a complex network of unusually large cardiac muscle fibers called **Purkinje fibers**, which stimulate individual groups of cardiac muscle fibers to contract.

The **SA node** is composed of a network of specialized cardiac muscle fibers which are much thinner than ordinary cardiac muscle fibers, having a diameter of only 3-4 μm. These muscle fibers do not exhibit intercalated discs and are joined to each other by desmosomes and gap junctions. They contain lesser number of myofibrils and lack an organized striated pattern. The specialized cardiomyocytes of the SA node are embedded in an enormous amount of fibrous connective tissue which contains abundant blood vessels and numerous nerve fibers.

The **AV node** is also composed of a meshwork of small cardiac muscle fibers embedded in bulky stroma of collagenous fibers, in which numerous blood vessels and nerve fibers are present. Histologically, the cardiomyocytes of the AV node are similar to those of the SA node.

Purkinje Fibers

As mentioned above, the branches of the atrioventricular bundle consist of specialized cardiac muscle fibers called Purkinje fibers or *conducting myofibers*. The Purkinje fibers have a larger diameter but contain fewer myofibrils than the ordinary cardiomyocytes (Fig. 13.6). Their cytoplasm is exceptionally rich in mitochondria and contains a large number of glycogen granules. The plasmalemma of the Purkinje fibers lodges numerous voltage-gated sodium channels to enable them to conduct electrical impulses rapidly.

ENDOTHELIUM OF THE BLOOD VASCULAR SYSTEM

All components of the blood vascular system (heart, arteries, veins, and capillaries) are lined by a special type of simple squamous epithelium which is called endothelium. The adjacent endothelial cells are joined to each other by adhering and occluding junctions. The luminal

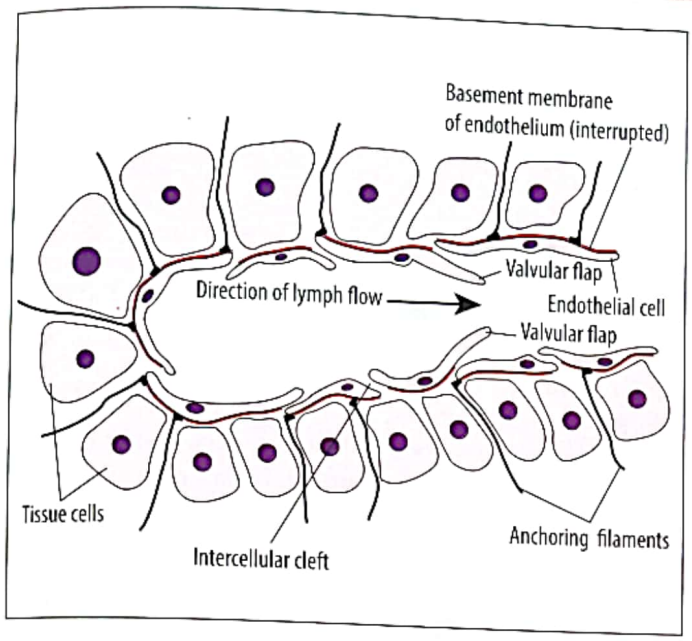

Fig. 13.7 Diagrammatic representation of the blind-ended beginning of a lymphatic capillary.

anastomose freely to form extensive networks in the spaces between blood capillaries.

The lymphatic capillaries are lined by a single layer of endothelial cells with an incomplete basal lamina (Fig. 13.5). The endothelial cells partly overlap each other but do not make occluding junctions. A special feature of the lymphatic capillaries is the presence of clefts between the lining endothelial cells. These **intercellular clefts** allow the entry of macromolecules from the interstitial fluid into the lumen of the lymphatic capillaries. Very thin filaments called *anchoring filaments* extend between the basal lamina of the endothelium and the surrounding connective tissue. These filaments are composed of elastin and play an important role in maintaining the patency of the intercellular clefts. The back flow of the lymph into the tissue spaces id prevented by the valvular mechanism created by the overlapping of the endothelial cells (Fig. 13.7).

The special structural features of lymph capillaries (wide intercellular clefts and incomplete basal lamina) make them much more permeable than the ordinary blood capillaries. The greater permeability of the lymph capillaries enables the lymphatic system to carry proteins and large particulate matter away from the tissue spaces. The lymphatic capillaries of the small intestine convey proteins as well as fats (because the fat molecules are too large to cross the pores in the endothelium of the fenestrated capillaries of the small intestinal villi).

LYMPHATIC VESSELS

These vessels resemble the veins in structure but have much thinner walls. In the larger lymphatic vessels, the three tunics, i.e., intima, media and adventitia may be distinguished, but usually there is no clear cut demarcation between the successive coats. The **tunica intima** is lined by endothelium beneath which is present a network of delicate elastic and collagen fibers. The **tunica media** is composed of circularly running smooth muscle fibers; some delicate elastic fibers may be present between the smooth muscle fibers. The **tunica adventitia** is the thickest coat and consists of longitudinally running collagenous and elastic fibers with a few smooth muscle cells.

LYMPHATIC DUCTS

These vessels, which include the thoracic duct and right lymphatic duct, have a structure similar to a vein of equal size except for the presence of a greater amount of smooth muscle in the media.

The **intima** of lymphatic ducts consists of endothelium and subendothelial layer of delicate connective tissue containing a few smooth muscle fibers; an internal elastic lamina may be distinguishable. The **media** is the thickest coat. It is made up of longitudinal and circular bundles of smooth muscle fibers. Between the muscle bundles is present abundant connective tissue composed principally of collagen fibers. The **adventitia** is poorly defined and consists of longitudinally arranged coarse collagen fibers with a few smooth muscle cells.

Like the veins, the lymphatic vessels and ducts also possess valves, which prevent back flow and assist in unidirectional flow of the lymph.

The Immune System and Lymphoid Organs

14

The body is under constant threat of invasion by viruses, unicellular organisms, e.g., bacteria and protozoa, multicellular organisms, e.g., helminths (worms), and other foreign bodies, which may gain entry into the body through the skin, gastrointestinal tract, respiratory tract, urinary tract, and reproductive tract, etc. To protect itself from these hazardous agents, the body employs a defense system which is called **immune system**. This system consists of two components:

1. Innate immune system
2. Adaptive immune system

THE INNATE IMMUNE SYSTEM

This system acts rapidly against the foreign agents which invade the body. It neutralizes or destroys the invading agents by different means which include compliment, antimicrobial peptides, cytokines, neutrophils, macrophages, and natural killer cells. This system has no immunological memory and responds in a nonspecific manner, whatever the initiating agent. A brief description of various components of the innate immune system is given below.

The **compliment** is a cascade of about 20 serum proteins that attack those microorganisms which manage to enter the bloodstream. The **antimicrobial peptides (AMPs)** are small molecular weight proteins which have broad spectrum antimicrobial activity against bacteria, fungi, and viruses. The major AMPs in humans include defensins and cathelicidins. The defensins are produced by the neutrophils, macrophages, and epithelial lining of the skin, respiratory tract, gastrointestinal tract, and genitourinary tract. The cathelicidins are produced by the neutrophils, macrophages, and epithelial cells (keratinocytes) of the epidermis. The **cytokines** are low molecular weight peptides or glycoproteins that are produced by the cells of the immune system. They act in a paracrine manner and serve to coordinate the activities of the cells involved in the immune response. Examples of the cytokines are various types of interleukins, lymphokines, chemokines, and interferon. The *neutrophils* of the blood and *macrophages* of the connective tissues engulf the microorganisms and kill them intracellularly. The **natural killer (NK) cells** are a type of lymphocytes having the special capability of killing those body cells which have become infected by a virus or have undergone cancerous change.

THE ADAPTIVE IMMUNE SYSTEM

The response of the adaptive immune system is slower than that of the innate immune system but is highly specific, i.e., it responds against specific molecules (antigens) contained in the invading organism or foreign particle. This system employs B lymphocytes, T lymphocytes, and antigen-presenting cells to mount the immune response. An encounter of the target antigen with the effecter cells (B and T lymphocytes) frequently leads to a state of *immunological memory*. This memory enables the adaptive immune system to recognize the specific antigen upon a subsequent exposure to the same antigen and, thus, to mount an immune response more quickly than that initiated upon the first exposure.

The adaptive immune system operates through two types of immune responses: (i) humoral immune response, and (ii) cellular immune response.

The **humoral immune response** depends on the production of *antibodies* by the activated B lymphocytes (plasma cells). The antibodies are proteins of the immunoglobulin family and have the capability of neutralizing the foreign agents by binding to the antigens carried by these agents.

The **cellular immune response**, also called *cell-mediated immune response*, causes the destruction of foreign cells, and those body cells which have been infected by a virus or have undergone a cancerous change.

ANTIGENS

Any foreign structure or substance, against which an immune response is generated, is known as an *antigen*. Generally, the antigen is either a soluble foreign substance or an infectious microorganism. A transplanted foreign tissue or organ (e.g., transfused blood or transplanted kidney) is also recognized by the immune system as an antigen. The transformed cells of the body (e.g., cancer cells) also act as antigens. Occasionally, the immune system of the body may react against its own normal molecules, cells or tissues; this abnormal reaction of the immune system to the "self" materials produces pathological conditions called *autoimmune diseases*.

The cells of the immune system do not respond to the whole antigen molecule but react to small molecular regions (domains) of the antigen, which are called **epitopes** or *antigenic determinants*. Biochemically, the epitopes may be proteins, sugars (polysaccharides), or lipids.

ANTIBODIES

The antibodies are immunoglobulins which have the ability to combine chemically with the epitopes of antigens. They are secreted by the activated B lymphocytes (plasma cells) which release them into the blood or lymph vascular system.

against the virus-infected cells or cancer cells when the viral or cancer-specific peptides are displayed (along with MHC I molecules) on the surface of the virus-infected or transformed (i.e., cancerous) cells.

The MHC II molecules are expressed only on the surface of the antigen-presenting cells (APCs), including the B lymphocytes. These molecules function in presenting polypeptide fragments derived from exogenous proteins, which were phagocytosed from the extracellular space and processed in the lysosomal system of the B lymphocytes or antigen-presenting cells. When these foreign (non-self) antigenic epitopes are co-presented with MHC II molecules, the T lymphocytes react against such epitopes and the cells infected by that antigen (either B lymphocytes or other types of APCs) are killed by the action of the cytotoxic T lymphocytes.

CELLS OF THE IMMUNE SYSTEM

The cells of the innate and adaptive immune system can be divided into two major categories: (1) lymphocytes, and (2) antigen-presenting cells (APCs).

LYMPHOCYTES

Three functional types of lymphocytes take part in immune reactions: B lymphocytes, T lymphocytes, and natural killer (NK) cells.

B LYMPHOCYTES

These cells originate and become immunocompetent in the bone marrow. During the processes of achieving the immunocompetence, the B lymphocytes express monomers of IgM and IgD on the external surface of their plasmalemma. These immunoglobulin molecules are called **B-cell receptors (BCRs)**, as these molecules serve as receptors for various types of antigens. The antigen can be free-floating or presented by an APC. The B lymphocytes also express MHC II molecules on their surface.

When a B cell receptor reacts with an antigen, activation of the B lymphocyte takes place. This activation leads to mitotic division of the B lymphocyte and results in the formation of the following two varieties of cells:

1. **Plasma cells** which synthesize and secrete antibodies against the antigen that resulted in the formation of the plasma cells.
2. **Memory B cells** which are lesser in number than the plasma cells, but are long-living cells. The memory B cells have the capability to produce a more rapid and extensive immune response upon a subsequent exposure to the same specific antigen.

T LYMPHOCYTES

These cells originate in the bone marrow but become immunocompetent in the thymus. During the process of becoming immunocompetent, the T cells do not manufacture surface immunoglobulins but acquire **T-cell receptors (TCRs)** which are generally composed of two glycoprotein chains (an alpha chain and a beta chain). Two other important characteristics of the T cells are worth noting: (i) the T lymphocytes recognize only those epitopes that are presented to them by the antigen-presenting cells, and (ii) these cells respond only to protein antigens.

Functionally, the T lymphocytes are classified into 5 types: (1) cytotoxic T cells, (2) helper T cells, (3) suppressor T cells, (4) gamma delta T cells, and (5) memory T cells.

1. The **cytotoxic T lymphocytes (CTLs)** are also called *effector T cells* or *killer T cells*. In addition to T cell receptors (TCRs), these cells express possess CD8 markers on their surface. The CTLs kill virus-infected cells, cancerous cells and foreign cells by secreting mainly two cytotoxic proteins called perforins and granzymes. The **perforins** drill holes in the plasma membrane of the target cell. The **granzymes** are proteolytic enzymes which enter the target cell through the holes created by the perforins and induce death of the target cell by apoptosis.
2. The **helper T cells**, more commonly called **T helper cells (TH cells)**, express TCRs and CD4 markers on their surface. The TH cells help other cells of the immune system during the immune responses by producing different cytokines, particularly those of the interleukin family. Two subtypes of T helper cells are recognized: TH1 cells and TH2 cells. The **TH1 cells** secrete interleukin-2 (IL-2), interferon-α, and tumor necrosis factor-α. These cells play an important role in cell-mediated immune responses by interacting with the cytotoxic T lymphocytes, NK cells, and macrophages. The **TH2 cells** secrete cytokines of the interleukin family only; these cytokines include IL-4, IL-5, IL-10, and IL-13. The TH2 cells are essential for launching a humoral immune response as they interact with the B lymphocytes and activate them to develop into plasma cells and secrete antibodies.
3. The **suppressor T cells**, also called *regulatory T cells*, play an important role in the maintenance of the immunologic tolerance. Their main function is to regulate the cell-mediated immunity by shutting down the immune response when the purpose of an immune reaction has been achieved. The suppressor T cells also possess CD4 markers on their plasmalemma.
4. The **gamma delta T cells** (γδ T cells) represent a small population of T lymphocytes which have a TCR composed of a gamma and a delta glycoprotein chain (instead of the alpha and beta chains found in the TCRs of the other T lymphocytes). The gamma delta T cells are peculiar in that they do not require antigen processing and MHC presentation of epitopes. After developing in the thymus, these cells migrate to the skin and mucosal lining of the oral cavity, gastrointestinal tract, and vagina. In these

lymphatic tissue as well as lymphoid follicles. This lymphoid tissue is called ***mucosa-associated lymphoid tissue (MALT)***. The function of MALT is to provide protection against bacteria (and other microorganisms), which can easily approach and invade the mucosal linings. In the digestive tract the mucosa-associated lymphoid tissue is called *gut-associated lymphoid tissue (GALT)*, while in the bronchial tree of the lungs, it is titled as *bronchus-associated lymphoid tissue (BALT)*.

STRUCTURE OF LYMPHOID NODULES

The lymphoid nodules are dense accumulations of lymphocytes. They are sharply defined but not encapsulated and vary greatly in size, ranging from 0.2 mm to 1 mm in diameter. Before activation by an antigen, a lymphoid nodule is known as a **primary lymphoid nodule**, which consists mainly of B lymphocytes. However, after the arrival of an antigen, a primary lymphoid nodule quickly becomes converted into a **secondary lymphoid nodule,** which has a specific structure of its own. Most of the lymphoid nodules in the body are secondary nodules. Therefore, unless otherwise specified, the term lymphoid nodule refers to a secondary lymphoid nodule. Each secondary lymphoid nodule consists of two zones: a central light-staining zone called *germinal center* and a dark-staining outer region known as *mantle zone* (Fig. 14.2).

The **germinal center** is the central, lightly staining region of the lymphoid nodule. It develops when an antigen, either free-floating or carried by a dendritic cell, reaches the central region of the nodule and causes activation of the B lymphocytes in this region. The B lymphocytes respond to this activation by undergoing mitotic division to produce a large number of immature lymphocytes, which are either *plasmoblasts* or *lymphoblasts*. The plasmoblasts, also called centroblasts, differentiate into plasma cells, while the lymphoblasts differentiate into B memory cells. Being immature and differentiating cells, the plasmoblasts and lymohoblasts have nuclei which contain finely dispersed chromatin (euchromatin). Therefore, they stain much lighter than the nuclei of the mature B lymphocyte (which chiefly contain heterochromatin). Consequently, the germinal center of a lymphoid follicle stains much lighter than its mantle zone. The germinal center also contains macrophages and mature dendritic cells called *interdigitating dendritic cells*.

The **mantle zone** (or mantle) is the peripheral part of a secondary lymphoid follicle. It contains densely packed B lymphocytes along with a few mature dendritic cells and macrophages. The mantle zone is also known as corona of the lymphoid follicle.

LYMPHOID ORGANS

The lymphoid organs are classified into two major groups:

1. ***Primary lymphoid organs***, which are concerned with the production and development of the cells involved in the immune response of the body; these organs include the bone marrow and the thymus (microscopic structure of the bone marrow has already been described in chapter 9).

2. **Secondary lymphoid organs,** which include **lymph nodes, spleen**, and the **tonsils**. Whenever there is an antigenic stimulation, the lymphocytes of the secondary lymphoid organs increase in number and take part in the immune response launched against the concerned antigen.

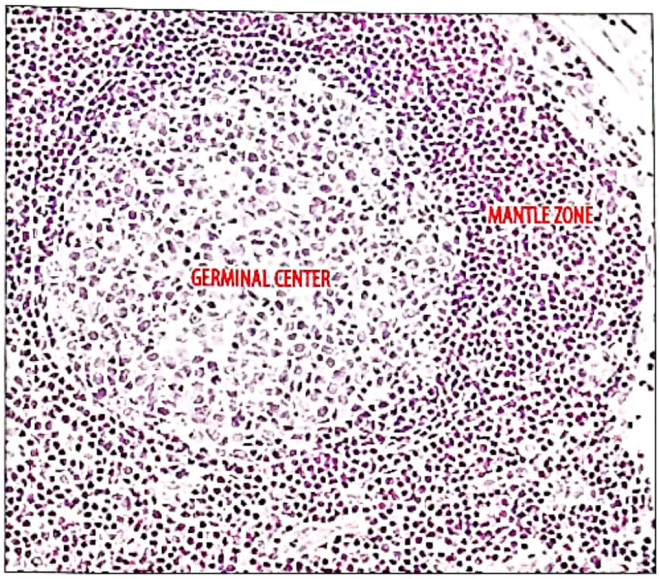

Fig. 14.2 A section through a secondary lymphoid nodule showing the lightly staining germinal center and darkly staining peripheral mantle zone.

THYMUS

The thymus is a primary lymphatic organ which is responsible for the supply of T lymphocytes to the body. It provides a special microenvironment in which the precursors of the T cells develop, differentiate, and undergo the unique process of "T cell education".

The thymus is located in the superior mediastinum, consists of two lobes, and is enclosed within a capsule of dense irregular connective tissue. Knowledge of the development of thymus during the embryonic period is important to understand the histological structure and functions of this organ. The thymus develops from the endoderm of the third pair of pharyngeal pouches. The endodermal cells derived from the ventral wings of the third right and left pharyngeal pouches give rise to the bilobed thymic primordium which migrates caudally into the superior mediastinum. The endodermal cells differentiate into epithelial cells which are called **thymic epithelial cells (TECs)**, *epithelioreticular cells,* or *epitheliocytes.* Lymphocyte precursors (lymphoblasts) originating in the bone marrow reach and invade the developing thymus. These cells, that are destined to develop into immunocompetent T lymphocytes, occupy the spaces between the thymic epithelial cells, and thus, the thymus is converted into a lymphoepithelial organ.

barrier. These cells form occluding junctions with each other and thus serve to isolate the developing T lymphocytes from the connective tissue of the thymus and from the blood circulating in the cortical blood vessels.

The **type II thymic epithelial cells** are located in the midcortex. These are stellate cells having long, wide, sheet-like processes. Cytoplasmic processes of neighboring cells are joined to each other by desmosomes. In this way, these cells form a cytoreticulum that divides the thymic cortex into small, isolated compartments that are filled with the developing T lymphocytes. The type II TECs, also called *thymic nurse cells*, surround and envelop the developing T cells and, thus, play a very important role in T cell education.

The **type III thymic epithelial cells** are located at the junction of thymic cortex and medulla. These cells also have sheet-like cytoplasmic processes. The cytoplasmic processes of the neighboring type III cells are joined together by occluding junctions and, thus, these cells perform the function of isolating the thymic cortex from the medulla. In addition, these cells take part in T cell education.

The type II and type III thymic epithelial cells carry both MHC class I and class II molecules on their cell membrane and, therefore, also function as antigen-presenting cells during the process of thymic cell education.

THYMIC MEDULLA

The thymic medulla, i.e., the central region of each thymic lobule, stains much lighter than the cortex because it lodges much fewer T lymphocytes than the cortex. The thymic medulla contains dendritic cells and thymic epithelial cells of type IV, V, and VI.

The **type IV thymic epithelial cells** also possess sheet-like cytoplasmic processes and are present at the corticomedullary junction in close association with the type III TECs of the thymic cortex. The adjacent type IV TECs are bound to each other and to the type III TECs of the thymic cortex by occluding junctions, so that a barrier is created at the corticomedullary junction.

The **type V thymic epithelial cells** form the cytoreticlum of the medulla. The cytoplasmic processes of neighboring type V cells are joined by desmosomes to form small compartments that contain groups of T lymphocytes. Nuclei of the type V TECs contain a large amount of euchromatin and, therefore, stain much lighter than the lymphocyte nuclei.

The **type VI thymic epithelial cells** constitute the most distinguishing feature of the thymic medulla. These cells form **thymic corpuscles** (also called *Hassall corpuscles)* which are isolated masses of closely packed type VI cells. The thymic corpuscles are spherical or oval structures ranging from 30 to 100 µm in diameter. The closely packed and concentrically arranged type VI TECs have flattened nuclei and their cytoplasm is markedly eosinophilic (Fig. 14.3 & 14.4). The adjacent cells are joined to each other by desmosomes. The central cells of a Hassall corpuscle may become keratinized or even calcified. The functional importance of the thymic corpuscles has been a subject of controversy, but now it is agreed that they produce cytokines (especially interleukin-4 and interleukin-7) which play an important role in T cell education.

VASCULAR SUPPLY OF THE THYMUS AND BLOOD-THYMUS BARRIER

The arteries supplying the thymus enter the organ by penetrating the connective tissue capsule. These arteries are distributed throughout the thymus via the connective tissue trabeculae. Branches of these arteries enter the thymic lobules at the corticomedullary junction and give off branches that run in an outward direction and give rise to capillaries that form branching and anastomosing arcades in the cortical region of the lobule. The arteries running at the corticomedullary junction also give rise to arterioles that pass into the medullary region of each thymic lobule. These arterioles give rise to fenestrated capillaries in the medulla, which finally drain into post-capillary venules. The descending parts of the cortical arterial arcades return to the corticomedullary junction and drain into postcapillary venules that continue into the medulla. The postcapillary venules of medulla join to form larger venules, which leave the thymus via the connective tissue trabeculae.

Blood-Thymus Barrier

The developing T lymphocytes of the thymic cortex are prevented from contacting the blood-borne antigens by a physical barrier called blood-thymus barrier. This barrier consists of three major components:

1. **The lining endothelium of cortical capillaries.** The endothelial cells of these continuous capillaries are bound to each other by occluding junctions and, therefore, no intercellular gaps are present. The basal lamina of the endothelium is also unusually thick. These features make the lining endothelium of cortical capillaries highly impermeable to macromolecules.
2. **The type I thymic epithelial cells.** These cells surround the cortical capillaries to form a continuous sheath around these vessels. Presence of occluding junctions between the adjacent type I TECs provides further protection to the developing thymocytes.
3. **Macrophages.** The macrophages located around the cortical capillaries phagocytose all those antigenic molecules that manage to escape from these capillaries into the thymic cortex.

It is to be noted that the blood-thymus barrier does not exist in the medullary region of the thymic lobules, although the medulla has a richer blood supply than that of the cortex.

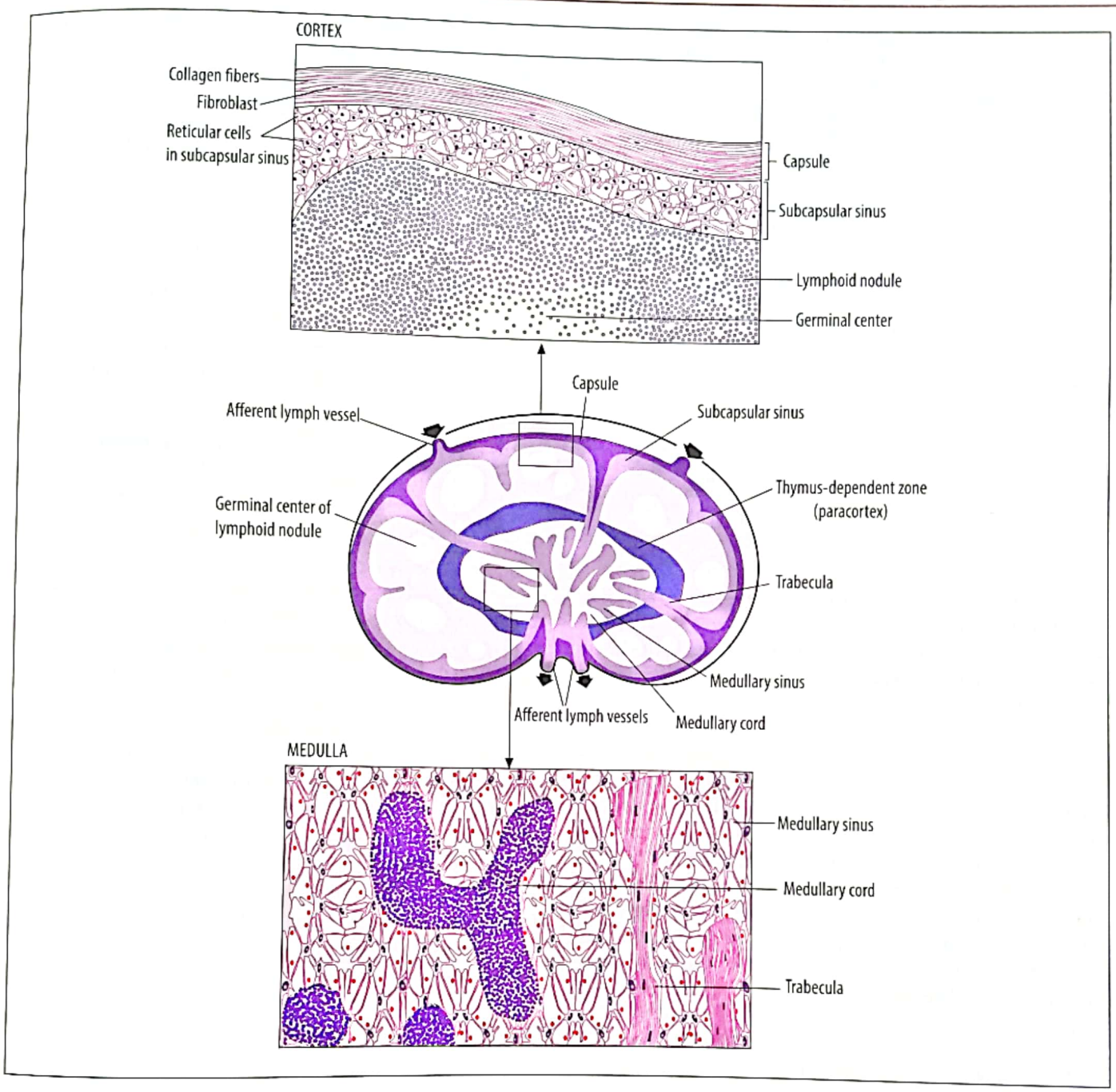

Fig. 14.5 A diagrammatic representation of the microscopic structure of a lymph node.

receives afferent lymphatics and is continuous with radially arranged *cortical sinuses* that run parallel to the trabeculae. The cortical sinuses (also called paratrabecular or radial sinuses) deliver the lymph to the medullary sinuses.

The cortex of a lymph node can further be divided into two regions. The outer region contains lymphatic nodules and is known as ***nodular cortex*** or *superficial cortex*. The inner region is free of lymphatic nodules and is called ***paracortex*** or *deep cortex*.

The Nodular Cortex

The nodular cortex (superficial cortex) contains primary as well as secondary lymphatic nodules. The primary nodules are compact masses of B lymphocytes which take a uniform, deeply basophilic stain and do not exhibit any germinal center. Each secondary lymphatic nodule exhibits a light-staining *germinal center* and a darkly staining peripheral region called *mantle zone or corona*. The mantle zones of the lymphoid nodules consist of compactly arranged B lymphocytes, while their germinal centers contain plasmoblasts (differentiating into plasma cells) and lymphoblasts (differentiating into B memory cells).

The Paracortex

The paracortex or *deep cortex* is the region between the nodular cortex and medulla. It contains loosely aggregated T lymphocytes, and therefore, is also known as ***thymus-dependent zone***. No lymphoid nodules are present in the paracortx.

A special feature of the paracortex is presence of a special

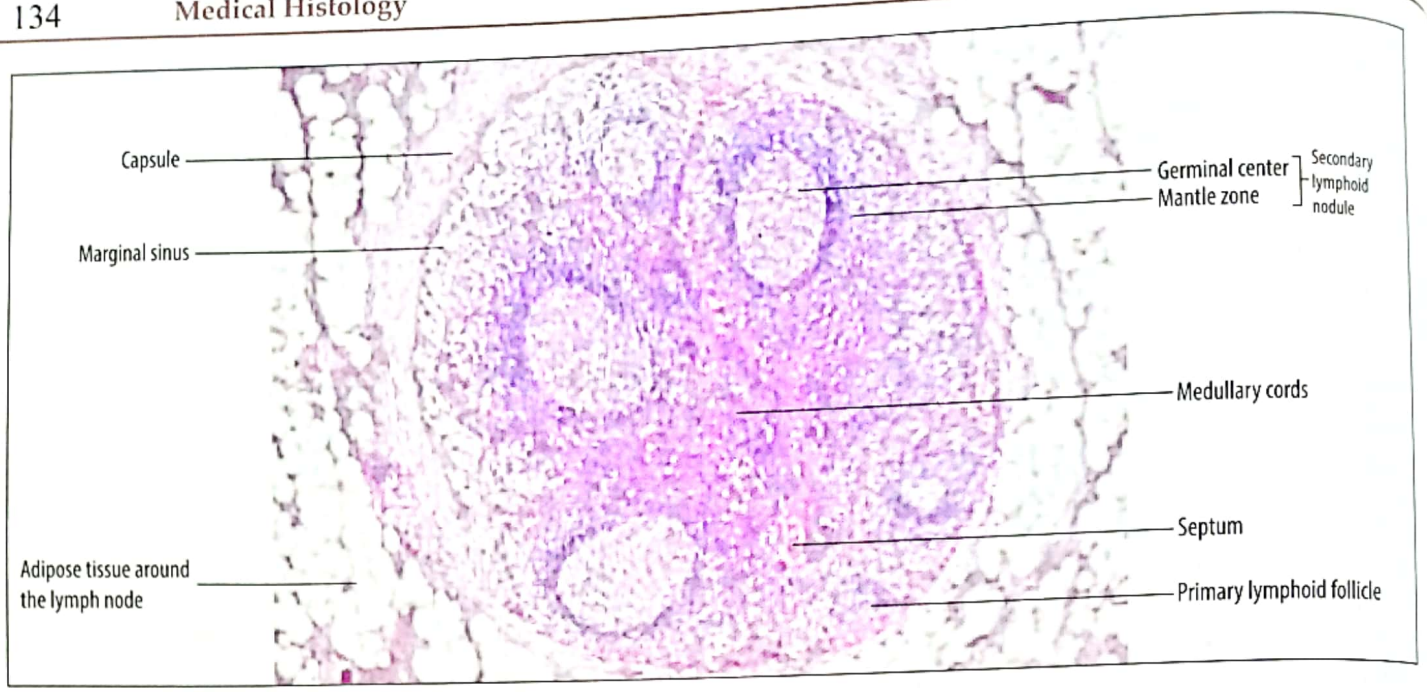

Fig. 14.6 A section through the lymph node as seen under the low power of light microscope.

type of postcapillary venules called **high endothelial venules (HEVs)**. Instead of the flat endothelial cells which line the ordinary capillaries and venules, the HEVs are lined by cuboidal epithelial cells. These cells possess receptors which interact with the lymphocytes and facilitate their migration from the blood into the paracortex. About 90% of the lymphocytes enter the lymph node through the HEVs (remaining 10% arrive through the afferent lymphatics). After their migration, the lymphocytes of B type move to the nodular cortex, while the T lymphocytes remain in the paracortex. Plasmalemma of the endothelial cells of the HEVs also contains a large number of water channels (aquaporins) which enable these venules to concentrate the lymph entering the lymph node. It is estimated that about 35% of the water and electrolytes from the lymph are returned to the blood through the high endothelial venules.

MEDULLA OF THE LYMPH NODE

In the medulla of the lymph node, the trabeculae become highly branched and finally fuse with the connective tissue of the hilum. The medullary lymphoid tissue is arranged chiefly in the form of irregular anastomosing cords, called *medullary cords*. These cords consist of B lymphocytes and plasma cells lying in a network of reticular fibers and reticular cells. Some of the medullary cords are continuous with the lower poles of the cortical nodules but no lymphatic nodules are present in the medulla. Lymphatic sinuses, called *medullary sinuses,* intervene between the medullary cords and medullary trabeculae. The antibodies produced by the plasma cells of the medullary cords pass into the efferent lymphatics of the nodes and finally reach the general circulation.

Role of the Lymph Nodes in the Filtration of Lymph

The lymph nodes serve as immunologic filters and one of their chief functions is to remove bacteria and other foreign materials from the lymph.

The afferent lymphatics penetrate the capsule and deliver the lymph into the *subcapsular sinus.* Valves in the afferent lymph vessels prevent flow of the lymph in the reverse direction. From the subcapsular sinus the lymph passes into the *cortical* sinuses, which deliver the lymph to the *medullary sinuses.* All the medullary sinuses converge upon one or two efferent lymphatic vessels, through which the filtered lymph leaves the lymph node. The efferent lymphatics are also provided with valves that ensure unidirectional flow of lymph.

The **lymphatic sinuses** of the lymph nodes are lined by the reticular cells and endothelium-like simple squamous epithelium. This epithelium is continuous where it directly lines the connective tissue capsule or trabeculae, but shows discontinuities where it faces the lymphatic parenchyma of the lymph node. A large number of macrophages reside in the walls of the lymphatic sinuses and send their cytoplasmic processes into the sinus cavity through the discontinuities in the lining simple squamous epithelium. These macrophage processes continuously monitor the lymph as it flows through the lymphatic sinuses.

In addition to the cytoplasmic processes of macrophages, the cavity of the lymphatic sinuses is crossed by reticular fibers, which are covered by the processes of the reticular cells. Consequently, a criss-crossing meshwork is created in the lumen of the lymph node sinuses, which retards the free flow of lymph and promotes its filtration.

As the lymph slowly percolates through the sinuses of a lymph node, the bacteria, antigenic materials and metastatic cancer cells are trapped by the mechanical barrier created by the meshwork of reticular fibers and processes of macrophages spanning the lumen of sinuses. After being trapped, the bacteria, antigens and transformed cells of the metastatic cancer are phagocytosed by the macrophages.

Role of the Lymph Nodes in the Immune Response

The dendritic cells located in the skin and mucous membranes capture the antigens and migrate through the lymph vessels to the lymph nodes and present their epitope-MHC II complex to the helper T lymphocytes in the paracortical region of the lymph node. If the helper T cells of the paracortex become activated, they divide mitotically to produce more helper T cells. The newly formed helper T lymphocytes pass into the medulla and finally leave the lymph node through the efferent lymphatic vessels. As all the lymph from the body is finally drained into the blood, the helper T lymphocytes travel in the blood and ultimately reach the area of antigenic activity to neutralize the antigen.

As the lymph nodes are interposed in the path of lymph vessels, the lymph collected from the tissues must cross at least one lymph node before reaching the bloodstream. If any bacteria or other antigenic substances are present in the lymph, these percolate through the cortical sinuses and penetrate the lymphoid follicles in the cortical region of the lymph node. Some of these antigens become attached to the processes of the follicular dendritic cells, while others are phagocytosed and processed by the macrophages. The processed antigen is then presented to the B lymphocytes of the cortical lymphatic nodules.

Presentation of an antigen to the B lymphocytes of the cortical nodules causes activation of these lymphocytes. The activated B lymphocytes migrate to the center of a primary lymphoid nodule and proliferate there to form a germinal center, so that the primary nodule is converted into a secondary lymphoid nodule. The cells produced in the germinal center differentiate into plasma cells and memory B lymphocytes. The plasma cells migrate to the medullary cords of the lymph node and release antibodies into the lymph flowing through the medullary sinuses. These antibodies leave the lymph node through the efferent lymphatics and finally reach the bloodstream to increase the level of circulating immunoglobulins.

Some of the memory B lymphocytes remain in the cortical nodules, but most of them leave the lymph node to reach the bloodstream. These memory B cells finally settle down in other secondary lymphoid organs of the body, so that they can proliferate upon a subsequent exposure to the same antigen and enable the body to launch a prompt and potent secondary immune response.

The lymph nodes draining an infected area become enlarged because of the formation of germinal centers and proliferation of lymphocytes in the lymphoid nodules. Such lymph nodes usually appear as painful swellings in the neck, axilla, groin or other regions of the body.

In the event of development of a malignant tumor in a body part, the cancerous cells may break off from the primary tumor and enter the lymphatic vessels and then metastasize to the nearby lymph nodes. The cancerous cells may continue to grow within the lymph nodes as secondary tumors and produce painless enlargement of these nodes. Presence of metastatic cancer cells in the lymph nodes is used by the surgeons and pathologists as one of the most important deciding factors in the "cancer-staging systems" and in estimating the prognosis of the disease.

SPLEEN

The spleen is the largest lymphoid organ in the body. It is located in the upper left quadrant of the abdominal cavity. The spleen is the only lymphoid organ interposed in the blood circulation. Its sinuses are filled with blood and contain macrophages and dendritic cells in their walls, due to which the spleen carries out immunologic filtration of blood by removing blood-borne antigen. The spleen is also the site for destruction of the old and worn out erythrocytes. Being a lymphoid organ, the spleen is also a site of production of activated lymphocytes.

The spleen is covered by a **capsule** of dense irregular connective tissue which also contains some myofibroblasts. From the capsule, **trabeculae** (septa) extend into the substance of the organ and divide its parenchyma, called *splenic pulp*, into incomplete compartments.

Although the storage capacity of the human spleen is quite limited, the myofibroblasts in the capsule and trabeculae enable the spleen to contract and discharge the stored blood components (mainly erythrocytes and platelets) into the systemic circulation when the blood volume suddenly falls (e.g., due to severe bleeding as a result of trauma).

SPLENIC PULP

The spaces between the trabeculae are occupied by a soft sponge-like tissue known as the splenic pulp. If a freshly cut spleen is examined by naked eye, the splenic pulp is seen to exhibit different colors (red or whitish gray) in various regions. On this basis, the splenic pulp is conventionally divided into two types: red pulp and white pulp (Fig. 14.7 & 14.8)

THE RED PULP

The red pulp appears reddish in color in the fresh state as well as in routine histological sections because it contains huge numbers of erythrocytes. The microscopic examination of the red pulp reveals that it consists of cellular cords called ***splenic cords***, which are separated from each other by sinusoidal capillaries which are called ***splenic sinusoids***.

The **splenic cords** contain cells of many different kinds including erythrocytes, T lymphocytes, B lymphocytes,

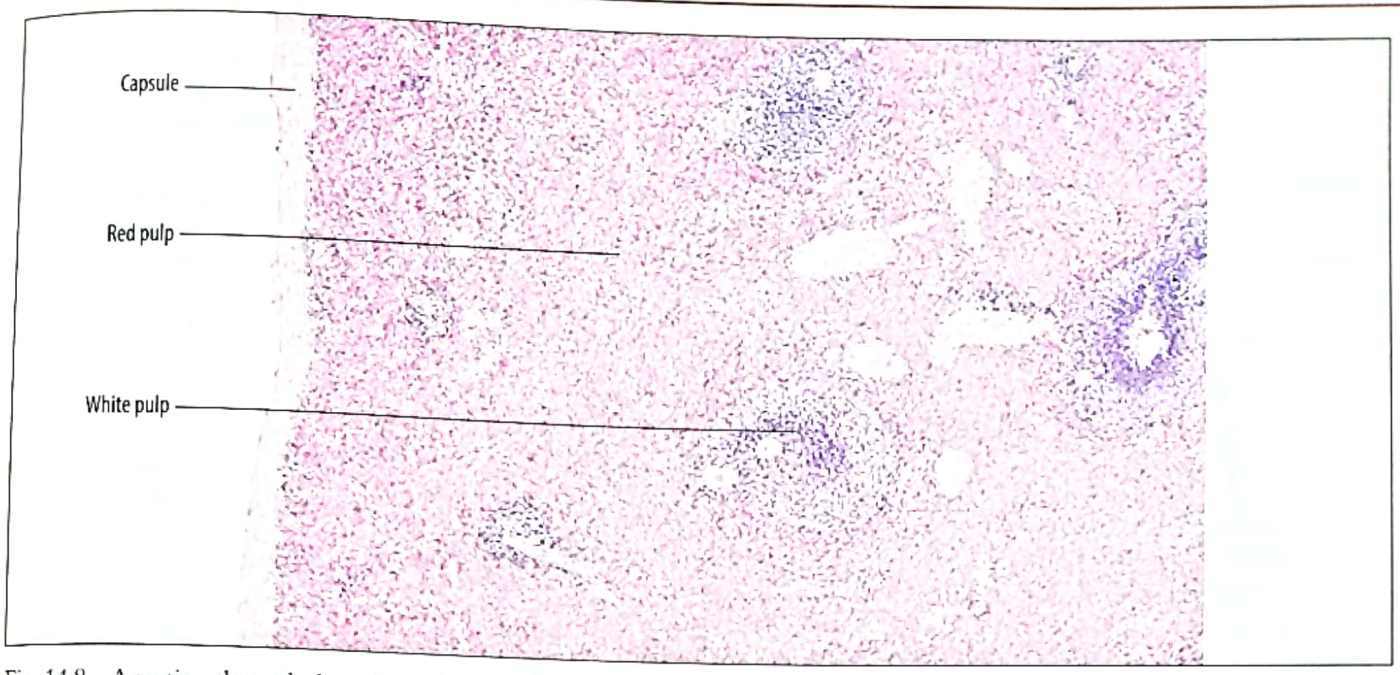

Fig. 14.8 A section through the spleen showing its structure under the low power magnification of the light microscope.

of the sinusoids. Processes of these macrophages extend through the intercellular gaps into the lumen of the splenic sinusoids to monitor the passing blood for antigenic materials.

THE WHITE PULP

This part of splenic pulp consists of typical lymphoid tissue that surrounds and follows branches of the splenic artery. This lymphoid tissue forms a cylindrical **periarterial lymphatic sheath (PALS)** around each branch of the splenic artery. The periarterial lymphatic sheaths are composed chiefly of T lymphocytes and may be considered equivalent to the thymus-dependent paracortex of the lymph nodes. At places, enclosed within the PALS are lymphoid nodules that are composed of B lymphocytes. At these places the periarterial lymphatic sheaths appear to be expanded in the form of ovoid or fusiform masses. Most of these lymphatic nodules are secondary nodules that exhibit germinal centers (indicative of antigenic challenge). Each branch of the splenic artery that runs through a periarterial lymphatic sheath is known as a *central artery*. Histologically, the central arteries are arterioles.

It is important to note that, contrary to its whitish appearance in fresh condition, the white pulp takes a darker color than the red pulp in the stained sections of splenic tissue. The white pulp stains deeply basophilic (purplish) because it consists mainly of densely packed lymphocytes. The red pulp contains fewer lymphocytes and, therefore, stains comparatively lighter. Careful observation under the LM shows that the red pulp exhibits a mixture of eosinophilia (because of the erythrocytes) and basophilia (because of the lymphocytes).

Blood Supply of the Spleen (Fig. 14.9)

A thorough knowledge of vascular arrangements in the spleen is of fundamental importance in understanding the microscopic structure of the organ.

The splenic artery enters the spleen through the hilum and divides into branches that course in the trabeculae for some distance and are known as **trabecular arteries.** These arteries branch and re-branch within the trabeculae and leave the trabeculae when their diameter is reduced to 0.2 mm (i.e., when they become arterioles). After leaving the trabeculae, the arterioles enter the white pulp, and are now known as **central arterioles.** As described above, each central artery is surrounded by a periarterial lymphatic sheath which is expanded in those regions which contain lymphatic nodules. It is to be noted that in the region of a lymphatic nodule the central artery usually occupies an eccentric position since it lies away from the germinal center.

After a number of divisions, the central arteries become reduced in size, lose the investment of white pulp, enter the red pulp and divide into a number of short but relatively straight arterioles, called **penicillar arterioles.** The penicillar arterioles divide and give rise to arterial capillaries, which convey the blood to the splenic sinusoids. Some of these capillaries are surrounded by a sheath of APCs (mainly macrophages) and, therefore, are called ***sheathed capillaries***. The APCs of the sheathed capillaries play an important role in immune surveillance of the blood.

The manner in which blood flows from the arterial capillaries to the interior of the splenic sinusoids has been a subject of considerable controversy. Two models were proposed in this regard: (i) closed circulation, and (ii) open circulation. According to the **closed circulation** model, the terminal arterial capillaries are connected to the splenic sinusoids and deliver the blood directly into these sinusoids. According to the **open circulation** model, the

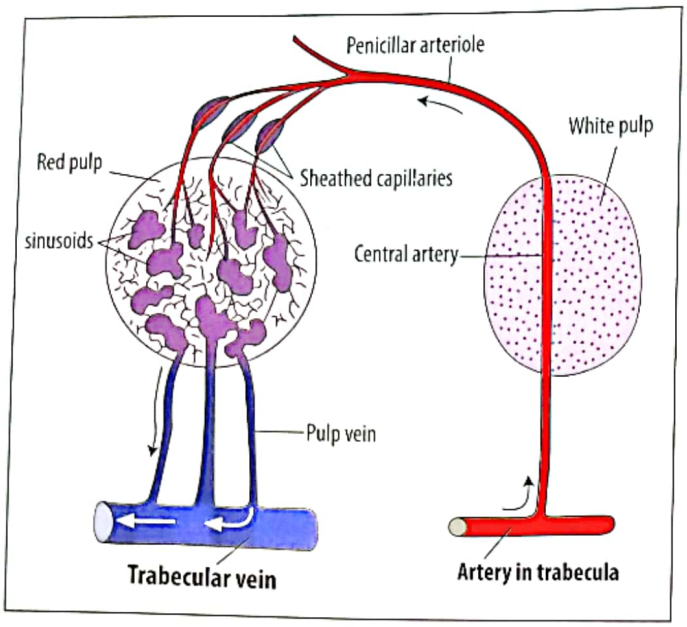

Fig. 14.9 A schematic diagram to show the blood circulation through the spleen.

terminal arterial capillaries release their blood into the splenic cords from where the blood slowly percolates into the sinusoids. It is now generally agreed that only the open circulation route exists in humans, whereas the closed circulation route is present in the splenic tissue of other mammals like the rat and dog.

The splenic sinusoids pour their blood into the pulp veins which unite to form larger trabecular veins that run in the connective tissue trabeculae. These veins consist only of endothelium supported by fibromuscular tissue of the trabeculae. The trabecular veins drain into the splenic vein.

Functions of the Spleen

1. **Functions relating to the Immune system.** As the lymph nodes filter the lymph, the spleen filters the blood. The APCs (macrophages and dendritic cells) capture the bacteria and other antigens floating in the blood, process these antigens and present their epitopes to the B and T lymphocytes, resulting in initiation of immune response against the blood-borne antigens.
2. **Destruction of old and worn out erythrocytes and platelets.** Those erythrocytes and platelets which have completed their normal life span, are removed from the circulation by the macrophages of the spleen. The hemoglobin of the RBCs is degraded in the cytoplasm of the macrophages and the iron so liberated is released into the circulation for re-use.

MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT)

This variety of lymphoid tissue consists of nonencapsulated lymphoid tissue found in the mucosa of the digestive, respiratory and urogenital tracts. The MALT contains diffuse lymphoid tissue containing B lymphocytes, plasma cells, helper T lymphocytes, macrophages, and dendritic cells. In certain locations, lymphoid follicles are present in the MALT. The mucosa-associated lymphoid tissue plays an important role in protecting the body against bacteria and other antigenic agents that manage to penetrate the mucosal epithelia.

In the urogenital tract the MALT is not very prominent and, hence, is not given any specific name. In the mucosa of the gastrointestinal and respiratory tracts, the MALT is well-developed and is titled as gut-associated lymphoid tissue (GALT) and bronchus-associated lymphoid tissue (BALT), respectively.

THE TONSILS

The tonsils are masses of lymphoid tissue that are arranged in the form of a ring (called Waldeyer's ring) around the entrance into the oropharynx. They are incompletely encapsulated aggregates of lymphoid tissue and their free surface is covered by mucosal epithelium. The tonsils have no afferent lymphatics and contain no sinuses. The lymph from these lymphoid aggregates drains into efferent lymph vessels.

The Waldeyer's ring consists of paired (i.e., bilateral) palatine tonsils, a single pharyngeal tonsil, and a single lingual tonsil. Unless otherwise specified, the term 'tonsil' customarily implies the palatine tonsil.

The tonsils are large and well-developed in children but undergo involution after the person attains the age of puberty.

PALATINE TONSILS (Fig. 14.10)

These are ovoid masses of lymphoid tissue located between the palatoglossal and palatopharyngeal folds of the pharyngeal wall. The superficial surface of each palatine tonsil is covered by stratified squamous nonkeratinized epithelium which is continuous with the lining epithelium of the rest of the oropharynx. The surface epithelium of the tonsil dips into the underlying lymphoid tissue at numerous places to form deep invaginations called **tonsillar crypts**. The tonsillar epithelium is usually infiltrated by lymphocytes.

The parenchyma of the tonsils consists of a dense accumulation of lymphoid follicles, most of which show germinal centers. The space between the follicles is occupied by diffusely scattered lymphocytes.

The deep aspect of the palatine tonsil is isolated from the subjacent structures by a band of dense connective tissue that forms the *capsule* of the tonsil. It is to be noted that this capsule is incomplete because it only covers the deep aspect of the tonsil.

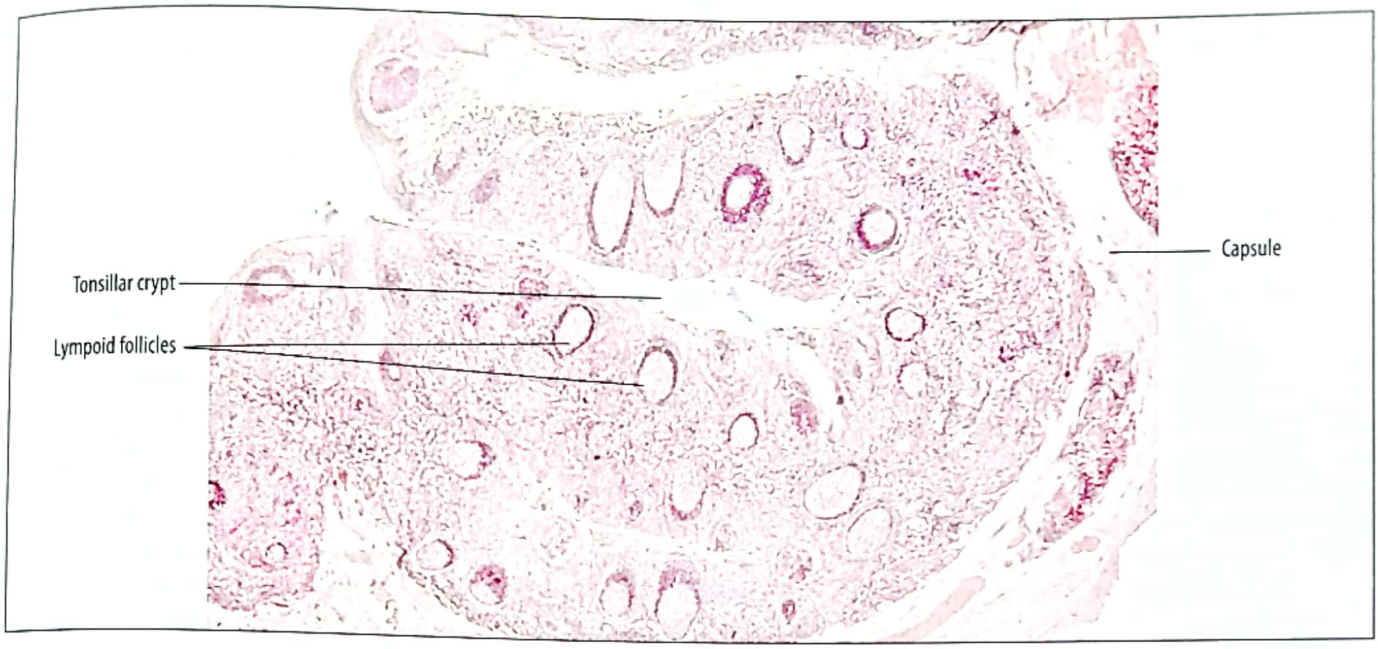

Fig. 14.10 A section through the palatine tonsil to show its microscopic structure.

The tonsillar crypts frequently contain exfoliated epithelial cells, dead leukocytes, food debris, and bacteria. In palatine tonsils there is no mechanism to keep the crypts clean and, therefore, the material collected in the crypts may result in chronic infection of the tonsils. Persistent infection causes inflammation of the tonsils (tonsillitis) leading to their enlargement. Unremitting infection and inflammation of the tonsils may require their surgical removal (tonsillectomy).

PHARYNGEAL TONSIL

This is an accumulation of lymphoid tissue in the posterosuperior wall of the nasopharynx. The free surface of the pharyngeal tonsil is covered by pseudostratified columnar ciliated epithelium interspersed with patches of stratified squamous nonkeratinized epithelium. No crypts are present. However, the epithelium shows longitudinal folds (pleats). Beneath the epithelium is present a diffuse mass of lymphoid tissue in which are embedded many lymphatic nodules. The deeper surface of the pharyngeal tonsil is also covered by a thin capsule of fibrous connective tissue.

Hypertrophy of the pharyngeal tonsil, with consequent obstruction of the nasal passages, is a common condition in children and is known as *adenoids*.

LINGUAL TONSIL

This mass of lymphoid tissue is located in the posterior one-third (root) of the tongue. It is covered on its superficial aspect by stratified squamous epithelium. The lingual tonsil has many crypts, each lined by a continuation of the epithelium covering the tongue root (satartified squamous nonkeratinized epithelium). Beneath the epithelium is present diffuse lymphatic tissue with lymphatic nodules containing germinal centers. A very thin capsule of connective tissue separates the lingual tonsil from the underlying structures.

It is to be noted that mucous glands of the tongue root open into the crypts of the lingual tonsil. Secretion of these glands cleans these crypts and keeps them free of debris. Therefore, the lingual tonsils are not subject to persistent infection and inflammation.

Integumentary System 15

The skin and its certain specialized derivatives, called *appendages*, constitute the integumentary system. The appendages of skin include hair, nails, sebaceous glands, and sweat glands.

THE SKIN (Fig. 15.1)

The skin (also called integument or cutis) forms the external covering of the body. It consists of two layers of completely different types of tissues that are attached to each other over their entire extent. The superficial layer, known as **epidermis,** is a stratified squamous keratinized epithelium. Developmentally, the epidermis is derived from the surface ectoderm. The deeper layer of skin is called **dermis** or *corium*. It is composed of dense, irregular connective tissue and is derived from the mesoderm.

Under the dermis lies hypodermis which consists of loose connective tissue containing variable amounts of adipose tissue. The hypodermis binds the skin loosely to the underlying deep fascia (or underlying muscles where the deep fascia is absent, e.g., the face and anterior abdominal wall)) and thus allows the skin to move over the deep fascia (or underlying muscles). Generally, the hypodermis is not considered to be a part of the skin, but some histologists regard it as the third layer of skin because it lodges deeper parts of the appendages of skin like hair follicles and sweat glands (Fig. 15.1).

Taking into account the thickness of the epidermis, the skin is classified into two types: thick skin and thin skin. The **thick skin** covers palms and soles, whereas the **thin skin** is found on remainder of the body. In the thick skin the epidermal thickness ranges from 400 to 1400 µm, while in the thin skin it varies from 75 to 150 µm.

EPIDERMIS

The epidermis is a continuously self-replacing stratified squamous keratinized epithelium. It contains four types of cells:

1. Keratinocytes.
2. Melanocytes.
3. Langerhans cells.
4. Merkel cells.

KERATINOCYTES

These cells constitute the principal cells of the epidermis. The most characteristic feature of the keratinocytes is that their cytoplasm is rich in keratin intermediate filaments (also known as *tonofilaments*). The protein keratin is synthesized in the rough endoplasmic reticulum of the keratinocytes and the number of tonofilaments increases as the cells move outwards.

The keratinocytes undergo constant renewal throughout life by continuous production new cells by the mitotic division of the keratinocytes located in the basal layer (stratum basale) of the epidermis. The newly produced keratinocytes constantly move outward into the more superficial layers of the epidermis. As they move away from the basement membrane of the epidermis, the keratinocytes undergo progressive changes in size, shape and content, eventually transforming from cuboidal living cells to dead flat cells which are constantly shed off (desquamated) from the skin surface.

As the keratinocytes leave the stratum basale and move outwards, the number of keratin filaments increases and they become aggregated into bundles called *tonofibrils*. In the mature keratinocytes, which occupy the most superficial layers of the epidermis, tonofibrils occupy almost 85% of the cell volume.

In the stratum granulosum of the epidermis, the keratinocytes begin to form small, oval, membrane-bound granules called *lamellar granules*. These granules consist of concentric lamellae which contain pro-barrier lipids which are secreted into the intercellular spaces in the stratum corneum. These lipids serve to make the epidermis impermeable to water.

MELANOCYTES

The melanocytes are stellate cells with oval bodies and long dendritic processes. These cells are found to be scattered between the keratinocytes of the stratum basale and stratum spinosum of the epidermis. The melanocytes are not identifiable in the routine H&E stained sections and special staining procedures are needed to visualize these cells under the light microscope. Although the melanocytes lie among the keratinocytes but no desmosomes or any other type of intercellular junctions are present between the melanocytes and keratinocytes. The melanocytes synthesize the brownish black pigment **melanin,** which protects the skin from the harmful effects of the ultraviolet radiation (received mainly from the sun).

Formation of the melanin pigment occurs within membrane-bound bodies called *melanosomes*. The melanosomes are derived from the Golgi apparatus of the cell and contain the enzyme tyrosinase. This enzyme acts on tyrosine to transform it into 3, 4-dihydroxyphenyl-alanine (DOPA) which is subsequently converted into melanin. The melanin-packed mature melanosomes are called *melanin granules*. These brownish black granules are transferred to the keratinocytes (of the skin and hair) through the dendritic processes of the melanocytes.

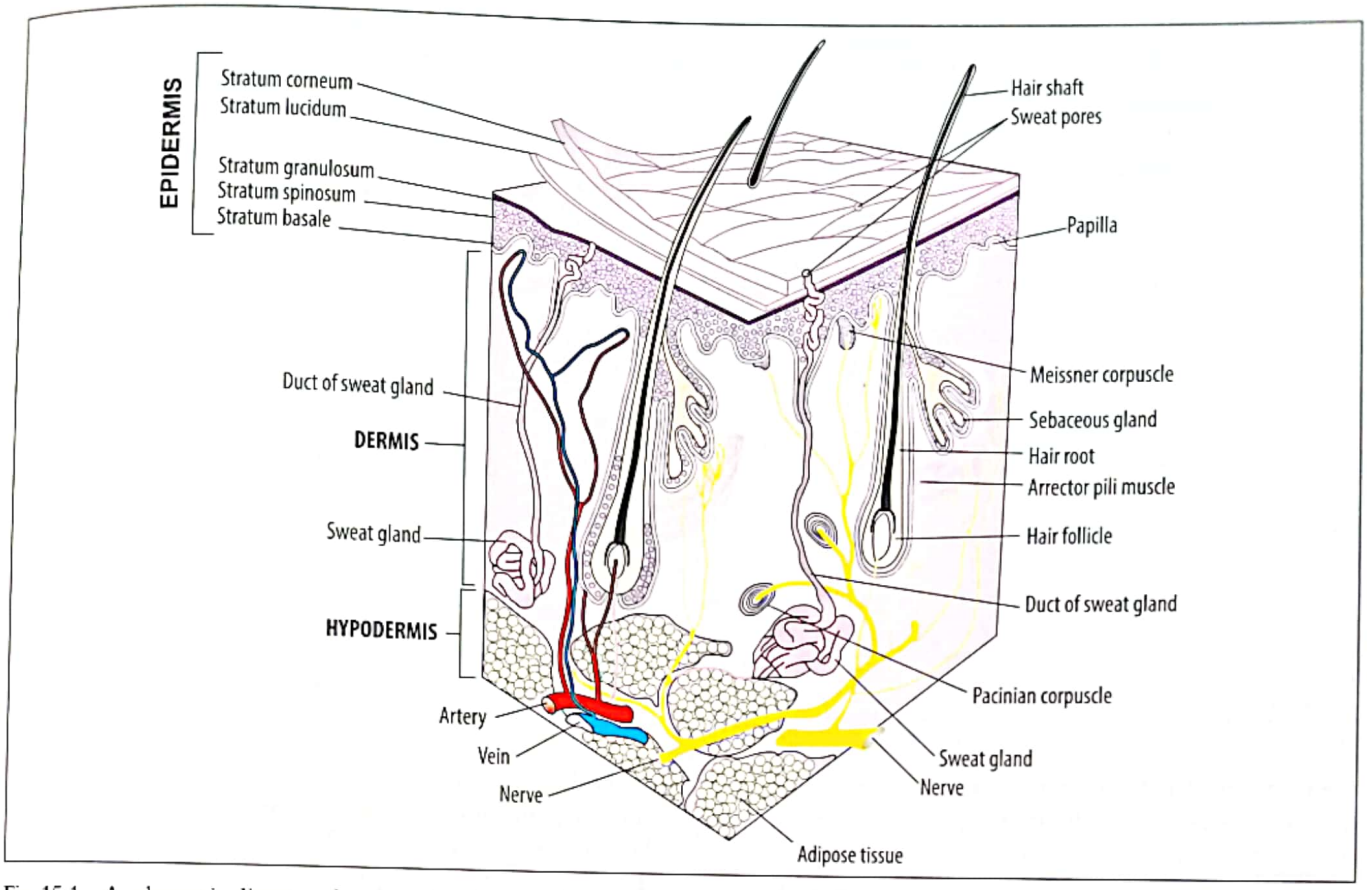

Fig. 15.1 A schematic diagram showing the microscopic structure of the skin.

with each other and thus enable them to resist abrasion. In addition to the keratinocytes, the stratum spinosum contains Langerhans cells and occasional lymphocytes.

In the cytoplasm of keratinocytes of the stratum spinosum, the tonofilaments begin to aggregate to form bundles called ***tonofibrils***. The cytoplasm of the keratinocytes of the stratum spinosum also contains variable number of melanin granules which are transferred to these cells by the dendritic processes of the melanocytes. The number of melanin granules in the keratinocytes of the stratum spinosum varies in different persons, being very high in the dark-skinned people.

STRATUM GRANULOSUM

The stratum granulosum consists of 3 to 5 layers of flattened rhomboid keratinocytes with their long axes parallel to the surface of the skin. The cytoplasm of these cells contains a large number of coarse, intensely basophilic granules called ***keratohyalin granules***. These granules are irregular in shape and are not bounded by a membrane. They contain *keratohyalin* which is a protein complex consisting of *filaggrin* and some other proteins. These proteins function as promoters of aggregation of tonofilaments into tonofibrils.

Electron microscopic examination of the keratinocytes of the stratum granulosum shows that they also contain small, oval, membrane-bound granules which exhibit a lamellar (multilayered) structure and, therefore, are called ***lamellar granules***. The lamellar granules, also called *membrane-coating granules*, are produced by the Golgi apparatus of the cell and contain various lipids (chiefly phospholipids, glycoshingolipids, and ceramides). These granules release their contents by exocytosis and the special lipids so released diffuse into the intercellular space. Presence of these lipids between the cells of the stratum corneum of the epidermis creates a barrier which is impermeable to water.

The lamellar granules also contain proteolytic enzymes which are released in the most superficial layers of the epidermis. These enzymes cause a regulated breakdown of the desmosomes of the keratinocytes, so that these cells can exfoliate from the skin surface.

As the keratinocytes move to the outer layer of the stratum granulosum, keratinization becomes progressively more pronounced and number of tonofibrils in the cytoplasm rapidly increases. With increase in the number of the tonofibrils, the nuclei of the cells of stratum granulosum show degenerative changes and gradually become pale and indistinct. The cell organelles are lost as a result of autophagy by lysosomal enzymes. Consequently, the cells become dead before moving into the more superficial layers.

STRATUM LUCIDUM

The stratum lucidum is identifiable only in the thick skin covering the palms and soles (Fig. 15.3) and is not seen in

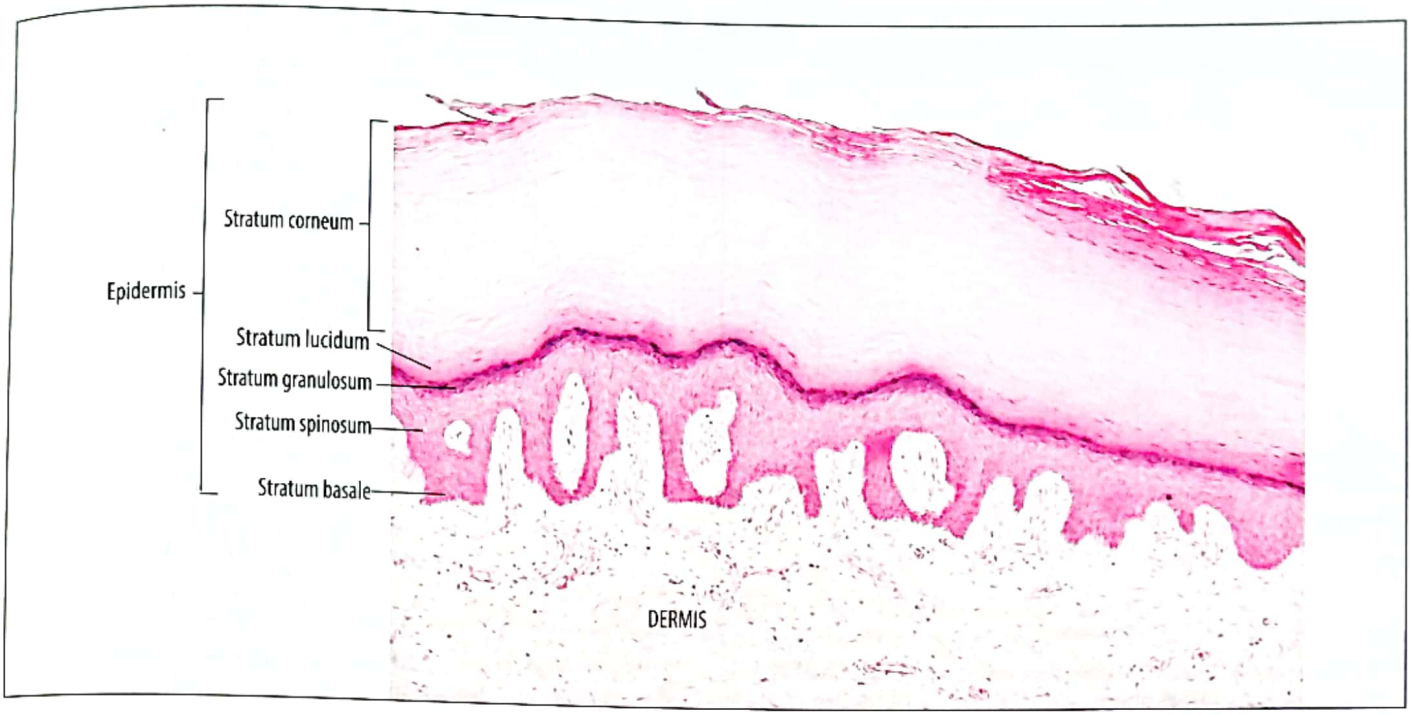

Fig. 15.3 A section through the thick skin of the palm. Note the great thickness of the stratum corneum and presence of stratum lucidum.

PAPILLARY LAYER

This layer is so named because it bears projections, called dermal *papillae that* protrude into the overlying epidermis. The dermal papillae interdigitate with reciprocal projections of epidermal epithelium, which are called *epidermal ridges.*

The papillary layer of the dermis is thinner than the reticular layer and consists of loose connective tissue composed of a network of fine collagen type I fibers, reticular fibers, and elastic fibers. Fibroblasts, macrophages and mast cells are also present in the connective tissue of the papillary layer. Special fibrils, called *anchoring fibrils,* which are composed of collagen type VII, extend from the basal lamina of the epidermal epithelium into the papillary layer. The anchoring fibrils serve to bind the epidermis to the dermis.

The papillary layer is richly vascular and contains numerous capillary loops. The capillaries of the papillary layer regulate the body temperature and provide nourishment for the overlying epidermis (which is avascular). This layer also contains sensory nerves and sensory receptors. The receptors are mostly tactile corpuscles of Meissner that are located in the dermal papillae. These receptors are more commonly found in those areas of skin that are particularly sensitive to tactile stimulation, e.g., finger tips and lips. Some papillae also contain Krause end bulbs.

RETICULAR LAYER

This layer is thicker and consists of dense, irregularly-arranged connective tissue. It is composed mainly of interlacing bundles of collagen fibers. Many thick elastic fibers are also present. The intervals between the interlacing bundles of connective tissue fibers are filled with the glycosaminooglycan dermatan sulfate. The reticular layer of the dermis is less cellular than the papillary layer; the cells found in this layer include fibroblasts, mast cells, macrophages, T lymphocytes, and adipocytes. The reticular layer of the dermis generally contains two types of encapsulated sensory receptors which are Pacinian corpuscles and Ruffini corpuscles.

Scattered smooth muscle fibers are found in the deeper part of the reticular layer of the dermis in the skin covering the areolae of nipples, penis, and scrotum. Such portions of the skin become wrinkled when these muscle fibers undergo contraction.

THE HAIR

Hairs are thread-like, keratinized structures that project from the epidermis. Thickness of the hairs varies considerably; in some regions of the body they may be as thin as 18 μm in diameter, while in other regions they may be as thick as 180 μm. The hairs are found over the entire skin except on the palms, soles, lips, dorsal surfaces of the distal phalanges, glans penis, glans clitoridis, labia minora, and vestibular aspect of the labia majora. Embryologically, each hair is derived from an invagination of the ectodermal epithelium of the epidermis into the underlying mesenchyme.

Each hair consists of two portions: a *shaft* and a *root.* The shaft projects above the skin surface, while the root is embedded in the skin. Surrounding the hair root is a tubular *hair follicle* (Fig. 15.4) which consists of epidermal (epithelial) and dermal (connective tissue) elements. At its deep end, the hair follicle is expanded into a *hair bulb.* Here the hair root and its epithelial sheath blend in a mass of cells called *matrix.* Proliferation of the matrix cells is responsible

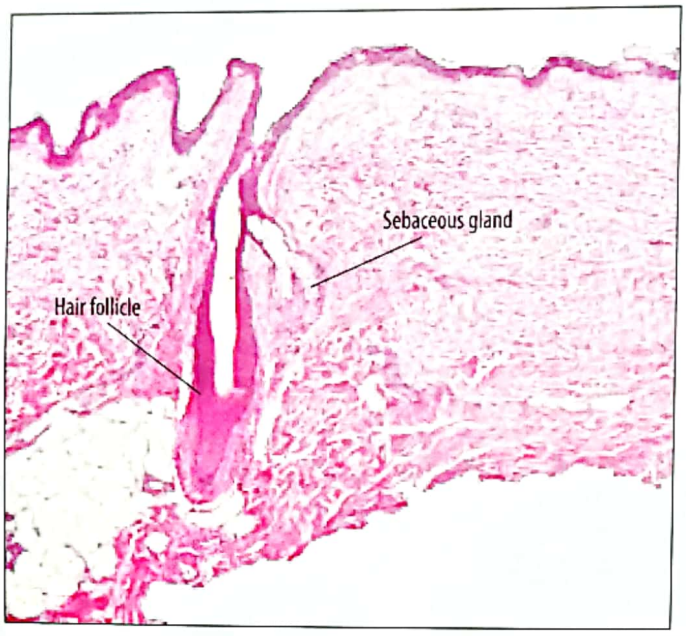

Fig. 15.4 A section through the thin skin showing a hair follicle and a sebaceous gland opening into the neck of the hair follicle. The lowermost part of the hair follicle is extending into the hypodermis.

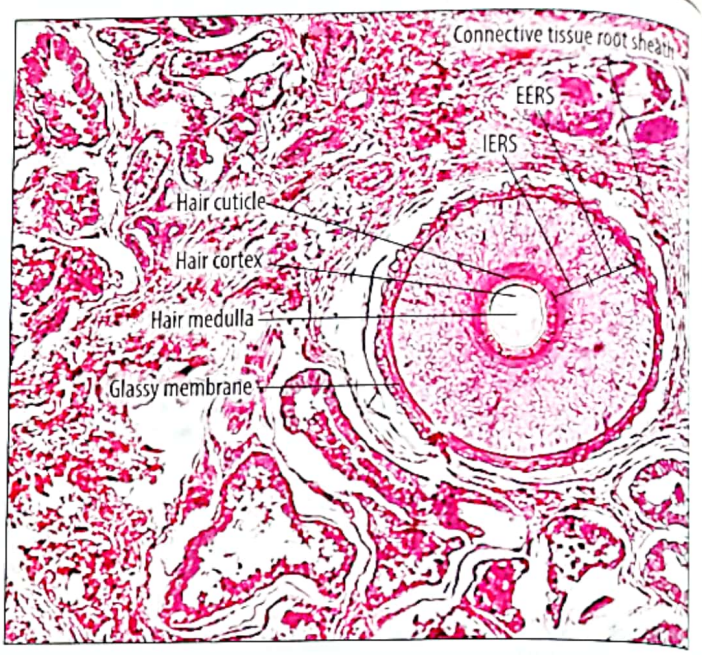

Fig. 15.5 A section through the dermis of skin showing a transversely cut hair follicle. IERS: Internal Epithelial Root Sheath, EERS: External Epithelial Root Sheath.

for the growth of the hair and, therefore, these cells can be regarded to be homologous to the stratum basale of the epidermis. Melanocytes are scattered among the matrix cells, especially in the region immediately around the hair papilla. The melanocytes have long dendritic processes which transfer melanin granules to the cells of the hair cortex.

There is an indentation at the basal end of the hair bulb which is occupied by a tuft of vascularized loose connective tissue called hair *papilla*. Associated with each hair follicle are one or more sebaceous glands and a bundle of smooth muscle fibers which constitutes the erector muscle of the hair and is called arrector pili (*plural* arrectores pilorum).

STRUCTURE OF THE HAIR

The hair shaft and root consist of epithelial cells arranged in three concentric regions: medulla, cortex and cuticle (Fig. 15.5).

The **medulla** forms the central axis of the hair and consists of two or more layers of shrunken, moderately keratinized, cuboidal cells. The medulla is absent in very thin hair.

The **cortex** forms the main bulk of the hair. It is composed of long, flattened, fusiform, heavily keratinized cells which are very closely packed. In dark hair, the cells of the cortex contain granules of melanin pigment.

The **cuticle is the outermost covering of the hair and** consists of a single layer of heavily keratinized, squamous cells which partially overlap each other. The cells are non-nucleated except for those present in the lower part of the root.

The *color* of the hair depends on the type and quantity of melanin produced by the melanocytes located between the epithelial cells of the hair root. From the melanocytes, the pigment is transferred to the cortex of hair. The melanocytes of the hair root produce eumelanin or pheomelanin. The eumelanin makes the hair appear brown or black, whereas the pheomelanin colors the hair blond or red.

Whitening of the hair, which normally begins around 40 years of age, occurs due to a gradual decrease in in melanin formation by the melanocytes and ultimately death and disappearance of these cells from the hair root.

HAIR FOLLICLE

The hair follicle surrounds the root of the hair and is responsible for the production and growth of the hair. The hair follicles lie in the dermis but frequently invade the hypodermis.

Each hair follicle consists of three parts: infundibulum, isthmus, and inferior segment. The ***infundibulum*** is the segment from the surface opening of the follicle to the level of opening of the duct of the sebaceous gland associated with the hair follicle. The infundibulum forms a part of the *pilosebaceous canal*, which serves as a route for the discharge of the sebum (secretion of the sebaceous glands). The ***isthmus*** is the part that lies between the opening of the sebaceous gland and attachment of arrector pili muscle. The ***inferior segment*** is the part from the arrector pili attachment to the proximal end (base) of the follicle where it expands to form the *hair bulb*. Each hair bulb is indented at its base by a dermal papilla consisting of loose, vascularized connective tissue. The capillaries of the dermal papilla provide oxygen and nutrients for the cells of the hair follicle.

Each hair follicle (Fig. 15.5) is composed of the following two major coats:

1. *Epithelial root sheath*, which is derived from the epidermis. This sheath surrounds the hair root and lies immediately next to the hair cuticle.
2. *Connective tissue root sheath*, which is derived from the dermis, lies outer to the epithelial root sheath.

The Epithelial Root Sheath

This sheath is further composed of two coats: (1) internal epithelial root sheath, and (2) external epithelial root sheath.

The Inner Epithelial Root Sheath

This sheath represents the stratum cornеum and stratum granulosum of the epidermis. It does not extend above the point of entry of the duct of the sebaceous gland into the follicle.

The External Epithelial Root Sheath

This sheath corresponds to the stratum spinosum and stratum basale layer of the epidermis. It lies outer to the internal epithelial root sheath and covers it up to the epidermis of skin where the epithelial layers of this sheath become continuous with the stratum basale and stratum spinosum of the epidermis. Merkel cells are located among the cells of the outer epithelial root sheath especially in the large hair follicles. The external epithelial root sheath is separated from the connective tissue root sheath by an acelluar hyaline layer called *glassy membrane*. This membrane is actually the thickened basement membrane of the epithelium of the epithelial root sheath.

The Connective Tissue Root Sheath

This sheath lies outer to the epithelial root sheath. It consist of dense irregular connective tissue belonging to the dermis of skin and extends up to the level where the arrector pili muscle is attached to the hair follicle.

THE HAIR GROWTH CYCLE

Between starting to grow and falling out years later, each hair passes through three phases: anagen, catagen, and telogen. At any time, every hair is at a different stage of the growth cycle. The length of the hair growth cycle is variable on different parts of the body. For eyebrows, this cycle is completed in about 4 months. For the scalp hair, the length of the growth cycle is around 7 years. The following description of various phases of the hair growth cycle is regarding the scalp hair.

The **anagen** or *growing phase* is a long phase which lasts for 2-7 years and determines the length of the hair. The rate of the growth of the scalp hair is about 1.25 cm (1/2 inch) per month. This implies that a newly growing scalp hair would attain a length of approximately 15 cm (6 inches) in one year.

The **catagen** or *regression phase* lasts for about 10 days. During this phase, the hair follicle shrinks and detaches from the dermal papilla.

The **telogen** or *resting phase* lasts for 1-4 months. During this phase, the hair follicle remains dormant for many weeks and, at some point, begins to grow again. As the new growth cycle begins, the old hair breaks free from the follicle and is shed. Within 2 weeks of the shedding of the old hair, the new hair shaft makes its appearance on the surface of the skin.

ARRECTORES PILORUM

These are oblique bundles of smooth muscle fibers found in the dermis in relation to the hair follicles. An arrector pili muscle is attached at one end to the papillary layer of the dermis and at the other end to the connective tissue root sheath of a hair follicle at about middle of the follicle. Arrectores pilorum are innervated by the sympathetic nerve fibers.

As indicated by their name, action of arrectores pilorum is to cause erection of the hair. Normally a hair is not erect (i.e., not set perpendicularly to the skin surface) but rather slopes at an obtuse angle. When arrectores pilorum contract, they cause erection of the hairs and elevation of the skin around the hair shafts, producing tiny bumps on the skin and thus giving the skin the appearance of "goose flesh". When the skin surface is exposed to excessive cold, the arrectores pilorum undergo contraction producing erection of the hair in an attempt to create a pocket of warm air over the skin. The arrectores pilorum also contract when there is excessive sympathetic activity in the body, e.g., when a person is suddenly frightened.

Function of the Hair

As opposed to the hair of the animals, human hairs do not perform the insulatory function against the heat loss. However, the human hairs play an important role in tactile sensation. Any stimulus that results in the deformation of a hair is transmitted along the shaft of the hair to the free nerve endings that surround the hair follicle.

NAILS (Fig. 15.6)

The nails consist of hard, translucent, roughly rectangular, horny plates, called nail plates, which cover the dorsal surfaces of the terminal phalanges of the fingers and toes. Microscopically, the nails are homologous with the stratum corneum of the epidermis and, therefore, the *nail plate* consists of extremely compact, highly keratinized epithelial cells. Each nail plate rests on a *nail bed* that consists of the skin under the nail.

Each **nail plate** consists of three parts:

1. **Body**. This is the uncovered and visible part that rests on the nail bed.
2. **Free edge**. This is that part of the nail which projects beyond the skin distally.
3. **Root**. This is the proximal part of the nail that lies beneath a fold of the epidermis.

breakdown into a fatty mass and cellular debris. This oily material, called ***sebum***, is passed through the duct of the gland into the infundibulum of the hair follicle and thence onto the skin surface.

Secretion of the sebaceous glands is under the control of androgens both in the male and female. In males the controlling hormone is testosterone, whereas in the females it is a combination of the ovarian and adrenal androgens.

Function of the sebum. The oily sebum ensures the maintenance of proper skin texture and hair flexibility. It also has weak antifungal and antibacterial properties and thus contributes to the first line of defense against the harmful microorganisms.

Acne vulgaris

At the time of puberty there is an increase in androgen production both in the males and females (testosterone in the males and adrenal androgens in the females). This is associated with an excessive keratinization in the pilosebaceous unit and a heightened activity of the sebaceous gland acini. Sometimes, the pilosebceous canal becomes blocked due to excessive keratinization of its lining cell. In such case, large amounts of the sebum accumulate in the gland acini. The accumulated sebum usually becomes invaded by bacteria. This leads to the formation of localized areas of chronic inflammation, which may appear as small cysts, papules, or pustules on the skin, especially on the face, chest, and back. This common clinical condition is called *acne vulgaris* (or simply *acne*).

SWEAT GLANDS

The sweat glands, also called *sudorific glands,* are generally classified into two types: (i) eccrine sweat glands, and (ii) apocrine sweat glands. The apocrine sweat glands are limited to certain specific regions of the body, whereas the eccrine sweat glands are distributed almost over the entire body surface. Because of the overwhelmingly greater number of the eccrine sweat glands, the term 'sweat gland' implies the eccrine sweat gland, unless otherwise specified.

ECCRINE SWEAT GLANDS

The eccrine sweat glands are simple tubular glands having a diameter of 0.4 mm. These glands are distributed throughout the skin except that covering the glans penis. They are especially abundant in the skin of the palms and soles.

The terminal secretory portion of a sweat gland is coiled and lies deeply in the dermis, commonly extending into the hypodermis. The duct of the gland travels through the dermis, joins the epidermis and spirals through it to reach the free surface, where it terminates at a small opening called *sweat pore.*

The secretory portion of a sweat gland is lined by a simple cuboidal or low columnar epithelium surrounded by a basement membrane. Two varieties of cells can be distinguished in this epithelium: (1) clear cells, and (2) dark cells.

The **clear cells** stain very lightly because these cells do not contain secretory granules. These are pyramidal cells, each having a broad basal region that lies on the basal lamina, and a narrow apex, which does not reach the lumen. The apical parts of the clear cells communicate with lumen through narrow intercellular canaliculi present between the dark cells. EM reveals that the clear cell cytoplasm contains organelles similar to those of the dark cells except that the RER and free ribosomes are scarce in these cells. These cells also show infoldings of the basal plasmalemma (which is a characteristic feature of those cells which are engaged in transepithelial fluid and electrolyte transport). The clear cells produce a serous (watery) secretion. This secretion reaches the lumen of the sweat gland through the intercellular canaliculi.

In each sweat gland, a layer of *myoepithelial cells* is present between the bases of the lining secretory cells and their surrounding basement membrane. These cells are fusiform in shape and show cytoplasmic processes. Their cytoplasm contains actin and myosin filaments. The myoepithelial cells are supplied by sympathetic nerve fibers and their contraction results in the discharge of secretion from the secretory cells into the lumen of the sweat gland.

The **dark cells** are so named because they stain darkly in routine histological sections prepared for light microscopy. These cells have a narrow basal area and a broader apical part lining the lumen. Their basal end rarely touches the basal lamina. EM shows that these cells contain a prominent Golgi apparatus, long mitochondria, many cisternae of RER and abundant free ribosomes. Their broad apical portion contains dense secretory granules containing glycoproteins. The function of the dark cells is not very clear. It has been proposed that the secretion produces by these cells has bactericidal properties and thus plays a role in the innate immunity of the body.

The **duct** of a sweat gland is highly tortuous and is of smaller diameter than the secretory portion. It is lined by stratified cuboidal epithelium consisting of two layers of cuboidal cells. As the duct joins the epidermis, it loses its own wall and becomes a specialized passage through the stratified squamous epithelium.

The sweat glands are innervated by cholinergic sympathetic nerve fibers. Their secretion is produced in response to heat and nervous strain.

Sweat

The sweat is a clear, odorless fluid with an acidic pH (ranging from 4 to 6.8). The chief content of the sweat is water (98-99%). Apart from water, the sweat contains sodium, chloride, potassium, urea, ammonia, uric acid, and lactic acid, etc.

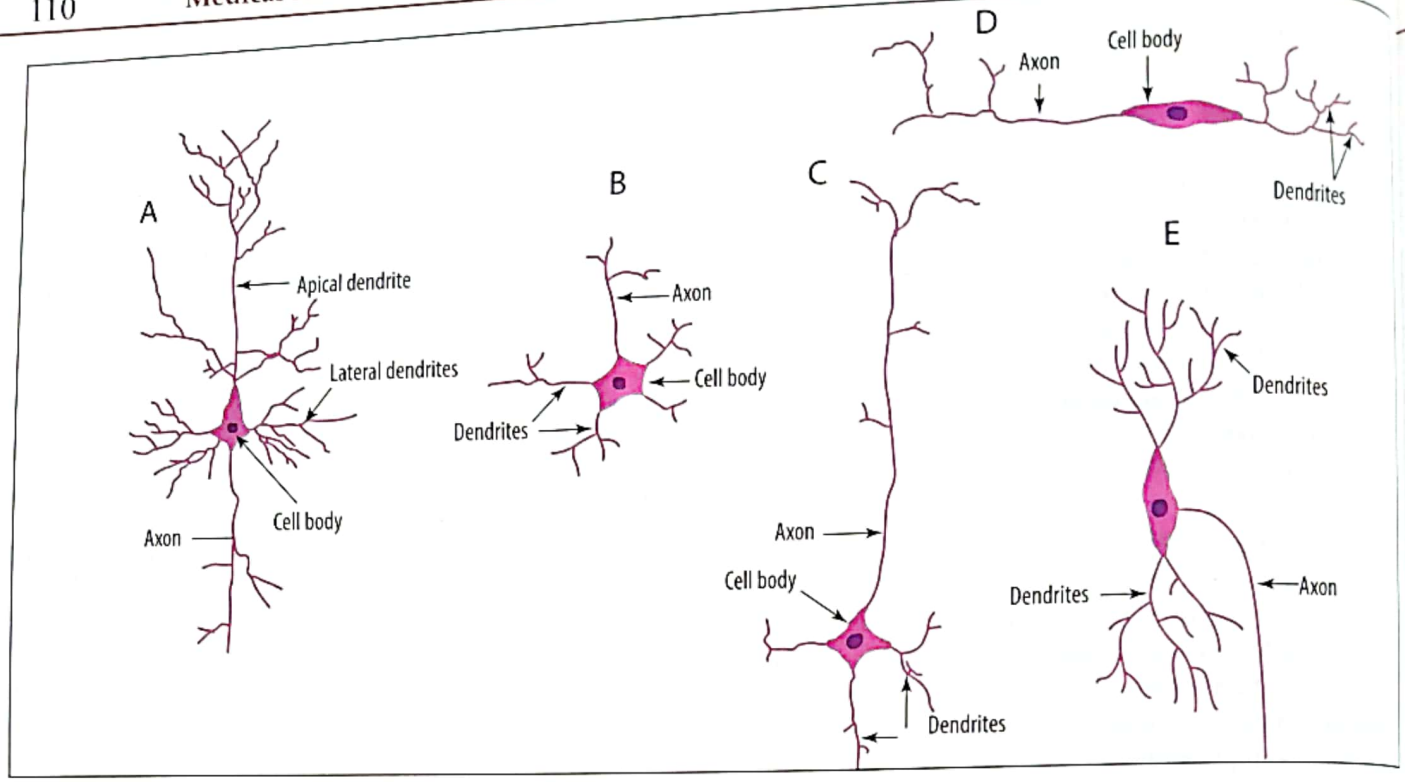

Fig. 12.1 A diagrammatic representation of various types of cells found in the cerebral cortex. A. Pyramidal cell, B. Granule cell, C. Martinotti cell, D. Horizontal cell. E. Fusiform cell.

"molecular" appearance to this layer. The molecular layer is essentially a synaptic zone of the cerebral cortex. Neuroglial cells and some horizontal cells are also present in this layer.

2. **External Granular Layer**. This layer consists mainly of closely packed granule cells. In addition, many small pyramidal cells are also present.
3. **External Pyramidal Layer**. This layer is composed mainly of medium pyramidal cells, which increase in size from the external border to the internal border of the layer. Some granule cells and Martinotti cells are also found in this layer.
4. **Internal Granular Layer.** This layer consists mainly of closely-packed granule cells; many small pyramidal cells are also present.
5. **Internal Pyramidal Layer**. This layer consists mainly of large pyramidal cells. In addition, it contains fusiform cells, granules cells, and Martinotti cells.
6. **Multiform layer**. This is the deepest (innermost) layer of the cerebral cortex. It contains neurons of various shapes and, therefore, is also known as *polymorphic layer*. This layer lodges fusiform cells, pyramidal cells, stellate cells, and Martinotti cells.

Allocortex

The allocortex has a much simpler structure and consists only of three layers. These layers are: (i) molecular layer, (ii) pyramidal cell layer, and (iii) multiform layer.

CEREBELLUM

The cerebellum consists of two hemispheres with a central vermis. The surface of the cerebellum shows many transverse fissures. These fissures divide the substance of the cerebellum into lobules. The gray matter of the cerebellum is located on the surface as a layer of cerebellar cortex which overlies the centrally placed white matter. Masses of gray matter, called *deep cerebellar nuclei*, are located within the white matter in the deep central parts of the cerebellar hemispheres.

CEREBELLAR CORTEX

The cerebellar cortex on section shows three layers; from without inwards, these layers are: (1) molecular layer, (2) Purkinje cell layer, and (3) granular layer (Fig. 12.3, 12.4 & 12.5).

1. **Molecular Layer.** This layer is mainly a synaptic zone and primarily contains the dendritic arborizations of the Purkinje cells (which lie in the second layer of the cerebellar cortex). The molecular layer also contains many unmyelinated axons which course parallel to the surface of the cortex. Cell population of the molecular layer is very low. Two varieties of neurons may be observed in this layer: stellate cells and basket cells.

   The ***stellate cells*** have small, star-shaped perikarya. Each cell has many short thin dendrites and a fine unmyelinated axon. The dendrites ramify near the cell body, whereas the axon extends transversely to make synapses with the dendrites of the Purkinje cells.

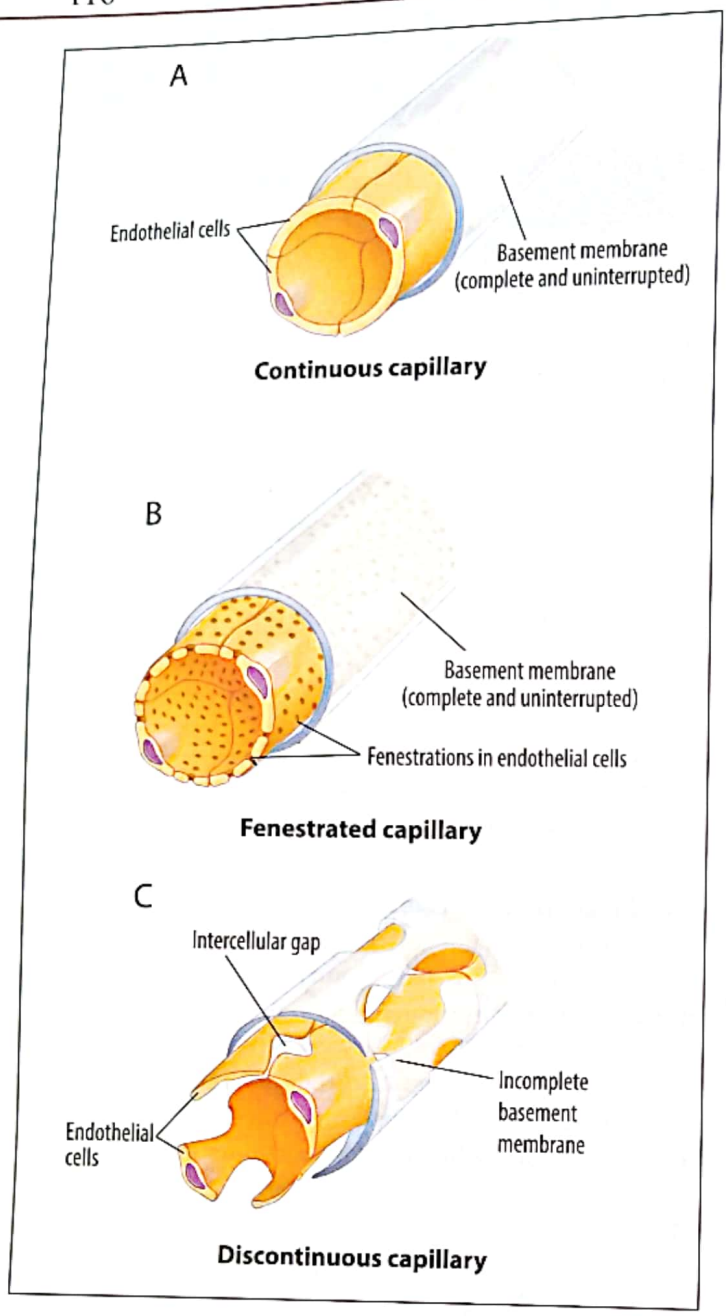

Fig. 13.2 Diagrammatic representation of the structure of the three types of capillaries.

the endothelial cells lining the continuous capillaries.

The continuous capillaries are widely distributed in the body. The capillaries present in the muscles, lungs, skin, and central nervous system are typical examples of continuous capillaries.

FENESTRATED CAPILLARIES (Fig. 13.2B)

The fenestrated capillaries have nearly the same diameter as that of the continuous capillaries but they are characterized by the presence of numerous, small circular pores (fenestrations*) in the lining endothelial cells. These intracellular pores measure 60-80 nm in diameter and most of them are spanned by very thin diaphragms composed of proteoglycans.

As in the continuous capillaries, the endothelial cells of the fenestrated capillaries are joined to each other by occluding junctions and no intercellular gaps are present in the wall of these capillaries. It is also to be noted that the basement membrane of the endothelium of the fenestrated capillaries is an uninterrupted and continuous layer and covers the endothelial pores on the external surface of the endothelium.

The fenestrated capillaries are more permeable than the continuous capillaries and allow the passage of small molecules and limited amounts of protein to pass through the capillary wall. These capillaries are found in those organs and tissues where there is extensive molecular exchange with the blood, e.g., intestinal villi, renal glomeruli, and endocrine glands.

DISCONTINUOUS CAPILLARIES (Fig. 13.2C)

Some organs of the body contain capillaries that have large diameter and show wide gaps between the lining endothelial cells. Due to the presence of intercellular gaps in their walls, these capillaries are called *discontinuous capillaries*. Because of their large lumen (diameter 30-40 μm), these capillaries are also called *sinusoidal capillaries* (or simply *sinusoids*). The endothelial cells of the discontinuous capillaries also exhibit pores which are not spanned by any diaphragms. The basement membrane of endothelial cells of the discontinuous capillaries is also interrupted and incomplete, being absent in the region of the intercellular gaps.

The discontinuous capillaries serve two major functions: (1) they allow maximal exchange of macromolecules between the blood and tissues, and (2) they permit the passage of cells from the blood into the tissues and vice versa.

The discontinuous (sinusoidal) capillaries are found primarily in liver, spleen, and bone marrow. Some endocrine glands also contain sinusoidal capillaries, e.g., the suprarenal gland.

GENERAL STRUCTURAL PATTERN OF ARTERIES AND VEINS

All arteries and veins (and also lymphatic vessels) exhibit a generalized structural pattern as regards the composition of their walls. Though there are important structural differences between the blood vessels of different types and sizes, the basic design remains the same.

Generally, the wall of a blood vessel is composed of three concentric layers: tunica intima, tunica media and tunica adventitia.

1. The **tunica intima**, or simply *intima*, is the innermost layer. It consists of a single layer of endothelial cells lining the internal surface of the vessel. Beneath the basal lamina of the endothelium is present a thin layer of delicate loose connective tissue known as the subendothelial layer. In some

* *Fenestra* is a Latin word meaning "window".

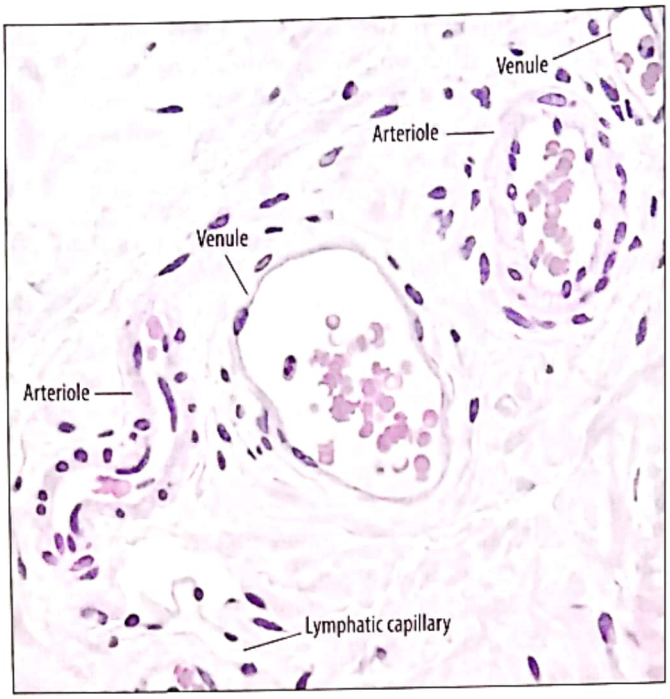

Fig. 13.5 A tissue section showing arterioles, muscular venules and lymphatic capillaries.

HEART

The heart is a four-chambered pumping organ of the cardiovascular system. Essentially, the heart may be regarded as a blood vessel with very wide lumen and extremely thick muscular walls. From within outwards, the wall of the heart consists of the following three coats:

1. Endocardium
2. Myocardium
3. Epicardium

ENDOCARDIUM

The endocardium is homologous to the tunica intima of blood vessels. It is lined by a layer of *endothelium* beneath which is present a thin layer of *subendothelial connective tissue* composed of very fine collagen fibers, elastic fibers and some smooth muscle cells. Deeper to the subendothelial connective tissue is present a layer of loose connective tissue called *subendocardial layer*. This layer contains small veins, nerves, and branches of the impulse conducting system of the heart. The subendocardial layer also serves to attach the endocardium to the myocardium.

MYOCARDIUM

The myocardium corresponds to the tunica media but contains the cardiac muscle which is striated but involuntary. The thickness of the myocardium varies in different parts of the heart, being thinnest in the atria and thickest in the left ventricle.

The cardiac muscle fibers, also called cardiomyocytes, are elongated, branching cells which show cross-striations due to the presence of alternating light and dark bands (Fig. 10.10). Each cardiomyocyte contains only one nucleus.

The adjacent cardiac muscle cells are joined end-to-end to form cardiac muscle fibers. In stained sections, the junctional regions between the adjacent cardiac muscle cells are seen as darkly stained cross bands which are called *intercalated discs*. The intercalated discs are special junctional regions which contain three types of cell junctions: desmosomes, fasciae adherentes, and gap junctions.

In the atria the myocardium is disposed in two layers which are not clearly demarcated from each other. In the outer layer, the cardiac muscle bundles run chiefly in an oblique or transverse direction and continue over both the atria. In the deeper layer, the muscle bundles are confined to each atrium separately and run at right angles to muscle fibers of the outer layer.

In the ventricles the muscular bundles take origin from the dense fibrous connective rings (annuli fibrosi) surrounding the atrioventricular valves. The cardiac muscle of the ventricles is also disposed in two layers. Fibers of the superficial layer follow a spiral course from the base of the ventricles to the apex. Muscle fibers of the deeper layer pursue a circular course on each ventricle. However, some fibers of this layer form an S-shaped pattern as they pass from one ventricle to the other through the interventricular septum.

EPICARDIUM

This is actually the visceral layer of pericardium. It is lined externally by simple squamous epithelium (mesothelium). Beneath the mesothelium is a thin layer of connective tissue rich in elastic fibers. The epicardium is attached to the myocardium by a subepicardial layer of loose connective tissue, which contains blood vessels, nerves, and fat.

Cardiac Skeleton

The heart contains a skeleton (supporting framework) of dense connective tissue, which consists of three main components: (1) two **annuli fibrosi,** which are rings of dense connective tissue; one annulus fiborsus encircles the two atrioventricular (AV) valves, whereas the other surrounds the aorta and pulmonary trunk at their origin, (2) a **septum membranaceum** in the upper part of interventricular septum, and (3) two **trigona fibrosa** that connect the annuli fibrosi with each other. The atrial musculature is inserted into the annulus fibrosis surrounding the aortic and pulmonary valves, whereas the musculature of the ventricles is attached to the annulus fibrosis of the AV valves. It is to be noted that the presence of dense collagenous tissue between the atrial and ventricular musculature creates an electrically non-conductor barrier which allows the atrial and ventricular myocardia to contract independent of each other.

Impulse Generating and Conducting System of the Heart

The rhythmic contraction of the atria and ventricles results

plasmalemma of the endothelial cells is covered by a thick glycocalyx coat which primarily consists of a meshwork of proteoglycans and glycoproteins. Molecules derived from the plasma as well those secreted by the endothelium integrate into this meshwork.

Functions of the Endothelium

The endothelium lining the blood vascular system performs a variety of functions. Some of these functions are general in nature and are performed by the endothelial cells in all locations, whereas certain functions are carried out exclusively in the endothelial lining of blood vessels in specific organs or regions, e.g., secretion of the angiotensin-converting enzyme occurs almost exclusively in the capillaries of the lungs. The main functions of the endothelium are discussed below:

1. **The endothelium acts as a selective permeability barrier** that controls the movement of small and large molecules from the blood to the tissues and also in the reverse direction. With the exception of the discontinuous capillaries, the endothelial cells are firmly bound to each other by adhering and occluding cell junctions which seal the intercellular space and, therefore, the exchange of substances between the blood and tissues can occur only through the endothelial cells. Oxygen and carbon dioxide pass through the epithelium by simple diffusion. Amino acids, glucose, and electrolytes cross the endothelium by active transport. Water, small molecules and soluble proteins are transported through the endothelium by transcytosis. Low-density lipoproteins, cholesterol antibodies, MHC complexes, and transferrin cross the endothelium by receptor mediated endocytosis. In the fenestrated capillaries, the larger molecules pass through the pores in the endothelial cells.
2. **Maintenance of a smooth, anticoagulant and antithrombotic surface** for the blood. The luminal plasmalemma of the endothelial cells is covered by a thick glycocalyx coat which contains anticoagulant and antithrombotic substances secreted by the endothelium. The chief anticoagulants present on the endothelial surface are thrombodulin and tissue factor pathway inhibitor, while the antithrombogenic agents found on this surface include prostacyclin, tissue plasminogen activator, antithrombin III, and heparin.
3. **In the event of injury**, the **endothelial cells secrete prothrombogenic agents** like von Willebrand factor, tissue thromboplastin, and plasminogen activator inhibitor. These agents cause platelets to aggregate and result in the formation of a clot (thrombus) at the site of injury to prevent the blood loss.
4. **The endothelium regulates blood flow and tissue perfusion** by regulating the tone of the arterioles feeding a capillary bed and that of the venules draining this bed. This is achieved by the secretion of vasoconstrictor and vasodilator substances according to the need. The vasoconstrictors include a family of peptides called *endothelins*, and the angiotensin-converting enzyme (ACE). The ACE converts the angiotensin I into angiotensin II which is a potent vasoconstrictor. The vasodilators produced by the endothelium include nitric oxide (NO) and prostacyclin (which is a prostaglandin that serves as an antithrombotic agent and a vasodilator).
5. **The endothelium regulates the immune responses** by secreting the cytokines of the interleukin family (IL-1, IL-6, and IL-8). The endothelial cells also secrete cell adhesion molecules (CAMs), mainly selectins and adherins, which modulate leukocyte migration from the blood to the tissue where an immune response is needed against an antigen.
6. **Oxidation of lipoproteins** is also a function of the endothelial cells. The lipoproteins, mainly LDLs (low density lipoproteins) and VLDLs (very low density lipoproteins), are oxidized into triglycerides and fatty acids by the enzyme lipoprotein lipase, which is associated with the surface of the endothelial cells in the capillaries of adipose tissue, lactating breast, heart, and skeletal muscle.

The endothelial cells also secrete **proteins which maintain the extracellular matrix and basement membrane** of the endothelium; these proteins include collagen type IV and various types of laminins.

THE LYMPH VASCULAR SYSTEM

The hydrostatic pressure in arteries forces the plasma from the arterial side of the capillary beds into the interstitial space of the tissues. The fluid so filtered out is not exactly equal to the amount of fluid returning from the tissues to the blood under the influence of the osmotic pressure. The surplus interstitial fluid that remains in the interstitial compartment is called lymph. The lymph is returned to the bloodstream by the lymph vascular system that consists of lymph capillaries, lymphatic vessels and lymphatic ducts. Lymph drainage is a one-way flow (from the periphery toward the heart) and is not a circulation in the strict sense. Lymph nodes are situated along the course of lymphatic vessels; these nodes serve to remove the unwanted particulate matter (e.g., bacteria and cancer cells) from the lymph.

LYMPH CAPILLARIES

These are delicate endothelial tubes that originate in various tissues as blind-ended vessels. They have slightly larger lumen than that of the blood capillaries but their caliber is not uniform. The lymph capillaries branch and

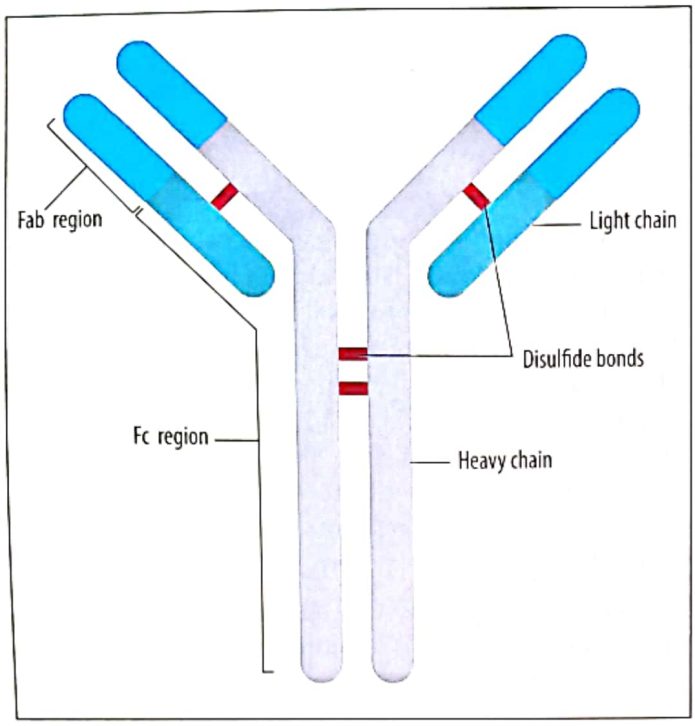

Fig. 14.1 Diagram showing the molecular design of an antibody.

All types of immunoglobulins have a common molecular design. It has been found that each Ig monomer is a Y-shaped molecule, consisting of two identical long polypeptides called **heavy chains** and two shorter identical polypeptides called **light chains** (Fig. 14.1). The four chains are connected and bound to each other by disulfide bonds in such a way that the stem of the Y is composed only of heavy chains, whereas the diverging arms consist of both heavy and light chains.

The base of the Y plays a role in modulating immune cell activity. The amino acid sequence of this region is same (constant) in different antibody molecules and, therefore, it is called **Fc region** (fragment, constant region). The amino acid sequence of the initial 100 or so amino acids of the light and heavy chains near the terminal ends of the arms of the Y is not constant and varies widely among different antibody molecules. This region that can recognize and bind to specific epitopes, is called *variable region* or **Fab region** (fragment, antigen-binding region).

Types of Immunoglobulins

The immunoglobulins (antibodies) are classified into different types on the basis of number of Ig units (molecules) present in the antibody.

IgG. This is the monomeric form of immunoglobulins (i.e., it consists of a single Ig molecule). The IgG constitutes the most abundant variety of antibodies and represents about 75% of serum immunoglobulins.

IgA. This antibody is found chiefly in the secretions produced by the mucous membranes the digestive, respiratory, urinary, and reproductive tracts, although it is also found in the serum in small quantities. The IgA secreted by the lining epithelial cells is called **secretory IgA**. It is secreted by the epithelial cells lining the mucous membranes and acini of glands like salivary glands, lacrimal glands, and the prostate gland, etc. The secretory IgA consists of 2 or 3 monomeric molecules of IgA united by a polypeptide chain called ***J chain*** (joining chain). These polymers (dimers or trimers) of IgA are combined with a polypeptide of large molecular mass which is called ***secretory component***. The IgA molecules and J chain are secreted by the plasma cells located in the connective tissue lying beneath the epithelial cells, while the secretory component is synthesized by the epithelial cells and added to the IgA polymers as they pass through the mucosal epithelial lining cells or the epithelial acinar cells of glands. The massive secretory component protects the immunoglobulin from being broken down by the proteolytic enzymes. Therefore, the secretory IgA can survive even harsh environments (e.g., that of the stomach) and provide protection against the proliferatin of microbes in the body secretions.

IgM. In the plasma, this immunoglobulin exists in a pentameric form (five IgM molecules bound to each other). It represents nearly 10% of the antibodies circulating in the blood. The IgM is the first antibody expressed by the B lymphocytes in response to initial exposure to an antigen. Because of its pentameric nature, the IgM has multiple antigen-binding sites which simultaneously interact with the target antigen epitopes, and therefore this antibody is highly effective in activating the complement system.

IgE. The IgE is also a monomeric immunoglobulin. Its concentration in the serum is very low. It is chiefly expressed on the surface of basophils and mast cells. The IgE plays an important role in allergic reactions because when this antibody binds with the antigen which caused in its production, the resulting antigen-antibody complex stimulates the release of histamine, heparin, and various leukotrienes from the mast cells and basophils.

IgD. This is a special immunoglobulin of the monomeric variety and is found in very low concentrations in the serum. The IgD molecules are expressed on the surface of the B lymphocytes during their maturation.

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

The major histocompatibility complex is a group of genes that code for special cell surface proteins, called **MHC molecules**, which are unique to each individual. These proteins play an important role in controlling the adaptive immune response of the body. The MHC molecules are of 2 types: MHC I and MHC II.

The **MHC I molecules** are present on the surface of all nucleated cells of the body as well as on platelets. These molecules function in presenting short peptide fragments derived from the cytosolic proteins (i.e., the endogenous or "*self*" proteins). The T lymphocytes are capable of recognizing the MHC I molecules of an individual as belonging to that specific individual and do not react against the *'self'* proteins. However, the T lymphocytes do react

strategically important locations, where the interface between the internal and external environments exists, they serve as the first line of defense against the invading organisms. The gamma delta T cells encounter the antigens on the surface of the epithelial cells, i.e., before these antigens can enter the body. They secrete a number of cytokines and other factors to neutralize the invading antigens on the skin or mucosal surfaces. The factors secreted by the gamma delta T cells include interleukins, defensins, and granzymes, etc.

5. The **memory T cells** are a subset of antigen-specific cells that persist for a long time after cell-mediated immune response against an antigen has been over. Upon a re-exposure to the same specific antigen, these cells quickly proliferate and transform into cytotoxic T cells to destroy the invading pathogen.

NATURAL KILLER (NK) CELLS

This variety of lymphocytes has the natural ability to destroy the virus-infected cells. They also have the capability to kill the tumor cells. Like the cytotoxic T lymphocytes, the NK cells also kill their target cells by secreting perforins and granzymes. The perforins form pores in the cell membrane of the target cells through which the granzymes enter the cell and kill it by inducing apoptosis. The NK cells serve to restrain the viral infections while the adaptive immune response is generating specific cytotoxic T lymphocytes to clear the infection.

ANTIGEN-PRESENTING CELLS

The antigen-presenting cells (APCs) represent a diverse variety of cells which have two properties in common: (i) they can engulf and process antigens, and (ii) they carry MHC II molecules on their plasmalemma. The APCs endocytose antigens and digest them in their lysosomal system, so that an antigen is finally reduced to peptide fragments only. These peptide fragments (epitopes) are returned to the surface of cell and presented, in combination with MHC II molecules, either to the B lymphocytes or T lymphocytes, depending on the type of the antigen.

Most of the APCs belong to the mononuclear phagocyte system (MPS) of the body, but some of them are not a part of this system. The APCs belonging to the MPS include the macrophages, dendritic cells of spleen and lymph nodes, Langerhans cells of the epidermis, and Kupffer cells of the liver. The APCs not belonging to the MPS include the B lymphocytes, and the type II and type III epitheliocytes of the thymus.

Dendritic Cells

The dendritic cells (DCs) are antigen-presenting cells which originate from progenitor cells in the bone marrow. They derive their name from the fact that these cells possess branching cytoplasmic projections. The immature dendritic cells, which have the capability to capture an antigen but cannot process it, are abundantly found in all those tissues that are in contact with the external environment, such as the skin, and the mucosal linings of the digestive and respiratory tracts. In the skin, the immature dendritic cells are titled *Langerhans cells*. The cytoplasmic processes of an immature dendritic cell are few and short. When an immature dendritic cell has engulfed an antigen, it migrates through the lymphatics to a secondary lymphoid organ, usually a lymph node, and then undergoes maturation. The mature dendritic cells possess a large number of long, branching dendritic processes and have the capability to process the antigen. After processing, the epitope of the antigen is presented, in combination with the MHC II molecules, to the B or T lymphocytes. In this way, an immune response is initiated against the concerned antigen.

The antigen-presenting dendritic cells (DCs) should not be confused with *follicular dendritic cells (FDCs)* which are found in in the lymphoid follicles of the secondary lymphoïd organs. The FDCs, mostly located in the germinal centers of the lymphoid follicles, are not antigen-presenting cells because they lack MHCII surface markers. The structure and functions of the FDCs are described later.

LYMPHOID TISSUE

The lymphoid tissue, also called *lymphatic tissue*, can be regarded as a special variety of connective tissue which is characterized by the presence of a large number of lymphocytes. Generally, the supporting framework of the lymphatic tissue is formed by reticular connective tissue consisting of reticular cell and reticular fibers. Due to preponderance of lymphocytes, the lymphatic tissue exhibits basophilia and takes a dark blue or purple stain in the ordinary H&E stained tissue sections.

The lymphoid tissue is generally classified into the following three categories: diffuse lymphoid tissue, nodular lymphoid tissue, and lymphoid organs.

The **diffuse lymphoid tissue** is characterized by a loose and random distribution of lymphocytes. No specific masses or aggregations of lymphocytes can be seen in this tissue.

The **nodular lymphoid tissue** is characterized by the presence of localized aggregations of lymphocytes in the form of small, roughly spherical masses that are called *lymphoid nodules* (also called *lymphatic follicles, lymphatic nodules, or lymphoid follicles*).

The **lymphoid organs** are masses of lymphatic tissue, surrounded by a connective tissue capsule that isolates the lymphatic tissue from the adjoining tissues or organs. Generally, the diffuse and nodular lymphoid tissues are present in each lymphoid organ in variable proportions.

The mucosal lining of the respiratory, digestive and urogenital tracts contains non-encapsulated diffuse

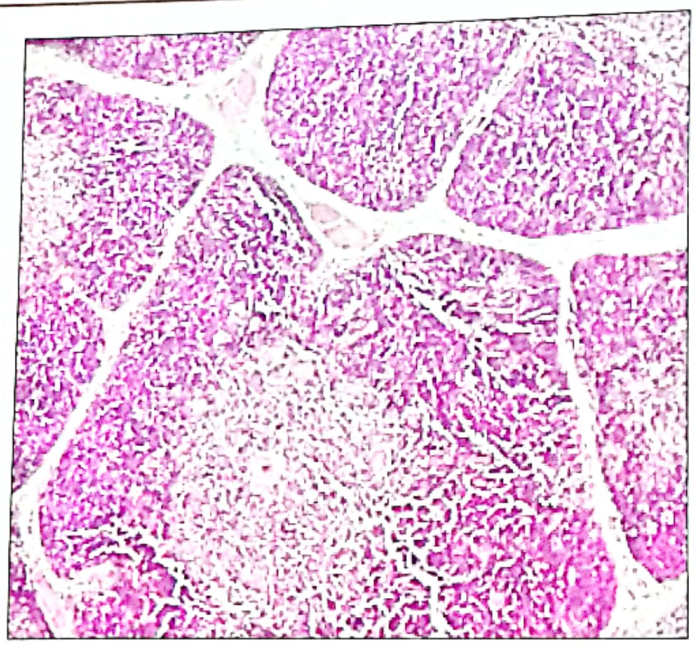

Fig. 14.3 A section of the thymus of a child (low power magnification) showing trabeculae dividing thymic substance into lobules. Each lobule is seen to consist of a darkly staining cortex and a lightly staining medulla which is characterized by the presence of strongly eosinophilic thymic corpuscles.

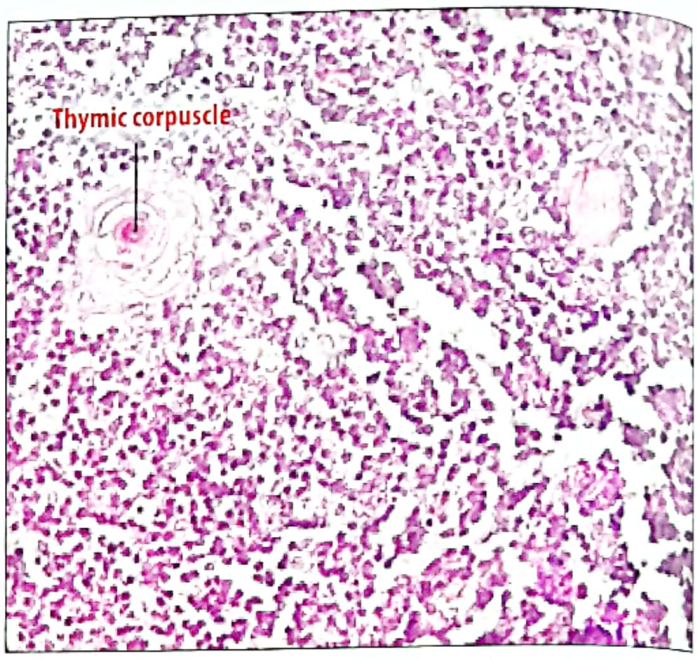

Fig. 14.4 A section of the thymus showing the medulla of a thymic lobule (high power magnification); concentric layers of thymic epithelial cells forming a thymic corpuscle can be seen.

The thymus is well-developed before birth and continues to enlarge in size and actively perform its function during the childhood and puberty. In the adulthood, the thymic activity gradually declines and it undergoes a steady process of regression (involution). As a result of the involutory process, the thymus gradually decreases in size and most of the lymphatic tissue is replaced by fatty tissue.

The capsule of the thymus consists of dense connective tissue. Numerous septa (trabeculae) extend from the capsule into the substance of each lobe and subdivide the thymic parenchyma into a number of incomplete lobules (Fig. 14.3). These lobules appear polyhedral under LM and range from 0.5 to 2 mm in diameter.

Each **thymic lobule** consists of a peripheral zone known as *cortex*, and a central region called *medulla*. Because the lobules are not completely separated from each other, the cortical regions of the adjacent lobules are frequently continuous with each other. It is to be noted that no lymphatic nodules are present in the thymus. Both the cortex and medulla of a thymic lobule contain T lymphocytes and TECs but the relative density of these two cell types varies in these two regions. The cortex contains very high population of lymphocytes that constitute about 95% of the total lymphocytes of the thymus. Understandably, the cortex contains a small number of TECs. The medulla contains much lesser number of T lymphocytes but lodges a large number of TECs. The cortex as well as medulla contain a considerable number of macrophages.

THYMIC CORTEX

The cortex of each thymic lobule stains darkly basophilic because it contains densely packed, immature T lymphocytes known as *thymocytes*. The thymocytes lie within a network (cytoreticulum) constituted by the thymic epithelial cells.

The peripheral region of the thymic cortex in each lobule contains larger cells which are actually lymphoblasts that originate in the bone marrow and reach the thymus through the blood vessels. These cells undergo extensive proliferation and give rise to huge number of small lymphocytes called *thymocytes*. The thymocytes, which are immature T lymphocytes, gradually move toward the central (i.e., medullary) region of the thymic lobules. During their movement through the thymic cortex, the thymocytes receive instruction (education) to develop into immunocompetent T lymphocytes; this process is called *thymic cell education*.

It is important to note that approximately 95% of the thymocytes produced in the thymic cortex fail to become properly immunocompetent. These cells die by apoptosis and are phagocytosed by the macrophages present in the thymic cortex. The surviving thymocytes (about 5% of the total) are supposed to have received proper thymic cell education and thus become properly immunocompetent T lymphocytes. These cells enter the thymic medullary region and finally leave the thymus through the postcapillary venules and efferent lymphatics.

The thymic cortex contains type I, type II, and type III thymic epithelial cells.

The **type I thymic epithelial cells** are flattened cells located at the outer boundary of the thymic cortex and separate it from the connective tissue capsule and trabeculae. The type I TECs also surround the small arteries and capillaries of the thymic cortex and play an important role in the formation of the blood-thymus

Lymphatics of the Thymus

The thymus receives no afferent lymphatic vessels and has only efferent lymphatics that transport lymph and lymphocytes away from this primary lymphoid organ. These efferent lymphatics follow the course of the arteries and vein of the thymus.

Functions of the Thymus

The primary function of the thymus is to instruct the immuno-incompetent T cells to develop into immunocompetent T cells. This process, called **thymic cell education**, involves expression and deletion of specific CD markers on the plasmalemma of the T cells.

During the fetal life, lymphocyte precursors (lymphoblasts) originating in the liver reach the developing thymus via the blood vessels. The lymphoblasts proliferate and give rise to large numbers of smaller prolymphocytes in the cortical region of the thymic lobules. The prolymphocytes differentiate into immuno-incompetent T lymphocytes known as *thymocytes*. The thymocytes, also called **Pre-T lymphocytes** move inwards through the cortex toward the thymic medulla and during this journey, which takes about 2 weeks, they receive the thymic cell education.

The immuno-incompetent thymocytes have neither T cell receptors (TCRs) nor CD markers on their surface. During their movement through the cortex, these cells receive *thymic cell education*, which makes these cells immunocompetent. During this process, the developing T cells are continuously tested and all those cell that fail to receive proper thymic cell education die by apoptosis. The dead cells are phagocytosed by the cortical macrophages. As already mentioned, 95% of the developing T cells die during their movement from the thymic cortex to the medulla.

As the pre-T lymphocytes move into the midcortical region, they express T cell receptors (TCRs) and CD4 and CD8 molecules on their surface. The state of having both the CD4 and CD8 surface markers is called *double positive stage*.

During further differentiation, the differentiating T cells undergo a very strictly controlled ***two stage selection process***. In the first stage, the type II and type III thymic epithelial cells of the thymic cortex present to the pre-T lymphocytes the MHC I and MHC II molecules bound to various foreign-antigens and self-antigens. Only those pre-T lymphocytes which recognize the foreign and self-antigens bound to the MHC molecules survive, while all others die by apoptosis. This is called ***positive selection*** and it indicates that the TCR proteins of the surviving T cells duly recognize the MHC molecules. The cells which have passed the positive selection test enter the medulla where the macrophages and dendritic cells present to them various self-antigens, i.e., antigenic determinants derived from body's own proteins. All those T cells which recognize and bind to the self-antigens die by apoptosis and are engulfed by the macrophages. This process is called ***negative selection***. The negative selection ensures that no surviving T cell reacts against body's own proteins.

Those double positive T cells which have passed both the positive and negative selection tests, finally retain either CD4 or CD8 surface markers; this is called *single positive stage*. Those T cells that lose CD4 and retain CD8 surface markers become the cytotoxic T lymphocytes, whereas those cells which lose CD8 and retain CD4 markers become helper T lymphocytes.

LYMPH NODES

The lymph nodes are small oval or bean-shaped bodies that are interposed in the path of large lymphatic vessels and serve as immunologic filters of the lymph. They range in size from about 1 mm to 20 mm. Each node has a convex contour except at an indented region on one side which is known as *hilum*. The arteries enter while the veins and efferent lymphatic vessels leave the node at the hilum. Each lymph node is supplied by a large number of afferent lymphatic vessels that enter the lymph node at various points along its convex surface.

Each lymph node is covered by a connective tissue *capsule* composed mainly of densely packed collagen fibers. *Trabeculae* (septa) arise from the inner aspect of the capsule and extend into the interior of the node. (Fig. 14.6)

A meshwork of reticular fibers and reticular cells extends throughout the substance of the lymph node. In addition to the reticular cells, this meshwork contains dendritic cells, follicular dendritic cells, and macrophages.

Follicular Dendritic Cells (FDCs)

These cells are found in large numbers in the germinal centers of the lymphoid follicles. These cells also possess many thin, branching processes but, unlike the dendritic cells (DCs), the FDCs are not antigen-presenting cells because they do not carry MHC II molecules on their surface. Antigen-antibody complexes adhere to the dendritic processes of the FDCs by means of the antibody's Fc receptors. The FDCs do not engulf these antigen-antibody complexes but can retain them on their surface for long periods. By virtue of their capacity to trap immune-complexed antigens in the germinal centers of the lymphatic follicles and their ability to produce chemokimes, the FDCs play and important role in shaping of the B lymphocyte responses.

The parenchyma of a lymph node can be divided into two regions: an outer darkly staining *cortex* and an inner lightly staining *medulla* (Fig. 14.5 & 14.6).

LYMPH NODE CORTEX

In the cortex of the lymph node, the connective tissue trabeculae run perpendicular to the surface and divide this region into incomplete compartments. The cortical lymphoid tissue is separated from the capsule of the lymph node by a narrow space called *subcapsular sinus*. This sinus

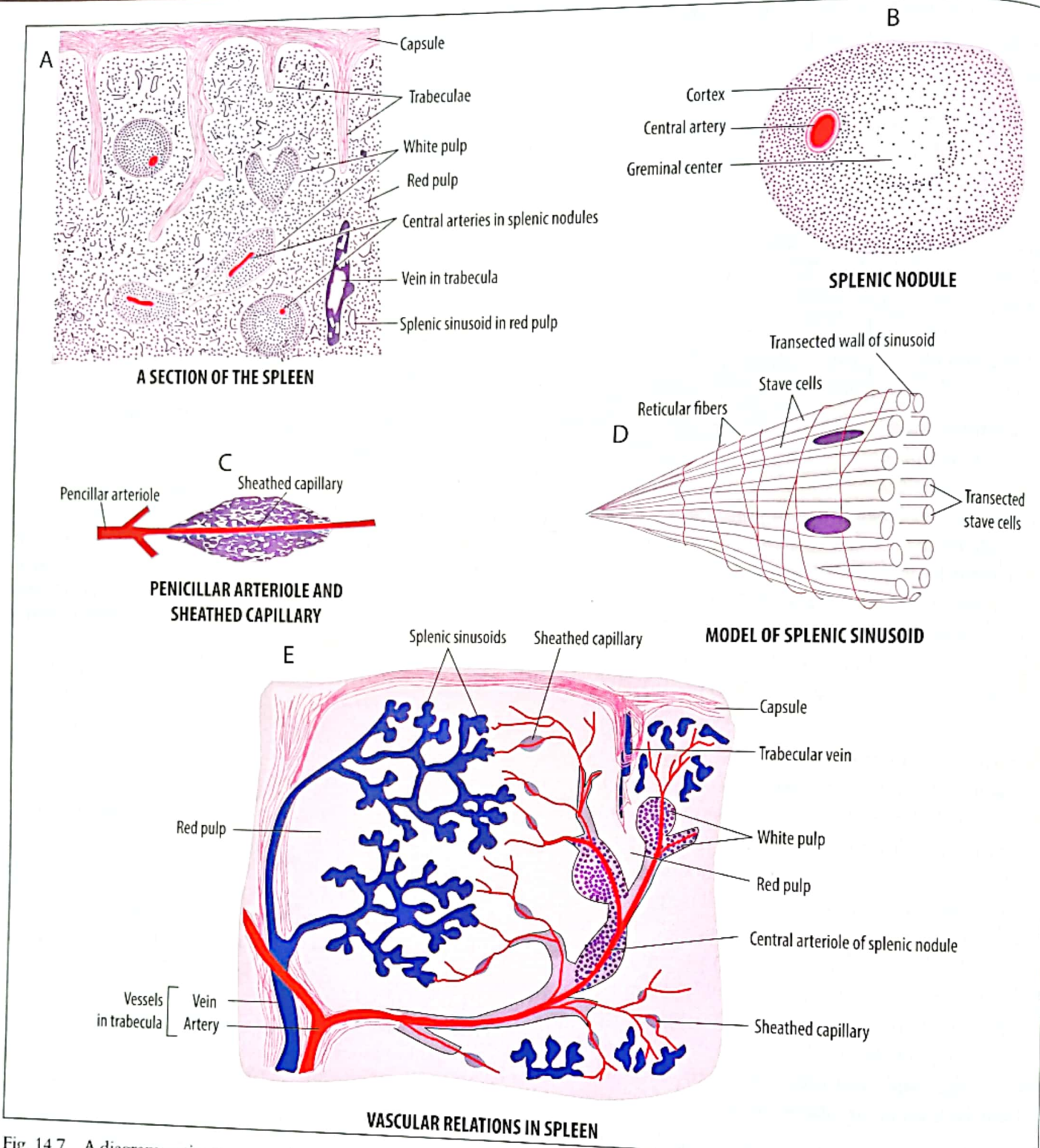

Fig. 14.7 A diagrammatic representation of various histological features of the spleen.

plasma cells, granular leukocytes, platelets, macrophages, and dendritic cells. All these cells are supported by a fine meshwork of reticular fibers and reticular cells.

The **splenic sinusoids** (also called *splenic sinuses*) are wide sinusoidal capillaries which lie between the splenic cords. The splenic sinusoids have a special structure and differ from ordinary sinusoidal capillaries in the following respects:

1. The splenic sinuses are lined by fusiform endothelial cells, known as *stave cells*, which are extremely long and run parallel to longitudinal axis of the sinus (Fig. 14.7D).
2. Large gaps (2 to 3 µm wide) are present between the adjacent endothelial cells which allow the exchange of formed elements of blood between the sinusoids and adjoining splenic cords.
3. The endothelial cells rest on an incomplete basal lamina and are supported by transversely arranged reticular fibers.

The macrophages of the splenic cords lie in the vicinity

In the dark-skinned people, the melanocytes are larger in size, possess more dendritic processes, and their cytoplasm contains larger and more numerous melanosomes as compared to the melanocytes in the epidermis of the white-skinned people.

LANGERHANS CELLS

The Langerhans cells are antigen-presenting dendritic cells located in the epidermis and are not identifiable in the ordinary H&E stained sections. With special staining techniques, such as gold impregnation, these cells are found to be located mainly in the stratum spinosum of the epidermis and each of them is seen to possess several dendritic processes. Electron microscopic studies reveal that the cytoplasm of a Langerhans cell contains a small number of mitochondria, sparse RER, but many lysosomes and vesicles.

The Langerhans cells constitute an important component of the adaptive immune system of the body. As they are present in the epidermis, their location is strategically extremely important. The skin is continuously exposed to microorganisms and also to various antigenic molecules. However, these antigens cannot penetrate the skin without encountering the Langerhans cells which are actually immature dendritic cells which can engulf an antigen but cannot process it unless they migrate to a secondary lymphoid organ. Upon capturing an antigen, the Langerhans cells retract their processes, move through the dermis and enter the lymphatic vessels to reach the nearest lymph node where they undergo maturation, process the engulfed antigen and present its epitope to the B and T lymphocytes, so that an appropriate immune response against the concerned antigen is initiated.

MERKEL CELLS

These cells, also called *tactile epithelial cells*, are found to be scattered among the cells of stratum basale of epidermis. Fine dendritic processes of the Merkel cells extend between the overlying keratinocytes. The Merkel cells are especially abundant in the skin of the highly sensitive areas like fingertips and lips. A special feature of these cells is the presence of dense-cored granules (80-100 nm in diameter) which contain neurosecretory peptides. Their cytoplasm also contains closely-packed keratin intermediate filaments.

The basolateral surface of each Merkel cell makes a synaptic junction with the expanded terminal of an afferent nerve fiber. The afferent (sensory) nerve fibers reach the stratum basale by penetrating the basement membrane of the epidermis. Combination of the expanded terminal of the afferent nerve fiber and a Merkel cell is referred to as a *Merkel cell-neurite complex*.

The Merkel cell-neurite complexes serve as mechanoreceptors which are concerned with the sensation of light touch. These sensory nerve endings play an important role in the perception of the texture and shape of various objects.

LAYERS OF THE EPIDERMIS

It is important to realize that the structural organization of the epidermis into layers reflects successive stages in the life of a keratinocyte, involving *proliferation growth, outward displacement*, and *differentiation*, followed by death and *desquamation*.

When a stained section of the thick skin is examined under LM, the epidermis is seen to consist of five layers (Fig. 15.3). From deep to superficial, these layers are:

1. Stratum basale
2. Stratum spinosum
3. Stratum granulosum
4. Stratum lucidum
5. Stratum corneum

STRATUM BASALE

The stratum basale, i.e., basal layer, of the epidermis consists of a single layer of basophilic cuboidal or low columnar keratinocytes resting on a basement membrane, which lies on the dermis. Each cell contains a large, oval nucleus. Desmosomes occur frequently at the lateral and outer surfaces of the keratinocytes of the stratum basale and serve to bind these cells to each other and to the keratinocytes of the stratum spinosum. Hemidesmosomes are found on the basal plasmalemma of the stratum basale keratinocytes; these hemidesmosomes serve to bind these cells to the basement membrane.

EM shows that the cytoplasm of the keratinocytes of the stratum basale contains a small Golgi complex, a few mitochondria, a small amount of RER, and abundant free ribosomes. The cytoplasm also contains keratin intermediate filaments (***tonofilaments***). The tonofilaments pass through the plaques of the laterally placed desmosomes, while they end in the plaques of the hemidemosomes.

New keratinocytes are produced in the stratum basale by the mitotic division of the keratinocytes of this layer and, therefore, this layer is also known as *stratum germinativum*. The newly produced cells are constantly displaced into the stratum spinosum. In addition to the keratinocytes, the stratum basale contains Merkel cells and cell bodies of the melanocytes. Dendritic processes of the melanocytes extend outward in between the cells of the stratum spinosum.

STRATUM SPINOSUM

The stratum spinosum consists of several layers of keratinocytes. The cells in the deeper layers are polyhedral in shape, while in the superficial layers the keratinocytes are more or less flattened. A special feature of the cells of this layer is the presence of numerous fine cytoplasmic processes. Early histologists named these as 'spines' and consequently this layer was named stratum spinosum. EM shows that the cytoplasmic processes of neighboring cells are joined to each other by desmosomes. These desmosomes strongly bind the cells of stratum spinosum

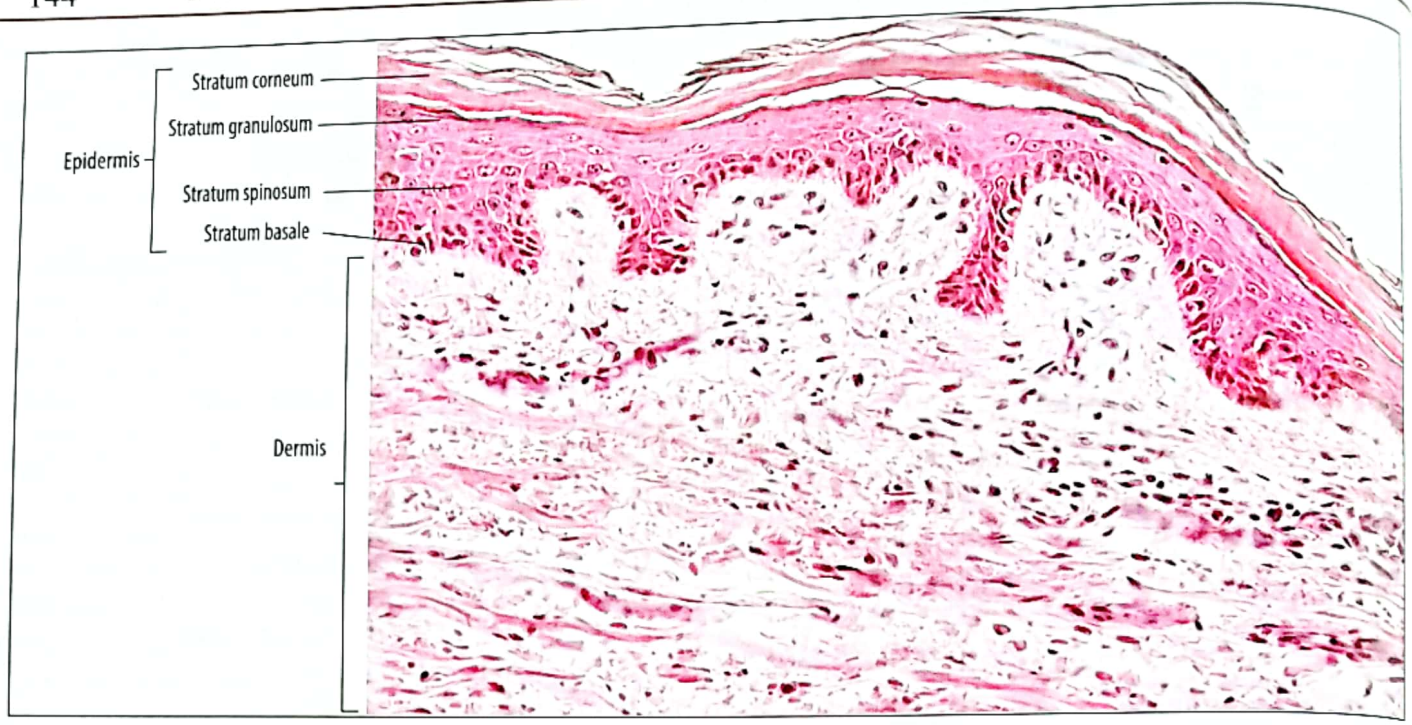

Fig. 15.2 A section showing the microscopic structure of the thin skin

the thin skin (Fig. 15.2). Many histologists do not recognize the stratum lucidum to be an independent layer but regard it as a subdivision of the stratum corneum of the thick skin.

In the ordinary histological sections examined under low power of LM, the stratum lucidum appears as a thin, translucent, and rerfractile layer that stains poorly. Examination under the high power of the light microscope reveals that this layer is composed of three to five layers of closely packed and extremely flattened eosinophilic keratinocytes, which are still bound to each other by desmosomes. The nuclei and organelles of these cells have been lost and their cytoplasm consists chiefly of densely packed keratin filaments embedded in a protein called *eleidin*, which is a transformation product of keratohyalin.

STRATUM CORNEUM

The stratum corneum, i.e., *cornified layer,* consists of many layers of flattened, highly keratinized keratinocytes called *corneocytes*. These cells are devoid of nuclei and organelles. The cytoplasmic space of the corneocytes is almost completely occupied by the bundles of tonofibrils embedded in a dense amorphous matrix. The keratinized cells become more and more flattened as the surface is approached. Simultaneously, their plasmalemma becomes very thick. Presnce of flat, highly keratinized corneocytes (with a thick plasmalemma) on the surface of the epidermis provides the skin a great capacity of resistance against friction and abrasion.

The phospholipids, glycosphingolipids, and ceramides released by the lamellar granules of the keratinocyte of the stratum granulosum diffuse into the intercellular spaces of the cells of the stratum corneum. Presence of these pro-barrier lipids in the straum cormeum makes the epidermis impermeable to water and water-soluble substances.

In the deeper layers of the stratum corneum the corneocytes retain desmosomes. In the most superficial layer the cells become extremely thin and flat and are now called *squames.* The desmosomes of these cells are degraded and broken down by the special proteolytic enzymes released from the lamellar granules of the keratinocytes. Consequently, the dead corneocytes and are constantly exfoliated from the skin surface; this process is called *desquamation.*

The thickness of the stratum corneum varies considerably in the skin over different parts of the body. It is the thickness of this layer that determines the overall thickness of the epidermis and differentiates the thick skin from thin skin. The stratum corneum is broadest in the thick skin covering the palms and soles (Fig. 18.3).

The stratum corneum becomes abnormally thickened in those regions of the non-hairy, glabrous skin that are subjected to excessive friction; such abnormally thickened patches of skin are called *callosities.*

DERMIS

The dermis, also called **corium,** is a sheet of connective tissue that supports the epidermis and gives the skin its flexibility and strength. Thickness of the dermis varies in different parts of the body; it ranges from 0.5 mm in the eyelids to almost 3 mm in the palms and soles. The dermis also lodges hair follicles, sweat glands and sebaceous glands, which are epidermal derivatives but project into the connective tissue of the dermis.

The dermis is composed of two layers with rather indistinct boundaries. The outer layer, which lies just beneath the epidermis, is called *papillary layer,* while the deeper layer is known as *reticular layer.*

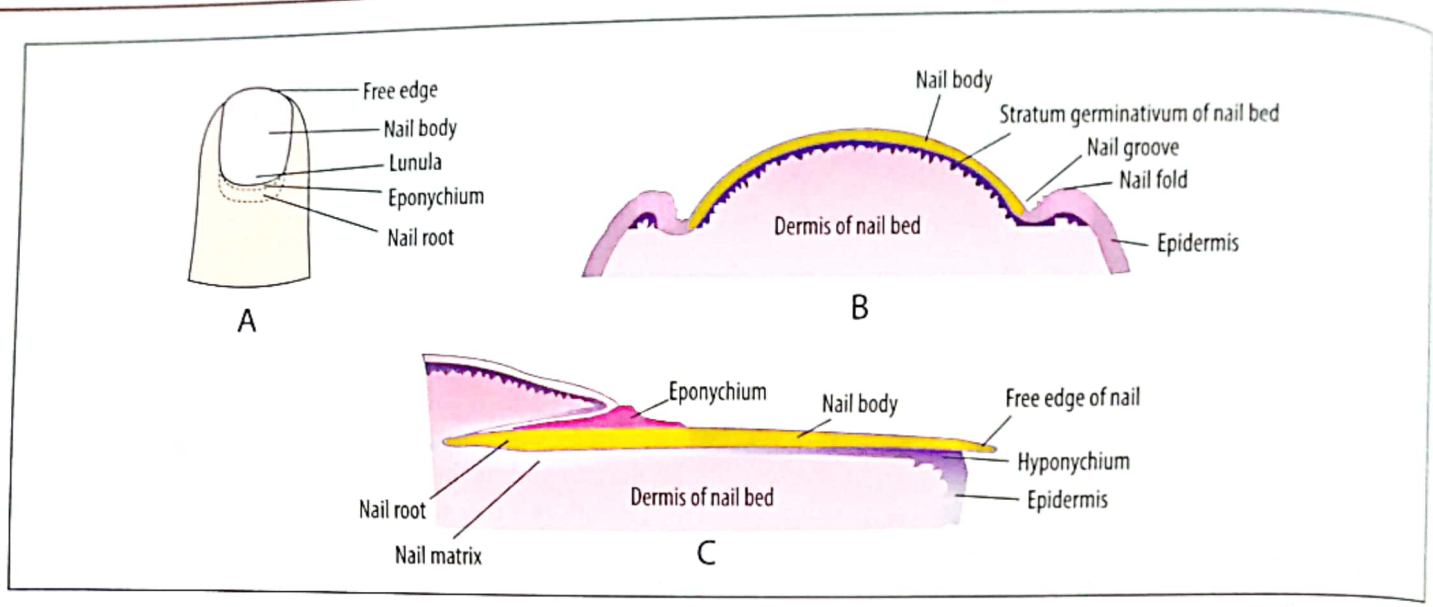

Fig. 15.6 Structure of a fingernail. A. Surface view, B. Cross section through the nail and its bed, and C. Longitudinal section section through the nail and its bed.

The body of the nail is translucent and appears pink because it transmits the color of blood present in the underlying vascular tissue. The crescent-shaped whitish area on the proximal part of the nail body is known as *lunula*. It is a reflection of the partially keratinized cells in this region of the nail.

The fold of the epidermis present around the proximal and lateral borders of the nail is known as *nail fold*. The furrow between the nail fold and nail bed is called *nail groove*.

The epidermis of the **nail bed** consists only of the stratum basale and stratum spinosum. The dermis of the nail bed does not bear typical papillae but, instead, shows longitudinal ridges. The epidermis of the nail bed is very thick proximally and here it is known as *nail matrix*.

The **nail matrix** is the germinative zone that exhibits a high rate of cell division and is responsible for the growth of the nails. The epithelial cells of the matrix proliferate constantly, move distally and eventually become keratinized. These keratinized cells are continuously added to the proximal part of the nail plate. As a result, the nail plate constantly slides forwards on the nail bed. The growth rate of the fingernails is about 0.5 mm per week, whereas the toenails grow at a much slower rate.

The nail keratin is a hard keratin that consists of compactly packed keratin filaments lying in a matrix of amorphous keratin. This keratin has a high sulfur content, which imparts hardness to the nail plate. Unlike the keratinized cells of the ordinary epidermis, the keratinized cells of the nail plate do not desquamate.

Epidermis of the nail bed is continuous distally with epidermis of the fingertip under the free edge of the nail. At this junction, the stratum corneum of the epidermis is thickened and is known as *hyponychium*. The stratum corneum of the proximal nail fold extends for a short distance over the free surface of the nail plate as *eponychium*.

GLANDS OF THE SKIN

The skin contains two types of glands: sebaceous glands and sweat glands.

SEBACEOUS GLANDS (Fig. 15.4)

The sebaceous glands are spherical or ovoid structures enclosed in a thin connective tissue capsule. They lie in the dermis and their ducts open into the infundibular parts of the hair follicles. A hair follicle and its associated sebaceous gland are collectively known as a *pilosebaceous unit*. The duct of a sebaceous gland and the infundibulum of the hair together form the *pilosebaceous canal*. In certain locations, such as the glans penis, lips, labia minora and tarsal plates, the sebaceous glands are not associated with hair follicles and open directly upon the free surface of the skin. Sebaceous glands are very abundant in the skin of the face and scalp, but completely absent in the skin of the palms and soles.

The sebaceous glands are simple branched acinar glands of the holocrine variety. Usually several (5 to 20) acini open into the short wide duct. The acini are filled completely with a stratified epithelium. The most peripheral (basal) cells of an acinus are low cuboidal in shape and rest on a delicate basal lamina. The basal cells contain a spherical nucleus and their cytoplasm shows mitochondria, SER, glycogen granules and lipid droplets. These cells undergo mitosis to produce more basal cells and larger round cells. The round cells have more abundant SER and their cytoplasm contains more lipid droplets.

When a sebaceous gland has released its secretion, the newly produced round cell gradually move toward the center of the acinus, where they attain a polyhedral shape and become still larger in size. Their cytoplasm gradually accumulates more and more fat (mostly cholesterol and triglycerides). Nuclei of these cells become pyknotic and then disappear. Finally, all of the centrally located cells

The sweat performs three main **functions** for the body: (i) it plays an important role in temperature regulation by producing a film of moisture for evaporative cooling, (ii) it is a source of excretion of waste products like ammonia, urea, and uric acid, and (iii) due to its acidic pH and antimicrobial contents, the sweat protects the skin against colonization by bacteria and fungi, etc.

APOCRINE SWEAT GLANDS

The apocrine sweat glands are found only in certain specific locations in the body which include axilla, areola of the breast, circumanal region, and labia majora. These glands are also simple coiled tubular glands. The secretory portion is located deep in the dermis or in the upper region of the hypodermis. The duct of an apocrine sweat gland does not open on to the skin surface directly, but instead, it opens into the canal of a hair follicle just distal to the entry of the duct of the sebaceous gland.

The lumen of the secretory portion of an apocrine sweat gland is larger than that of an eccrine sweat gland and is lined by a simple epithelium consisting of a single layer of cuboidal or low columnar cells.

The lining cells contain a round or oval nucleus, abundant mitochondria, a large Golgi apparatus, numerous lysosomes, and many lipofuscin pigment granules. Myoepithelial cells are present between the secretory cells and their basal lamina.

The apocrine sweat glands become functional at puberty. They produce a slightly viscous fluid, which contains proteins, carbohydrates, ammonia, lipids, and certain other organic compounds that may color the secretion. This secretion is odorless when it is released on to the skin surface, but quickly acquires a distinctive musky odor as a result of decomposition by bacteria present on the skin surface. The apocrine sweat glands are innervated by adrenergic sympathetic nerve fibers and they secrete in response to emotional and sensory stimuli.

Previously it was held that these glands produce their product by an apocrine method of secretion and their product contains a portion of the apical parts of the secretory cells. However, EM studies have revealed that this concept is wrong. It has been found out that these special sweat glands also release their secretion by a merocrine method. It has been proved that the contents of the apical granules of the lining cells are released into the lumen of the gland by exocytosis and no part of the cell cytoplasm is lost into the secretion. However, conventionally but erroneously, these special large sweat glands are still titled as 'apocrine sweat glands'.

HYPODERMIS

The hypodermis, also called subcutis, subcutaneous tissue, or superficial fascia, is a layer of loose connective tissue that lies under the dermis of the skin (Fig.15.1). It contains variable amounts of fat which is a major factor in determining contours of the body. In women, the amount of fat in the hypodermis is greater and is also more evenly distributed than in men.

The hypodermis is more distinct in the lower part of the anterior abdominal wall, perineum and the limbs. However, it is very thin over the dorsal aspect of the hands and feet, face, and sides of the neck.

The hypodermis contains collagen and elastic fibers that anchor the dermis of the skin to the deep fascia. The fat of the subcutaneous tissue is composed of unilocular adipocytes that are grouped together to form lobules, which are separated from each other by connective tissue septa. The number of adipocytes varies in different regions of the body and their size depends on the nutritional status of the individual. The fat in the hypodermis acts as a padding and serves as an energy store. This fat also serves to conserve the body heat and thus helps in thermoregulation.

The hypodermis also lodges the mammary glands, deeper parts of the sweat glands, and lowermost portions of the hair follicles. It also contains blood vessels, lymph vessels, cutaneous nerves, Ruffini corpuscles and Pacinian corpuscles. The subcutaneous tissue also contains skeletal muscle in the face, neck, and scrotum.

cells *called melanotrophs*. Research has revealed that the melanotrophs synthesize a major protein called pro-opiomelanocortin (POMC) which generates, by proteolytic cleavage, several peptide hormones; these hormones include ACTH, endorphins, and various subtypes of melanocyte-stimulating hormone (MSH), which include α-MSH, β-MSH, and γ-MSH.

PARS TUBERALIS

The pars tuberalis, also called *pars infundibularis,* surrounds the infundibulum in the form of a collar. It consists mainly of groups or cords of faintly basophilic cuboidal cells containing lipid droplets and glycogen granules. Immunohistochemistry of the pars intermedia reveals that it also contains a sparse population of gonadotrophs which secrete LH and FSH.

THE NEUROHYPOPHYSIS

The neurohypophysis consists of the infundibulum and pars nervosa. The infundibulum, also called infundibular stalk, is the short stalk by which the hypophysis is attached to the hypothalamus. It contains the hypothalamo-hypophyseal tract which consists of the axons of the supraoptic and paraventricular nuclei of the hypothalamus. Through the infundibulum, these unmyelinated axons, ensheathed by the astrocytes, reach the pars nervosa. The blood capillaries of the hypothalamo-hypophyseal portal system also pass along the infundibulum from the median eminence to the anterior pituitary.

The **pars nervosa** consists of unmyelinated nerve fibers (axons), fenestrated capillaries, and specialized glial cells called *pituicytes*. The unmyelinated nerve fibers, about 100,000 in number, are the axons of neurosecretory neurons located in the supraoptic and paraventricular nuclei of the hypothalamus. These axons converge at the median eminence to form a bundle of nerve fibers called *hypothalamo-hypophyseal tract*. This tract passes through the infundibulum to the pars nervosa, where the axons terminate in dilated terminals close to a rich plexus of fenestrated capillaries. The dilated axonal terminations contain membrane-bound secretory granules which measure 100-200 nm in diameter.

The neurosecretory material produced in the perikarya of the neurons of the supraoptic and paraventricular nuclei of the hypothalamus moves along the axons of the hypothalamo-hypophyseal tract into the pars nervosa. This material, consisting of granules containing hormones, is stored in the dilated axonal terminals and released into circulation as and when needed. Two hormones are released from the pars nervosa: (1) **oxytocin**, and (2) **vasopressin**, which is also called **antidiuretic hormone (ADH)**.

It is to be understood that the neurohypophysis is merely a depot for the storage of hormones and not an endocrine organ in the strict sense because its hormones originate elsewhere (i.e., in the neurons located in the hypothalamus).

In the ordinary stained preparations examined under LM, the structural features of the neurohypophysis cannot be easily distinguished, because the axons and axonal terminals do not stain well. However, large accumulations of the neurosecretory material, called ***Herring bodies***, can be seen as basophilic masses of irregular shapes and sizes located close to the blood capillaries. Under EM, the Herring bodies are seen as exceptionally large axonal swellings which contain large numbers of neurosecretory granules.

The **pituicytes** are specialized glial cells which resemble the astrocytes. They are abundant in the pars nervosa and play an important supportive role for the axons present in this part of the pituitary gland. Each pituicyte has a small polygonal cell body which is drawn out into a number of cytoplasmic processes. Like the astrocytes, the cell bodies and processes of the pituicytes contain abundant glial intermediate filaments. Some of these processes are wrapped around the axons and axonal terminals, while others end in close relation to blood vessels. In ordinary stained sections of the neurohypophysis, the round, pale-staining nuclei of the pituicytes (with the surrounding scanty cytoplasm) can be seen but their processes are not visible in routinely prepared histologic sections.

ADRENAL GLANDS

The adrenal glands (also called *suprarenal glands*) are paired endocrine glands; one gland lying on the superior pole of each kidney. Each adrenal gland is surrounded by a thick connective tissue capsule from which fine septa (trabeculae) penetrate into the substance of the gland (Fig. 16.3), carrying blood vessels and nerves along with them.

On gross inspection of a freshly cut suprarenal gland, two concentric regions can be distinguished:

1. **Cortex**, which is the yellowish outer region that constitutes about 90% of the mass of the gland.
2. **Medulla**, which is the smaller inner (i.e., central) region that appears reddish to the naked eye.

Although located together anatomically, the adrenal cortex and medulla have different embryologic origins. The adrenal cortex is derived from mesoderm, whereas the parenchymal cells of the adrenal medulla are derived from the neural crest cells, which migrate into the developing adrenal gland.

THE ADRENAL CORTEX

The parenchymal cells of the adrenal cortex synthesize and secrete steroid hormones. Like all the steroidogenic cells found elsewhere in the body, the cells of the adrenal cortex also show three special features: (i) abundant fat droplets, (ii) an exceptionally large amount of SER, and (iii) abundant mitochondria, many of which have tubular cristae. Due to the abundance of SER and mitochondria, the cytoplasm of these steroidogenic cells

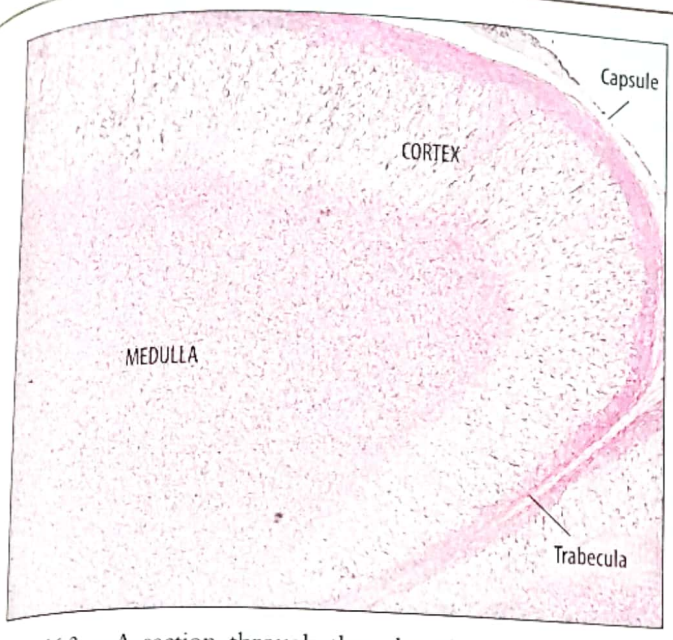

Fig. 16.3 A section through the adrenal gland showing its cortical and medullary regions.

The lipid in the fat droplets of the cells of the adrenal cortex consists of cholesterol which is kept in store in the cytoplasm of these cells. As and when required, the cholesterol is released from the fat droplets to be used as raw material for the synthesis of adrenal cortical hormones. The secretory activity of the cells of the adrenal cortex is under the control of adrenocorticotrphic hormone (ACTH) which is secreted by the corticotrophs of the adenohypophysis.

The hormones synthesized by the cells of the adrenal cortex are not stored in the cytoplasm of the streroidogenic cells but, being lipid-soluble and hydrophobic in nature, they diffuse out freely through the plasmalemma. The released hormones quickly enter the bloodstream through the fenestrated capillaries, which are always present in close association with the endocrine cells.

The suprarenal cortex is divided into three zones on the basis of arrangement of its cells:

i. Zona glomerulosa

ii. Zona fasciculata

iii. Zona reticularis

ZONA GLOMERULOSA

The zona glomerulosa is the outermost zone and lies just beneath the capsule. It constitutes about 15% of the cortical volume and is composed of columnar or pyramidal cells which contain darkly-staining, spherical nuclei. These cells are arranged as arched columns or rounded clusters (glomeruli) which are surrounded by fenestrated capillaries (Fig. 16.4). In stained sections examined under the LM, it is seen that the cytoplasm of these cells stains eosinophilic and contains an extensive SER, numerous mitochondria, a well-developed Golgi apparatus, and many lipid droplets.

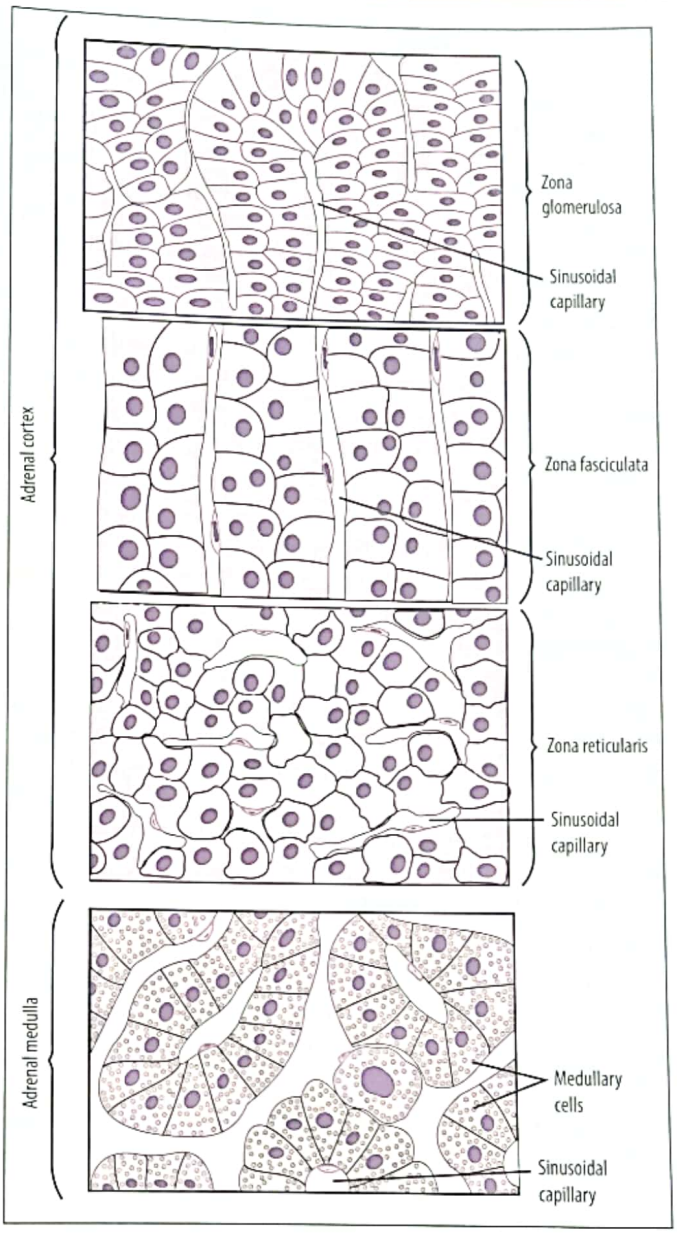

Fig. 16.4 A diagrammatic representation of the microscopic structure of various regions of the adrenal gland.

The zona glomerulosa cells secrete mineralocorticoids, chiefly ***aldosterone***.

ZONA FASCICULATA

The zona fasciculata is the thickest zone and accounts for 70-80% of the cortical volume. This zone consists of large polyhedral cells arranged in long straight cords that are one or two cell thick and run at right angles to the surface of the gland (Fig. 16.4). The adjacent cellular cords are separated from each other by fenestrated capillaries.

The cells of the zona fasciculata are larger in size than those of the zona glomerulosa. Each cell generally contains a single, spherical, lightly staining nucleus, but binucleate cells are also commonly seen. The cytoplasm of the zona fasciculata cells also shows the typical features of steroid-secreting cells, i.e., an extensive smooth endoplasmic

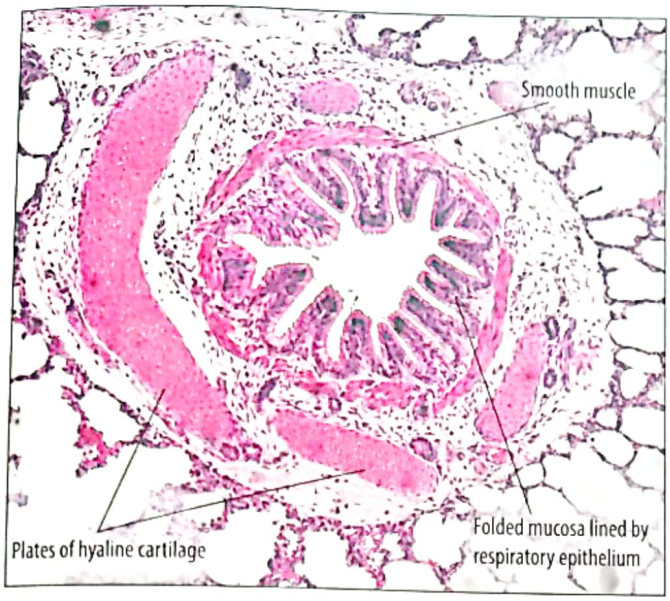

Fig. 17.4 A section through lung tissue showing an intrapulmonary bronchus.

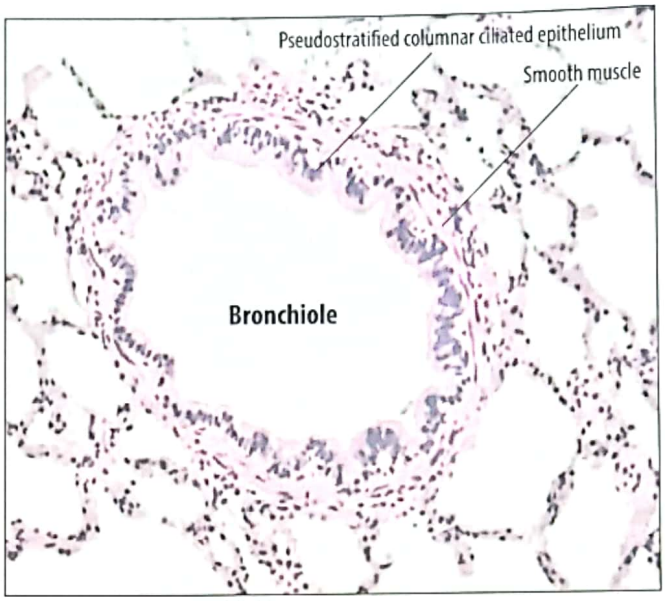

Fig. 17.5 A section of the lung tissue showing a bronchiole. Notice that there are no glands and no cartilage in the wall of the bronchiole.

loose connective tissue. The submucosa also contains many seromucous glands. Outer to the submucosa, the hyaline cartilage is present in the form of irregular plates. In larger intrapulmonary bronchi these plates completely encircle the lumen. As the bronchial diameter diminishes, the amount of cartilage gradually decreases. In the wall of the smaller bronchi the hyaline cartilage is present as small isolated plates which, in sections, appear as small *islands* of cartilage (called *insulae cartilagenae*). All bronchi are surrounded by a layer of connective tissue which is continuous with the connective tissue of the hilum.

BRONCHIOLES

The bronchioles are conducting tubes, measuring 1 mm or less in diameter. Apart from their small diameter, the bronchioles differ from bronchi in the following two respects: (1) there is **no cartilage** in the wall of a bronchiole, and (2) the bronchiolar mucosa contains **no glands** (Fig. 17.5).

Outer to the mucosa of the bronchioles is present a layer of smooth muscle. External to the smooth muscle lies a layer of fibrous connective tissue.

In the larger bronchioles the lining epithelium is of pseudostratified columnar ciliated variety, showing many goblet cells. As the bronchioles divide into smaller divisions, the goblet cells decrease in number and finally disappear. The lining epithelium also gradually changes its form, first becoming simple columnar ciliated and then simple cuboidal ciliated in the terminal bronchioles, which represent the last component of the conducting division of respiratory passages.

TERMINAL BRONCHIOLES

These bronchioles are 0.5 mm or less in diameter. They are lined by a simple cuboidal epithelium. Most of the cells of this epithelium are ciliated but scattered among the ciliated cell are some nonciliated cells which are called Clara cells. No goblet cells are present in the lining epithelium of the terminal bronchioles.

The **Clara cells** (also called *club cells*) have dome-shaped apices which protrude into the lumen of the bronchiole. Their cytoplasm contains extensive RER, well-developed Golgi apparatus, abundant mitochondria, and some profiles of SER. The apical regions of these cells contain numerous secretory granules. The Clara cells perform the following functions:

i. These cells secrete surfactant*, acting as a secondary source of this material (the primary source being the type II pneumocytes of the pulmonary alveoli).
ii. These cells secrete certain antimicrobial peptides which protect the bronchiolar epithelium against the microorganisms present in the inhaled air.
iii. The Clara cells detoxify the pollutants contained in the inhaled air. The toxic materials are absorbed by the Clara cells and then metabolized in their cytoplasm by means of the cytochrome P-450 enzyme.
iv. The Clara cells serve as stem cells. They divide, differentiate and replace the damaged cells of the bronchiolar epithelium.

It is to be noted that the cilia extend further down the respiratory tree than do the glands and goblet cells. This arrangement serves to prevent the terminal respiratory passages from becoming occluded by the mucus.

* The surfactant is a material consisting mainly of phospholipids but also containing some proteins and neutral lipids; it serves to reduce the surface tension of the pulmonary alveoli.

iii. Capillary endothelial cells and their basement membrane.

The basement membrane of the type I pneumocytes and that of the capillary endothelium lie in close apposition and are often fused with each other.

Pleura

The pleura is the serous membrane that invests the lungs and lines the walls of the thoracic cavity. It consists of a thin layer of connective tissue stroma covered by a layer of simple squamous epithelium (known in this location as *mesothelium*). The stroma consists of collagen fibers, elastic fibers, and contains blood capillaries, lymph vessels, nerve fibers, some macrophages, and a few fibroblasts

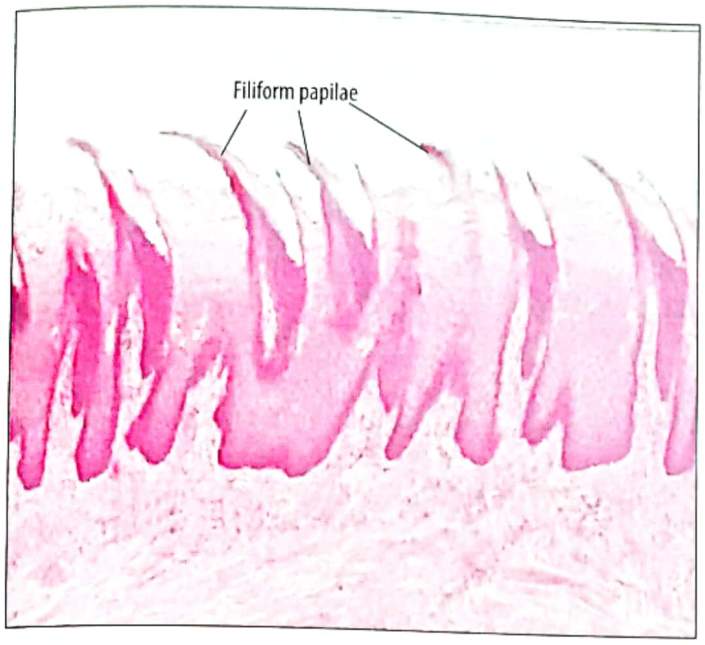

Fig. 18.3 A section of the dorsal surface of the tongue showing filiform papillae.

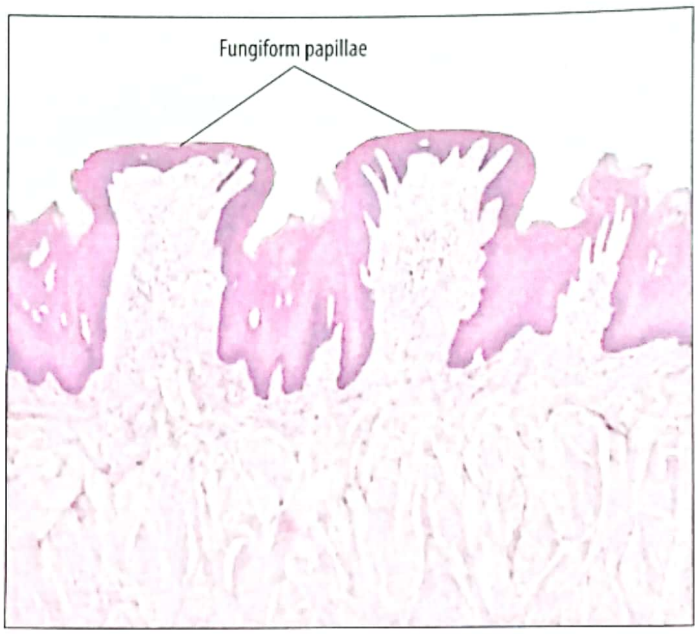

Fig. 18.4 A section of the dorsal surface of the tongue showing two fungiform papillae.

to their slender form that these papillae are called *filiform*, i.e., thread-like (*filum* is a Latin word meaning thread). The filiform papillae do not bear taste buds.

Fungiform Papillae

As indicated by their name, the fungiform papillae are mushroom-shaped structures (Fig. 18.4). Each of them has a dilated upper part and a lower narrow, stalk-like portion by which the papilla is attached to the tongue. These papillae are scattered singly among the filiform papillae, being more abundant in the region close to the tip of the tongue. The fungiform papillae are covered by stratified squamous nonkeratinized epithelium and each papilla bears a large number of taste buds.

Circumvallate Papillae

These papillae (also called *vallate papillae*) are 8-12 in number and are located just anterior to the V-shaped sulcus terminalis. Each circumvallate papilla (Fig. 18.5) is sunk into the lingual mucosa and is surrounded by a deep circular, moat-like groove. The margins of this moat are elevated to form a wall (which is the reason why the circumvallate papillae are so named; in Latin, *circumvallate* means 'surrounded by a wall'). Each circumvallate papilla and the groove surrounding it are lined by stratified squamous nonkeratinized epithelium. Numerous taste buds are located in the epithelial lining of the groove and on the sides (but not on the dorsum) of each circumvallate papilla. Numerous serous glands, called von Ebner glands or *gustatory glands*, lie embedded in the base of the tongue. Ducts of these glands open into the base of the grooves surrounding the circumvallate papillae. The watery secretion of these glands serves to flush food materials out of these grooves, so that taste buds can respond to the changing taste stimuli rapidly.

Foliate Papillae

The foliate (i.e., leaf-like) papillae are well-developed in many mammals, but are minimally represented in the human tongue. The foliate papillae occur on the sides of the tongue as parallel low ridges separated by deep mucosal furrows. They are covered by nonkeratinized stratified squamous epithelium and lodge many taste buds. The foliate papillae are easily identifiable on the tongue of young children but, with progressing age, they undergo a gradual atrophy and in the old people these papillae may not be recognizable at all.

Functions of the Papillae

The lingual papillae increase the surface area of the dorsal surface of the tongue and also serve to make this surface rough. Thus, the area of contact and the friction between the food and tongue are increased. The lingual papillae also increase tongue's ability to manipulate food bolus and to position the food between the teeth during mastication. All lingual papillae, except the filiform papillae, bear taste buds and thus play an important role in the perception of the taste of the materials taken into the mouth.

Glands of the Tongue

Three main groups of simple tubular or tubuloacinar glands occur in the tongue:

1. **Anterior lingual glands.** These constitute a paired group of seromucous glands located under the tip of the tongue. They are embedded in the muscle and their ducts open on to the ventral surface of the tongue.
2. **von Ebner's glands.** As mentioned before, many serous glands, called von Ebner's glands or *gustatory glands*, are embedded in the substance of the tongue

2. A *root* which is buried in the bony socket (alveolus).
3. The region where the crown and root meet is referred to as *neck*.

In the center of each tooth is present an elongated cavity known as *pulp cavity*. This cavity contains loose connective tissue called *dental pulp*. The pulp cavity extends to the apex of the root, where it opens to the exterior through the apical foramen which is a small opening at the tip of each tooth root.

The **hard tissues** of the tooth are ***dentin***, ***enamel*** and ***cementum***. The dentin surrounds the pulp cavity and forms bulk of the tooth. The enamel covers the dentin of the crown, whereas the cementum overlies the dentin of the root. The **Soft tissues** of the tooth include the dental pulp and periodontal ligament. The dental ***pulp*** occupies the pulp cavity, while the ***periodontal ligament*** surrounds the cementum and serves to fix the tooth firmly in the bony socket.

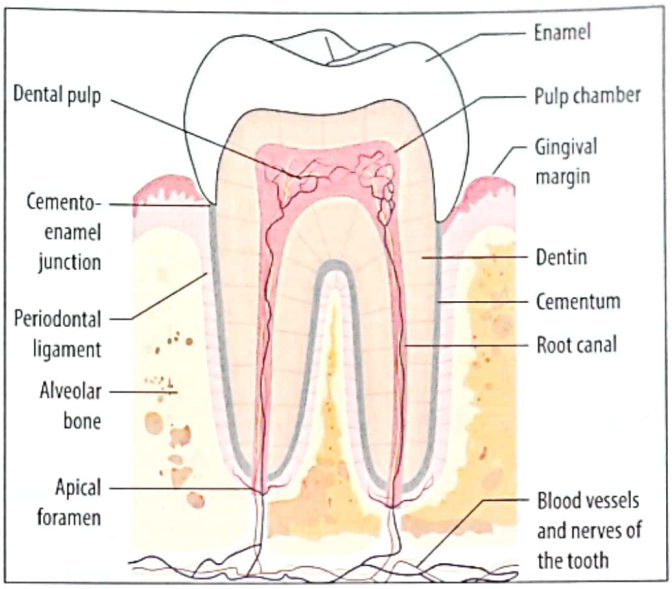

Fig. 18.7 Sagittal section through a molar tooth showing structure of the tooth and various tissue associated with it.

DENTIN

The dentin is a calcified tissue similar to bone, but it is much harder because it contains larger quantities of minerals which constitute about 70% of its dry weight (in the bone, the mineral salts are approximately 50%). As in bone the calcium salts are present in the form of hydroxyapatite crystals. The organic component (about 30%) consists of collagen fibers, proteoglycans, and glycoproteins.

There are no Haversian systems or any cell bodies in the dentin. However, the dentin lodges cytoplasmic processes of cells called *odontoblasts* whose bodies lie in the pulp cavity. These processes lie within the dentine in narrow canals called *dentinal tubules*.

The **odontoblasts** are dentin-forming cells which persist throughout life. These are tall columnar cells lying as a single layer on the inner aspect of the dentin, i.e., at the periphery of the dental pulp. The basal region of an odontoblast contains a large nucleus. In the apical region of the cell are present a large Golgi apparatus and abundant RER, indicating that these cells are engaged in the synthesis and secretion of large amounts of protein. The odontoblasts continue to form dentin into the late adult life. The dentin is first laid down as *predentin*, which consists of type I collagen fibers and glycosaminoglycans and is deposited on the inner surface of the pulp cavity. Later on, the predentin is converted into dentin by the deposition of mineral salts, mainly calcium phosphate. Due to continuous formation of the dentin, the size of the pulp cavity is gradually reduced throughout life.

The dentin is sensitive to heat, cold, trauma and acidic pH. These stimuli are received by the processes of the odontoblasts and conveyed to nerve fibers of the dental pulp. In the CNS, all these stimuli are perceived as pain.

ENAMEL

The enamel covers the crown of the tooth. It is the hardest substance in the body and consists of 96% hydroxyapatite and 4% organic material and water. The organic component consists of some proteins and polysaccharides. However, no collagen is present in the enamel.

The structural units of the enamel are roughly hexagonal *enamel rods* which are bound together by an *inter-rod substance*.

The **enamel rods** are composed of tightly packed hydroxyapatite crystals. The enamel rods measure about 4 μm in diameter but have much greater length. Each rod extends from the dentin-enamel junction to the tooth surface, thus spanning the entire enamel layer which averages 2 mm in thickness.

The *inter-rod substance* of the enamel consists of calcified organic material and lies between the enamel rods as a very thin layer.

In contrast to other structures of the tooth which are derived from the mesoderm, the enamel is ectodermal in origin. During development of the tooth, the enamel is formed by ectoderm-derived cells known as *ameloblasts*. After the enamel is fully formed, the ameloblasts persist for a short while as a part of the dental cuticle covering the enamel surface. The dental cuticle is shed off with tooth eruption and, therefore, no further enamel formation is possible in later life.

even if the sympathetic and parasympathetic nerve supply of the alimentary canal is completely cut. Therefore, the enteric nervous system is considered to be the third division of the autonomous nervous system.

ESOPHAGUS

The esophagus is a muscular tube whose function is to transport food materials from the oropharynx to the stomach. In cross sections, the lumen of the esophagus is seen to be collapsed. This appearance is due to the presence of longitudinal mucosal folds with intervening grooves. However, when water or a bolus of food passes through the esophagus, the mucosal folds disappear and the lumen becomes patent. The wall of the esophagus consists of all the four typical coats of the digestive tube, i.e., mucosa, submucosa, muscularis externa, and adventitia (Fig. 18.9).

The **mucosa** of esophagus is lined by stratified squamous nonkeratinized epithelium. The lamina propria consists of fine connective tissue in which are found scattered lymphatic nodules. In the upper and lower thirds of the esophagus, the lamina propria contains mucus-secreting simple branched tubular glands. These glands are called *esophageal cardiac glands* because, structurally, they resemble the glands in the cardial region of the stomach. The muscularis mucosae consists of smooth muscle fibers running mainly in a longitudinal direction. This coat is thicker in the esophagus than in any other segment of the digestive tract. The thick muscularis mucosae is responsible for the presence of longitudinal folds in the mucosa of the esophagus.

The **submucosa** consists of dense irregular connective tissue that contains blood vessels, lymphatics, and Meissner plexus of nerves. It also contains diffuse lymphatic tissue and lymphoid nodules. The special feature of the submucosa of the esophagus is that it lodges mucus-secreting, branched tubuloacinar glands called *esophageal glands proper* (Fig. 18.10). The ducts of these glands passes through the mucosa to open onto the luminal surface of the esophagus. The mucus secreted by these glands lubricates the mucosal surface of the esophagus and makes it slippery. The submucosa also contains the Meissner's plexus of nerves.

The **muscularis externa** of esophagus consists of the inner circular and outer longitudinal layers but differs from the muscularis externa of rest of the digestive tract in that it contains smooth muscle as well as skeletal muscle. In the upper third of the esophagus only skeletal muscle is present (which is a continuation of the pharyngeal musculature). In the middle third of the organ, muscularis externa contains a mixture of skeletal and smooth muscle. However, only smooth muscle is present in the lower third of the esophagus. The myenteric plexus of nerves lies between the inner circular and outer longitudinal layers of the muscularis externa.

In the cervical and thoracic regions, the esophagus is surrounded by **adventitia** of loose connective tissue containing blood vessels, nerves and lymphatics. The abdominal part of the esophagus is covered by a *serosa*.

STOMACH

The stomach is the most dilated segment of the digestive tube which is capable of considerable distension to accommodate two to three liters of material when full. The food that enters the stomach as small masses (*boli*) of semisolid masticated material leaves it as a viscous, pulp-like mass called *chyme*. The thick muscular wall of the stomach churns the contained material, mixing it thoroughly with the gastric juice secreted by the gastric mucosa. The gastric juice contains hydrochloric acid, enzymes, and mucus. The enzymes include pepsin and gastric lipase.

Additional functions of the stomach include secretion of *intrinsic factor* (a glycoprotein which is essential for the absorption of vitamin B12), and production of hormones especially *gastrin*. Absorption of water, salts, glucose and alcohol occurs in the stomach. Certain drugs are also absorbed through the stomach.

The stomach consists of 4 parts: cardia, fundus, body, and pylorus (Fig. 18.12). The ***cardia*** is the narrow region surrounding the inlet (cardial orifice) of the stomach. The ***fundus*** is the dome-shaped, uppermost part of the stomach which lies above the horizontal plane of the cardial orifice. The ***body*** is the main part of the stomach that lies between the fundus and pyloric antrum. The ***pylorus*** is the funnel-shaped outflow region of the stomach which is divided further into two parts: (i) the proximal wider part called *pyloric antrum*, and (ii) the distal narrower part known as *pyloric canal*. The pyloric canal ends at *pylorus* where the circular layer of the smooth muscle of stomach is thickened to form the *pyloric sphincter*.

The wall of the stomach consists of the usual four coats: mucosa, submucosa, muscularis externa, and serosa.

MUCOSA

The mucosa of the empty stomach is thrown into numerous longitudinal folds called *rugae*. However, the rugae disappear in the distended stomach. The mucosal surface is also divided by shallow grooves into small irregular areas, 1 to 5 mm in diameter, called *mamillated areas*. Each mamillated area shows numerous depressions, called *foveolae* or ***gastric pits***, which extend for a variable distance into the mucosa (Fig. 18.15). At the bottom of each pit are several openings of the glands lying in the lamina propria. In the fundus and body of the stomach, the foveolae are shallow and occupy only the inner one-third of the mucosal thickness and, therefore, the glands are longer and occupy the outer two-thirds of the mucosal thickness (Fig. 18.11 A & 18.13). In the cardiac and pyloric regions of the stomach, the gastric pits are deeper and extend through the inner two-thirds of the mucosal thickness and, consequently, the glands occupy only the outer third of the gastric mucosa (Fig. 18.11 B & 18.14).

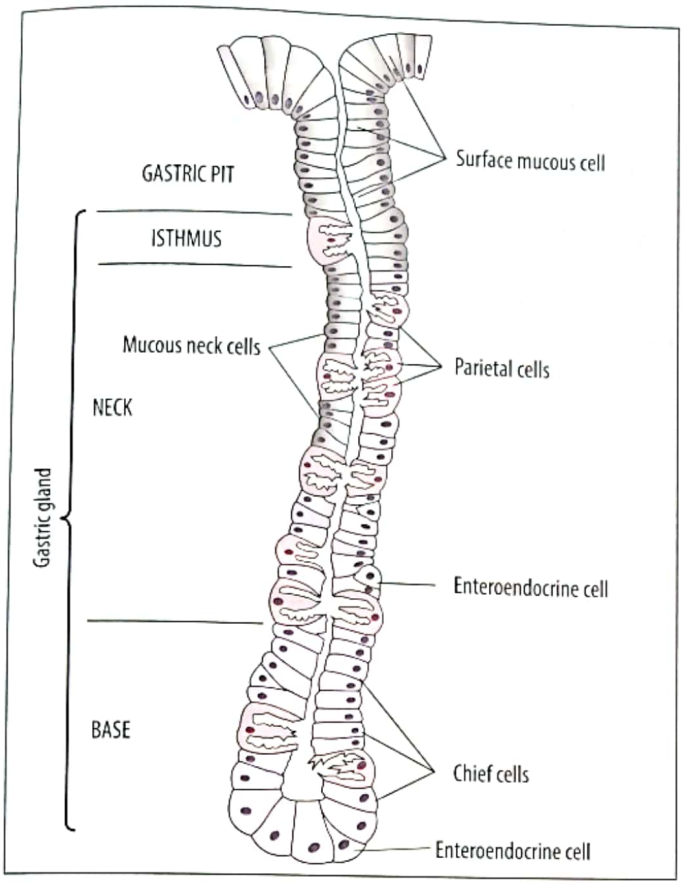

Fig. 18.15 Diagram showing the cell types of the stomach surface, gastric pit, and gastric gland.

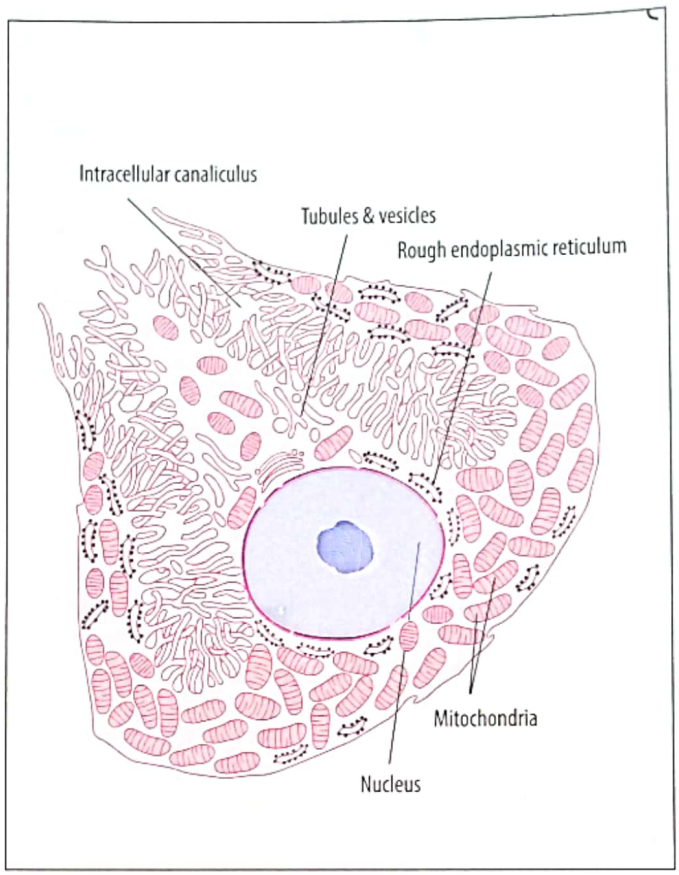

Fig. 18.16 Diagram showing the EM structure of a parietal cell of a gastric gland.

in the most peripheral part of the gland and, therefore, appear to bulge into the surrounding lamina propria. Their peripheral location, close to the outer wall of the gland is the basis upon which these cells have been named parietal cells (in Latin, *parietis* means wall).

Each parietal cell contains a centrally located, rounded nucleus, a large Golgi apparatus, some profiles of RER, and a great number of mitochondria. Due to presence of abundant mitochondria the cytoplasm appears intensely eosinophilic in stained sections examined under LM. The presence of plentiful mitochondria in the cytoplasm of the parietal cells correlates with the high levels of energy needed by these cells for acid secretion.

Electron microscopic studies reveal that a special feature of the parietal cell is the presence of an ***intracellular canaliculus***, which communicates with the lumen of the gastric gland. This canaliculus is actually a deep invagination of the luminal plasmalemma of the parietal cell into the interior of the cell and is meant to increase the surface area available for HCl secretion. The plasmalemma of the intracellular canaliculus contains numerous H^+/K^+-ATPase pumps, called proton pumps, which pump H^+ ions from the cytoplasm of the parietal cell into the lumen of the intracellular canaliculus.

In the resting parietal cells, the intracellular canaliculus is generally shallow and partially encircles the nucleus, but in the actively secreting oxyntic cells this canaliculus is very deep and extends almost to the base of the cell. Numerous microvilli project from the plasmalemma into the cavity of the intracellular canaliculus; these microvilli further increase the surface area available for secretion.

EM also shows that the cytoplasm of a parietal cell contains membrane-bound tubules and vesicles. In an inactive (resting) parietal cell the number of these tubulovesicular structures is very high. However, when the parietal cells are stimulated to produce HCl, the cytoplasmic tubules and vesicles of these cells fuse with the plasmalemma of the cell, which results in: (i) deepening of the intracellular canaliculus, and (ii) an increase in the number of microvilli projecting into the intracellular canaliculus. Therefore, the number of tubules and vesicles is very low in the cytoplasm of an active parietal cell.

Secretion of the HCl by the parietal is stimulated by the hormone gastrin. The gastrin is secreted by the enteroendocrine cells located in the pyloric glands of the stomach and intestinal glands of the proximal duodenum.

In addition to HCl, the parietal cells secrete a glycoprotein called *intrinsic factor*, which binds with vitamin B12 in the stomach and facilitates the absorption of this vitamin in the small intestine.

Chief Cells

The chief cells secrete the proteolytic enzyme pepsin. These cells, also called *zymogenic cells,* are located in the

circumference. The plicae circulares increase the surface area of the mucosa by about three times. They are most pronounced in the jejunum and are absent in the proximal part of the duodenum and distal half of the ileum.

Villi

A further ten-fold increase in surface area is obtained by the presence of villi, which are finger-like projections of the small intestinal mucosa (Fig. 18.17 A). The villi cover the entire surface the mucosa and give it a characteristic velvety appearance in the freshly opened organ. Each intestinal villus is approximately 1 mm in length and 0.1 mm in diameter. About 10 to 40 villi are present per square millimeter of the mucosal surface.

The shape of the intestinal villi varies in different parts of the small intestine. There is a mixture of leaf-shaped and finger-shaped villi in the duodenum. Traced distally, the number of leaf-shaped villi gradually decreases. The jejunal mucosa has a preponderance of finger-shaped villi but many leaf shaped villi are also present. The mucosa of the ileum bears only finger-shaped villi.

Under LM, each intestinal villus is seen as an evagination of the intestinal mucosa having a core of loose connective tissue covered by the intestinal epithelium. This connective tissue is an extension of the lamina propria of the mucosa and contains a network of fenestrated capillaries, a blind-ending central lymphatic vessel called *lacteal,* and a few strands of smooth muscle fibers. The intestinal villi contract and shorten intermittently owing to the activity of the smooth muscle fibers, which continue into the muscularis mucosae of the small intestinal mucosa. These rhythmic movements of the villi are important for absorption.

Microvilli

The enterocytes (absorptive cells) lining the intestinal villi bear microvilli on their apical surface. These microvilli further increase the surface area of the small intestinal mucosa by a factor of 20.

Crypts of Lieberkuhn

A further increase in mucosal surface area is achieved due to the occurrence of about 0.4 mm deep invaginations of mucosa between the bases of the villi. These invaginations are called crypts of Lieberkuhn, intestinal crypts, or ***intestinal glands***. The crypts of Lieberkuhn (Fig. 18.17 A & B) are actually simple tubular glands that lie in the lamina propria and may extend as far as the muscularis mucosae (but do not penetrate it).

Lamina Propria

Beneath the lining epithelium of the small intestine is present the lamina propria which is a layer of loose connective tissue which also extends into the cores of the intestinal villi. Most of the space in the lamina propria is occupied by the intestinal glands (crypts of Lieberkuhn). The connective tissue of lamina propria is heavily infiltrated with lymphocytes and other cells of immune system (plasma cells, macrophages, mast cells, and eosinophils). At places, the lymphocytes accumulate to form lymphoid follicles. In the ileum the lymphoid follicles aggregate to form big lymphoid masses called *Peyer's patches*, which may extend into the submucosa by piercing the muscularis mucosae (Fig. 18.20 & 18.21). The diffuse and nodular lymphatic tissue in the lamina propria of the gastrointestinal tract constitutes the GALT (gut-associated lymphatic tissue).

The Peyer's patches are always located opposite to the attachment of mesentery and each of them may contain more than 50 lymphoid follicles. The mucosal surface over the Peyer's patches is smooth and does not show villi or crypts.

Muscularis Mucosae

The muscularis mucosae of the small intestine consists of the usual inner circular and outer longitudinal layers of smooth muscle. From the inner circular layer, delicate strands of smooth muscle cells extend into the core of each intestinal villus.

EPITHELIUM OF THE SMALL INTESTINE

The mucosa of small intestine is lined by a simple columnar epithelium. This epithelium that lines the crypts and covers the villi is a continuous sheet, which is constantly being renewed. The intestinal epithelium contains six types of cells: (1) enterocytes, (2) goblet cells, (3) enteroendocrine cells, (4) Paneth cells, (5) stem cells, and (6) M cells.

ENTEROCYTES

The enterocytes, also called *absorptive cells,* are tall columnar cells, each having a basally located oval nucleus. Apical surface of an enterocyte is characterized by the presence of about 3,000 closely packed **microvilli.** When routine sections are examined under LM, the microvilli, arranged parallel to each other, impart a striated appearance to the free border of the absorptive cells and, therefore, this border is known as *striated border* (also called *brush border).* EM shows that each microvillus is about 1 μm in length and 0.1 μm in diameter. The core of each microvillus is formed by a bundle of about 20 parallel actin filaments. Just beneath the brush border the cell exhibits *terminal web*, which is a layer of transversely oriented cytoplasmic filaments. Interaction between actin filaments of the microvillus and myosin filaments of the terminal web results in an oscillatory movement of the microvilli, which facilitates the absorptive process.

EM studies also reveal that the tips of microvilli are covered by a **thick glycocalyx coat.** This coat contains enzymes that hydrolyze the disaccharides and dipeptides into monosaccharides and amino acids, respectively, which are readily absorbed by the enterocytes.

EM also shows that the apical region of an enterocyte contains a large amount of SER. The cytoplasm also contains

(0.2-1 μm in diameter) which are called *chylomicrons*. The chylomicrons are secreted by the enterocytes into the paracellular space of the plasmalemma of the basolateral domain of the enterocytes, from where they cross the basement membrane of the intestinal epithelium to enter the lamina propria of the mucosa. Here, only the smallest of these enter the blood capillaries but, due to their large size, most of the chylomicrons enter the lymph capillaries (lacteals) present in the intestinal villi.

GOBLET CELLS

The goblet cells are mucus-secreting, unicellular glands which are scattered among the enterocytes. Their number increasing from the duodenum to the terminal ileum.

These cells have the typical shape of a goblet (Fig. 4.1 A). The expanded, cup-shaped apical part of each goblet cell is occupied chiefly by mucin granules. These secretory granules are surrounded by a thin peripheral rim of cytoplasm called theca. The narrow and elongated basal part of the cell contains the nucleus and cell organelles and extends downwards to rest on the basal lamina.

EM shows that the luminal surface of the goblet cells bears a few microvilli around the periphery, but is smooth and convex over the secretory granules that nearly fill the apical part of the cell. Mitochondria, abundant RER, and numerous free ribosomes are scattered throughout the lateral and basal cytoplasm. The nucleus lies in the basal region of the cell and a large Golgi complex is seen to be present between the nucleus and secretory granules (Fig. 4.1 B).

In the ordinary H&E stained sections the narrow basal part of a goblet cell stains basophilic because of the presence of the heterochromatic nucleus, RER and ribosomes. However, the apical part of the cell appears empty and unstained because the mucin granules are washed away during processing of the tissue for microscopy. When special methods of processing and staining are employed, the secretory granules of goblet cells are preserved and are found to be basophilic, metachromatic, and PAS positive.

The goblet cells secrete **mucin** which represents a group of strongly hydrophilic glycoproteins. After release from the cell, the mucin molecules come in contact with water and become hydrated to form **mucus**, which is a thick and viscous, gel-like material whose chief functions are protection and lubrication.

ENTEROENDOCRINE CELLS

The enteroendocrine cells present in the crypts of Lieberkuhn of the small intestine also belong to the diffuse neuroendocrine system (DNES). Enteroendocrine cells of many varieties, producing different hormones, have been identified in the small intestine. The names of these cells and the hormones secreted by them are given below:

1. **S cells** produce secretin.
2. **K cells** secrete gastric inhibitory peptide.
3. **L cells** secrete glucagon-like peptide-1 (GLP-1).
4. **I cells** produce cholecystokinin.
5. **EC cells** secrete serotonin and substance P.
6. **MO cells** produce motilin.
7. **D1 cells** produce vasoactive intestinal peptide (VIP).
8. **G cells** (which are present in the proximal duodenum only) secrete gastrin.

PANETH CELLS

These cells occur only in the bottom of the intestinal crypts. They are pyramidal cells having the cytological characteristics of zymogen cells. The Paneth cells have a strongly basophilic basal cytoplasm, a large supranuclear Golgi complex, and large acidophilic granules in the apical cytoplasm. The basal cytoplasm contains abundant RER. The secretory granules of the Paneth cells contain the bactericidal agent lysozyme, defensive proteins called defensins, zinc, and an arginine-rich protein (which is responsible for the intense acidophilia of the granules). The secretory product of Paneth cells has the ability to kill certain bacteria and protozoa and, therefore, these cells are supposed to play an important role in regulating the normal intestinal flora (i.e., the bacteria which are normally present in the lumen of the intestine).

STEM CELLS

The stem cells of the intestine are undifferentiated cells which are located in the lower half the crypts of Lieberkuhn. These cells undergo regular mitotic division to produce more undifferentiated cells which move in an upward direction along the wall of the intestinal crypt to reach the epithelium covering the intestinal mucosal surface and intestinal villi. As the newly produced undifferentiated epithelial cells move upwards, they differentiate into absorptive cells, goblet cells, and other component cells of the intestinal epithelium. In this way, a constant turnover of all types of the intestinal epithelial cells is ensured as the old epithelial cells are regularly replaced by new cells produces as a result of mitotic activity of the stem cells located in the lower parts of the crypts of Lieberkuhn.

M CELLS (Fig. 18.22)

These cells, also called microfold cells, constitute a special variety of epithelial cells in the intestinal mucosa. The M cells are chiefly located in the ileum where they are found in the epithelium overlying the Peyer's patches (which are aggregations of the lymphoid follicles). These cells are broad and flat and have dome-like apices. These cells show the following two important structural features: (1) their free (apical) surface exhibits microfolds, and (2) their basal plasmalemma is invaginated to form deep pockets or recesses; these recesses are occupied by the dendritic cells and lymphocytes.

The M cells are antigen-transporting cells. They endocytose those antigens (mostly bacteria) that manage to reach the intestinal lumen and pass these on to the dendritic cells

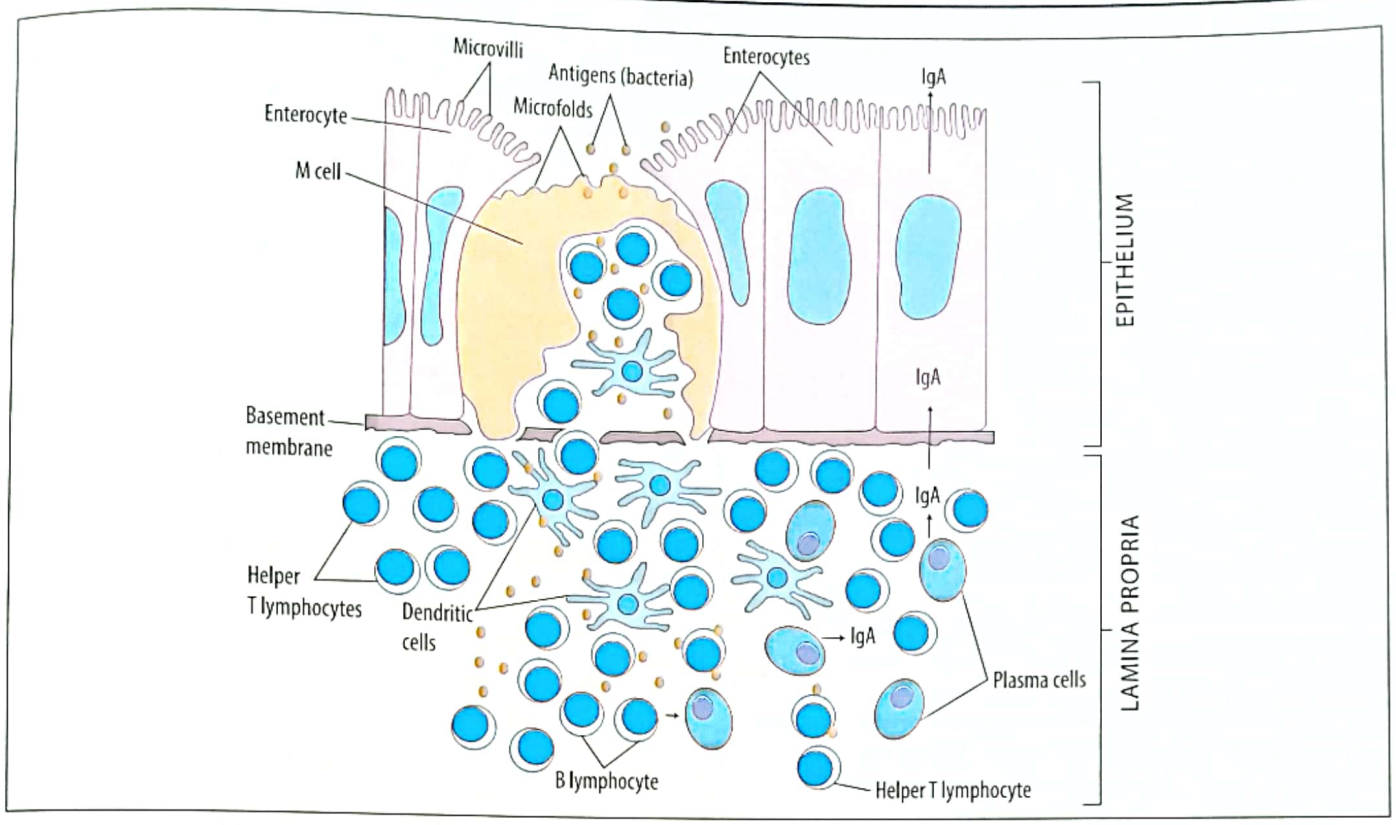

Fig. 18.22 Diagram depicting an M cell in the small intestinal mucosa. The cells related to the functioning of the M cell are also shown.

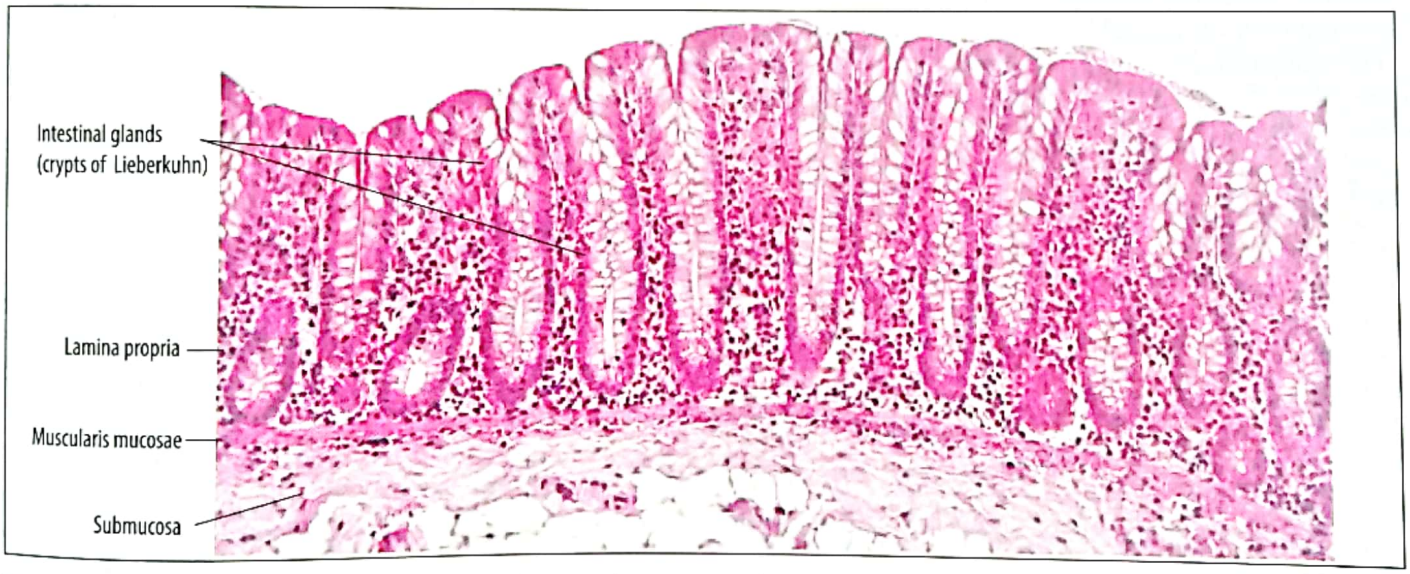

Fig. 18.23 A section through wall of the colon showing the mucosa and submucosa. Note that the intestinal glands are lined mainly by the goblet cells.

smooth muscle. Teniae coli are absent. Serosa forms the outermost coat of the appendix.

RECTUM

Histologically, the rectum resembles the colon except for the following features: (i) the rectal mucosa contains fewer but deeper crypts of Lieberkuhn, (ii) the outer longitudinal layer of the muscularis externa is of uniform thickness (and does not show teniae coli), and (iii) most of the rectum is covered by a connective tissue adventitia; only the anterior and lateral surface of the upper third of the rectum have a serosal covering (visceral layer of peritoneal).

ANAL CANAL

The anal canal is the terminal part of the alimentary tract. It begins where the rectum suddenly narrows. It is about 4 cm long and ends at the anus.

The ***mucosa*** of the anal canal shows a number of longitudinal folds called *anal columns*. The furrows between anal columns are called *anal sinuses*. The lower ends of

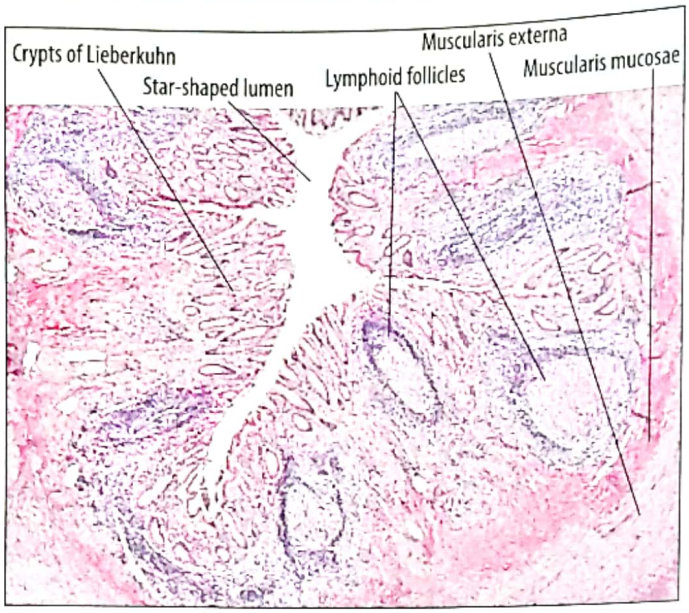

Fig. 18.24 A section through the vermiform appendix showing its microstructure.

the anal columns are joined to one another by transverse mucosal folds known as *anal valves*. Above the level of anal valves, the mucosa is lined by the same epithelium as rest of the large intestine, i.e., simple columnar epithelium consisting of absorptive cells and goblet cells. Below the level of the anal valves the lining epithelium is of stratified squamous nonkeratinized variety which becomes continuous with the epidermis of skin at the anal orifice. The lamina propria contains crypts of Lieberkuhn above the level of anal valves, but below this level the crypts are completely absent. The muscularis mucosae becomes gradually indistinct and cannot be recognized in the lower part of the anal canal.

The ***submucosa*** of the anal canal consists of dense irregularly arranged connective tissue. At the level of anal valves, the submucosa lodges about six simple branched tubular glands that usually extend into the muscularis externa. These glands, called *anal glands,* secrete mucus onto the mucosal surface by means of long ducts that are lined by stratified columnar epithelium. The submucosa of the anal canal also contains a plexus of small veins.

The ***muscularis externa*** of the anal canal consists of the inner circular and outer longitudinal layers of smooth muscle. At the level of anal columns, the inner circular layer is thickened to form the *internal anal sphincter.* The *external anal sphincter* consists of skeletal muscle that belongs to the musculature of the pelvic floor.

The anal glands are clinically important because an inf of them may become infected to form an ***anal abscess***. Such an abscess may ultimately lead to formation of a *fistula* between the anal canal and perianal skin, which is commonly called ***fistula-in-ano***. Such a fistula usually requires treatment by surgery.

Pathological dilatation of the submucosal veins of the anal canal produces a clinical condition known as piles or *hemorrhoids*.

Endocrine System 16

The control and integration of cellular and tissue function is carried out by the nervous system. The endocrine system can be regarded as a far-reaching extension of nervous system. The chemical signaling molecules (hormones or cytokines) produced by various endocrine cells and organs regulate a diverse variety of important cellular activities to ensure a coordinated control of the internal environment, in which the cells and tissues function appropriately. The hormones either have a stimulatory or an inhibitory effect on the target cells.

A **hormone** is defined as a biologic substance that acts on a specific target cell. Generally, the hormones pass into the circulatory system (i.e., blood or lymph) to reach their target cells at remote sites in the body. This common mode of transport of hormones to their target cells is called **endocrine communication** (or endocrine control).

It is to be noted that two other ways of intercellular communication also exist: paracrine communication and juxtacrine communication. The **paracrine communication** is a localized type of humoral communication, in which the hormones or cytokines diffuse in the extracellular fluid to reach and affect the neighboring target cells, which may be located some distance away. In **juxtacrine communication**, the chemical messengers produced by one cell directly interact with the receptors located on an adjacent cell.

Another mode of action of hormones is the **autocrine mode,** in which chemical messengers produced by a cell bind to receptors located on the surface of the same cell.

HORMONES

Chemically, the hormones can be classified into four types: (1) **steroids hormones**, e.g., estradiol, testosterone, and cortisol, (2) **protein hormones**, e.g., insulin, oxytocin, and growth hormone, (3) **amino acid-derivatives**, e.g., epinephrine (derived from tyrosine), and serotonin (derived from tryptophan), and (4) **arachidonic acid-derivatives** which include prostaglandins and leukotrienes.

Hormone Receptors

The hormones act upon their target cells by binding to specific hormone receptors. The fat-soluble hormones, e.g., steroid hormones and thyroid hormones, easily pass through the plasma membrane of the target cells and bind to **intracellular receptors** to produce their effect. The intracellular receptors may be located in the cytoplasm or nucleus of the cell. The water-soluble hormones, e.g., protein hormones, cannot cross the cell membrane and, therefore, act by binding to the **cell surface receptors.**

Distribution of Endocrine Cells in the Body

The endocrine cells occur in the following three ways:

1. In the mucosa of the digestive and respiratory tracts and in some other organs in the body, the endocrine cells are found to be scattered among the exocrine secretory cells. Collectively, such endocrine cells constitute the *diffuse neuroendocrine system* (described below).
2. In many locations in the body, the endocrine cells are organized to form discrete endocrine glands, e.g., the pituitary gland, suprarenal glands, and thyroid gland.
3. The endocrine cells may occur as clusters of cells within the parenchyma of certain organs, e.g., islets of Langerhans of the pancreas and interstitial cells of the testes.

Diffuse Neuroendocrine System (DNES)

This system consists of a network of cells which are distributed throughout the body. The term 'neuroendocrine' refers to 2 special features of these cells: (i) their structure resembles that of the neurons, and (ii) they secrete hormones (or neurotransmitters). Developmentally, the cells of the DNES are derived from the neural crest cells.

The DNES cells located in the mucosa of the stomach and intestine are called **enteroendocrine cells**. The secretory granules of many of these cells show affinity for chromium salts and take a brown color when the tissue sections are stained by potassium dichromate; such cells are called **enterochromaffin cells**.

Many cells of the DNES have the capability to take up amine precursors (like tryptophane) and to decarboxylate these into biologic amines (like serotonin). Accordingly, these cells are also called **APUD cells** (the acronym APUD stands for Amine Precursor Uptake and Decarboxylation).

THE DISCRETE ENDOCRINE GLANDS

General microscopic structure of these glands is fairly simple. They are usually composed of cords or plates of endocrine cells, supported by a delicate stroma of loose connective tissue. Fenestrated blood capillaries are interposed between the cords of the endocrine cells. This arrangement ensures that every endocrine cell has a direct access to a blood capillary, so that it can release its secretion directly into the circulation. Each endocrine gland is surrounded by a connective tissue capsule.

HYPOPHYSIS

The hypophysis, or **pituitary gland,** is the master endocrine gland that produces several hormones which

influence the activity of other endocrine glands. This small gland is suspended from the hypothalamus by means of a short stalk called *infundibulum*. The hypophysis is divided into two main subdivisions: (1) adenohypophysis, and (2) neurohypophysis. These two parts develop from different embryonic sources and also have different functions.

The neurohypophysis is derived from neuroectoderm and arises as a ventral evagination from the floor of the developing diencephalon (a part of the forebrain). The adenohypophysis is derived from oral ectoderm and originates as an outpocketing from the primitive oral cavity (stomodeum) of the embryo. This outpocketing, called Rathke's pouch, grows cranially and becomes closely applied to the neurohypophysis, which maintains its connection with the forebrain by means of infundibulum. The Rathke's pouch becomes detached from the stomodeum completely and its anterior wall becomes greatly thickened, so that lumen of the pouch is finally reduced to a narrow fissure.

The **adenohypophysis** (also called *anterior pituitary)* is divided by the fissure representing the residual lumen of the Rathke's pouch into two unequal parts: pars distalis and pars intermedia (Fig. 16.1). The thicker ***pars distalis*** develops from the anterior wall of the Rathke's pouch and, therefore, lies anterior to the fissure. An extension of the pars distalis, called *pars tuberalis,* surrounds the infundibulum. Posterior to the fissure lies the ***pars intermedia*** which develops from the posterior wall of the Rathke's pouch and exists in the form of a thin cellular partition between the pars distalis and neurohypophysis.

The **neurohypophysis** (also called posterior pituitary) consists further of two parts: infundibulum and pars nervosa (Fig. 16.1). The ***infundibulum*** is the short stalk by which the hypophysis is attached to the median eminence part of the hypothalamus (some authorities consider the median eminence to be a part of the neurohypophysis). The infundibulum conveys the axons of the hypothalamo-hypophyseal tract to the ***pars nervosa,*** *which* lies immediately posterior to the pars intermedia.

THE ADENOHYPOPHYSIS

The adenohypophysis comprises pars distalis, pars intermedia and pars tuberalis.

PARS DISTALIS

The pars distalis constitutes about 75% of the hypophysis. It is surrounded by a dense fibrous capsule. The parenchyma of the pars distalis consists of cords and clusters of cells which are supported by a scanty connective tissue stroma. Fenestrated capillaries are present between the cords and around the clusters of parenchymal cells.

The endocrine cells of the pars distalis are classified either on the basis of their staining properties or on the basis of their functional activity.

Classification of the pars distalis cells on the basis of staining properties of the cells

On this basis of their staining affinity exhibited in the ordinary stained sections, the cells of the pars distalis are classified into two major types: chromophils (those cells which stain well) and chromophobes (those cells which stain poorly). The chromophils are further divided into two subtypes: acidophils and basophils (Fig. 16.2).

Chromophils

The chromophils stain well because they contain stainable secretory granules in their cytoplasm. The contents of these granules have affinity either for acidic or for basic dyes. Accordingly, the chromophils are divided into two subtypes: acidophils and basophils.

The **acidophils** contain cytoplasmic granules that have affinity for acidic dyes and, therefore, these cells take a red or orange color in stained sections.

The **basophils** contain cytoplasmic granules which have affinity for basic dyes; these cells take a bluish color in stained sections.

Chromophobes

These cells exhibit little or no affinity for dyes and, therefore, stain poorly. EM studies reveal that the cytoplasm of these cells is devoid of secretory granules. The chromophobes are actually those chromophils which have released their secretory granules and are in a temporary resting phase.

Classification of the pars distalis cells according to their functional activity

Advent of the immunocytochemistry and other modern investigative techniques has made it possible to determine the function of various pars distalis cells. Taking into account the hormone (or hormones) secreted by these cells, they are classified into five varieties: (1) somatotrophs, (2) lactotrophs, (3) corticotrophs, (4) gonadotrophs, and (5) thyrotrophs.

Somatotrophs

The somatotrophs constitute about 50% of the cells of pars distalis. They are oval cells containing a centrally-located, spherical nucleus. EM shows that, in addition to the ordinary cell organelles, the cytoplasm of these cells contains a large number of secretory granules that measure 300 to 400 nm in diameter. The somatotrophs secrete **growth hormone** (somatotropin). *In the stained tissue sections examined under LM, the somatotrophs take an acidophilic stain.*

Lactotrophs

These cell (also called mammotrophs) are polygonal cells with oval nuclei. EM studies reveal that the cytoplasm of these cells contains large secretory granules having an average diameter of 600 nm. The lactotrophs constitute 15-20% of total cells of the pars distalis. In the ordinary

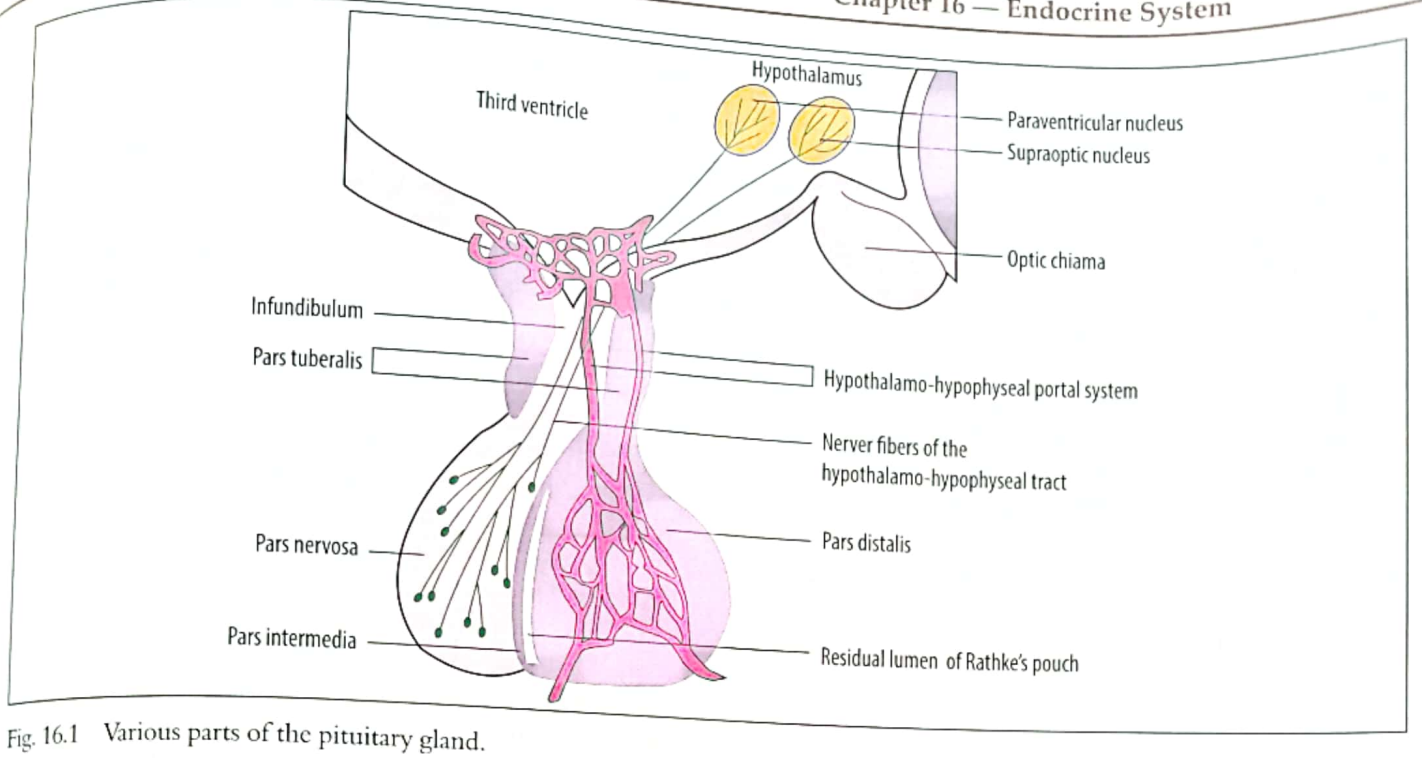

Fig. 16.1 Various parts of the pituitary gland.

stained sections of the pituitary gland examined under LM, these cells stain acidophilic. The lactotrophs secrete **prolactin,** which is a hormone that carries out a number of functions in the females as well as males (like control of the sexual desire and modulation of activity of the immune system). An important function of prolactin in the females is that it promotes development of the mammary gland during pregnancy and production of milk (lactation) after childbirth.

Corticotrophs

The corticotrophs are also polygonal cells, each having a round nucleus located in an eccentric position. EM shows that their cytoplasm contains secretory granules measuring 250-400 nm in diameter. In histologic sections prepared for light microscopy, the cytoplasm of these cells takes a basophilic stain. The corticotrophs also constitute 15-20% of the total pars distalis cells. These cells secrete **ACTH** (adrenocorticotropic hormone) and **β-lipotropin**. The β-lipotropin promotes beak down and mobilization of the fat stored in the adipocytes.

Gonadotrophs

These cells constitute approximately 10% of the total pars distalis cells. EM shows that the gonadotrphs are oval cells with round nuclei. Their cytoplasm contains secretory granules which measure 200-400 nm in diameter. These granules stain basophilic in tissue sections prepared for light microscopy. The gonadotrophs produce **FSH** and **LH**. The FSH stimulates the growth and development of ovarian follicles in the females, while in the males it supports the function of Sertoli cells of the testes. The LH stimulates ovulation and formation of corpus luteum in the females, whereas in the males this hormone stimulates the interstitial cells of the testes to secrete testosterone.

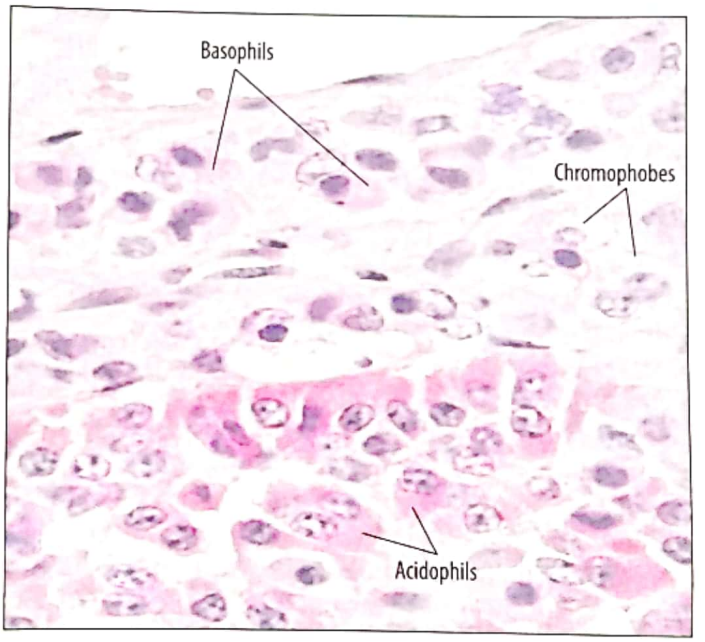

Fig. 16.2 A section through the pars distalis of pituitary gland showing acidophils, basophils, and chromophobes.

Thyrotrophs

The thyrotrophs constitute about 5% of the total pars distalis cells. EM studies show that the thyrotrophs are relatively large, oval cells with round, eccentrically-placed nuclei. They contain small cytoplasmic granules having an average diameter of 150 nm. In the ordinary stained sections examined under LM, the granules stain basophlic. The thyrotrophs secrete TSH (thyroid-stimulating hormone) which is also known as *thyrortropin*.

PARS INTERMEDIA

The pars intermedia of the adenohypophysis is a thin zone composed mainly of cords of basophilic polygonal

reticulum, numerous mitochondria, and abundant fat droplets of large size. A well-developed Golgi apparatus and numerous profiles of RER also present. The cytoplasm of these cells takes a general eosinophilic stain but some regions of the cell exhibit slight basophilia because of the concentration of the RER in those regions. In ordinary histological preparations, the cytoplasm of the zona fasciculate cells appears vacuolated because the fat stored in the cytoplasm as large droplets is dissolved out by the chemicals used for tissue processing. The cells of zona fasciculata produce glucocorticoids, mainly ***cortisol*** (hydrocortisone). This zone derives from its name from the arrangement of the component cells in the form of closely-packed cords (in Latin, *fascicle* means 'a small bundle').

ZONA RETICULARIS

The zona reticularis makes up about 10% of the cortex and lies adjacent to medulla. This zone consists of polyhedral or rounded cells arranged in branching and anastomosing cords (Fig. 16.4). Sinusoidal capillaries are present between the cellular cords. The cells of the zona reticularis are smaller than those of the other two zones and, therefore, the nuclei in this zone appear to be closely packed. The cytoplasm of these cells contains a large amount of SER, and abundant mitochondria. Fat droplets are present but in comparatively smaller numbers. A special feature of the cytoplasm of the zona reticularis cells is the presence of large quantities of the lipofuscin pigment.

The cells of zona reticularis secrete weak **androgens**, mainly *dehydroepiandrosterone (DHEA)*. The cells of this zone also produce small quantities of estrogens, progesterone, and cortisol.

The zona reticularis is so named because the branching and anastomosing cords of cells in this zone appear to form a reticulum (network).

THE ADRENAL MEDULLA

The adrenal medulla contains anastomosing cords of large, polyhedral cells called ***medullary cells*** (Fig. 16.4). Between the cellular cords are present sinusoidal blood capillaries. The cells are supported by a meshwork of reticular fibers. Due to their affinity for chromium salts, the medullary cells are also called *chromaffin cells*.

Developmentally, the medullary cells are derived from the neural crest and are regarded to be modified post-ganglionic neurons that lack dendrites and axons. These cells have assumed a secretory function and release their secretory products in response to stimulation by preganglionic sympathetic nerve fibers. Their secretory product consists mainly of two catecholamines: epinephrine (adrenaline) and norepinephrine (noradrenaline). Epinephrine is the chief hormone produced by the medullary cells and accounts for 80% of the total catecholamine output of the adrenal medulla.

EM studies reveal that the medullary cells contain extensive RER, abundant mitochondria, a well-developed Golgi apparatus, and numerous membrane-bound secretory granules measuring 100-300 nm in diameter. These granules contain either epinephrine or norepinephrine. In addition, these granules contain calcium ions, ATP, and a variety of proteins called *chromogranins*, all of which serve to bind and store the low molecular weight catecholamines in the granules till they are released by exocytosis.

Depending on the ultrastructure of secretory granules, the medullary cells can be divided into two types: *norepinephrine-producing cells* and *epinephrine-producing cells*. The secretory granules of norepinephrine-producing cells contain an electron-dense core surrounded by an outer zone of lower density. The secretory granules of the epinephrine-producing cells are homogeneous and less dense.

THYROID GLAND

The thyroid gland is located in the anterior neck region and consists of two lateral lobes connected by an isthmus. This endocrine gland secretes three hormones: tetraiodothyronine (thyroxine), triiodothyronine, and calcitonin.

The thyroid is covered by a thin capsule of dense irregular connective tissue. From the capsule, fine septa pass into the gland to divide it into indistinct lobules. These septa also serve as conduits for blood vessels, lymphatic vessels, and nerve fibers.

The parenchyma of the thyroid gland consists of 20 to 30 million spherical, cyst-like structures called **thyroid follicles**, which range from 0.2 to 1.0 mm in diameter. The wall of a thyroid follicle is generally lined by simple cuboidal epithelium (Fig. 16.5 & 16.6). The cavity of the follicle contains a glue-like material called *colloid*.

In between the thyroid follicles is present fine connective tissue consisting mainly of reticular fibers. This connective tissue is highly vascular and each follicle is surrounded by an extensive network of fenestrated capillaries. Blind-ended lymphatic capillaries are also present in the interfollicular connective tissue.

The lining epithelium of each thyroid follicle contains two types of cells: (i) follicular cells, and (ii) parafollicular cells.

FOLLICULAR CELLS

The follicular cells, also called thyrocytes or ***principal cells***, are generally cuboidal in shape. However, the cell shape depends on the state of functional activity of the gland. In an inactive gland the cells are low cuboidal, whereas in a hyperactive gland the cells assume a columnar shape (Fig. 16.7). In H&E stained sections, the follicular cells exhibit a lightly basophilic cytoplasm and a centrally placed, spherical nucleus that contains one or two prominent nucleoli.

EM reveals that the follicular cells exhibit the features of both secretory and absorptive cells. At their apical ends, the neighboring cells are joined to each other by typical

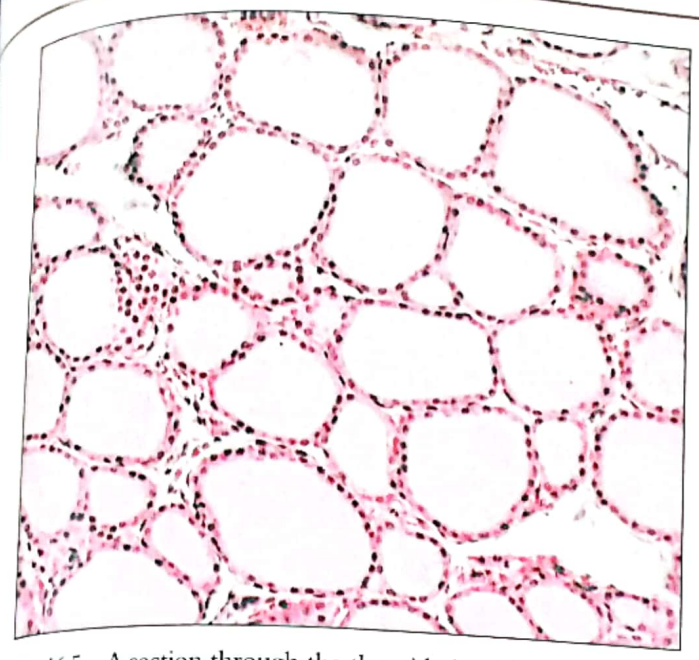

Fig. 16.5 A section through the thyroid gland (in resting state) as seen under low power magnification of light microscope. Multiple thyroid follicles containing colloid material are seen.

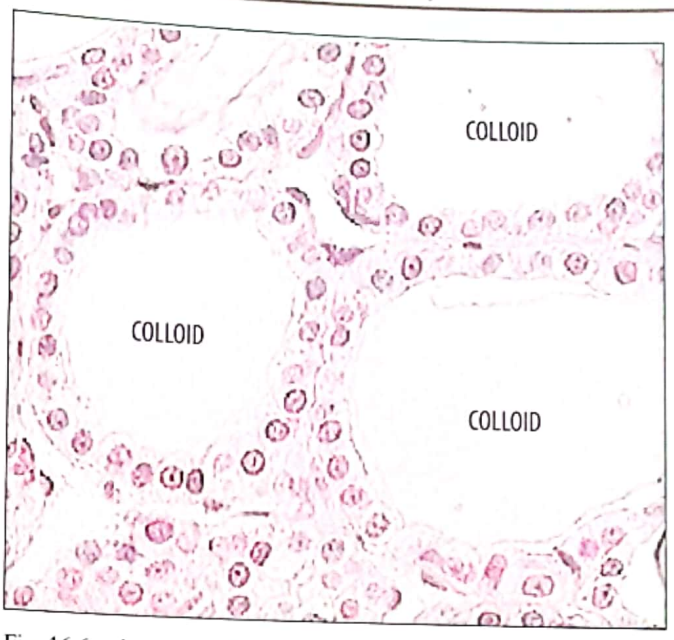

Fig. 16.6 A section of the thyroid gland (in resting state) as seen under high power magnification of light microscope. Thyroid follicles lined by simple cuboidal epithelium and filled by colloid are visible.

junctional complexes and their apical surface shows a moderate number of small microvilli (both being the characteristic features of absorptive cells). The cytoplasm of the thyrocytes contains abundant RER (especially in the basal region) and a well-developed Golgi apparatus located in the supranuclear region (features of the secretory cells). Mitochondria are also abundant and are dispersed throughout the cytoplasm. The apical region of the thyrocytes also contains secretory vesicles, endocytotic vesicles, and numerous lysosomes.

Synthesis, Storage and Secretion of the Thyroid Hormones

The follicular cells of thyroid gland synthesize a glycoprotein called *thyroglobulin* and secrete it into the follicular cavity. The follicular cells also take up iodide from the blood by means of iodide pumps located in their basal plasmalemma. The iodide passes through the cytoplasm of follicular cells to their apical region where oxidation of the iodide to iodine is carried out by the enzyme *thyroid peroxidase*, which is located in the apical plasmalemma of these cells. At the interface between the colloid and apical plasmalemma of follicular cells, *iodination* of the tyrosine residues of thyroglobulin molecules occurs. This iodination results in the formation of monoiodotyrosine (MIT) and diiodotyrosine (DIT). Oxidative coupling of iodotyrosine residues leads to the formation of two thyroid hormones: triiodothyronine (T3) and tetraiodothyronine (T4). The tetraiodothyronine is more popularly known as *thyroxine*. The T3 is formed by the coupling of one MIT molecule with one DIT molecule, whereas T4 is formed by the coupling of two DIT molecules with each other.

The thyroxine and triiodothyronine remain attached to the thyroglobulin molecules and are stored within the follicular cavity as *colloid*. Re-absorption of the colloid into the follicular cells occurs under the influence of the thyroid-stimulating hormone (TSH), which is produced by the thyrotrophs of the anterior pituitary. The microvilli (filopodia) of the apical plasmalemma of the follicular cells facilitate the endocytosis of the colloid as small membrane-bound vesicles. These vesicles move to the basal region of the cells and fuse with the lysosomes. The lysosomal proteases degrade the thyroglobulin molecules and release T3 and T4 in free form. These hormones diffuse through the basal plasmalemma and enter the blood and lymph capillaries surrounding the thyroid follicles. The thyroxine (T4) constitutes about 95% of the released hormones.

PARAFOLLICULAR CELLS

The parafollicular cells of the thyroid gland lie singly or in small groups among the follicular cells (Fig. 16.7A). They are also located within the basal lamina of thyroid follicles but do not extend to the lumen of the follicles.

The parafollicular cells are larger than the follicular cells but their cytoplasm stains poorly with routine staining methods and, therefore, these cells are also called **C cells** (i.e., clear cells). Electron microscopic studies show that the parafollicular cells contain a round nucleus, some RER, mitochondria, a well-developed Golgi apparatus, and numerous, dense, membrane-bound secretory granules, which are located in the basal region of the cell. The parafollicular cells secrete a hormone called **calcitonin**, which is released into the blood capillaries surrounding the thyroid follicles. The calcitonin lowers plasma calcium level, inhibits bone resorption, and acts as an antagonist to the parathyroid hormone.

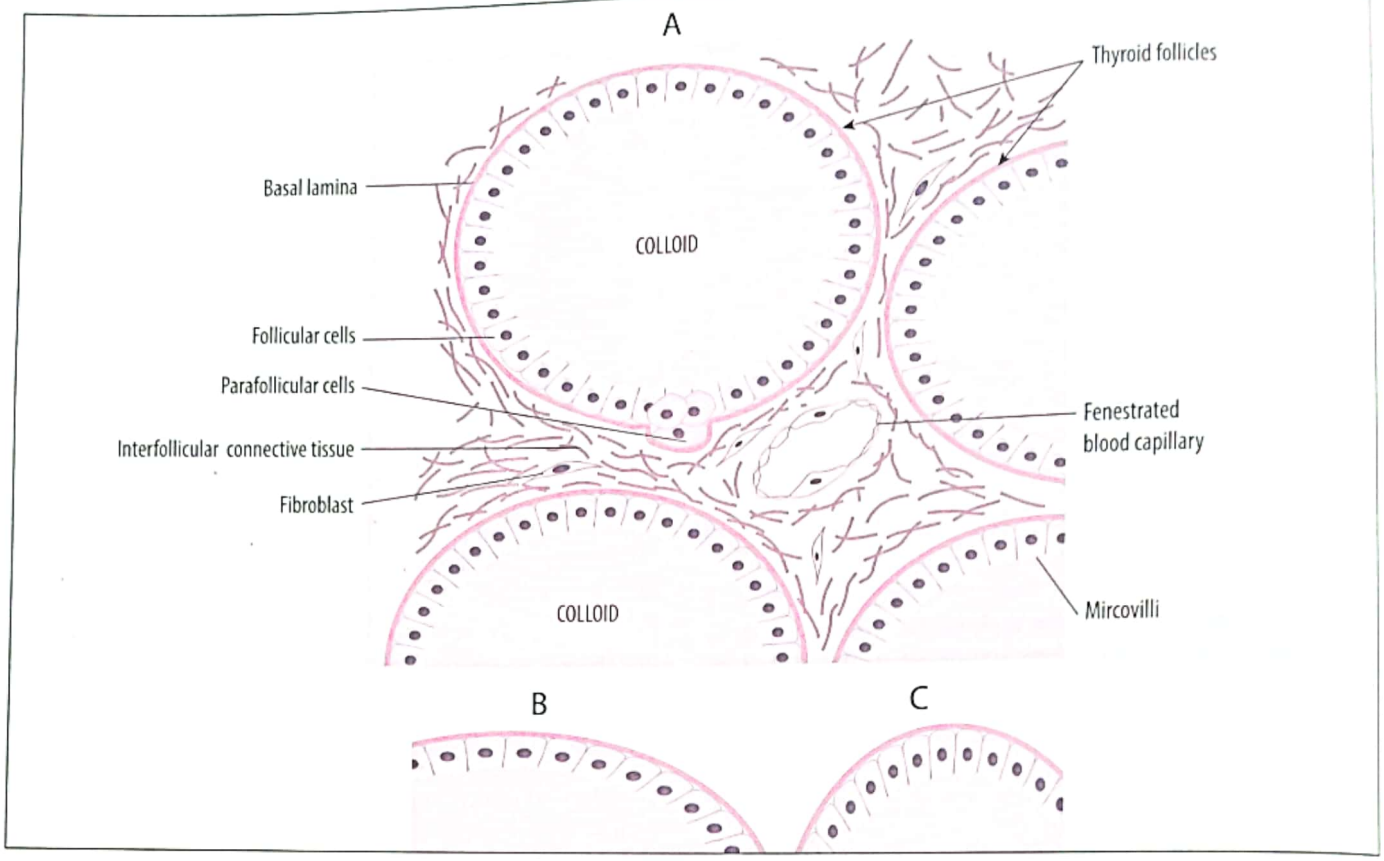

Fig. 16.7 Diagram depicting the microscopic structure of the thyroid gland. A. Under average physiological conditions, B. Resting state, C. Highly active state.

PARATHYROID GLANDS

Each parathyroid gland is covered by a connective tissue capsule, from which delicate septa penetrate into the gland. The parenchyma of this gland consists of anastomosing cords of cells; fenestrated capillaries lie between the cellular cords. Two types of cells are found in the parenchyma of the parathyroid gland of an adult: (1) chief cells, and (2) oxyphil cells (Fig. 16.8).

CHIEF CELLS

These cell, also called *principal cells,* are more numerous than the oxyphil cells. They are small, polygonal cells, measuring 6-8 µm in diameter. The cytoplasm of these cells contains the cell organelles, glycogen granules, lipid droplets, masses of lipofuscin pigment, and small membrane-bound secretory granules. These cells generally stain lightly acidophilc in the ordinary stained sections of the parathyroid gland.

The chief cells secrete **parathyroid hormone (PTH)**, which is also called *parathormone*. This hormone raises the blood calcium level by: (1) stimulating bone resorption by the osteoclasts, and (2) decreasing the renal excretion of calcium by stimulating resorption of calcium ions in the kidney tubules.

OXYPHIL CELLS

These cells appear around puberty and their number increases with age. The oxyphil cells are also roughly polygonal and but are generally larger in size than the chief cells. They generally occur in small clusters and stain intensely acidophilic. The cytoplasm of the oxyphil cells contains a very large number of mitochondria, most of which have bizarre shapes. No secretory granules are present in the cytoplasm of the oxyphil cells and their function remains unknown. The distinct acidophilia of the oxyphil cells, which is due to the presence of exceptionally numerous mitochondria, is the basis upon which these cells have been named (in Greek, *oxys* means acid).

The stroma of the parathyroid gland consists mainly of reticular connective tissue containing many fat cells. The number of fat cells increases with age; In elderly people, the adipocytes may occupy 60-70% of the glandular mass.

PINEAL GLAND

The pineal gland, also called epiphysis cerebri or pineal body, is a small, flattened structure having the shape of a pine cone (hence the name *pineal*). It is attached by a short stalk to the roof the third ventricle of brain. The pineal is covered by pia mater which forms a capsule of the organ and sends in septa which divide the gland substance into incomplete lobules. Two varieties of cells are found in the pineal gland: (i) pinealocytes, and (ii) interstitial cells (Fig. 16.9).

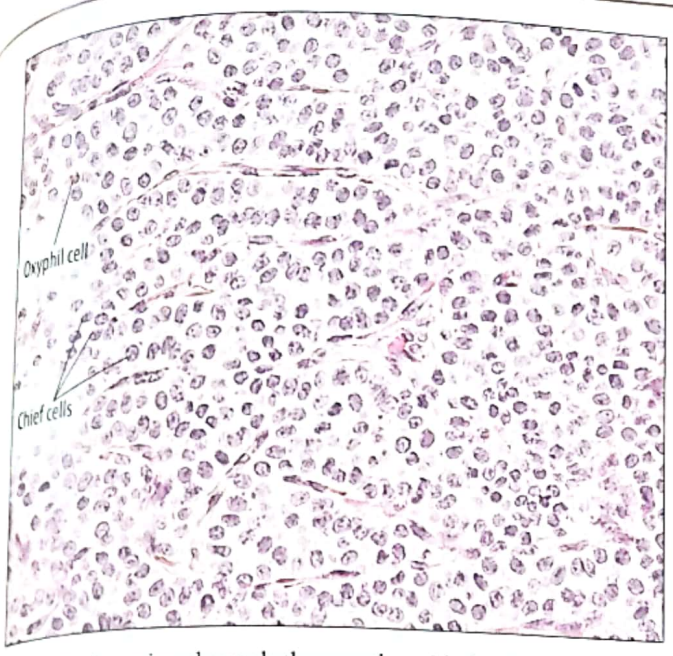

Fig. 16.8 A section through the parathyroid gland.

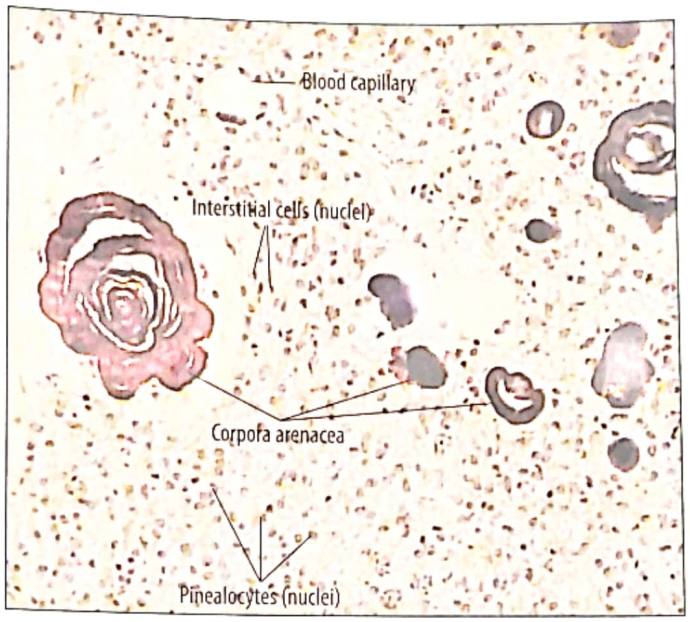

Fig. 16.9 A section through the pineal gland of a middle-aged person.

The **pinealocytes** are the parenchymal cells of the pineal body. They are arranged in cords or clusters in close relation to sinusoidal capillaries. The pinealocytes have a slightly basophilic cytoplasm and contain a lobated or deeply indented, vesicular nucleus with one or two prominent nucleoli. Each pinealocyte has two or more long processes, which end in flattened dilatations that are applied to blood capillaries. EM shows that, in addition to the typical cytoplasmic organelles, a pinealocyte contains many dense-cored, membrane-bound granules and large numbers of microtubules. The pinealocytes secrete **melatonin** and several other related substances.

The **interstitial cells** (also called *glial cells*) constitute only 5% of the cells in the pineal gland. These cells are modified astrocytes and possess long cytoplasmic processes. The nucleus of an interstitial cell is elongated and darkly-staining. These cells are found between the cords and clumps of pinealocytes and in perivascular regions.

Starting from the second decade of life, calcified concretions appear in the pineal extracellular matrix. These concretions are mulberry-shaped structures of variable size and are collectively titled as **corpora arenacea** or *brain sand*. The corpora arenacea exhibit a lamellar structure in ordinary histological sections (Fig. 16.9) and take a basophilic stain. The corpora arenacea consist of calcium phosphate and calcium carbonate deposited concentrically around an organic matrix; therefore, they exhibit a lamellated structure in sections. It has been found that melatonin is released from the pinealocytes by exocytosis in combination with carrier proteins. After release from the cell, the carrier proteins become dissociated from the hormone and attract calcium ions. The carrier proteins-calcium complex becomes deposited in the pineal gland as corpora arenacea. Understandably, the number of corpora arenacea increases with age.

Function of the Pineal Gland

Function of the pineal gland in humans is subject of great controversy. The secretion of melatonin (and other related substances) is controlled by amount of light seen by the eyes each day. The nervous pathway involved is as follows. Visual impulses are carried by a special inferior accessory optic tract to the suprachiasmatic nucleus of the hypothalamus. This nucleus sends fibers to the tegmental reticular nuclei, which are connected to the neurons of the lateral horn of the gray matter in the upper thoracic segments of the spinal cord. Fibers from the lateral horn neurons pass to the superior cervical ganglia of the sympathetic chains. Postganglionic fibers from these ganglia reach the pineal gland, where they terminate in close relation to the pinealocytes. Darkening has been found to increase the sympathetic input, resulting in an increase in the secretion of melatonin by the pinealocytes. On the other hand, exposure to light inhibits the production of melatonin. The melatonin passes either by the way of blood or through cerebrospinal fluid to the anterior pituitary gland to suppress the secretion of gonadotropic hormones. It is, therefore, supposed that the pineal gland plays some role in controlling sexual drive and reproduction in human beings.

ENDOCRINE PART OF THE PANCREAS

The endocrine part of the pancreas exists as numerous spherical or oval masses of light-staining endocrine cells lying between the darkly satining exocrine pancreatic acini; these masses of endocrine cells are called **islets of Langerhans** or ***pancreatic islets*** (Fig. 16.10). The islets of Langerhans vary from 100 to 200 µm in diameter and are scattered throughout the pancreatic tissue, being most abundant in the tail region. The pancreas contains more than one million islets of Langerhans, but they constitute only 1-2% of the total volume of the organ.

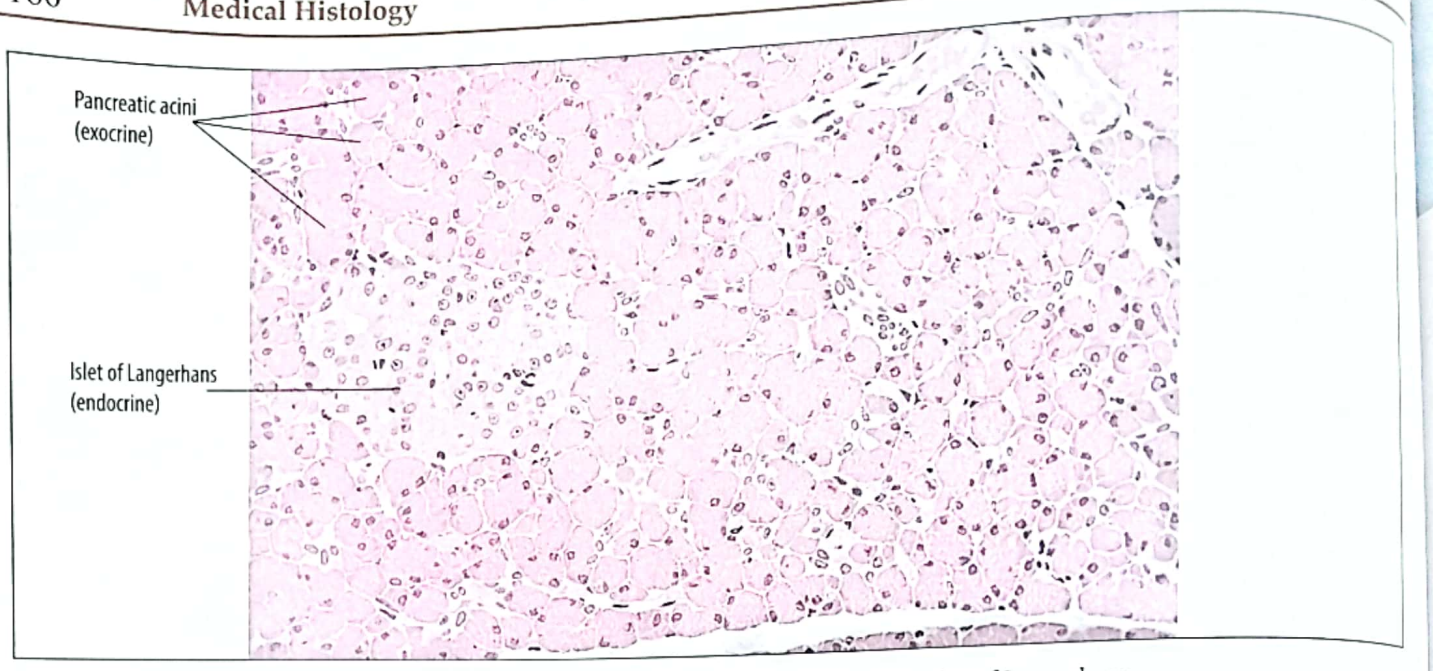

Fig. 16.10 A section through the pancreas showing numerous pancreatic acini and one islet of Langerhans.

Each pancreatic islet consists of a variable number of roughly spherical or polygonal cells arranged as irregular cords. A network of fenestrated blood capillaries intervenes between the cellular cords, so that each cell of the islet is in contact with at least one capillary. The endocrine cells of the pancreatic islets are smaller and much lighter-staining than the exocrine acinar cells. Each islet of Langerhans is separated from the surrounding acinar cells by a thin capsule of reticular fibers.

Cell Types in the Islets of Langerhans

The cytoplasm of the islet cells contains secretory granules which cannot be distinguished in the ordinary H&E stained sections. By employing special staining techniques and electron microscopy, four different types of cells can be identified in the pancreatic islets, each type producing a different hormone. These four cell types are named alpha cells, beta cells, delta cells, and PP cells.

1. The **alpha cells** (also called A cells) are located at the periphery of the islet and constitute about 20% of the total islet cells. These cells secrete glucagon.
2. The **beta cells** (also called B cells) are about 70% of the total cells and occupy the central region of the islet. These cells secrete insulin.
3. The **delta cells** (also called D cells) constitute 5-10% of the total cells and are found to be scattered among the alpha and beta cells. The D cells are considered to be a part of the diffuse neuroendocrine system (DNES). They secrete somatostatin.
4. The **PP cells** are only 1-2% of the total cells and are mostly found in the islets located in the head of the pancreas. These cells secrete pancreatic polypeptide.

Diabetes mellitus (DM) is a disease characterized by impaired carbohydrate, protein and fat metabolism because of insufficient insulin secretion by the pancreas or due to target tissue insulin resistance. The main symptoms of this disease are polyuria (excessive micturition), polydipsia (excessive thirst), and polyphagia (excessive desire to eat). Two main types of diabetes mellitus are recognized: type I and type II.

The **type I diabetes mellitus** is also known as ***juvenile-onset diabetes*** or insulin-dependent diabetes mellitus (IDDM). This type of diabetes develops in the childhood or adolescence due to the loss of beta cells of the pancreatic islets as a result of autoimmune destruction. Consequently, the body suffers from a deficiency of insulin, resulting in hyperglycemia and other metabolic complications which can only be treated by regular injections of insulin obtained from extraneous sources.

The **type II diabetes mellitus**, also called ***maturity-onset diabetes*** or non-insulin-dependent diabetes mellitus (NIDDM), is much more common than the type I diabetes mellitus. In this disease, either there is insufficient production of insulin by the beta cells of the pancreatic islets in response to hyperglycemia, or the target cells in the body tissues fail to respond to the insulin present in the blood (because these cells develop resistance to the effects of insulin). This type of diabetes usually becomes manifest around the 40 years of age and can be treated by oral intake of drugs which either stimulate the beta cells to produce more insulin, or decrease the resistance of the target cells to the action of insulin, or can have both of the aforementioned effects.

Respiratory System 17

The main function of the respiratory system is to provide oxygen to the blood and to eliminate carbon dioxide. Its additional functions include perception of smell (by means of the olfactory mucosa located in the nasal cavity) and production of sound (by means of the larynx).

The respiratory system consists primarily of a system of respiratory passages, collectively called **respiratory tract**. This tract is divided into proximal and distal parts. The gaseous exchange occurs in the distal part of the respiratory tract. The proximal part of the respiratory tract does not take part in gaseous exchange but forms a series of conducting passages by which the air passes from the atmosphere to the distal part of the respiratory tract where gas-exchange structures (pulmonary alveoli) are located.

The ***proximal respiratory tract*** consists of nasal cavities, pharynx, larynx, trachea, bronchi, bronchioles, and terminal bronchioles. As the air passes through the proximal part of the respiratory tract, it is warmed, moistened and freed of particulate matter.

The ***distal respiratory tract*** includes the respiratory bronchioles, alveolar ducts, alveolar sacs, and pulmonary atria with pulmonary alveoli.

THE RESPIRATORY EPITHELIUM

Most of the conducting division of the respiratory system is lined by a pseudostratified columnar ciliated epithelium resting on a thick basement membrane that separates the epithelium from the underlying connective tissue. This epithelium, customarily titled as *respiratory epithelium*, consists of five cell types: (1) ciliated columnar cells, (2) goblet cells, (3) basal cells, (4) brush cells, and (5) small granule cells.

The **ciliated columnar cells** constitute about 30% of the total cell population of the respiratory epithelium. These are tall, slender cells that extend through the full thickness of the epithelium. Each of them bears about 300 motile cilia (kinocilia) on its apical surface. The nucleus is located in the basal region of the cell. The apical cytoplasm of these cells also contains a large number of mitochondria. The ATP synthesized by these mitochondria is utilized to move the cilia. By the coordinated sweeping motion of their cilia, these cells move the mucus (and its trapped particulate matter) from the distal parts of the respiratory passages toward the pharynx for elimination.

The **goblet cells** are unicellular mucous glands that are interspersed among the ciliated columnar cells and extend through the full thickness of the epithelium. These also constitute about 30% of the total cell population. The wider apical portion of a goblet cells is filled with mucin granules, whereas its narrow basal portion contains the nucleus and cell organelles. The goblet cells secrete mucin which, after hydration, gives rise to mucus, which is a flexible, gel-like material. The mucus forms a continuous layer over the respiratory epithelium and, because of its sticky nature, serves to trap the particulate matter present in the inhaled air. The mucus, along with the trapped debris, is carried toward the larynx and laryngopharynx by the rhythmic beating of the kinocilia of the columnar cells. This contaminated mucus is either expectorated or swallowed.

The **basal cells** constitute about 30% of the total cell population of respiratory epithelium. These are short, pyramidal cells which rest on the basal lamina but do not reach the free surface of the respiratory epithelium. No granules are present in the cytoplasm of these cells. The basal cells constitute a reserve of stem cells which divide mitotically and produce cells that differentiate into other cell types.

The **brush cells** constitute about 5% of the total cell population. These are slender columnar cells, which have numerous microvilli on the apical surface. The brush cells have afferent nerve endings at their basal surfaces and are supposed to serve as to be sensory receptors.

The **small granule cells** (also called *Kulchitsky cells*) constitute about 5% of the total cell population. These cells resemble the basal cells in shape and occupy a similar location. However, their cytoplasm is characterized by the presence of numerous small, membrane-bound, granules with dense cores. The small granule cells belong to the diffuse neuroendocrine system (DNES), but the exact nature of the hormone produced by these cells is not well understood*.

NASAL CAVITIES

The **vestibule** of each nasal cavity is lined by skin which contains sebaceous and sweat glands and bears short, thick hairs known as *vibrissae*. These hairs function to filter out coarse particles from the inspired air. The **nasal cavity proper** (nasal fossa) is lined by nasal mucosa which shows structural specialization into two regions: (1) respiratory mucosa which lines most of the nasal fossa, and (2) olfactory mucosa which covers the roof of nasal cavity.

The **respiratory mucosa** is lined by a typical respiratory epithelium. Beneath the epithelium lies a thin layer of connective tissue (lamina propria) which lodges many simple branched tubuloacinar glands of the mixed

* Recently , some research worker have reported that the basal cells or the repiratory epithelium secrete a calcitonin-like polypeptide.

(seromucous) variety. The lamina propria also contains a rich plexus of blood vessels in which arteriovenous anastomoses are common. Over the conchae, especially over the inferior concha, the nasal mucosa contains large venous plexuses called *swell bodies* or *cavernous bodies*. Every 20 to 30 minutes, the swell bodies on one side of the nasal cavity become automatically engorged with blood, thus restricting air flow on that side. These periodic intervals of reduction in the air flow permit the respiratory epithelium to recover from desiccation. The deepest layer of the lamina propria blends with the periosteum of the bone or perichondrium of the cartilage forming the walls of the nasal fossae. The mucous and serous secretions keep the mucosal surface moist and also humidify the inspired air. This humidification is necessary to protect the delicate epithelium from desiccation. The mucous coat also entraps particulate matter and absorbs water-soluble pollutant gases like sulfur dioxide, ammonia and ozone. The mucus is constantly propelled by the cilia of the epithelial cells toward the pharynx, where it is either swallowed or expectorated. Blood in the vascular plexuses of the lamina propria warms the inspired air in the cold environment.

OLFACTORY MUCOSA

The roof of each nasal cavity, the superior concha, and the upper part of nasal septum are lined by olfactory mucosa. In the living, the olfactory mucosa can be distinguished by its yellowish color (in contrast to the pinkish color of the ordinary respiratory mucosa). The olfactory mucosa is lined by **olfactory epithelium** which serves as a receptor for the sensation of smell. The lamina propria of olfactory mucosa contains serous glands.

OLFACTORY EPITHELIUM

This epithelium is also of the pseudostratified columnar variety but lacks goblet cells. The olfactory epithelium is composed of three types of cells: (1) olfactory cells, (2) sustentacular cells, and (3) basal cells (Fig. 17.1).

Olfactory Cells

The olfactory cells are bipolar neurons possessing a cell body, a dendrite extending to the mucosal surface, and an axon which leaves the epithelial surface, enters the underlying connective tissue and joins the axons from other bipolar neurons to form the fascicles of the olfactory nerve. The apical dendrites are slender and pass between the sustentacular cells to the mucosal surface, where they terminate in small bulbous enlargements called *olfactory vesicles*. From each olfactory vesicle arise 6 to 8 *olfactory cilia* which run parallel to the surface of the olfactory epithelium.

The olfactory cilia are up to 200 µm long and have a short proximal segment which tapers to a much longer trailing portion. The proximal segment shows the typical ultrastructure of a cilium, i.e., nine peripheral doublet microtubules surrounding two central singlets. The distal longer segment of an olfactory cilium contains only the central singlets. The microtubules of the olfactory cilia are not associated with dyenin arms; therefore, these cilia are immotile. The plasmalemma of the olfactory cilia contains *odorant-binding proteins*, which function as *olfactory receptors*. Molecules of the odoriferous substances interact with the olfactory receptors to generate stimuli which are conveyed to the brain by the olfactory nerves.

Sustentacular Cells

The sustentacular cells (**supporting cells**) are tall columnar cells, relatively broad at their apices and tapering basally. They bear numerous microvilli on their free surface. The oval nuclei of the sustentacular cells are located in the apical parts of these cells and, hence, occupy a more superficial position in the epithelium than the nuclei of the olfactory cells. EM shows that junctional complexes are present between the supporting cells and olfactory cells. The sustentacular cells contain granules of a yellowish brown pigment which is responsible for the distinctive yellowish color of the olfactory mucosa.

Functionally, the sustentacular cells are comparable to glial cells. They provide nourishment, physical support, and electrical insulation to the olfactory cells.

Basal Cells

The basal cells are small, conical or spherical cells that form a single layer in the basal part of the olfactory epithelium and, hence, their nuclei lie at a level below the olfactory cell nuclei. The basal cells are reserve cells (stem cells) that exhibit mitotic activity. Both the olfactory and sustentacular cells have limited life spans and are continuously replaced by new cells which are produced by the mitotic division of the basal cells (the newly produced cells have the potential to differentiate into olfactory cells or sustentacular cells).

PARANASAL AIR SINUSES

The paranasal air sinuses (maxillary, frontal, ethmoidal and sphenoidal) are lined by a mucous membrane that resembles the mucosa of the nasal cavities. However, the pseudostratified columnar ciliated epithelium of the paranasal sinuses is shorter in height and exhibits fewer goblet cells. Also, the lamina propria is thinner, contains fewer glands, and does not lodge the large venous plexuses found in the nasal mucosa. As in the nasal fossae, the deeper layers of the lamina propria are fused with the underlying periosteum.

NASOPHARYNX

From within outwards, the wall of the nasopharynx consists of the following four layers:

1. Mucosa.
2. Submucosa.
3. Muscle layer.
4. Fibrosa.

The **mucosa** consists of epithelium and lamina propria. The epithelium is of respiratory type except for a small region over the posterior wall of the nasopharynx. This

region, with which the soft palate comes in contact during swallowing, is lined by stratified squamous epithelium. The lamina propria is rich in elastic tissue and contains a large number of simple branched tubuloacinar glands. Mostly these glands are of mixed (seromucous) variety, but in the area covered by stratified squamous epithelium some pure mucous glands are also present. Lymphatic tissue is scattered in the lamina propria throughout the nasopharynx. In the posterosuperior wall the lymphatic nodules are aggregated to form the *pharyngeal tonsil*.

A **submucosa** of loose connective tissue is present only in the superolateral part of the nasopharynx. The **muscle** forming the wall of the nasopharynx is of skeletal variety. The **fibrosa** is the outermost layer and consists of a thin layer of fibrous connective tissue.

LARYNX

The laryngeal wall consists of mucosa, intrinsic muscles and cartilages. The *mucosa* comprises epithelium and lamina propria. The lining epithelium varies in type in different parts of the larynx. The laryngeal inlet, most of the epiglottis, and the true vocal cords are covered by stratified squamous nonkeratinized epithelium. Rest of the larynx is lined by typical respiratory epithelium (i.e., pseudostratified columnar ciliated epithelium with goblet cells). The lamina propria consists of fine connective tissue. It contains scattered lymphatic nodules and many simple branched tubuloacinar glands of seromucous variety. The *intrinsic muscles* of the larynx are of skeletal type. The *larger laryngeal cartilages* (cricoid, thyroid and most of the arytenoids) are of hyaline variety. The *smaller laryngeal cartilages* (epiglottis, cuneiform and corniculate) and the tips of the arytenoid cartilages are of elastic type.

The **epiglottis** consists of a plate of elastic cartilage covered on both sides by mucosa. Its anterior (lingual) surface and upper part of the posterior (laryngeal) surface are covered by stratified squamous nonkeratinized epithelium. The lower half of the posterior surface is covered by respiratory epithelium. Beneath the epithelium lies the lamina propria which lodges mixed (seromucous) tubuloacinar glands. These glands are found mainly on the posterior surface, where they lie in irregular depressions in the underlying elastic cartilage. Scattered taste buds are also present between the epithelial cells on the posterior surface of the epiglottis.

TRACHEA

From within outwards, the tracheal wall consists of the following four components:

1. Mucosa.
2. Submucosa.
3. Cartilage and intrinsic smooth muscle.
4. Adventitia.

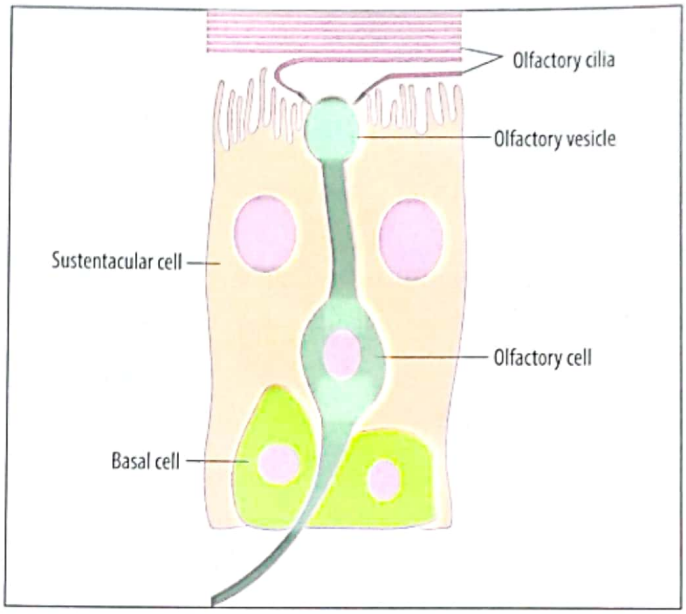

Fig. 17.1 A diagrammatic representation of the olfactory epithelium.

The **mucosa** is lined by pseudostratified columnar ciliated epithelium containing many goblet cells. The lamina propria is thin and contains many elastic fibers. Small aggregations of lymphocytes as well as diffusely scattered lymphocytes are common in the lamina propria; this lymphatic tissue is considered to be a part of the bronchus-associated lymphatic tissue (BALT).

The **submucosa** consists of loose connective tissue and contains numerous mixed (seromucous) simple branched tubuloacinar glands (Fig. 17.3). Ducts of these glands pierce the lamina propria to open onto the mucosal surface.

The lumen of the trachea is kept patent by 16 to 20 C-shaped **rings of hyaline cartilage** (Fig. 17.2) which are arranged one above the other. Open ends of these cartilages are located on the posterior surface of the trachea. The spaces between adjacent rings are filled by dense fibrous connective tissue which is fused with the perichondrium of the cartilaginous rings. The gap between the posterior free ends of each C-shaped cartilage is bridged by a band of fibroelastic connective tissue and a bundle of smooth muscle fibers known as **trachealis muscle** (Fig. 17.2). Contraction of the trachealis muscles decreases the tracheal diameter resulting in faster air flow through the trachea, which assists in dislodging of mucus (or any foreign material) from the larynx by coughing.

The **adventitia** forms the outermost covering of the trachea. It is composed mainly of loosely arranged collagen fibers. It lodges small blood vessels and autonomic nerves which supply the trachea.

THE BRONCHIAL TREE

The trachea divides into right and left principal (*primary*) bronchi. The principal bronchi give rise to lobar (*secondary*) bronchi which enter the lungs. Three lobar bronchi arise from the right principal bronchus, whereas the left

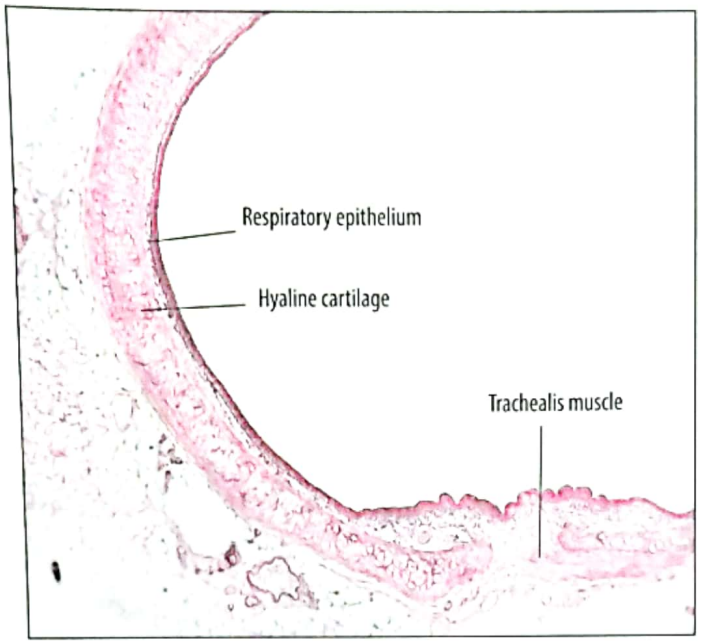

Fig. 17.2 A section of the wall of trachea under low power of light microscope.

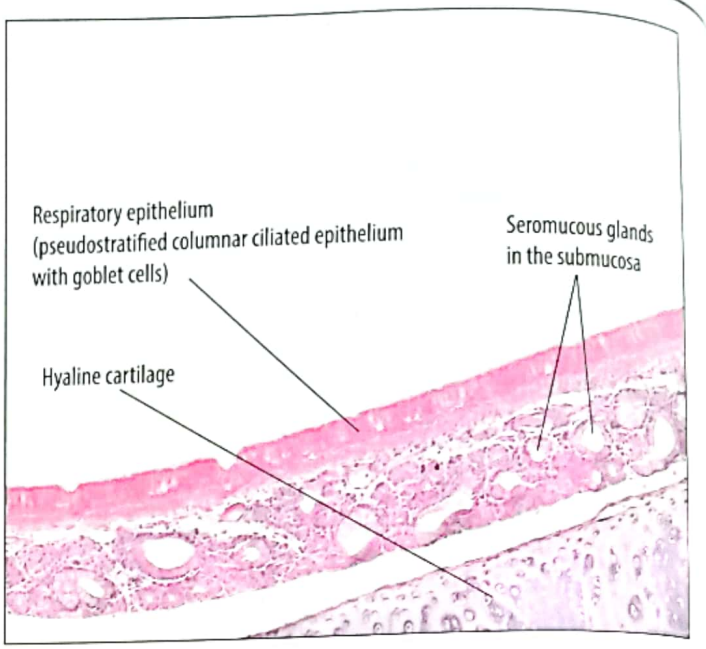

Fig. 17.3 A portion of a section through the tracheal wall as seen under high power magnification of light microscope. Note: The space between the tracheal cartilage and submucosa has been artificially created during the sectioning procedure.

principal bronchus divides into two lobar bronchi. Each lobar bronchus divides further into *segmental* (*tertiary*) bronchi which supply the bronchopulmonary segments. Within each bronchopulmonary segment, repeated divisions of bronchi occur in a dichotomous manner. After about 10 to 20 generations of divisions, the diameter of the tube is reduced to approximately 1 mm and now it is known as a *bronchiole*. Each bronchiole supplies a lung lobule which is the basic unit of the lung. These lobules are pyramidal in shape with their apices directed toward the hilum of the lung. Each lobule is delimited by a thin connective tissue septum. There are 30 to 60 lobules in each bronchopulmonary segment. Within a lung lobule, each bronchiole branches further and gives rise to 5 to 7 *terminal bronchioles*. Each terminal bronchiole branches into two *respiratory bronchioles* which divide further to give rise to *alveolar ducts*. These ducts lead into *pulmonary atria* which in turn open into *alveolar sacs* which bear numerous *pulmonary alveoli*. Gaseous exchange between air and blood occurs from the level of the respiratory bronchioles to the alveolar sacs.

Bronchus-associated Lymphatic Tissue (BALT)

The lamina propria of the trachea and bronchi contains lymphatic tissue in the form of small nodular aggregations of lymphocytes or as diffusely spread T lymphocytes, B lymphocytes, and plasma cells. This lymphatic tissue, called bronchus-associated lymphatic tissue (BALT), serves to provide protection against bacteria that enter the respiratory tract with the inspired air.

BRONCHI

The **extrapulmonary bronchi** resemble trachea in structure except for their smaller diameter. The intrapulmonary bronchi differ from the extrapulmonary bronchi in many respects. The main differences are:

1. The intrapulmonary bronchi have a smaller diameter and have a rounded contour as compared to the larger calibre and D-shaped contour of the extrapulmonary bronchi.
2. Smooth muscle appears in the form of a complete layer in the intrapulmonary bronchi.
3. The hyaline cartilage of the intrapulmonary bronchi is not present in the form of C-shaped rings, but occurs as irregular cartilaginous plates.

LUNGS

The lungs are essential respiratory organs and their chief function is to transport oxygen from the atmospheric air to the bloodstream and to convey carbon dioxide from the blood to the atmosphere. The lungs contain some parts of the proximal respiratory tract (intrapulmonary bronchi, bronchioles, and terminal bronchioles) and all parts of the distal respiratory tract (respiratory bronchioles, alveolar ducts, alveolar sacs, and pulmonary atria with pulmonary alveoli).

INTRAPULMONARY BRONCHI

The mucosa of the intrapulmonary bronchi is lined by pseudostratified columnar ciliated epithelium with goblet cells. The lamina propria is rich in elastic fibers. Beneath the lamina propria, smooth muscle is present in the form of a sheet in which muscle fibers are arranged as spirally coursing interlacing bundles. In histological sections, the bronchial mucosa appears characteristically folded (Fig. 17.4). This folding is caused by post-mortem contraction of the smooth muscle. Beneath the muscular layer is present submucosa consisting of a layer of relatively

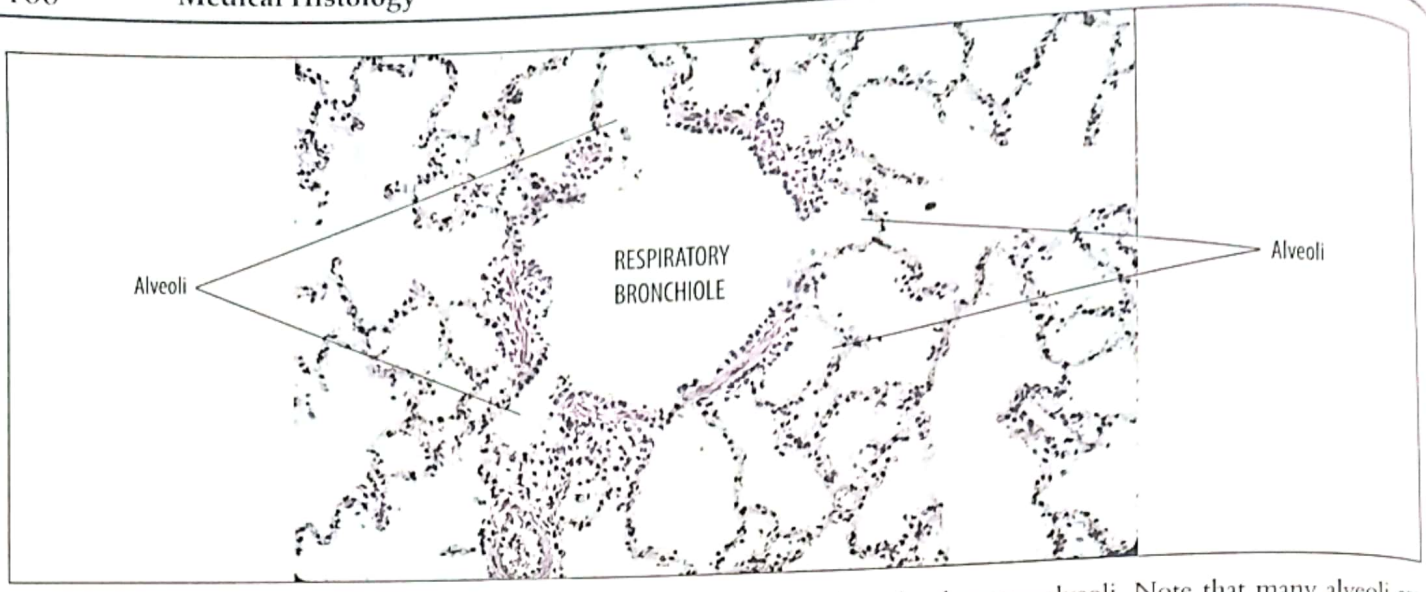

Fig. 17.6 A section through the lung tissue showing a respiratory bronchiole and pulmonary alveoli. Note that many alveoli are opening directly into the respiratory bronchiole.

RESPIRATORY BRONCHIOLES

The respiratory bronchioles are short, branching tubes which arise from the terminal bronchioles. Distinguishing feature of the respiratory bronchioles is that their wall is interrupted by thin-walled, saccular outpocketings called **alveoli** (Fig. 17.6). Between the alveoli, the bronchiolar wall is lined by simple cuboidal epithelium which bears cilia in the larger respiratory bronchioles but becomes completely nonciliated in the smaller ones. Many Clara cells are present but goblet cells and glands are entirely absent. At the margins of the alveolar openings, the simple cuboidal epithelium becomes continuous with the simple squamous epithelium of the pulmonary alveoli. Outer to the cuboidal epithelium, the wall of a respiratory bronchiole is composed of smooth muscle and connective tissue rich in elastic fibers. Functionally, the respiratory bronchioles constitute a transitional zone between the conducting and respiratory divisions of the respiratory tract.

ALVEOLAR DUCTS AND ALVEOLAR SACS

The respiratory bronchioles terminate in elongate airways called alveolar ducts. An **alveolar duct** is characterized by the presence of numerous pulmonary alveoli with almost no evidence of a bronchiolar wall. However, rings of smooth muscle fibers are present between the adjacent alveoli. In histological sections this smooth muscle is seen to bulge into the lumen of the alveolar duct as knob-like projections.

The alveolar ducts terminate in *pulmonary atria* which are the common chambers that give rise to the alveolar sacs. Two or more alveolar sacs arise from each atrium (Fig. 17.7). Each **alveolar sac** consists of a collection of alveoli opening into a central slightly larger chamber. A complex network of elastic and reticular fibers encircles the openings of atria, alveolar sacs and alveoli. The elastic fibers allow expansion of alveoli during inspiration and assist in passive recoil upon expiration. The reticular fibers serve as a support and prevent overdistension and damage to the delicate respiratory tissue. No smooth muscle is present in the wall of the pulmonary atria and alveolar sacs.

The alveolar ducts, pulmonary atria, alveolar sacs, and alveoli are all supplied by a rich network of blood capillaries.

PULMONARY ALVEOLI

The pulmonary alveoli are cup-shaped structures having very thin walls through which gaseous exchange between the blood and air takes place. Each pulmonary alveolus measures about 0.2 mm in diameter and opens either into a respiratory bronchiole, an alveolar duct, or an alveolar sac. Because of the interdigitating arrangement and tight packing, each alveolus does not have a completely separate wall. Rather, adjacent alveoli are separated from each other by *interalveolar septa*.

The pulmonary alveoli are lined by epithelial cells called *pneumocytes*. The pneumocytes are of two types: type I pneumocytes and type II pneumocytes. Both varieties of pneumocytes rest on a prominent basement membrane.

The **type I pneumocytes**, also called *type I alveolar cells* or ***squamous alveolar cells***, are extremely thin and flat epithelial cells. The simple squamous epithelium formed by these cells lines nearly 95% of the surface of each pulmonary alveolus. The total thickness of a type I pneumocyte is only 80 nm, except in the central, thicker region where a flat nucleus is present. The adjacent squamous alveolar cells are joined to each other by occluding junctions, which prevent the seepage of tissue fluid from the alveolar septa into the alveolar lumen. The principal function of these cells is to provide a barrier of minimal thickness that permits gaseous exchange between the air and blood. The type I pneumocytes cannot divide mitotically.

The **type II pneumocytes**, also called *type II alveolar cells* or ***great alveolar cells***, are cuboidal cells that occur singly or in small groups. Although these cells make up 60% of the pneumocytes, they cover only about 5% of the alveolar surface. Occluding junctions are present between

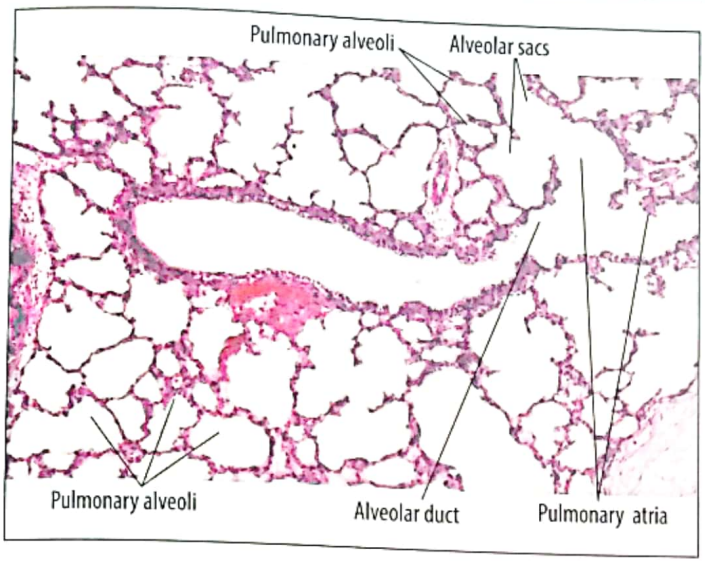

Fig. 17.7 A section of the lung tissue showing an alveolar duct, pulmonary atria, alveolar sacs, and pulmonary alveoli.

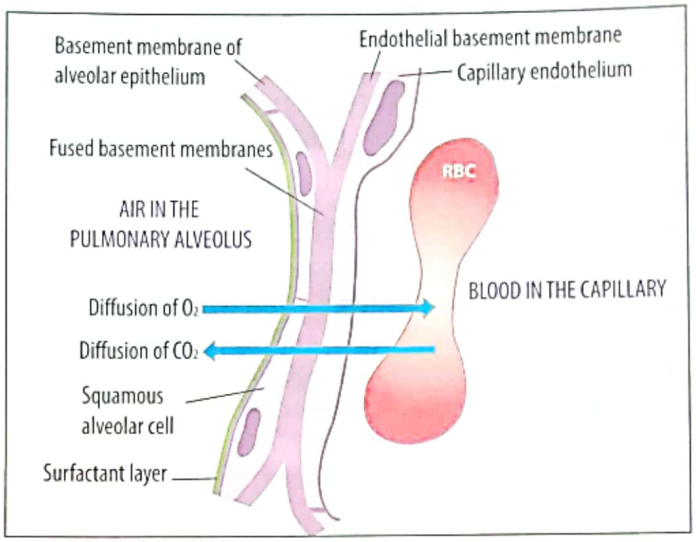

Fig. 17.8 A diagrammatic representation of the blood-air barrier.

the type II and type I alveolar cells. EM reveals that the free (luminal) surface of the type II pneumocytes bears short microvilli. In addition to the nucleus and the usual cell organelles, the cytoplasm of a type II pneumocyte contains secretory granules that exhibit stacks of parallel membranous lamellae and are, therefore, called *lamellar bodies*. The lamellar bodies do not stain well with ordinary stains. Consequently, the cytoplasm of a great alveolar cell gives a characteristic vacuolated appearance under the light microscope. The lamellar bodies have been found to contain a mixture of phospholipids, neutral lipids, and four types of unique proteins titled surfactant proteins A, B, C, and D. This oily material is secreted into the alveoli as *pulmonary surfactant* and it lines the internal surface of the alveoli in the form of a thin layer. Presence of microvilli on the free surface of the type II pneumocytes facilitates the release of the surfactant by increasing the surface available for secretion.

In addition to secreting the surfactant, the type II alveolar cells serve as stem cells for the alveolar epithelium. They divide mitotically to regenerate themselves as well as type I alveolar cells.

The **pulmonary surfactant** is a mixture of phospholipids and proteins which acts like a detergent. It serves to reduce the surface tension and thus prevents the walls of the pulmonary alveoli from collapsing and sticking together during expiration and thus facilitates alveolar expansion during inspiration.

It is important to know that, in the human fetus, production of the surfactant begins between weeks 24 and 28 of the intrauterine life. By about 35 weeks, the lungs of the fetus are producing enough surfactant to prevent the alveoli from collapsing. The premature babies born before 30 weeks of gestation have difficulty in breathing and have poor chances of survival unless they receive surfactant replacement therapy (by artificial surfactant materials) after birth, till their lungs begin to produce natural surfactant in sufficient quantities..

Interalveolar Septa

The adjacent pulmonary alveoli are separated from each other by partitions called interalveolar septa. Each interalveolar septum consists of two squamous epithelial layers between which lie blood capillaries. These continuous capillaries have wide lumina and anastomose freely to form an extensive network. The meshes of the vascular network contain reticular and elastic fibers, mast cells, wandering leukocytes, and alveolar macrophages.

The **alveolar macrophages**, also called ***dust cells***, are derived from the blood monocytes and belong to the mononuclear phagocyte system (MPS). They reside within the interalveolar septa but they also migrate into the lumen of the pulmonary alveoli. The alveolar macrophages maintain a sterile environment within the lungs by engulfing the inhaled particulate matter like dust, carbon particles, pollen, and bacteria. In histological sections, the cytoplasm of these cells is usually seen to be laden with phagocytosed carbon and dust particles (hence, the alternative name dust cells).

The interalveolar septa are not complete partitions and each is perforated by one or more pores which connect the adjacent alveoli. These *alveolar pores* measure about 10-15 µm in diameter in the expanded lung and serve to equalize the air pressure in pulmonary alveoli. The alveolar pores also permit collateral ventilation in case a bronchiole becomes occluded.

Blood-Air Barrier (Fig. 17.8)

The blood-air barrier includes those structures through which gases must pass during exchange between the blood (running in the pulmonary capillaries) and air (present in the pulmonary alveoli). This barrier consists of the following components:

i. A thin layer of the pulmonary surfactant.

ii. Type I pneumocytes and their basement membrane.

Digestive Tract 18

The digestive tract, also called digestive tube or *alimentary canal*, is a long passage that extends from the mouth to the anus. The associated large glands include the salivary glands, pancreas, and liver.

The digestive process begins in the mouth where food is cut and ground into small pieces by the teeth and is moistened by saliva, which also initiates the digestion of carbohydrates. The digestion continues in the stomach and small intestine where various digestive enzymes split the proteins, carbohydrates and fats into basic components such as amino acids, monosaccharides, and glycerides. These components are absorbed into circulation in the small intestine. Water absorption continues in the large intestine and the semisolid undigested contents are excreted as feces.

Anatomically, the digestive tract consists of the following two major components: (i) the oral cavity and oropharynx, and (ii) the tubular digestive tract (which includes the esophagus, stomach, small intestine, and large intestine).

THE ORAL CAVITY

The oral cavity is an irregular space that is bounded by the lips, cheeks and palate. It contains the tongue, gums and teeth.

LIPS (Fig. 18.1)

The substance or core of the lips is formed by skeletal muscle embedded in fibroelastic connective tissue. Each lip has three surfaces:

1. The cutaneous surface.
2. The vermilion zone.
3. The oral surface.

The **cutaneous surface** is covered by the ordinary skin containing hair follicles, sebaceous glands, and sweat glands.

The *red area* or **vermilion zone** is covered by stratified squamous epithelium which is very thinly keratinized. This epithelium is indented by numerous tall papillae of the underlying connective tissue. These papillae contain a large number of blood vessels, giving this area a reddish appearance. The vermilion zone contains no sebaceous glands or sweat glands; therefore, the lips are kept moist by saliva which is applied to them by the tip of the tongue. The epithelium and connective tissue of the red area is very richly supplied by sensory nerve endings.

The **oral surface** of the lip is covered by oral mucosa which consists of nonkeratinized stratified squamous epithelium and a connective tissue lamina propria. The lamina propria bears tall papillae which project into the overlying epithelium. Small groups of minor salivary glands, called *labial salivary glands,* are embedded in the lamina propria of the lips. These glands are simple acinar or tubuloacinar glands which produce a mucous secretion. Their secretion passes into the oral cavity through short ducts.

CHEEKS

The substance of the cheeks is also formed by skeletal muscle and fibroelastic connective tissue. External to the muscle core is present hairy skin containing sebaceous and sweat glands. Internally, the cheeks are lined by the oral mucosa which consists of stratified squamous nonkeratinized epithelium lying upon a connective tissue lamina propria which shows tall papillae. Beneath the mucosa is present a layer of loose connective tissue which constitutes the submucosa. This layer contains a large number of elastic fibers. Elasticity of this submucosal tissue prevents excessive folding of the mucosa, which could lead to injury while chewing if such folds were caught between the teeth. The submucosa also contains numerous minor salivary glands which are small glands of simple acinar or tubuloacinar glands of mucous variety. Secretion of these glands passes into the oral cavity via short ducts.

PALATE

The palate consists of two parts: (i) hard palate, and (ii) soft palate.

The **hard palate** contains bone and forms a rigid surface against which the tongue can apply force during mastication and deglutition. The mucosa of the hard palate is lined by stratified squamous keratinized epithelium which is supported by a connective tissue lamina propria. A distinct submucosa is present in some parts of the hard palate; elsewhere, the mucosa is attached directly to the underlying bone. The areas of direct mucosal attachment to the bone are along the perimeter of the hard palate, where it meets the gums, and along a midline band called *raphe.* The submucosa is composed mainly of coarse collagen fibers. In the anterior part of the hard palate, the submucosa also contains adipose tissue. In the posterior part of the palate, the submucosa lodges many simple tubuloacinar mucous glands.

The **soft palate** serves to close off the nasopharynx from the oropharynx during swallowing. Its inferior (oral) surface is covered by stratified squamous nonkeratinized epithelium, while the superior (pharyngeal) surface is lined by pseudostratified columnar ciliated epithelium.

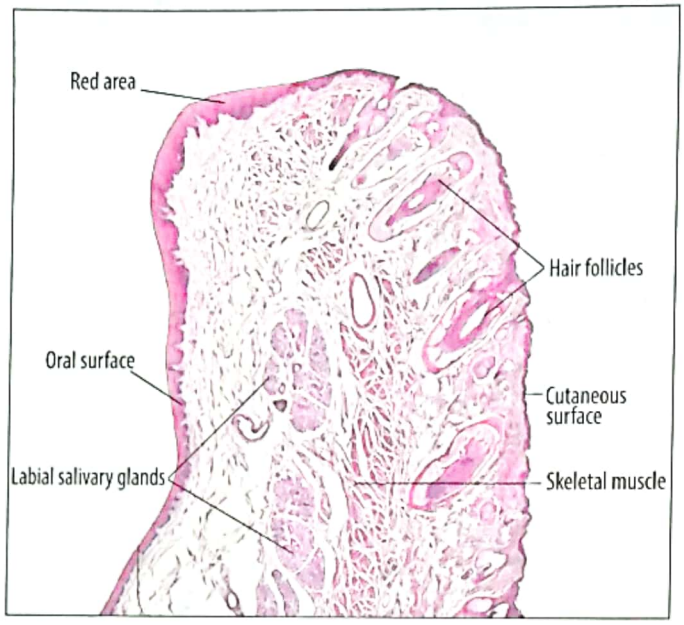

Fig. 18.1 A sagittal section through the lip showing its microscopic structure.

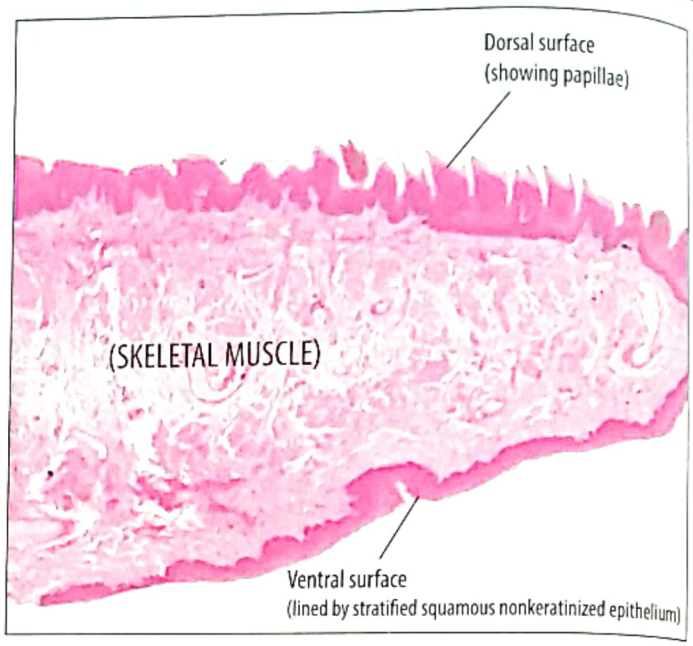

Fig. 18.2 A sagittal section through the tongue showing its microscopic structural features under low power magnification of light microscope.

Both varieties of the epithelium rest upon a connective tissue lamina propria which contains simple tubuloacinar mucous glands on the oral side and mixed glands on the pharyngeal side. The central substance or core of the soft palate is formed by skeletal muscle.

TONGUE

The tongue (Latin term: *lingua*) is a muscular organ covered by a thin mucous membrane (Fig. 18.2). It lies partly in the oral cavity and partly in the oropharynx. It is highly mobile and can be shifted into a number of different positions and can assume various shapes. The tongue has two surfaces: a superior surface (also called dorsal surface) and an inferior surface (also known as ventral surface).

Functions of the Tongue

1. **Taste.** The receptors for the taste sensation, called taste buds, are located on the tongue.
2. **Speech.** The movements of the tongue play a crucial role in speaking.
3. **Chewing and swallowing.** The tongue assists the teeth in chewing the ingested food and then passing it down the throat as the initial part of the swallowing process.
4. **Cleaning.** The movements of the tongue dislodge food particles stuck to the teeth, gums and cheeks, so that these can be spat out or swallowed.

Being a muscular organ, the bulk of the tongue consists of skeletal muscles. The muscles of the tongue can be divided into the intrinsic and extrinsic groups. The intrinsic muscles originate and terminate in the tongue and are not attached to any bone. Considering the direction of the muscle fibers, the intrinsic muscles can be divided into four groups: superior longitudinal, inferior longitudinal, transverse, and vertical. The extrinsic muscles of the tongue are genioglossus, styloglossus, palatoglossus, and hyoglossus.

The mucous membrane of the tongue is closely adherent to the muscle mass and consists of epithelium and lamina propria. The epithelium is of stratified squamous variety, being partially keratinized on the dorsal surface and nonkeratinized on the ventral surface of the tongue.

The dorsal surface of the tongue is rough and irregular and can be divided into anterior (2/3) and posterior (1/3) parts by a V-shaped groove called *sulcus terminalis*. The anterior two-thirds of the dorsal surface of tongue are rough due to the presence of numerous small projections called *lingual papillae*. The posterior one-third of this surface appears irregularly nodular because the root of tongue lodges aggregations of lymphoid nodules which constitute the *lingual tonsil*.

LINGUAL PAPILLAE

The lingual papillae are formed of a central core of connective tissue and a covering layer of stratified squamous epithelium. On the basis of their shape, these papillae are classified into **four types**: (1) filiform papillae, (2) fungiform papillae, (3) circumvallate papillae, and (4) foliate papillae.

Filiform Papillae

These papillae (Fig. 18.3) are the most numerous of the lingual papillae; they are also the smallest. They are distributed over the entire dorsal surface of the body (i.e., anterior two thirds) of the tongue. The filiform papillae are covered by stratified squamous keratinized epithelium that tapers to a point which is directed backwards. It is due

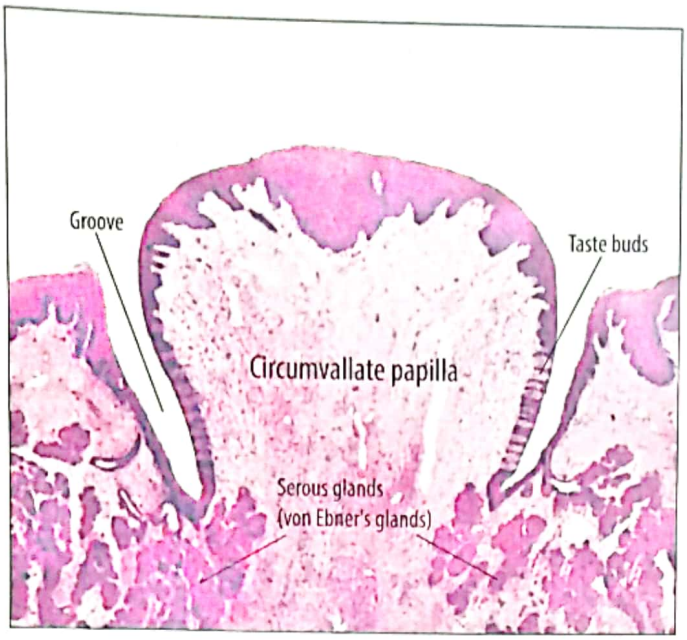

Fig. 18.5 A section through the dorsum of the tongue showing a circumvallate papilla and von Ebner's glands

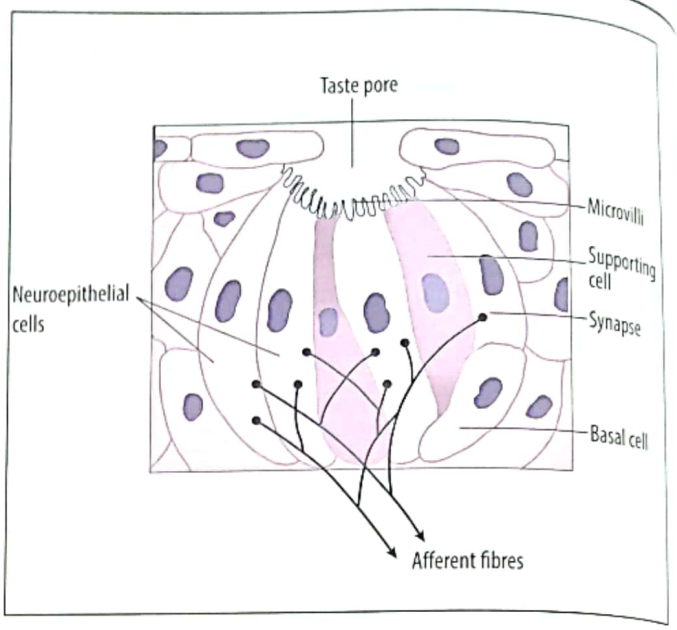

Fig. 18.6 A diagrammatic representation of the microscopic structure of a taste bud.

in the region of the circumvallate papillae. Ducts of these glands open into the grooves surrounding the circumvallate papillae. Watery secretion of the von Ebner's glands washes away food particles from these grooves, allowing reception of new gustatory stimuli by the taste buds of the circumvallate papillae.

3. **Mucous glands of the root.** These are numerous small purely mucous glands which lie in the posterior one third of the tongue. Their ducts open into the crypts of the lingual tonsil.

TASTE BUDS

The taste buds are receptors for the taste sensation. They are involved in the perception of five elements of the taste perception (sweet, saltish, bitter, sour, and umami). The taste buds are mainly located on the dorsal surface of the body of the tongue, but also occur on the soft palate and the laryngeal surface of the epiglottis. The lingual taste buds are embedded within the stratified squamous epithelium covering the fungiform, foliate and circumvallate papillae and rest on the basal lamina of the epithelium. In the ordinary (H&E) sections, a taste bud appears as an oval, pale-staining body which is 70-80 µm long and 40-50 µm wide. The long axis of the taste bud extends through the full thickness of the epithelium covering the papillae. In stained sections, the taste buds appear distinctly paler than the adjacent stratified squamous epithelium (Fig. 18.5). The apex of each taste bud communicates with the oral cavity through a small aperture called the *taste pore*.

A taste bud (Fig. 18.6) is composed of 50-90 cells which are classified into three types: (1) sustentacular cells, (2) neuroepithelial cells, and (3) basal cells.

The **sustentacular cells** (supporting cells) are elongated cells that extend from the basal lamina to the taste pore. At their apical end these cells bear long microvilli that project into the taste pore. Some histologists distinguish two varieties of sustentacular cells: *dark cells* and *light cells*, which are titled type I and type II cells, respectively.

The **neuroepithelial cells,** also called ***taste cells*** or type III cells, are the gustatory receptor cells. These are also elongated, tall columnar cells that extend from the basal lamina to the taste pore. They also bear long microvilli on their free surface which project into the taste pore. At their base, the taste cells form synapses with afferent nerve fibers through which the taste sensation is conveyed to the CNS. Near their apical ends, the neuroepithelial and sustentacular cells are joined to each other and to the surrounding epithelial cells by tight junctions.

The **basal cells** are small, roughly oval cells located close to the basal lamina. These cells serve as stem cells for other cells of the taste bud. They divide and differentiate into sustentacular cells and taste cells to ensure constant turnover of these cells.

GUMS

The gums (gingivae) are lined by a stratified squamous epithelium which is keratinized to a variable degree. Beneath the epithelium lies a lamina propria of connective tissue bearing tall papillae that extend into the covering epithelium. These papillae contain rich capillary plexuses. The blood in these plexuses is responsible for the reddish appearance of the gums. The lamina propria contains a large number of coarse, interweaving collagen fibers which bind the gums closely to the underlying alveolar bone. No submucosa and no glands are present in the gums.

TEETH (Fig. 18.7)

Each tooth consists of three parts:

1. A *crown* that projects (and is visible) beyond the gum margin.

Although enamel is the hardest substance in the body, it can be decalcified by the acids produced as a result of fermentation of sugars sticking to the teeth. This fermentation is caused by the bacteria which are normally present in the oral cavity. This destructive demineralization of the enamel, which results in the formation of cavities in the teeth, is called *dental caries*. Because no ameloblasts are present to restore the lost enamel, the damaged enamel needs to be replaced by artificial dental fillings.

PERIODONTIUM

The tissues and structures that help to maintain the teeth in their sockets are collectively known as periodontium. The periodontium includes the cementum, periodontal ligament, bone of the alveolar sockets, and gums.

CEMENTUM

This tissue covers the dentin of the root of the tooth. In composition, the cementum resembles ordinary bone in being made up of collagen fibers embedded in a calcified matrix, but it does not have a lamellar structure and there are no osteons. In the upper one third (or one half) of the length of the root, the cementum is devoid of cells and is referred to as *acellular cementum*. Remainder of the cementum contains cells called ***cementocytes***. These cells resemble osteocytes and lie in lacunae which are interconnected by canaliculi. A layer of cement-forming cells, called *cementoblasts,* is present on the outer surface of the cementum which continue to form cementum throughout the life of the tooth.

PERIODONTAL LIGAMENT

This is a layer of dense irregular connective tissue that lies between the alveolar bone and cementum of the tooth. The periodontal ligament fixes the tooth firmly to the bony wall of its socket. However, it permits slight movement of the tooth in every direction (thus helping to absorb masticatory forces).

The periodontal ligament consists of mainly of thick bundles of type I collagen fibers which pass obliquely from the wall of the alveolar socket to the cementum of tooth. The ends of the collagen fibers are anchored into the alveolar bone on one side and into the cementum on the other side. The space between the collagen fibers is filled with proteoglycans. The periodontal ligament is richly cellular and contains fibroblasts, undifferentiated mesenchymal cells, macrophages, mast cells, plasma cells, and lymphocytes. The fibroblasts not only produce new collagen fibers of the periodontal ligament but also cause dissolution and resorption of the old fibers. This ensures a continuous turnover of the collagen fibers in accordance with the demands of the tooth stress and movement. The undifferentiated mesenchymal cells differentiate into fibroblasts, cementoblasts, osteoblast, or osteoclasts, as and when needed. The macrophages, mast cells, plasma cells, and lymphocytes play an important role in defense against infection by the pathogenic microorganisms.

The periodontal ligament also contains blood vessels (capillaries) and nerve fibers. The nerve fibers are mainly of sensory variety and convey the pain and pressure sensations.

PULP CAVITY AND DENTAL PULP

The pulp cavity consists of an upper dilated portion called *pulp chamber* and a lower narrower tubular portion known as *root canal*. The root canal communicates with periodontal ligament through the *apical foramen*. The dental pulp is a soft jelly-like loose connective tissue. It consists of type I and type II collagenous fibrils and a ground substance composed of proteoglycans. It also contains odontoblasts, mesenchymal cells, fibroblasts, lymphocytes, plasma cells, and macrophages. The odontoblasts lie as a single layer at the periphery of the pulp and their processes extend into the adjacent dentinal tubules of the dentin. The pulp also contains blood vessels, lymph vessels, and nerve fibers that enter (or leave) the pulp cavity through the apical foramen.

Blood Supply of the dental pulp. The dental pulp is a highly vascular tissue. One or two arterioles enter the pulp through each apical foramen, travel through the pulp of the root canal and finally give rise to a rich capillary plexus in the pulp chamber immediately beneath the odontoblast layer. The capillaries are of fenestrated variety, indicating high metabolic activity of the odontoblasts. Usually two venules drain the capillary bed and leave through the apical foramen.

Nerve Supply of the dental pulp. Vasomotor sympathetic nerve fibers travel with the blood vessels of the pulp and supply the smooth muscle of the arterioles and thus function in regulating the blood flow in the capillary network. Somatic afferent (sensory) nerve fibers derived from the branches of the 5th cranial nerve also enter the dental pulp through the apical foramen. Most of these nerve fibers are myelinated, but upon reaching the pulp chamber, they lose their myelin sheath and give rise to a nerve plexus. A large number of free nerve endings arise from this nerve plexus. Some of these nerve endings terminate in close proximity to the cell bodies of the odontoblasts, while others extend into the dentinal tubules and make contact with the odontoblast processes. These processes are believed to perform a transducer function in conveying stimuli from the tooth surface to the nerves in the dental pulp. Excessive heat, cold or pressure is transmitted by the afferent nerves of the tooth to the brain as pain stimuli.

THE OROPHARYNX

That part of the pharynx which conducts the food from the oral cavity into the esophagus is called oropharynx. This part of the pharynx is covered internally by a mucosa which is lined by stratified squamous nonkeratinized epithelium. This epithelium rests on a thin lamina propria

of loose connective tissue. Outer to the mucosa is present the muscularis of the pharynx which consists of the skeletal muscles of the pharyngeal wall.

THE TUBULAR DIGESTIVE TRACT

The tubular digestive tract (alimentary canal) is a continuation of the oral cavity. It is about nine meters long and has four morphologically distinct portions: esophagus, stomach, small intestine, and large intestine. In the alimentary canal the food is churned, liquefied, and digested. The nutritive food elements and water are absorbed, whereas the indigestible components of the food are eliminated as fecal excretion.

General Structural Plan of the Alimentary Canal

(Fig. 18.8)

All the four portions of the tubular digestive tract have the same basic structural organization. Their wall comprises the following four concentric layers or coats:

1. Mucosa.
2. Submucosa.
3. Muscularis externa.
4. Serosa or, in certain places, adventitia.

MUCOSA

The mucosa (mucous membrane) is the innermost coat. It consists further of three layers: (i) epithelium, (ii) lamina propria, and (iii) muscularis mucosae.

The **epithelium** lines the luminal surface of the mucosa. It varies in different parts of the alimentary canal and is adapted to the specific functions performed by each part of the digestive tube. The epithelium also invaginates into the underlying connective tissue to form glands.

The **lamina propria** is a layer of loose connective tissue present beneath the mucosal lining epithelium. This layer is of variable thickness and contains mucosal glands, blood vessels, lymph vessels, and the mucosa-associated lymphoid tissue (MALT). The blood capillaries of the lamina propria are of fenestrated variety, so that the absorbed products of digestion can diffuse into the circulation easily. In the lamina propria of the small intestine, the lymphatic capillaries are very abundant and some absorbed lipids and proteins pass into these capillaries.

The MALT of the digestive tube consists of the diffuse as well as nodular lymphoid tissue. In the lamina propria of the ileum and vermiform appendix, the lymphatic nodules are aggregated to form masses of considerable size. In the lamina propria of the ileum, the aggregates of lymphatic nodules form small, roughly oval masses which are called Peyer's patches. The mucosa-associated lymphatic tissue serves as an immunologic barrier against those ingested antigens that manage to penetrate the mucosal epithelium from the lumen of the digestive tube.

The **muscularis mucosae** forms the boundary between the mucosa and submucosa. It consists of a thin layer of smooth muscle, in which the muscle fibers are arranged as two concentric layers. In the inner layer the smooth muscle fibers are circularly arranged, while in the outer layer they run longitudinally. Contraction of this smooth muscle causes independent local movement and folding of mucosa that facilitate digestion and absorption.

SUBMUCOSA

The submucosa is a layer of dense irregular connective tissue that lies between the mucosa and muscularis externa. The submucosa lodges comparatively larger blood vessels that send branches to mucosa and muscularis externa. The submucosa also contains lymphatic vessels and a nerve plexus. No glands are present in the submucosa except in the esophagus and duodenum.

The **submucosal nerve plexus** (also called *Meissner plexus*) is a network of neurons and interconnecting unmyelinated nerve fibers present in the connective tissue of the submucosa. Most of the nerve cells are motor neurons, but sensory neurons and interneurons are also present. Nerve fibers derived from the Meissner's plexus innervate the muscularis mucosae and intestinal glands. The submucosal nerve plexus constitutes a component of the enteric nervous system.

MUSCULARIS EXTERNA

The muscularis externa generally consists of two thick, concentric layers of smooth muscle. In histological sections, the muscle appears to be arranged as an inner circular layer and an outer longitudinal layer but actually the muscle fibers in both the layers are arranged in a spiral manner. The muscle fibers in the inner layer form a tight spiral, whereas those in the outer layer form a loose spiral. Contractions of the smooth muscle of muscularis externa mix and propel the contents of the digestive tract. Contraction of the inner layer of muscularis externa constricts the gut lumen, whereas contraction of the outer layer shortens the gut (and widens the lumen). Rhythmic contractions of the muscle layers of muscularis externa produce waves of contraction in the wall of the digestive tube which are called *peristalsis*.

At some locations along the digestive tube, the basic muscle pattern in the muscularis externa is modified by a localized increase in the circular layer of the smooth muscle; such localized thickenings of the circular smooth muscle are called *sphincters*. Contraction of the sphincters leads to occlusion of the gut lumen at particular locations, and thus delays the onward passage of the luminal contents. The pyloric sphincter, which is located at the junction of the stomach and duodenum, constricts to delay the gastric emptying and, thus, provides sufficient time for food breakdown in the stomach. The esophagogastric sphincter located at the lower end of the esophagus, prevents the reflux of the gastric contents into the esophagus. The ileocecal sphincter (ileocecal valve) delays the discharge of the small intestinal contents into the cecum. The internal

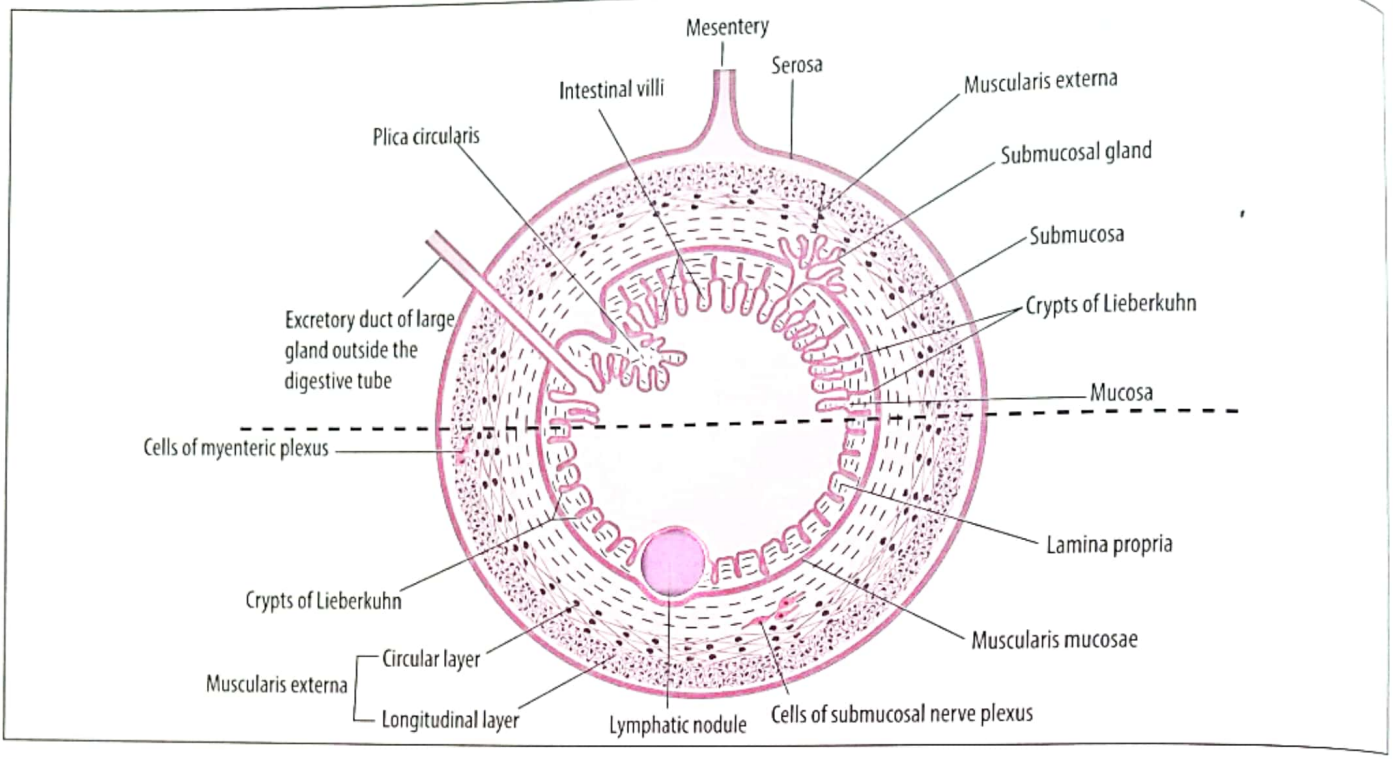

Fig. 18.8 Schematic representation of general structure of the tubular digestive tract. The upper half of the figure shows special structural features seen in the mucosa of small intestine. The lower half of the figure depicts structural features of the large intestinal mucosa.

anal sphincter of smooth muscle helps to retain the fecal material in the rectum until controlled defecation is possible.

Between the two smooth muscle layers of the muscularis externa is found a nerve plexus known as **myenteric nerve plexus** or *Auerbach plexus*. This plexus also consists of motor neurons, sensory neurons, and interneurons connected to each other by unmyelinated nerve fibers. The myenteric plexus innervates the smooth muscle of the muscularis externa and constitutes the second component of the enteric nervous system.

SEROSA AND ADVENTITIA

Mostly, the digestive tube is surrounded completely by serosa, which consists of: (i) a layer of simple squamous epithelium, called mesothelium, and (ii) a thin layer of loose connective tissue underlying the mesothelium. The serosa is actually the visceral layer of peritoneum described in gross anatomy. The serosa contains variable amounts of adipose tissue, the amount being very high in obese persons. Large blood vessels, lymphatics, and nerves of the digestive tube run in the serosa.

Some parts of the digestive tube are not covered by a serosa at all (e.g., the thoracic part of the esophagus and the anal canal), while some are only partially covered by the serosa (e.g., the duodenum, ascending colon, descending colon, and rectum). All surfaces of the digestive tube not covered by serosa are covered by a layer of connective tissue called adventitia. The adventitia also conducts large blood vessels, lymphatics, and nerves of the digestive tube. It blends with the adjacent connective tissue and thus serves to bind specific parts of the digestive tract to the abdominal wall.

ENTERIC NERVOUS SYSTEM

The submucosal and myenteric nerve plexuses have numerous interconnections and, together, these two nerve plexuses constitute the *enteric nervous system,* which is the self-contained nervous system of the alimentary tract. This system contains about 100 million motor neurons, interneurons, and sensory neurons. These neurons are present in the form of small clusters called ganglia. The neighboring ganglia are connected to each other by nerve fibers.

The enteric nervous system is self-sufficient but normally its functions are influenced and modulated by the sympathetic and parasympathetic nervous systems. The preganglionic parasympathetic fibers synapse on the motor neurons of the enteric nervous system which serve as postganglionic parasympathetic neurons. Post-ganglionic fibers from these neurons innervate the smooth muscle and glands of the alimentary canal. Parasympathetic input has a stimulatory effect on the enteric nervous system (i.e., it stimulates peristaltic contraction of the smooth muscle and secretory activity of the intestinal glands). Postganglionic sympathetic fibers (from sympathetic ganglia located outside the gut wall) also terminate on the motor cells of the enteric nervous system and exert an inhibitory effect on these neurons. The sensory neurons (and interneurons) are involved in the local reflex activity. However, the sensory neurons also send fibers to the parasympathetic neurons in the celiac and mesenteric plexuses (from where this visceral sensory information is conveyed to the CNS).

It is important to note that experiments have proved that the enteric nervous system can function autonomously

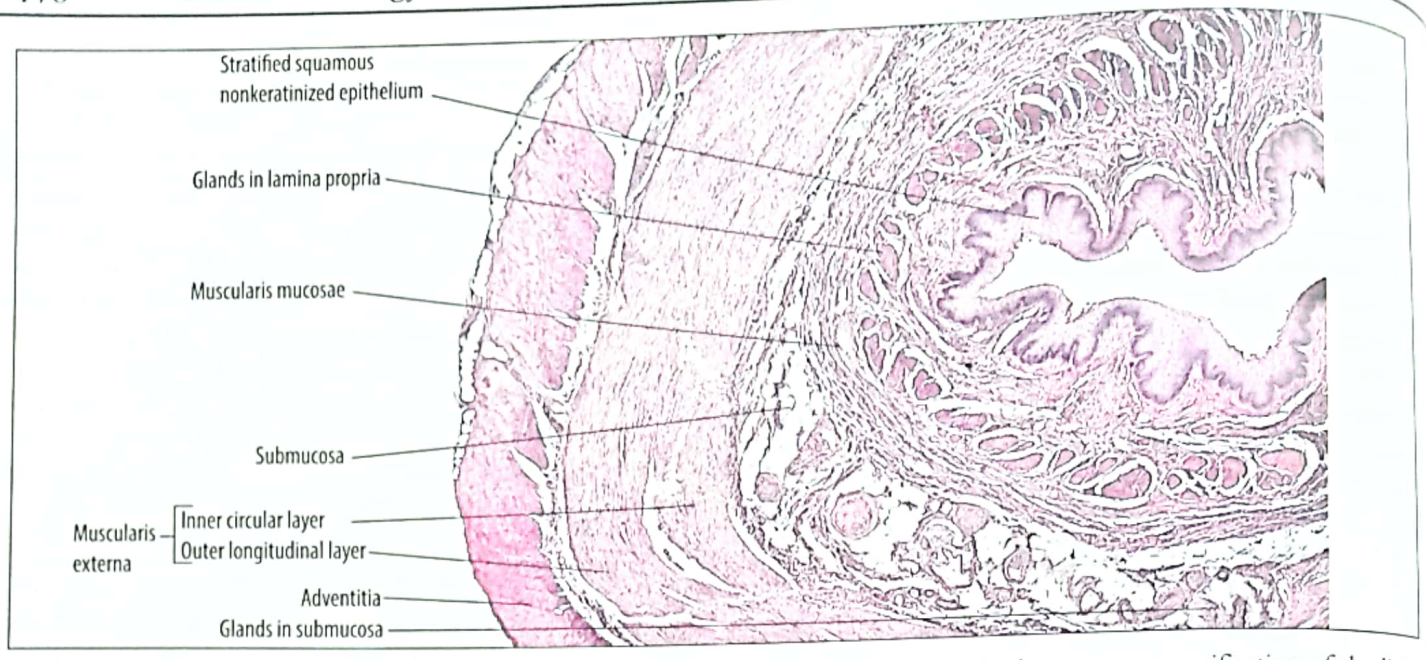

Fig. 18.9 A transverse section through the lower third of the esophagus as seen under the low power magnification of the light microscope.

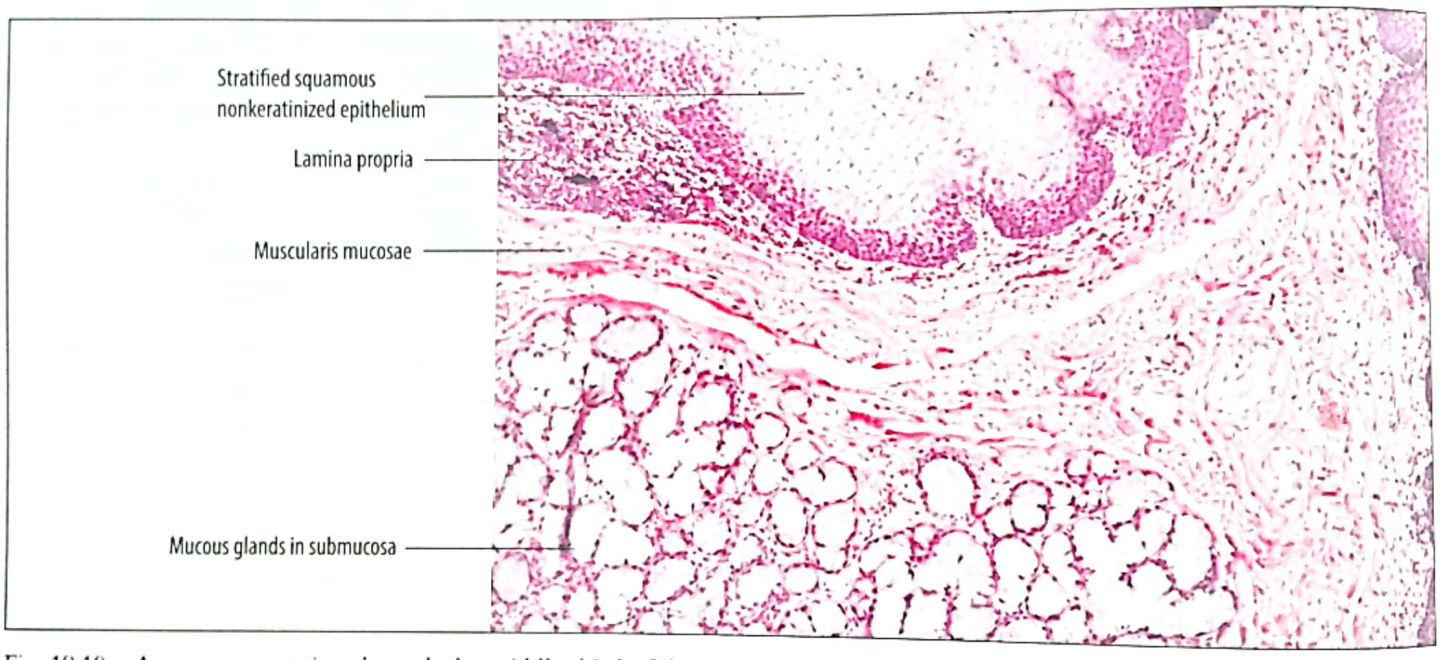

Fig. 18.10 A transverse section through the middle third of the esophagus as seen under the high power magnification of the light microscope.

The gastric mucosa consists of the usual three layers: epithelium, lamina propria, and muscularis mucosae.

EPITHELIUM OF THE STOMACH

The mucosal surface of the stomach and foveolae are lined by a mucus-secreting simple columnar epithelium. The columnar cells of this epithelium are called ***surface mucous cells*** or ***foveolar cells***. The apical cytoplasm of the surface mucous cells is filled with mucinogen granules. The mucin secreted by these cells becomes hydrated after its release from the cells and is transformed into mucus.

During preparation of tissue for light microscopy the mucinogen granules are washed away from the apical regions of the foveolar cells and, in the routine H&E stained sections, these regions appear empty and unstained. The nucleus of each surface mucous cell is roughly spherical in shape and is located in the basal region of the cell. EM studies reveal that the apical surface of the cell bears short microvilli. However, no brush border, like that of small intestinal epithelium, can be seen. A prominent Golgi apparatus is present between the nucleus and the apical cup of mucin granules. The basal cytoplasm of these cells contains mitochondria and a moderate amount of rough endoplasmic reticulum. The cells are attached to each other by juxtaluminal occluding junctions and have occasional desmosomes on their lateral surfaces.

The surface mucous cells also secrete bicarbonate, due to which the mucus produced by the cells becomes alkaline.

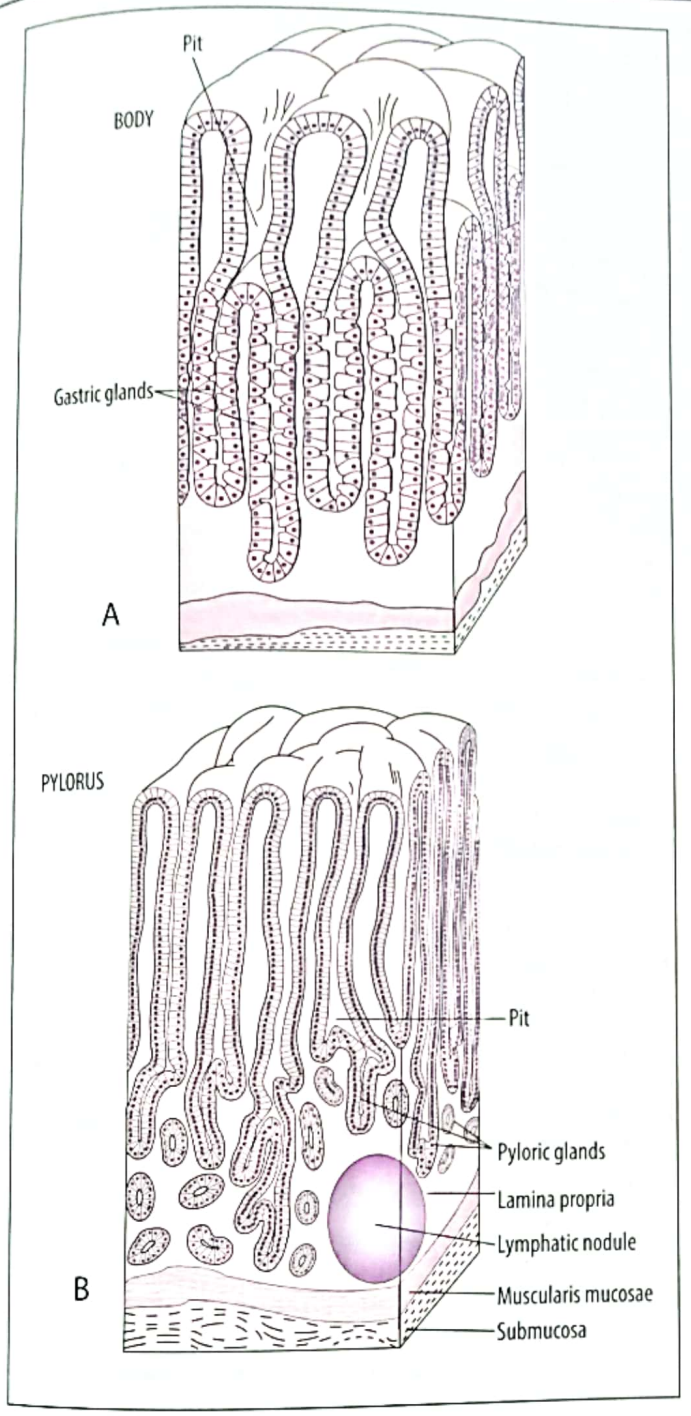

Fig. 18.11 Diagrammatic representation of the microscopic structure of different parts of the stomach: A. Fundus and body, B. Pylorus.

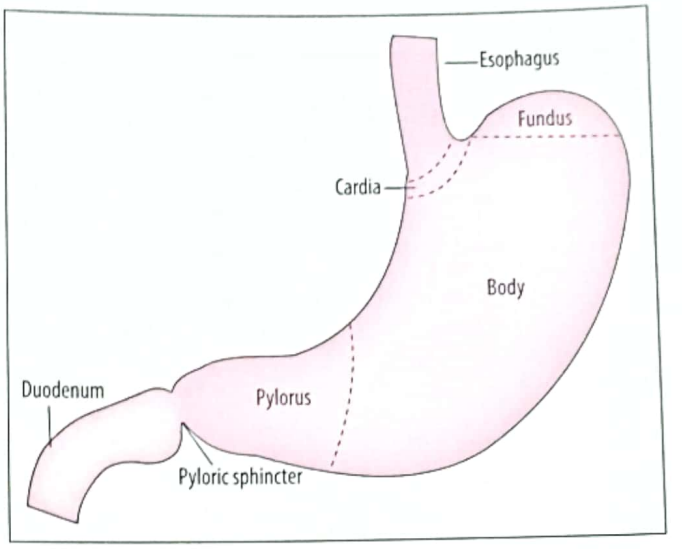

Fig. 18.12 Diagram showing different regions of the stomach.

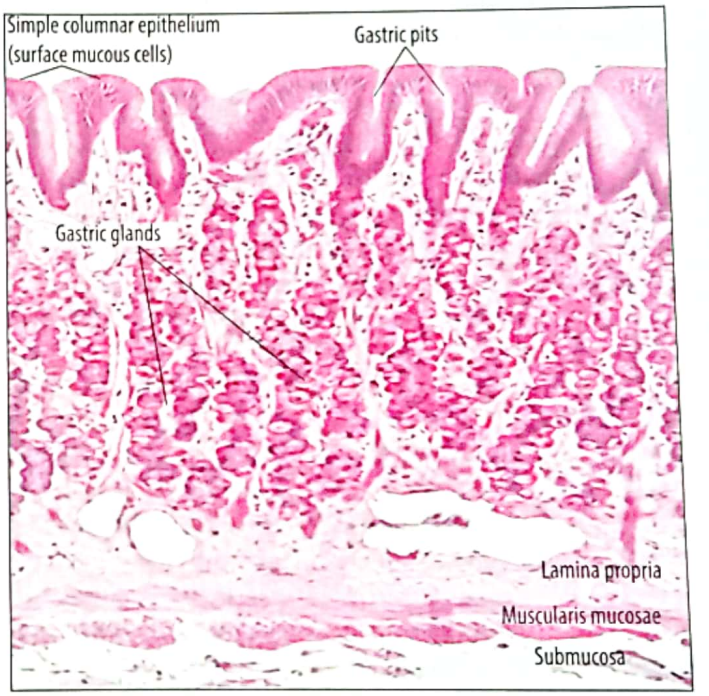

Fig. 18.13 A section through the mucosa of the body of stomach.

This alkaline mucus forms a thick and viscous, gel-like coat which adheres to the epithelial surface and provides protection to the gastric mucosa in two ways: (i) it protects the mucosa against the abrasive effect of the food, (ii) due to its alkaline nature, this mucus neutralizes the HCl present in the gastric juice and thus protects the gastric mucosa against the corrosive effect of the acid.

The **lamina propria** of the gastric mucosa is a loose, highly vascularized connective tissue that contains a rich population of fibroblasts, mast cells, plasma cells, and lymphocytes. Most of the space within the lamina propria is occupied by the glands of stomach. These glands have different structure in various regions of the stomach. A detailed description of the glands of the stomach is given later.

The **muscularis mucosae** of the stomach is a thin layer in which smooth muscle fibers are arranged into an inner circular and an outer longitudinal lamina.

SUBMUCOSA

The submucosa of the stomach consists of dense connective tissue containing variable amounts of adipose tissue. It also contains larger blood vessels and the submucosal plexus (Meissner's plexus) of nerves. No glands are present in the submucosa of the stomach.

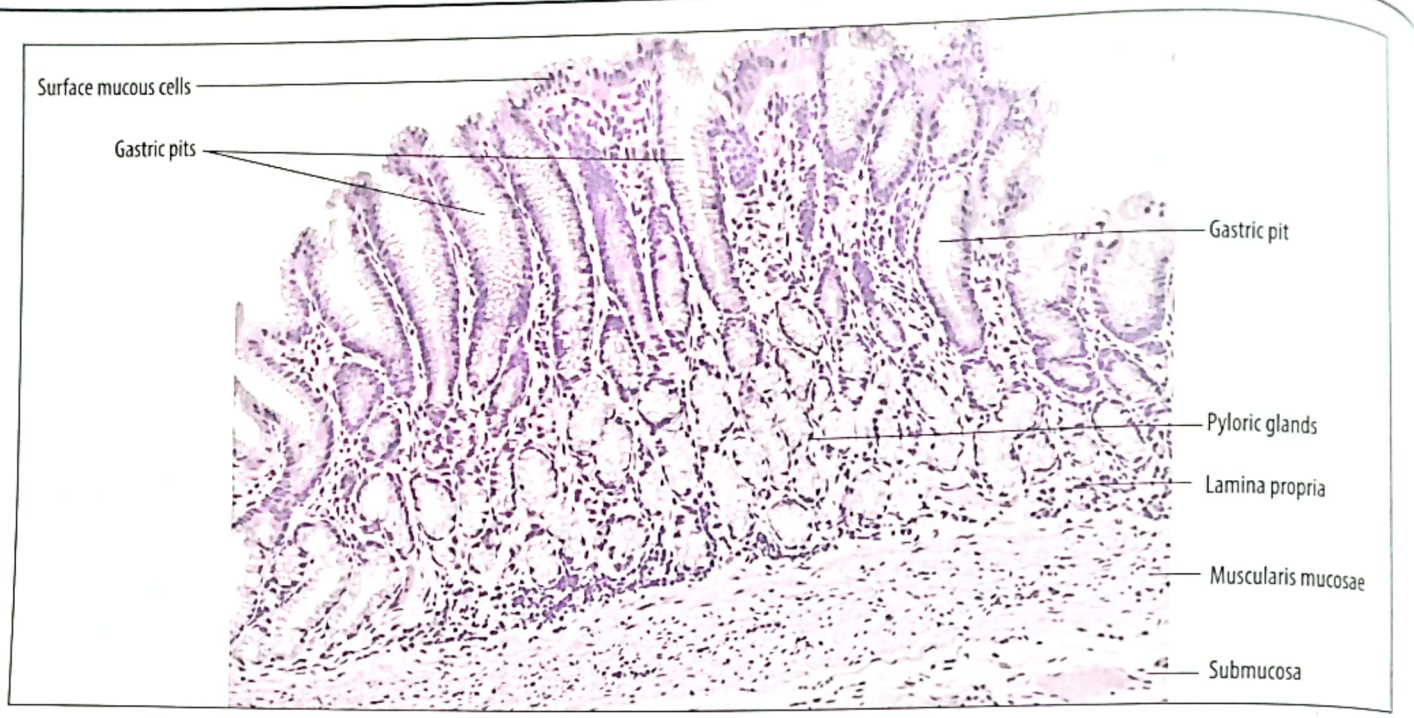

Fig. 18.14 A section through the mucosa of the pyloric part of stomach. Note the long and deep gastric pits lined by mucus-secreting cells and the mucous pyloric glands.

MUSCULARIS EXTERNA

The muscularis externa of the stomach is composed of *three layers* of smooth muscle: an inner oblique, a middle circular, and an outer longitudinal layer. The middle circular layer is greatly thickened at the pylorus to form the *pyloric sphincter*. The myenteric plexus of nerves is present between the middle circular and outer longitudinal layers of the muscularis externa.

SEROSA

The serosa is the visceral layer of peritoneum covering the outer surface of the stomach. It consists of a thin layer of connective tissue covered by mesothelium and provides a nearly friction-free environment for the churning movements of the stomach.

GLANDS OF STOMACH

The lamina propria of the gastric mucosa contains a large number of glands that open into the gastric pits. On the basis of distribution and difference in structure, these glands are classified into the following three varieties:

1. *Gastric glands* which are found in the fundus and body of the stomach.
2. *Cardial glands* which lie in the narrow region around the inlet of the stomach.
3. *Pyloric glands* which are located in the pyloric antrum and pyloric canal.

GASTRIC GLANDS

The gastric glands, also called ***fundic glands***, make the greatest contribution to the gastric juice and are responsible for the production of the acid and most of the enzymes secreted by the stomach. The gastric glands are of simple branched tubular variety and each of them is subdivided into three regions: isthmus, neck, and base (Fig. 18.15).

The *isthmus* is the upper short segment of the gland that opens into the gastric pit. The *neck* is the narrower, relatively long middle part of the gland. The *base* is the terminal, shorter and wider part of the gland which may be branched.

The epithelium lining the gastric glands contains four types of cells: (1) mucous neck cells, (2) parietal cells, (3) chief cells, and (4) enteroendocrine cells.

Mucous Neck Cells

The mucous neck cells are regarded to be extensions of the surface mucous cells into the necks of the gastric glands. The cells usually occur in small groups; parietal cells are interspersed between these groups. The mucous neck cells are generally shorter and contain a smaller number of mucinogen granules in their apical cytoplasm than the surface mucous cells. The mucus produced by these is also less alkaline. The mucous neck cells undergo active mitosis and serve as progenitor cells (stem cells) for the renewal of the surface mucous cells and gastric gland cells.

Parietal Cells (Fig. 18.16)

The parietal cells secrete hydrochloric acid and are also called *oxyntic cells*. These cells are mainly present in the isthmus and neck portions of the gastric glands where they are found to be dispersed among the mucous neck cells, but a few parietal cells are also seen to be located in the basal parts of these glands.

The parietal cells are large, round to pyramidal cells located

bases of the gastric glands. They are columnar or pyramidal cells, each having a spherical nucleus that lies toward the base of the cell. The chief cells exhibit the characteristic features of protein-secreting cells. The basal region of the chief cells contains abundant RER, due to which this region of the cell takes a basophilic stain. A well-developed Golgi apparatus is present in the supranuclear position. The apical region contains secretory granules that house the proenzyme **pepsinogen**; these granules impart acidophilia to the apical region of the cell. After its release into the lumen of the stomach, the pepsinogen is converted by the HCl of the gastric juice into *pepsin*, which is a highly active proteolytic enzyme. The chief cells also secrete ***gastric lipase***, which takes part in the breakdown of many lipids in the stomach.

Enteroendocrine Cells

These are small cells located in the necks and bases of the gastric glands. They rest on the basal lamina but usually do not reach the lumen of the gland. These cells belong to the diffuse neuroendocrine system (DNES). EM shows that the cytoplasm of these cells contains small membrane-bound secretory granules. The secretory granules of the enteroendocrine cells are released into the blood capillaries (and lymph capillaries) present in the lamina propria surrounding the gland.

Most of the enteroendocrine cells of the gastric glands are EC cells (enterochromaffin cells) and their function is to secrete serotonin, which enhances gastric motility and promotes gastric emptying.

CARDIAL GLANDS

These glands are found only in the *cardia* (the narrow region surrounding the esophageal orifice). Cardial glands are simple tubular glands which are lined by mucus-secreting cells. A few enteroendocrine cells and occasional parietal cells are found to be interspersed between the mucus-secreting cells, but no chief cells are present in these glands. Structurally, the mucus-secreting cells of the cardial glands resemble the mucous neck cells of the gastric glands.

The secretion of the cardial glands is contributed to the gastric juice. In addition, the mucous secretions of these glands, and those of the esophageal cardiac glands, provide protection to the esophageal epithelium against possible damage by reflux of the gastric juice. The mucous cells of the cardial glands also secrete lysozyme.

PYLORIC GLANDS

These are simple tubular branched glands located in the lamina propria of the pyloric antrum and pyloric canal. Because the gastric pits are deeper in these regions, the glands themselves are usually short, but they are very tortuous and in sections appear as transversely or obliquely cut tubules. Pyloric glands are lined mainly by mucus-secreting cells which are identical to mucous neck cells. A few parietal cells and many enteroendocrine cells are interspersed among the mucus-secreting cells. The pyloric glands contain enteroendocrine cells, most of which are **G cells** that secrete the hormone ***gastrin***, which stimulates HCl production by the parietal cells of the fundic glands.

THE INTESTINES

The intestines constitute a long continuous tube running from the stomach to the anus. Most of the nutrients and water are absorbed in the intestines. On the basis of difference in the diameter, the intestines are divided into two major segments: (i) the initial segment, titled small intestine, averages 2.5 cm in diameter, and (ii) the distal segment, designated large intestine, has an average diameter of 7.5 cm. It is important to note that the small intestine, although smaller in diameter, has a much greater length (7 meters) than the large intestine, which is only 1.5 meters long.

THE SMALL INTESTINE (Fig. 18.17)

The small intestine is a highly convoluted tube which is divided further into three segments: duodenum, jejunum and ileum. These segments exhibit nearly similar histological structure, but certain structural differences allow the identification of duodenum, jejunum, and ileum under the microscope.

Functions of the Small Intestine

1. The process of digestion is completed in the small intestine.
2. About 90% nutrients and 80% water in the chyme are absorbed in the small intestine.
3. The enteroendocrine cells of the small intestine secrete a number of gastrointestinal hormones (described later).

STRUCTURE OF THE SMALL INTESTINE

The wall of the small intestine consists of all the four typical coats of the digestive tube, i.e., mucosa, submucosa, muscularis externa, and serosa/adventitia.

MUCOSA

The mucosa of the small intestine has macroscopic and microscopic devices for increasing the surface area available for digestive and absorptive functions. These devices include plicae circulares, villi, microvilli, and crypts of Lieberkuhn.

Plicae Circulares

The plicae circulares, also called *valves of Kerckring*, are circular folds of the mucosa that are visible to the naked eye. Unlike rugae of the stomach, these crescentic folds are a permanent feature of the small intestinal mucosa. Each plica circularis also contains a core of submucosa and may extend about one half to two-thirds of the luminal

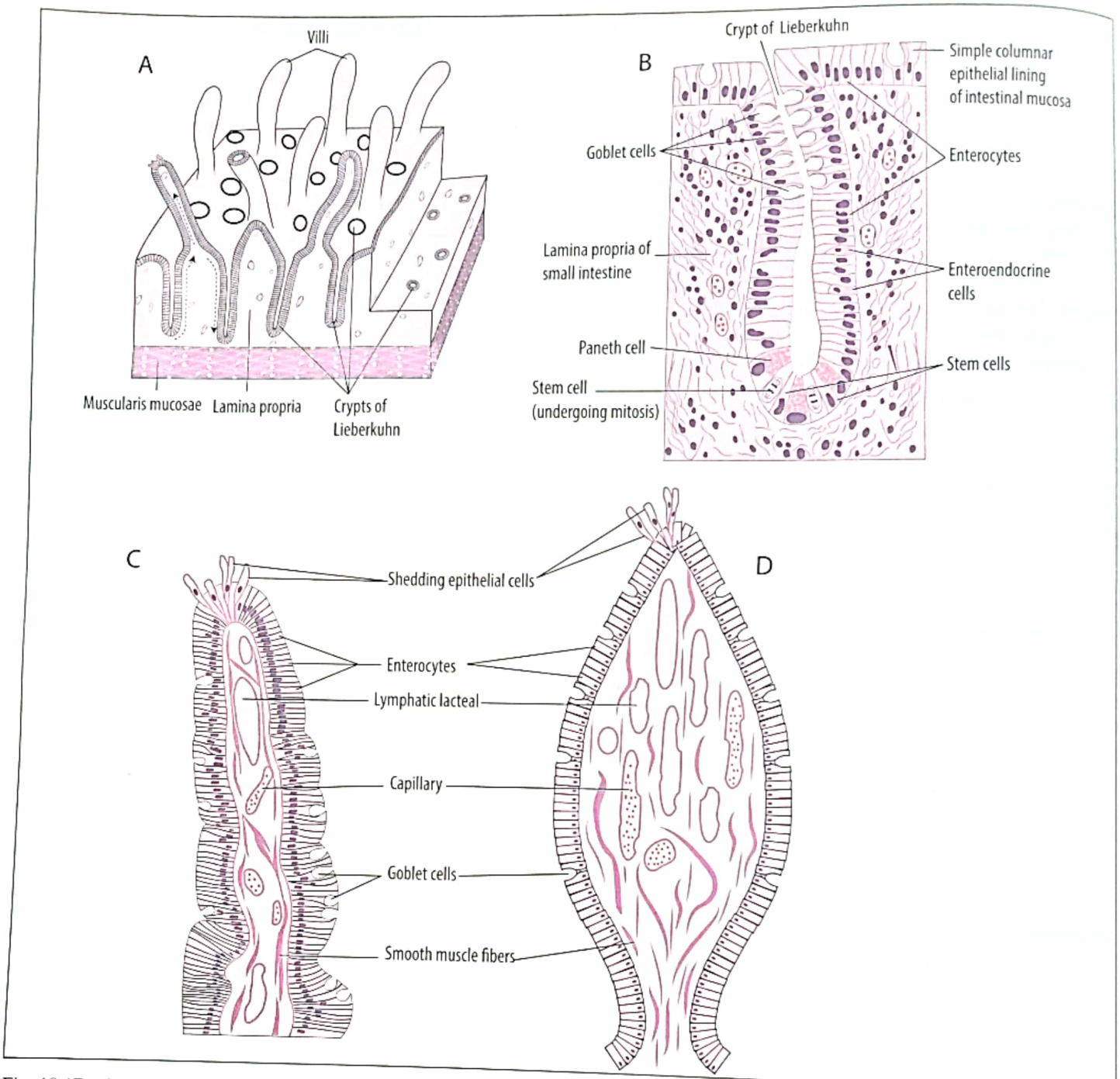

Fig. 18.17 Structure of the small intestine. A. Three-dimensional representation of the small intestinal mucosa (the arrows show the direction of movement of epithelial cells), B. Structure of a crypt of Lieberkuhn, C. A finger-shaped intestinal villus, D. A leaf-shaped intestinal villus.

RER, free ribosomes and numerous mitochondria. A large Golgi apparatus is present in a supranuclear position.

The enterocytes are closely bound to one another and to the other cells of the epithelium by junctional complexes, so that the products of digestion compulsorily pass through the absorptive cells and do not adopt a paracellular route.

The enterocytes are specialized for the absorption of nutrient molecules that are produced as a result of the digestive process. Water, ions, monosaccharides, amino acids, dipeptides, and tripeptides are absorbed by the enterocytes and released into the paracellular space through the basolateral plasma membrane of the cell. From the paracellular space these materials cross the basal lamina and enter the fenestrated blood capillaries present in the lamina propria.

The fatty acids and monoglycerides, which are the end products of lipid digestion, are emulsified by the bile salts into minute micelles which enter the enterocytes by passive diffusion by crossing the plasmalemma covering the microvilli on the apical surface of these cells. In the enterocytes, the fatty acids and monoglycerides are re-esterified in the SER of the cell to form triglycerides. The triglycerides are passed on to the Golgi apparatus, where they are combined with apoproteins to form small particles

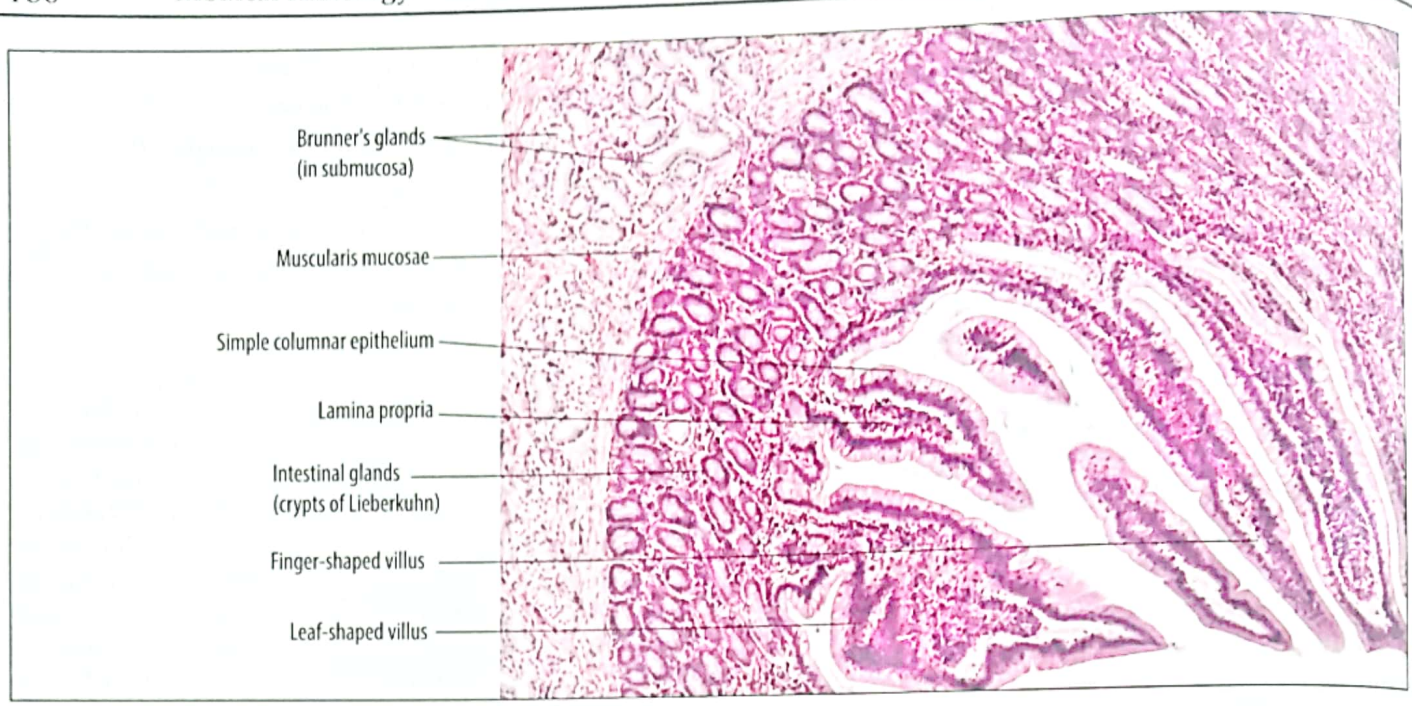

Fig. 18.18 A section through the wall of duodenum showing its microstructure as seen under the high power magnification of the light microscope.

lying in the pockets on the under surface of the M cells. The dendritic cells process the antigen and present its epitope to the neighboring helper T lymphocytes; some of these dendritic cells cross the basal lamina and present the antigenic epitope to the helper T cells located in the lamina propria of the intestinal mucosa. The activated helper T lymphocytes stimulate the B lymphocytes (present in the lamina propria) to produce IgA antibodies. These antibodies pass into the enterocytes surrounding the M cell. The enterocytes couple these antibodies to the secretory proteins and transport them into the intestinal lumen where these antibodies bind to the antigen (which caused the production of these antibodies) and neutralize it.

Crypts of Lieberkuhn and Renewal of Intestinal Epithelium

The epithelium covering the intestinal villi continues into the crypts of Lieberkuhn. The upper half of the wall of the intestinal crypts is lined by the absorptive cells, goblet cells, and enteroendocrine cells. The lower half of each crypt is lined almost exclusively by undifferentiated cells, except for a few Paneth cells which are present in the basal region of the crypt. The undifferentiated cells of the intestinal crypts serve as stem cells which undergo continuous mitotic division and thus ensure constant turnover of the epithelial cells lining the intestinal mucosa.

As the newly produced cells migrate upward along the wall of the crypt, they differentiate into enterocytes, goblet cells, and other cells of the intestinal epithelium. The newly differentiated enterocytes and goblet cells reach the base of the villi within 24 hours. Their upward migration continues and, during the next five to seven days, they ultimately reach the tip of the villus and are finally shed into the lumen of intestine. It is estimated that 20 to 50 million cells are shed off from the intestinal mucosa every minute.

SUBMUCOSA

The submucosa of small intestine consists of dense connective tissue and contains blood vessels, lymphatics and submucosal (Meissner's) plexus of nerves. In the proximal part of the duodenum, the submucosa also contains a large number of glands called Brunner's glands (Fig. 18.18).

The **Brunner's glands** (also called *duodenal glands*) are branched tubular, mucus-secreting glands. Ducts of these glands penetrate the muscularis mucosae and open into the intestinal crypts. The duodenal glands secrete large quantities of an alkaline mucus which protects the duodenal mucosa against damage by the acidic chyme entering the duodenum from the stomach. The alkaline secretion of these glands also helps to bring the intestinal contents at the optimal pH required for the activity of the pancreatic enzymes.

MUSCULARIS EXTERNA

The muscularis externa of small intestine consists of the usual inner circular and outer longitudinal layers of smooth muscle. Between these two layers is present the myenteric (Auerbach's) plexus of nerves.

SEROSA

Most of the small intestine is surrounded by a serosa consisting of a thin layer of connective tissue covered by a mesothelium. The posterior surface of the duodenum is covered by an *adventitia* of connective tissue.

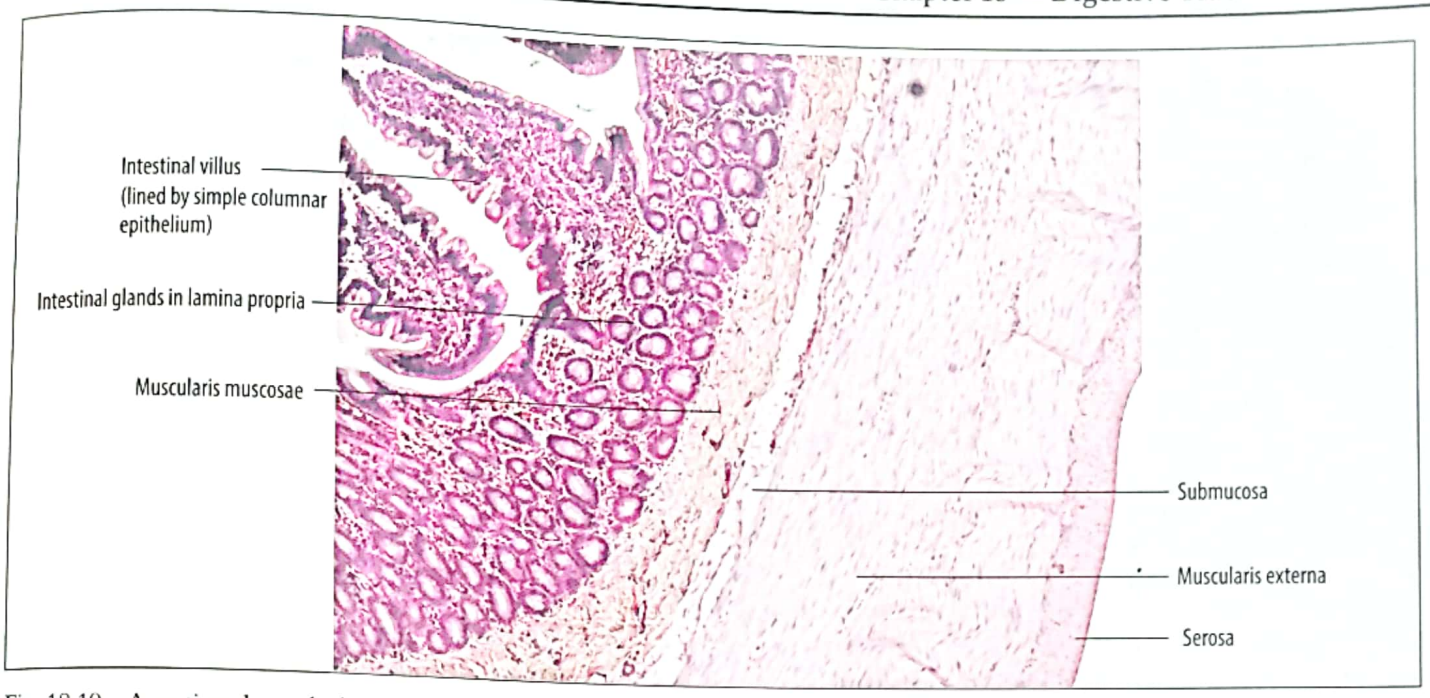

Fig. 18.19 A section through the wall of the jejunum.

REGIONAL DIFFERENCES

From the previous description it is obvious that the three segments of the small intestine (duodenum, jejunum and ileum) have common structural features, but there are important regional differences by which microscopic sections of different segments of small intestine can be distinguished from each other. The mucosa of the **duodenum** mostly bears tall and broad villi that appear leaf-shaped in sections However, some finger-shaped villi are also seen in the duodenum. The submucosa of the duodenum contains seromucous Brunner's glands which make the most distinguishing feature of this segment of the small intestine (Fig. 18.18). The **jejunum** is characterized by the presence of a mixture of finger-shaped and leaf-shaped villi on its mucosal surface (Fig. 18.19). Absence of aggregations of lymphatic nodules (Peyer's patches) in the lamina propria and absence of glands in the submucosa is also a distinguishing feature of the jejunum. The **ileum** is characterized by the presence of aggregations of lymphatic nodules, called *Peyer's patches,* in its lamina proria and a sparse number of finger-shaped villi on its mucosal surface (Fig. 18.20 & 18.21).

THE LARGE INTESTINE

The large intestine is subdivided into cecum, colon, rectum, and anal canal. The vermiform appendix is a small, blind outpocketing from the cecum. The colon and cecum are indistinguishable histologically and, therefore, will be discussed as a single entity.

Functions of the Large Intestine

1. *Absorption of water.* As the chyme reaches the large intestine, it is still in fluid in nature. However, most of the water from the chyme is absorbed back into the circulation which results in the formation of semisolid fecal mass that is eliminated from the body by excretion through the anus.
2. *Secretion of mucus* to lubricate and protect the mucosal surface from the damaging action of the harder (semisolid) fecal mass.
3. *Digestion.* Although the large intestine does not produce digestive enzymes, the digestive process continues in its lumen as a result of the activity of the enzymes already present in the food material as it reaches the large intestine. The bacteria which are normally present in the large intestine also facilitate the digestive process by decomposing some of those food materials which were not digested in the small intestine. The final products of digestion are absorbed by the enterocytes.
4. *Secretion of hormones.* The mucosa of the large intestine also contains enteroendocrine cells which secrete a number of gastrointestinal hormones. The enteroendocrine cells found in the large intestine include EC cell (which secrete serotonin and substance P), L cells (which secrete glucagon-like peptide-1), and D1 cells (which secrete vasoactive intestinal peptide).
5. *Production of vitamins.* The bacteria which normally reside in the lumen of the large intestine produce small quantities of many vitamins which include vitamin K and vitamins B1, B2, B6, and B12. From the lumen of the large intestine, these vitamins are absorbed into the circulation.

COLON

The wall of the colon also consists of the typical four coats of the digestive tube: mucosa, submucosa, muscularis externa, and serosa/adventitia.

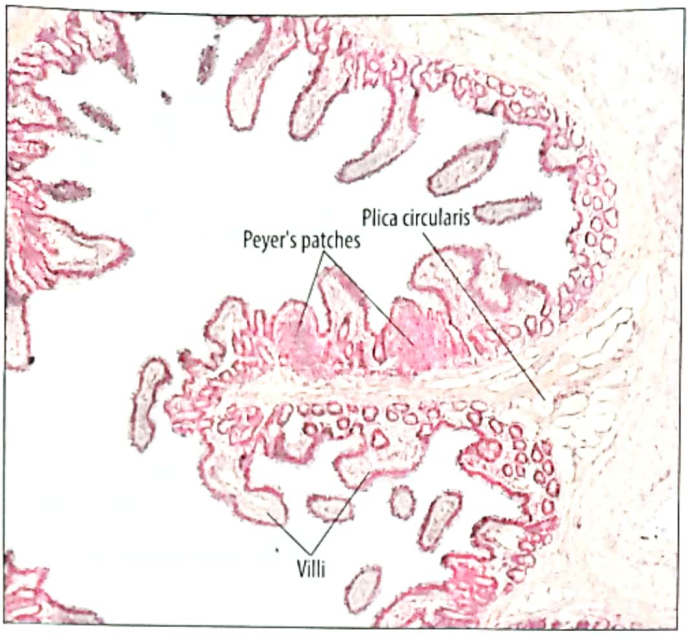

Fig. 18.20 A section of wall of the ileum as seen under low power magnification of the light microscope.

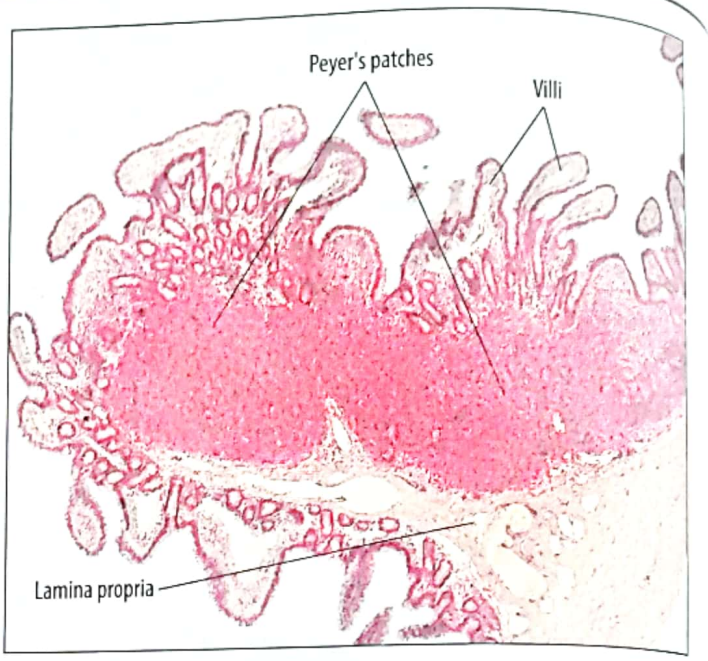

Fig. 18.21 A section of wall of the ileum as seen under high power magnification of the light microscope. Note the aggregations of lymphatic nodules (Peyer's patches) in the lamina propria.

The *mucosa* of the colon has no plicae circulares and no villi. It is line by simple columnar epithelium which contains columnar absorptive cells, called *colonocytes*, and goblet cells. The lamina propria contains a large number of crypts of Lieberkuhn. The upper one-third of each of these crypts is lined by colonocytes and goblet cells, while the lower two-thirds of these glands are lined mainly by the goblet cells (Fig. 18.23). Some enteroendocrine cells and undifferentiated stem cells are also present in the deeper parts of these glands, but Paneth cells are entirely absent. The lamina propria of the colon is rich in mucosa-associated lymphoid tissue (MALT), which occurs as diffusely spread lymphocytes as well as lymphoid nodules. The muscularis mucosae is a thin layer of smooth muscle consisting of an inner circular and an outer longitudinal layers.

The submucosa of the colon consists of dense connective tissue which contains blood vessels, lymphatics, and the Meissner's plexus of nerves.

The **muscularis externa** of the colon also consists of the inner circular and outer longitudinal layers of smooth muscle but is unusual in that most of the muscle fibers of the outer longitudinal layer are gathered into three thick, flat bands called **teniae coli.** These three muscular bands are equidistant and are separated from each other by a very thin layer of longitudinally running smooth muscle fibers. The constant tonus in the strong teniae coli produces folding of the colon into sacculations called *haustra coli.* The myenteric plexus of nerves lies between the inner and outer layers of the smooth muscle of the muscularis externa.

The transverse colon and sigmoid colon are completely surrounded by a serosa. The anterior surface of the ascending and descending colon is covered by serosa (visceral peritoneum), while their posterior surface is covered by an adventitia of dense fibrous connective tissue. The serosa of the colon is characterized by the presence of numerous small protuberances called *appendices epiploicae,* which are composed of adipose tissue.

APPENDIX

The vermiform appendix is a blind-ending tubular diverticulum of the cecum. The wall of the appendix consists of all the four typical coats of the digestive tube: mucosa, submucosa, muscularis externa, and serosa.

In cross sections, the appendix is characterized by a thick wall but a relatively narrow lumen which is star-shaped or irregular in outline. Intestinal villi are absent. The mucosa is lined mainly by absorptive cells; only a few goblet cells are present. The lamina propria contains crypts of Lieberkuhn which are lined by columnar absorptive cells as well as goblet cells.

In a newborn child, the mucosa of the appendix lodges MALT but does not exhibit nodular lymphoid tissue. The lymphoid follicles begin to develop soon after birth. The nodular lymphatic tissue in the lamina propria of the mucosa of the appendix of a child or young adult is present as large secondary lymphoid nodules showing germinal centers (Fig. 18.24). These nodules protrude toward the lumen of the appendix, due to which the appendicular lumen appears star-shaped in transverse sections. Some of the lymphatic nodules pierce the muscularis mucosae and project into the submucosa of the appendix.

The lymphoid tissue of the appendix gradually regresses after 20 years of age and only traces of the lymphoid tissue are present in the appendix of an elderly person.

The muscularis externa of the appendix is thin but shows the usual inner circular and outer longitudinal layers of

Organs Associated with the Digestive Tract

19

The digestive tract is associated with certain organs, most of which are glands, that help in digestion of food in the gastrointestinal tract. Some of them also carry out other functions which are not related to the digestive process. These organs are: the salivary glands, pancreas, liver, and gallbladder.

SALIVARY GLANDS

The salivary glands are divided into two main groups:

1. The ***major salivary glands*** which are positioned in and around the oral cavity and pour their secretions into this cavity by their excretory ducts. These glands include the paired parotid, submandibular and sublingual glands. The major salivary glands produce about 95% of the total salivary output.
2. The ***minor salivary glands*** are numerous (800-1000) small glands, most of which are located in the submucosa of the oral mucous membrane. In addition, they are buried in the connective tissue of the lips, cheeks, tongue and palate. About 5% of the total salivary output is produced by these glands. The minor salivary glands are 1-2 mm in diameter and generally consist of a small number of acini surrounded by a thin and ill-defined connective tissue layer. Each of the minor salivary gland may have its own, independent excretory ducts, or may share a common duct with neighboring minor salivary glands. The minor salivary glands are mucous glands, except the von Ebner's glands of the tongue which are serous glands whose ducts open into the grooves surrounding the circumvallate papillae.

THE MAJOR SALIVARY GLANDS

GENERAL STRUCTURAL PLAN

The major salivary glands are compound tubuloacinar glands, each of which is surrounded by a capsule of dense irregular connective tissue. From the capsule, connective tissue septa extend into the gland and divide it into lobe and lobules. The blood vessels, lymphatic vessels, nerves, and the excretory ducts of the gland course through the connective tissue of the septa. The secretory acini of these glands are surrounded and supported by very fine loose connective tissue, which contains a rich population of lymphocytes and plasma cells. The connective tissue surrounding the acini ultimately blends with the connective tissue of the septa. The plasma cells present in the connective tissue surrounding the secretory acini secrete IgA antibodies which are taken up by the acinar cells; these antibodies are then passed into the lumen of the acini along with the serous or mucous secretions of the acinar cells.

PARENCHYMA OF THE SALIVARY GLANDS

The parenchyma (functional part) of the major salivary glands is composed mainly of secretory acini, but because some portions of the ducts are also involved in the secretory process, these glands are included in the tubuloacinar variety of glands.

The acini are spherical structures, each consisting of secretory epithelial cells arranged around a central cavity which continues into that of the duct draining the acinus. The acini of salivary glands contain serous cells, mucous cells, or both. It should also be noted that while serous acini are generally spherical structures, the mucous end pieces are more often tubular. Nevertheless, the term alveolus/acinus is used to refer even to the more tubular mucous end pieces.

The basal parts of the secretory cells of the acini are embraced by *myoepithelial cells*, which lie between the basal plasmalemma and basal lamina of the secretory cells.

SEROUS CELLS

The serous cells of the salivary gland acini are generally pyramidal in shape, with a broad base facing the basal lamina and a relatively narrow apical surface facing the lumen of the acinus. Each cell contains a spherical nucleus located in the basal part of the cell which also contains numerous mitochondria, abundant RER, and many free ribosomes. The apical region of the serous cells contains numerous secretory granules called *zymogen granules*. A well-developed Golgi apparatus is present between the nucleus and the secretory granules.

Examination of the H&E-stained sections reveals that the basal region of a serous cell takes a basophilic stain, while the apical region stains acidophilic (Fig. 19.1). The basophilic staining of the basal part of a serous cell is due to abundance of RER and free polyribosome in this part. The apical portion of a serous cell stains acidophilic due to the presence of a large number of zymogen granules.

EM shows that the adjacent serous cells are joined to each other by junctional complexes, which occur some distance away from the apices of the cells. Apical to the junctional complexes, intercellular canaliculi exist between the adjacent serous cells; these canaliculi communicate with the acinar lumen. Basal to the junctional complexes, the plasma membranes of the serous cells exhibit folds in the form of processes that interdigitate with similar processes of the neighboring cells.

The serous cells of the salivary gland acini produce a thin, watery secretion which is rich in digestive enzymes.

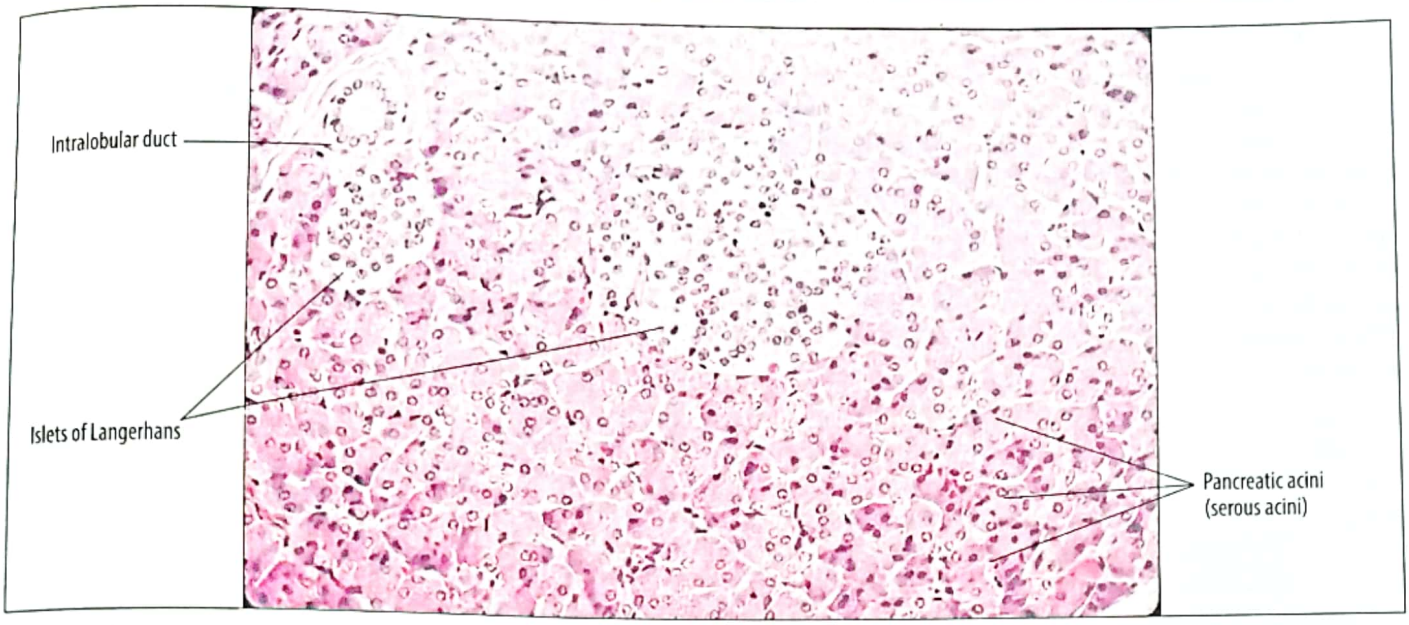

Fig. 19.8 A section of the pancreas showing serous acini and islets of Langerhans

SUBLINGUAL GLAND

The sublingual gland is also a compound tubuloacinar gland of mixed variety but its secretion is predominantly mucous in nature. The parenchyma of the sublingual gland consists mainly of mucous tubular secretory units or mucous acini (Fig. 19.6 & 19.7). Some of the mucous acini bear serous demilunes, but pure serous acini are not seen. Both intercalated and striated ducts are short and, hence, difficult to identify in sections. The connective tissue capsule is thin and poorly developed.

The sublingual gland produces only 5% of the total salivary output. Its secretory product contains mucin and α-amylase.

Saliva

The combined secretory output of all the major and minor salivary glands is called saliva. In humans, 0.75-1.5 liters saliva are produced per day. The saliva consists of 98% water and 2% other substances which include mucus, electrolytes (chiefly sodium, potassium, chloride, and bicarbonate), minerals (mainly calcium and phosphate), enzymes (chiefly α-amylase), antimicrobial compounds like lysozyme, lactoferrin, thiocyanate, and IgA.

The chief **functions** of the saliva are ***lubrication*** of the oral cavity, ***digestion*** of some components of the ingested food, and ***protection*** of the oral tissues especially teeth. The saliva constantly moistens the oral cavity and, because of its mucin content, acts as a lubricant during mastication, deglutition and speech. The salivary α-amylase initiates the digestion of the ingested carbohydrates. The saliva plays an important protective role for the oral tissues. The antimicrobial agents present in the saliva either kill or inhibit the growth of the microorganisms that enter the oral cavity.

Role of saliva in maintaining the dental health is important in many ways:

- As the saliva moves around the mouth, it helps sweep away small bits of food from the surface of the teeth and gums.
- The saliva buffers and neutralizes the acids in the mouth cavity produced during and after eating; these acids are produced by the metabolism of sugars by the oral bacteria and, if not neutralized early, can damage the tooth enamel.
- The thin film of saliva which constantly covers the teeth is very important in the prevention of *dental caries* (cavity formation in the teeth due to enamel damage). The antimicrobial agents in the saliva kill the bacteria and thus prevent the formation of acids from the sugars sticking to the teeth.

PANCREAS

The pancreas is a mixed exocrine and endocrine gland. The exocrine part of the pancreas constitutes major part of gland and consists chiefly of serous acini called pancreatic acini (Fig. 19.8). The exocrine pancreas synthesizes and secretes digestive enzymes that are conveyed to the small intestine by the duct system of pancreas. The endocrine part of the pancreas consists of numerous cell clusters called *islets of Langerhans* that are scattered among the exocrine secretory acini. By weight, the endocrine pancreas makes only 1-2% of the pancreatic mass. The endocrine component of pancreas secretes insulin, glucagon, and some other hormones.

A thin connective capsule surrounds the pancreas and sends in septa that divide the organ into lobules. The blood vessels, nerves, and ducts of the gland travel in the connective tissue septa.

EXOCRINE PANCREAS

The exocrine pancreas is actually a purely serous gland of compound tubuloacinar variety. The secretory units of the exocrine pancreas consist of serous acini, each of which is made up of 40 to 50 pyramidal serous cells enclosing a central cavity. LM shows that each serous cell has a spherical nucleus located in the central part of the cell. A well-developed Golgi apparatus is seen to be present in the supranuclear region. The basal region of the cell contains a large amount of rough endoplasmic reticulum and takes a basophilic stain. The apical region contains zymogen granules and, therefore, stains darkly eosinophilic.

Studies under the light microscope show that, in addition to the pyramidal serous cells, some sections of the pancreatic acini also exhibit pale, low cuboidal epithelial cells. These cells, called *centroacinar cells,* are found close to the apical parts of the serous cells and represent the initial segment of the intercalated duct.

The zymogen granules of the acinar cells of the pancreas contain a number of digestive enzymes which are released into the acinar lumen, from where they pass into the ducts of the pancreas and finally are delivered to the duodenum as the pancreatic juice. The enzymes produced by the pancreas include α-amylase, proteases, lipases, and nucleases. The secretion of the pancreas is regulated mainly by two hormones: secretin and cholecystokinin, both of which are produced by the enteroendocrine cells of the small intestine.

The **duct system** of the exocrine pancreas consists of intercalated ducts, intralobular ducts, interlobular ducts, and the main duct. Each *intercalated duct* begins within the center of the acinus (i.e., extends a short distance into the acinus). In histologic sections, the portions of intercalated ducts extending into the acini are seen as pale-staining, low cuboidal cells within the serous acini. As given above, these cells are called *centroacinar cells,* and they constitute a distinguishing histological feature of pancreas (Fig. 19.9). The intercalated ducts are short and their proximal segments (that extend from the acini to the intralobular ducts) are also lined by pale, low cuboidal epithelial cells. Several intercalated ducts join each other to form larger *intralobular ducts,* which converge to form *interlobular ducts* that run in the interlobular connective tissue. Both the intralobular and interlobular ducts are lined by a simple, low columnar epithelium. The interlobular ducts unite to form the *main pancreatic duct* which is lined by stratified columnar epithelium.

ENDOCRINE PANCREAS

The endocrine part of the pancreas consists of small, spherical clusters of cells called **islets of Langerhans** or *pancreatic islets* (Fig. 19.8). These islets range from 100 to 200 μm in diameter and consist of roughly spherical or polyhedral cells. The islets of Langerhans are scattered irregularly among the serous acini of the pancreas and are most numerous in the tail part of the organ. The cells of the pancreatic islets take a much lighter stain than those of the pancreatic acini. Therefore, in an ordinary H&E stained section of the pancreas, the islets of Langerhans can easily be distinguished because they appear as isolated clusters of light-staining cells scattered among the dark-staining pancreatic acini (which constitute the main bulk of the pancreatic tissue).

A thin layer of reticular connective tissue surrounds each pancreatic islet, separating it from the surrounding exocrine pancreatic tissue. The islet cells are arranged in short, irregular cords separated from each other by fenestrated capillaries. In the ordinary (H&E) sections, the cells of pancreatic islets stain lightly eosinophilic and no cytoplasmic granules can be distinguished. However, cytoplasmic granules can be seen by special staining techniques and by electron microscopic examination. Depending on the morphology, size and distribution of the cytoplasmic granules, the pancreatic islet cells are classified into four types, designated as A (alpha) cells, B (beta) cells, D (delta) cells, and PP cells. The **A cells** produce glucagon. The **B cells** are the predominant cell type, making up about 70% of the islet mass; they produce insulin. The **D cells** produce somatostatin. The **PP** cells secrete pancreatic polypeptide.

LIVER

The liver is the largest gland and chief metabolic organ of the body. It can be regarded as a huge biosynthetic chemical factory which synthesizes large complex molecules from the materials transported to this organ by the blood.

The chief functions of the liver can be grouped into five main categories as given below:

- **Carbohydrate metabolism**, which plays a major role in the regulation of the blood glucose level (by glycogenesis, glycogenolysis and glucogenesis). In addition, the liver converts carbohydrates into fatty acids.
- **Fat metabolism**, which includes synthesis of lipoproteins and cholesterol, and oxidation of triglycerides.
- **Protein metabolism**, which includes synthesis of plasma proteins and clotting factors, synthesis of non-essential amino acids, and deamination of amino acids (which results in the production of urea)
- **Storage functions**. The liver serves as a storehouse of many nutrients which are released into the blood as and when needed. These nutrients include glucose, triglycerides, vitamins A, D, E, K, and B_{12}.
- **Detoxification of the ingested toxins and drugs**. The toxins and most of the drugs ingested by an individual are first oxidized and then conjugated (with glucronic acid, glycine, or taurine) in the liver. The resulting water-soluble end products are passed into the blood, which are removed from the circulation by the kidneys and eliminated from the body in the urine.

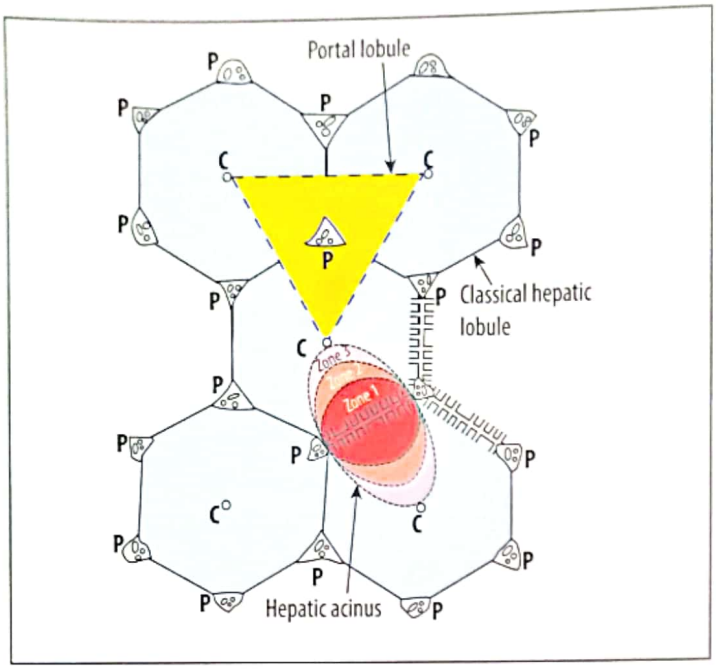

Fig. 19.13 Diagram showing the three concepts of liver structure: the classical hepatic lobule, the portal lobule, and the hepatic acinus. (P: portal tract, C: central vein).

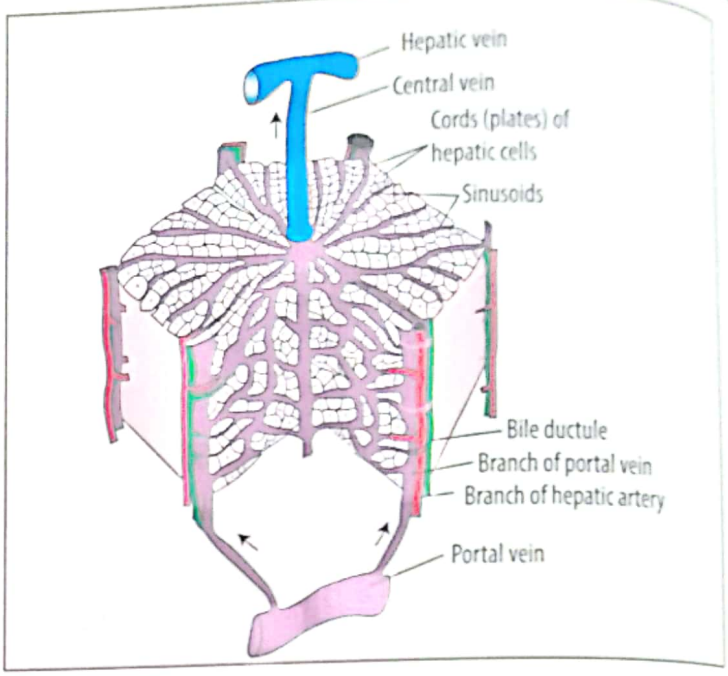

Fig. 19.14 Diagrammatic representation of blood flow through a hepatic lobule. Arrows indicate the direction of flow of blood.

In H&E stained sections examined under LM, the cytoplasm of hepatocytes generally stains acidophilic because of the presence of abundant mitochondria and SER. However, some basophilic regions can also be distinguished which represent cisternae of RER. The cytoplasm also contains a large number of glycogen granules and fat droplets, but in routine histological preparations these substances are washed out, leaving empty spaces in the cytoplasm.

As the hepatocytes are arranged in plates that are one cell thick (like the bricks of a wall), therefore they do not have apical or basal surfaces corresponding to the epithelial cells of other exocrine glands. However, the hepatocytes are highly polarized and, due to their peculiar arrangement, these cells possess three domains or surfaces, which are: (i) sinusoidal surface, (ii) canalicular surface, and (iii) intercellular surface.

As the name indicates, the ***sinusoidal surface*** of a hepatocyte lies in close association to the sinusoids of the liver. It accounts for nearly 70% of the total surface of the cell and bears short microvilli. Exchange of materials between the liver cell and the blood plasma (present in the perisinusoidal space) occurs through this surface; the microvilli increase the surface area available for this exchange.

The ***canalicular surface*** accounts for about 15% of the total surface of each hepatocyte. The canalicular surfaces of the adjacent hepatocytes lie in close contact with each other except where a bile canaliculus is present. A bile canaliculus is a tubular channel formed by the exact opposition of two shallow gutters, which are located on the canalicular surfaces of the opposing hepatocytes. The canalicular surfaces of the adjacent hepatocytes are bound to each other by junctional complexes.

The ***intercellular surface*** of a hepatocyte makes up the remaining 15% of the total cell surface. The intercellular surfaces of the hepatocytes are specialized for attachment with neighboring hepatocytes. The intercellular surfaces of adjacent hepatocytes are joined to each other by numerous maculae communicantes (gap junctions).

The Perisinusoidal Space

Adjacent to a blood sinusoid, the surface of a hepatocyte is separated from the wall of the sinusoid by a narrow space that mainly contains reticular fibers and blood plasma. This space, called perisinusoidal space or **space of Disse**, is the site where exchange of materials between the plasma and hepatocytes takes place. As already explained, the sinusoidal surface of the hepatocytes bears microvilli which protrude into the perisinusoidal space. the perisinusoidal space also contains special cells called hepatic stellate cells.

Hepatic Stellate Cells

The hepatic stellate cells (HSCs) are also called *hepatic lipocytes* or **Ito cells**. Normally, these cells store vitamin A as retinyl esters within the cytoplasm in the form of lipid droplets. In chronic liver diseases, the HSCs undergo a change called 'activation'. The activated HSCs lose their vitamin A and transform into fibroblast-like cells. These cells secrete large quantitits collagen fibers.

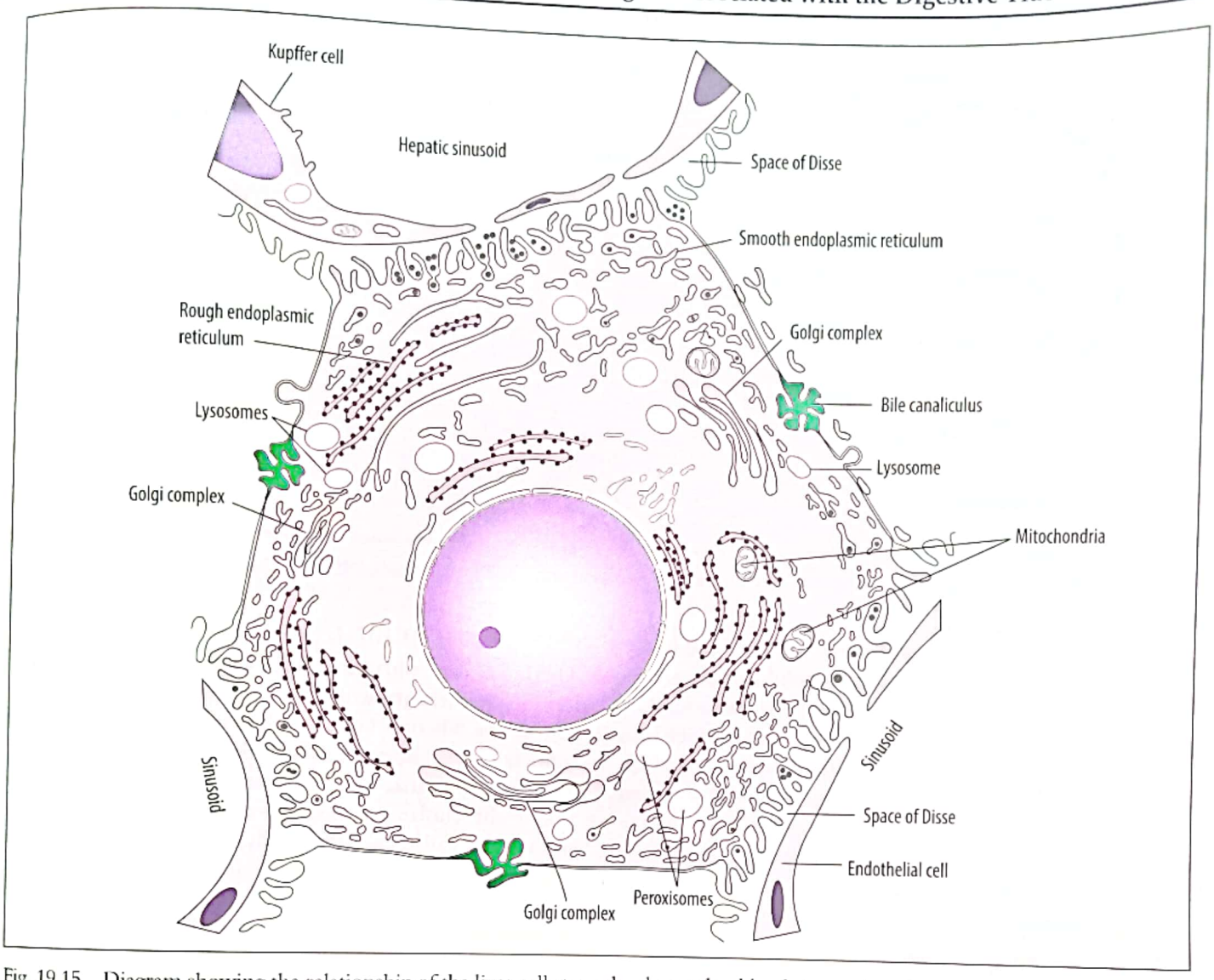

Fig. 19.15 Diagram showing the relationship of the liver cells to each other and to blood sinusoids; principal contents of a hepatocyte (as seen under EM) are also illustrated.

Cirrhosis of Liver

Chronic damage to the liver parenchyma (e.g., due to prolonged alcohol overuse, hepatitis B, and hepatitis C) leads to activation of the hepatic stellate cells which begin to produce excessive amounts of collagen fibers. Formation of large quantities of collagenous tissue leads to fibrosis of the liver which is called cirrhosis of liver (also called *heptic cirrhosis* or *portal cirrhosis*). Gradually, the liver parenchyma (consisting of hepatocytes) is destroyed and replaced by scar tissue (i.e., fibrous tissue). As a result of cirrhosis, flow of blood through the liver is gradually blocked resulting in portal hypertension (i.e., increase in blood pressure in the hepatic portal system). Finally, the hepatic cirrhosis progresses to the point where the liver loses most or all of its function - a condition called "hepatic failure". The only treatment available for the patients with advanced hepatic cirrhosis is a liver transplant.

DUCT SYSTEM OF THE LIVER

The system of channels through which the bile flows from the hepatocytes to the gallbladder, and then to the duodenum, is called *biliary tree*. This system is divided into two parts: intrahepatic part and extrahepatic part. The intrahepatic part of the biliary tree consists of bile canaliculi, canals of Hering, bile ductules, interlobular (trabecular) ducts, and the right and left hepatic ducts. The extrahepatic part of the biliary tree consists of the common hepatic duct and the common bile duct (CBD). The common hepatic duct is formed by the union of the right and left hepatic ducts. It becomes the common bile duct beyond the point where the cystic duct from the gallbladder joins it. The CBD opens into the second part of the duodenum at the ampulla of Vater.

CHOLANGIOCYTES

All tubular passages of the biliary tree, except bile canaliculi, are lined by a single layer of a special variety of epithelial cells called *cholangiocytes*. In the smaller ducts the cholangiocytes are cuboidal in shape but their height

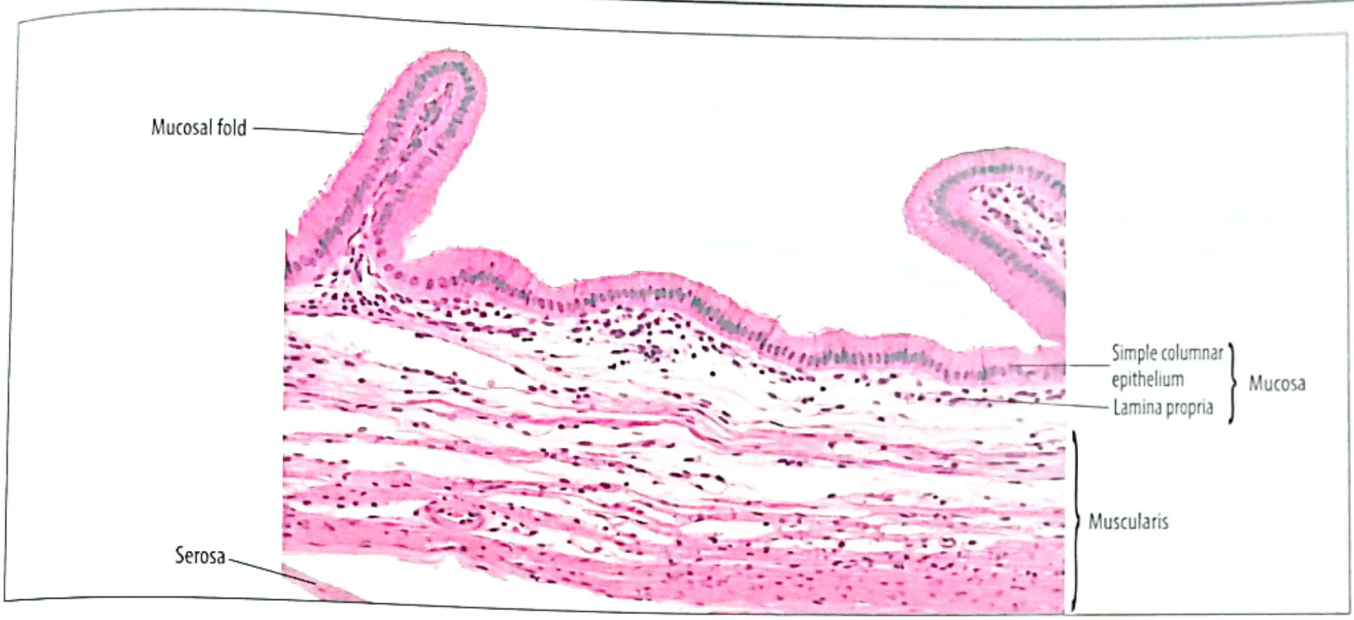

Fig. 19.17 A section through the gallbladder wall.

is present. The submucosa is also thin and consists of loose connective tissue. In the cystic duct, the submucosa contains mucous glands. The muscularis consists of a thin layer of smooth muscle fibers. Outer to the muscularis is present the adventitia which consists of a thin layer of connective tissue rich in collagen fibers.

HEPATIC SINUSOIDS

The spaces between the plates of hepatocytes are occupied by sinusoidal capillaries called hepatic sinusoids. These sinusoids have an incomplete basal lamina and are lined by two types of cells: (i) endothelial cells, and (ii) phagocytic cells which are called Kupffer cells.

The **Endothelial cells** show fenestrations (pores) which are not closed by diaphragms. Intercellular gaps (about 0.5 μm wide) are also present between adjacent endothelial cells. The basement membrane of the sinusoidal endothelium is also interrupted and is extremely thin.

The **Kupffer cells,** also called *stellate macrophages,* are large, star-shaped, phagocytic cells that belong to the mononuclear phagocyte system (MPS). These cells have multiple, long processes and are interspersed among the endothelial cells lining the hepatic sinusoids. The processes of the Kupffer cells extend into the lumen of the hepatic sinusoids. The major function of the Kupffer cells is to engulf and break down the damaged and aged erythrocytes. This function is greatly enhanced after splenectomy (surgical removal of the spleen). The Kupffer cells also serve as antigen-presenting cells by engulfing those bacteria which manage to enter the portal circulation by penetrating the intestinal wall.

Regenerative Capacity of the Liver Tissue

The liver tissue has a unique ability to regenerate after being damaged. This regeneration occurs by two mechanisms: (1) mitotic division of the surviving hepatocytes, and (2) stimulation of the hepatic stem cells.

The hepatocytes normally remain quiescent and divide only occasionally, but after injury due to blood-borne toxins, acute viral infections or surgical resection of a liver segment, the surviving hepatocytes are stimulated to undergo rapid mitotic division, so that the lost liver tissue is regenerated.

Liver injury results in the stimulation of the hepatic stem cells (oval cells) which normally lie dormant in the bile ductules. The hepatic stem cells give rise to progenitor cells which differentiate into hepatocytes as well as cholangiocytes.

The remarkable regenerative capability of the liver tissue has a great application in liver transplantation. A patient needing a liver transplant can get a considerable part (e.g., complete left lobe) of the liver from a close relative by surgical transplant, with complete restoration of the liver function in the recipient as well as donor.

GALLBLADDER

The gallbladder is a distensible, muscular sac which is attached to the visceral surface of the liver. The chief functions of the gallbladder are concentration and storage of the bile. The maximum storage capacity of the gallbladder is 50 mL. The wall of the gallbladder consists of three coats: mucosa, muscularis, and adventitia/serosa (Fig. 19.17).

The **mucosa** consists of epithelium and lamina propria and is thrown into numerous folds that are particularly prominent in the empty gallbladder. The lining **epithelium** is of simple columnar variety. The tall columnar cells of this epithelium exhibit the features of absorptive cells. The free surface of these cells bears numerous, short microvilli and junctional complexes bind the apical parts of the adjacent

cells with each other tightly to create an impermeable barrier between the gallbladder lumen and intercellular compartment. The lateral walls of the adjacent cells also show interdigitations. The basolateral plasmalemmas of these cells lodge numerous sodium pumps.

The **lamina propria** consists of loose connective tissue rich in elastic and collagen fibers. It lodges fenestrated capillaries, venules, lymphocytes, and plasma cells. The lamina propria of the gallbladder is devoid of glands, except in the neck region of the organ where simple tubuloacinar glands are present which secrete mucus into the bile.

The **muscularis** of the gallbladder consists of a meshwork of interlacing bundles of smooth muscle fibers. Collagenous and elastic fibers are present between the bundles of smooth muscle fibers.

Outer to the muscularis is a thick layer of dense connective tissue that contains large blood vessels, lymphatics, and autonomic nerves that innervate the muscularis. This connective tissue layer is referred to as *adventitia* over that surface of the gallbladder which attaches to the liver. The non-attached surface of the gallbladder is covered by visceral peritoneum that constitutes serosa of the organ.

Functions of the Gallbladder

The gallbladder receives dilute, watery bile from the common hepatic duct and releases a thick concentrated bile into the common bile duct. The concentration of the bile is brought about by osmotic absorption of water secondary to active transport of sodium by way of the sodium pumps, which are located in the basolateral plasmalemma of the columnar epithelial cells lining the mucosa of the gallbladder. The stored bile is passed into the duodenum through the common bile duct as a result of contractions of the smooth muscle in the wall of the gallbladder. These contractions are induced by the hormone cholecystokinin, which is produced by the enteroendocrine cells of the duodenum in response to a fatty meal.

Urinary System 20

The urinary system consists of a pair of kidneys, a pair of ureters, a urinary bladder, and a urethra. The chief function of the urinary system is to produce, store, and eliminate urine, which is a fluid that consists of water and several different types of waste products of the body (for example, urea, creatinine, ammonia, and uric acid). The kidneys produce urine which passes through the ureters to the bladder for temporary storage and periodic release to the exterior through the urethra.

THE KIDNEYS

The kidneys are two bean-shaped organs located in the back part of the abdominal cavity. These vital organs filter the blood, form and excrete urine, regulate water and electrolyte balance of the body, and also act as endocrine glands.

Each kidney* is covered by a thin but tough capsule of dense irregular connective tissue consisting of collagen fibers, fibroblasts, and myofibroblasts. On the medial side of each kidney is present a depression called *hilum,* through which the renal artery enters and the renal vein and ureter leave the organ. The upper part of the ureter is expanded to fill the hilum of the kidney; this part is known as *renal pelvis*. The renal pelvis has large and small cup-like extensions called *major calyces* and *minor calyces,* respectively (Fig. 20.1 A).

On naked eye inspection of a coronal hemisection of a kidney, the substance (parenchyma) of the kidney exhibits two zone: an outer reddish brown, granular zone called *cortex* and an inner striated zone of lighter color called *medulla.* The renal **cortex chiefly** contains renal corpuscles and convoluted parts of the renal tubules. As these structures have a round shape, the cortex appears granular upon eye examination of a freshly cut section of kidney. The reddish brown color of the cortex is due to the fact that, at any time, it contains about 90% of blood flowing through the kidney. The **renal medulla** lodges longitudinally arranged blood vessels and straight parts of the renal tubules which run toward the pelvis of kidney in a radiating manner (therefore, the medulla appears striated in a freshly cut section of kidney). The renal medulla contains only 10% of the blood passing through the organ and, therefore, exhibits much lighter color than the renal cortex.

The medullary region of each kidney shows 8-18 conical masses called *renal pyramids*(Fig. 20.1 B). The base of each renal pyramid lies adjacent to the cortex, whereas the apex, known as *papilla,* projects into a minor calyx of the renal pelvis. Each renal pyramid with its associated overlying cortex is regarded as a *lobe of kidney.*

The neighboring renal pyramids are separated from each other by **renal columns**, which are extensions of the cortical tissue into the medullary part of the kidney. From the base of each renal pyramid a series of longitudinal striations radiate into the renal cortex. These striations are called **medullary rays** and they represent the extensions of medullary tissue (collecting ducts and straight parts of the renal tubules) into the cortex.

Blood Supply of the Kidneys

Each kidney is supplied by a renal artery arising from the abdominal aorta. After entering the kidney through its hilum, the *renal artery* divides into two or three main branches which divide further and give rise to *interlobar arteries* which run between the medullary pyramids. At the corticomedullary junction each interlobar artery divides into *arcuate arteries* that arch over the bases of the pyramids and run parallel to the surface of the kidney (Fig. 20.8). From the arcuate arteries originate branches called *interlobular arteries,* which travel through the cortex toward the renal capsule. The interlobular arteries give rise to *intralobular* branches, each of which becomes an afferent arteriole which supplies the glomerular capillaries of a renal corpuscle. It is to be noted that the arterial blood reaching the kidney first passes through the glomerular capillaries and then supplies the other structures in the kidney substance.

The glomerular capillaries reunite to form the efferent arteriole. Most of the efferent arterioles branch again to form a *peritubular capillary plexus* which nourishes the proximal and distal convoluted tubules in the renal cortex. However, the efferent arterioles arising from the glomeruli located at the corticomedullary junction descend into the medullary pyramids alongside the loops of Henle; these long, straight arteries are known as *arteriolae rectae.*

The arteriolae rectae break up into smaller vessels which continue toward the apex of the pyramid but make hairpin turns at various levels to return to the corticomedullary junction as *venulae rectae,* which drain into arcuate veins. The arteriolae rectae and venulae rectae are collectively known as *vasa recta* (Fig. 20.8).

The cortical capillaries collect to form small veins called *stellate veins.* These veins converge in a star-like pattern and form interlobular veins which drain into the arcuate veins. Blood from the arcuate veins flows into interlobar veins which pass toward the hilum and finally join to form the renal vein.

* The Greek term for kidney is nephros, while the Latin term for this organ is renalis.

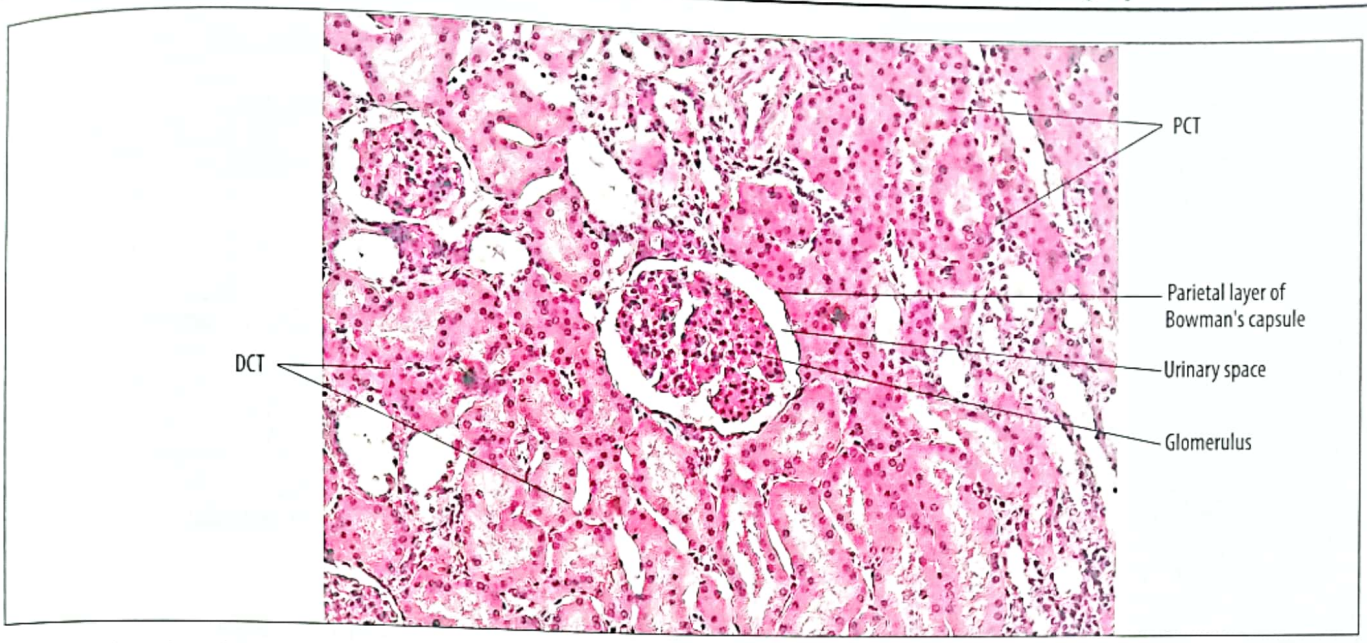

Fig. 20.4 A section showing the histological features of a portion of the renal cortex as seen under high power magnification of the light microscope (PCT: proximal convoluted tubules, DCT: distal convoluted tubules).

of the glomerular capillaries is called glomerular basement membrane. It is to be noted that this is a common basal lamina for the podocytes and capillary endothelium. In children the thickness of GBM is only 150 nm, but in the adults it is much thicker and has an average thickness of 300 nm. It is a continuous, uninterrupted layer, showing no gaps or pores. EM studies reveal that the glomerular basement membrane consists of three layers: lamina rara externa, lamina densa, and lamina rara interna.

The ***lamina rara externa*** is the electron-lucent layer adjacent to the podocyte processes. It mainly contains the glycosaminoglycan *heparan sulfate*. Due to its negative charge, the heparan sulfate repels the negatively charged organic molecules, especially the low molecular weight proteins, and thus blocks their passage through the GBM. The lamina rara externa also contains adhesive glycoproteins, mainly laminin and fibronectin, which help in anchoring the podocyte processes to the GBM.

The ***lamina densa*** is the comparatively electron-dense middle layer of GBM. Its chief component is collagen type IV, which is organized into a network that serves as a sieve-like filter which does not allow large molecules to pass through it.

The ***lamina rara interna*** is the electron-lucent layer bordering on the capillary endothelium. Its composition is the same as that of the lamina rara externa. Heparan sulfate is a major component of this layer as well and, due to its negative charge, repels the albumen and other plasma proteins and thus prevents them from crossing the GBM. The adhesive glycolproteins of this layer help the endothelial cells of glomerular capillaries to keep their attachment to the GBM.

THE GLOMERULUS

The glomerulus is a tuft of fenestrated capillaries connecting an *afferent arteriole* with an *efferent arteriole*. The two arterioles usually lie close together at the vascular pole of the renal corpuscle. As the afferent arteriole enters the glomerulus, it divides into four or five main branches. Each branch further divides into a number of capillaries which follow an irregularly looped course in their way toward the efferent arteriole. The looped capillaries from each main branch of the afferent arteriole tend to be grouped together; these groups are referred to as *lobules* of the glomerulus. Anastomoses occur between the capillaries of a lobule and also between the capillaries of adjacent lobules. All the capillaries eventually join to form the efferent arteriole.

It is to be noted that, instead of draining into a venule, the glomerular capillaries drain into an arteriole. It is also to be noted that the efferent arteriole is smaller in diameter than the afferent arteriole. This difference of calibre between the afferent and efferent arterioles ensures a substantial hydrostatic pressure in the glomerular capillaries, which facilitates the glomerular filtration.

The endothelial cells lining the glomerular fenestrated capillaries have large pores which are not covered by any diaphragms. The luminal surface of the lining endothelium is covered by a thick glycocalyx coat. The glomerular fenestrated capillaries restrict the blood cells and platelets but allow the components of the blood plasma to pass through their walls freely.

MESANGIAL CELLS

In addition to the podocytes and endothelial cells of the glomerular capillaries, each renal corpuscle also contains a special variety of cells called mesangial cells. These cells and their extracellular matrix are collectively known as *mesangium*. The mesangial cells lie in between the glomerular capillaries and cover those regions of these capillaries which are not covered by the podocyte

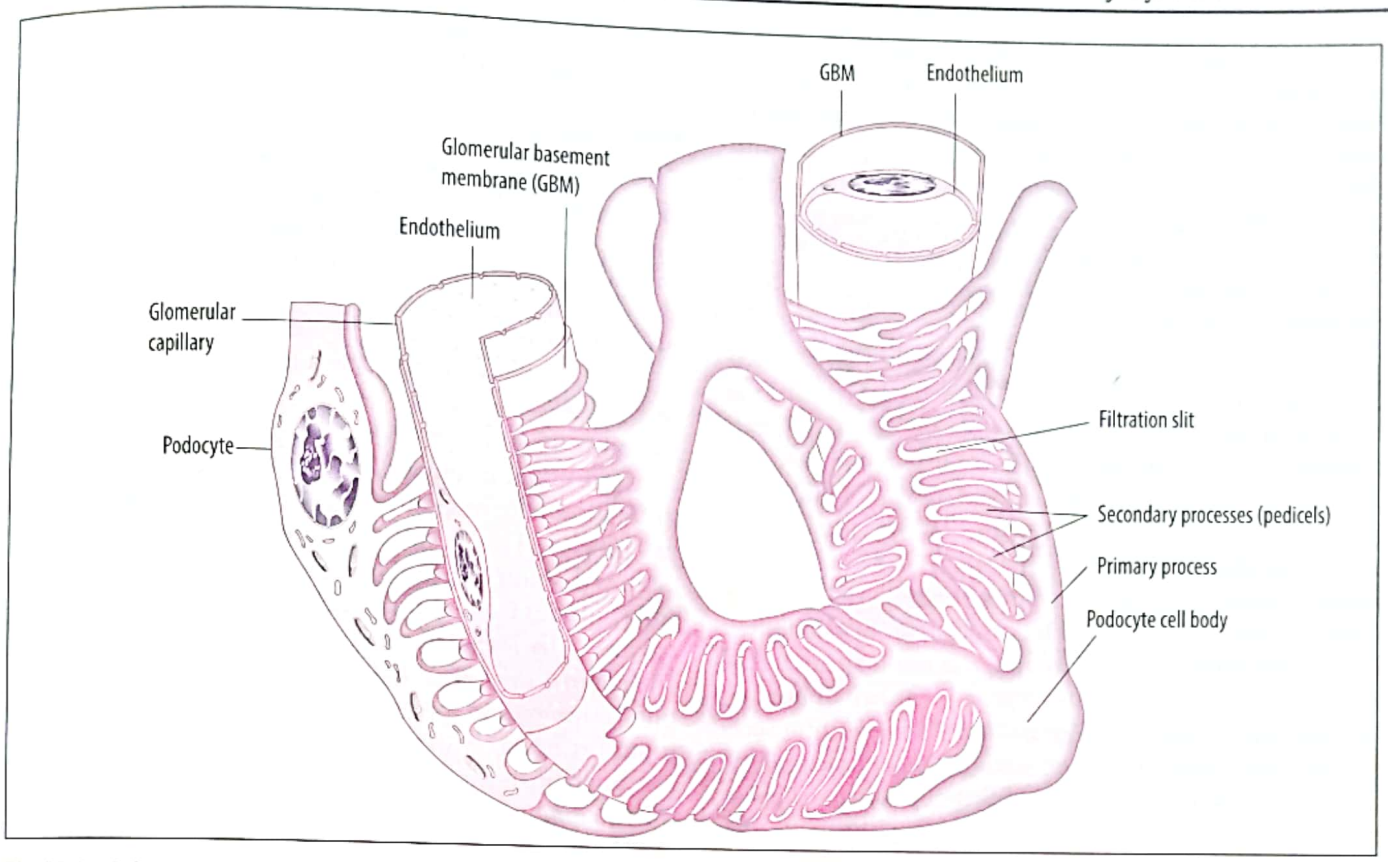

Fig. 20.6 Schematic representation of a glomerular capillary and podocytes.

capillaries. The mesangial cells are supposed to regulate the blood flow through the glomerular capillaries (and, hence, the GFR) by their contractile activity.

THE FILTRATION BARRIER

The filtration barrier consists of those structures which intervene between the blood in the glomerular capillaries and the filtrate in the capsular space. This barrier consists of three components:

1. The **endothelium** of the glomerular capillaries.
2. The glomerular basement membrane (**GBM**).
3. The **slit diaphragms** covering the filtration slits.

The glomerular capillaries are of fenestrated variety and are lined by endothelial cells which have large pores without any diaphragms. This fenestrated endothelium allows the rapid flow of plasma across the capillary wall but prevents the passage of formed elements of blood.

The blood plasma next encounters the GBM. This common basal lamina stops the passage of molecules according to molecular size, molecular weight, and electrostatic charge. Molecules having a size of 10 nm or more cannot pass through the GBM. Similarly, molecules near the molecular weight of albumin (69K Daltons) or larger are also restricted. However, smaller molecules like simple sugars, metabolites, amino acids, and even small peptides can pass easily through the GBM. Presence of negatively charged heparan sulfate in the glomerular basement membrane restricts the passage of negatively charged molecules i.e., anions (most importantly albumin); however, the cations (e.g., sodium ions) can pass through the GBM easily.

The last component of the filtration barrier is constituted by the slit diaphragms which span the filtration slits between the interdigitating pedicels of the podocytes. The slit diaphragms are porous but allow the passage of very small molecules only. These diaphragms do not allow those macromolecules that manage to cross the GBM (like serum albumin and gamma globulin) to pass into the urinary space of the Bowman's capsule, and thus ensure that these molecules remain in the blood.

PROXIMAL TUBULE

As explained earlier, this part of the nephron consists of two parts: a longer, coiled and convoluted part known as **proximal convoluted tubule,** and a shorter, uncoiled part called **proximal straight tubule**.

THE PROXIMAL CONVOLUTED TUBULE (PCT)

The glomerular filtrate passes from the urinary space of Bowman's capsule into the proximal convoluted tubule at the urinary pole of the renal corpuscle. At the junctional region, the simple squamous epithelium of the parietal layer of Bowman's capsule becomes continuous with the simple cuboidal epithelium of the PCT.

The proximal convoluted tubule is the longest and most tortuous part of the nephron. It is approximately 14 mm

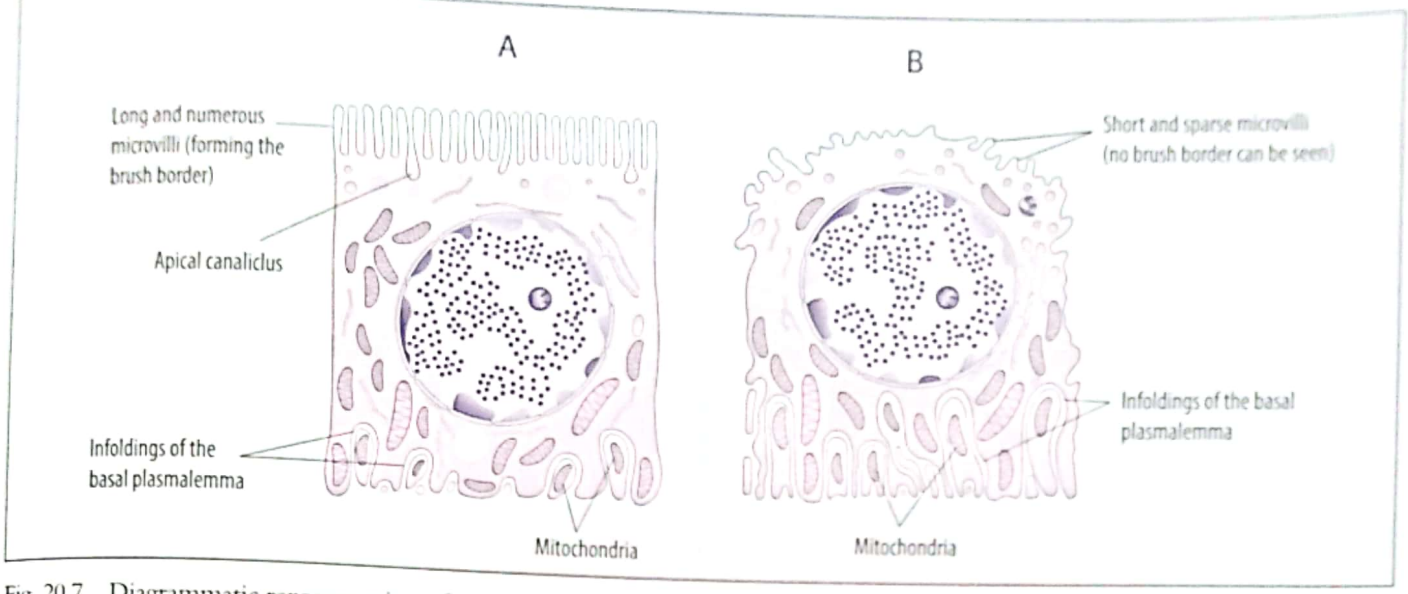

Fig. 20.7 Diagrammatic representation of electron microscopic appearance of a cell of: A. Proximal convoluted tubule B. Early distal convoluted tubule.

medulla, the proximal straight tubule suddenly narrows to form the descending thin segment of Henle's loop.

LOOP OF HENLE

The loop of Henle, also called nephron loop, is a U-shaped structure having a descending limb and an ascending limb. As discussed earlier, the length of the nephron loops differs according to the location of the nephrons in the renal cortex. The juxtamedullary nephrons have the longest loops and their loops are considered to be typical loops of Henle. A typical loop of Henle consists of 3 parts: (1) a thin descending segment, (2) a thin ascending segment, and (3) a thick ascending segment (Fig. 20.1C & 20.2).

Soon after entering the renal medulla, the proximal straight tubule narrows abruptly to an external diameter of approximately 30 µm to become the *thin descending segment* of the loop of Henle. It descends into the renal medulla for a variable distance, makes a hairpin loop and ascends toward the cortex as the thin ascending segment of Henle's loop. As it reaches near the cortex, the thin ascending segment gradually increases in diameter to form the thick ascending segment of the nephron loop. This segment continues into the cortex to become the straight part of the distal tubule.

Both the thin descending and thin ascending segments of the Henle's loop are lined by simple squamous epithelium. The thick ascending segment is lined by simple cuboidal epithelium. The cuboidal cells lining the thick ascending segment possess short microvilli on their free surface and the basal plasmalemma of these cells exhibits numerous infoldings.

Functions of the Loop of Henle

The descending thin segment of the Henle's loop is freely permeable to water and about 20% of the water in the glomerular filtrate is reabsorbed in this segment. The ascending thin and thick segments of the nephron loop are virtually impermeable to water. The cuboidal epithelial cells lining the thick ascending segment of the Henle's loop actively reabsorb sodium, chloride and potassium ions from the urinary filtrate. The sodium pumps located in the basolateral plasmalemma of these cells pump sodium (and chloride) ions into the interstitial tissue surrounding the nephron loops. Accumulation of sodium ions in the interstitial tissue of the renal medulla creates a hypertonic environment which builds up an osmotic pressure which, in turn, draws more water from the descending thin limb of the nephron loop into the medullary interstitial space (from where it is absorbed into the peritubular blood capillaries).

DISTAL TUBULE

The nephron loop continues into the distal tubule of the nephron which consists further of two parts: an uncoiled part called **distal straight tubule,** and a highly coiled and convoluted part known as **distal convoluted tubule.**

The Distal Straight Tubule

The thick ascending limb of the nephron loop becomes the straight part of the distal tubule. The distal straight tubule is lined by cuboidal cells that stain lightly with eosin. These cells contain a spherical, centrally located nucleus and possess a few club-shaped microvilli. They show extensive infoldings of basal plasmalemma and a large number of mitochondria associated with these infoldings. The cells interdigitate in the lateral domain and are joined to each other by tight junctions. The terminal part of the distal straight tubule takes part in the formation of *macula densa* which is a component of the *juxtaglomerular apparatus* (described later).

THE DISTAL CONVOLUTED TUBULE (DCT)

Near the vascular pole of the renal corpuscle, the distal straight tubule ends and the distal convoluted tubule

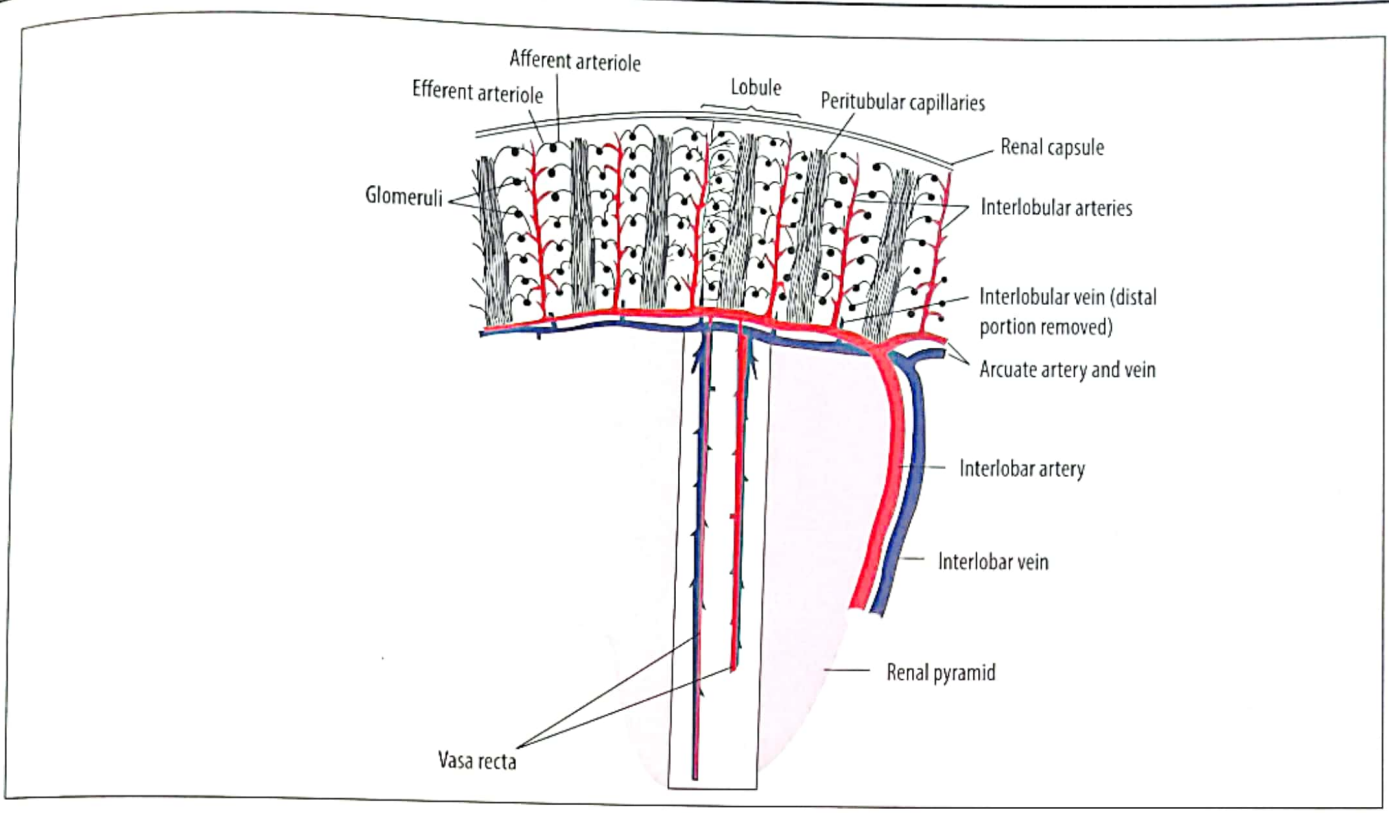

Fig. 20.8 Diagram showing arrangement of renal blood vessels.

and cortical collecting ducts, so that the volume of urine being produced by the kidneys is reduced (i.e., diuresis is inhibited).

COLLECTING DUCTS OF KIDNEY

In the medullary rays of the renal cortex, the connecting tubules (the terminal parts of the nephrons) join cortical collecting ducts which average 40 μm in diameter and are lined by a simple cuboidal epithelium. These collecting tubules enter the medulla and join with each other to form long and straight medullary collecting ducts which average 200 μm in diameter and are lined by simple columnar epithelium. Near the apex of each renal pyramid, many medullary collecting ducts merge with each other and give rise to a papillary duct, also called duct of Bellini, which is lined by a simpel columnar epithelium. The papillary duct opens at the apex of the pyramid and delivers urine into a minor calyx(Fig. 20.1C & 20.2).

JUXTAGLOMERULAR APPARATUS

At the vascular pole of the renal corpuscle, there is an association of three structures which are known collectively as juxtaglomerular apparatus or juxtaglomerular complex. The components of the juxtaglomerular apparatus are: macula densa, juxtaglomerular cells, and extraglomerular mesangial cells.

MACULA DENSA

The macula densa is a specialized region of the distal straight tubule where the tubule comes in contact with the afferent and efferent arterioles at the vascular pole of the renal corpuscle of its own nephron. In this region the simple cuboidal epithelium of the distal tubule shows several modifications.

The cells in the region of the macula densa are taller, closely packed, and have more prominent nuclei than the surrounding cells of the distal tubule and, therefore, this region of the tubule appears darker in histological sections. The basal lamina of these cells is thin and discontinuous and blunt processes of these cells extend through this basal lamina toward the juxtaglomerular cells.

The macula densa functions as a sensor of osmolarity of fluid in the distal convoluted tubule. A decrease in the sodium ion concentration in the tubular fluid, which generally occurs due to a decrease in the glomerular filtration rate (GFR), stimulates the macula densa cells to produce chemical mediators which have two effects: (i) the resistance to blood flow in the afferent arterioles of the kidney is decreased, which increases the glomerular hydrostatic pressure and thus helps to return the GFR back to normal, and (ii) the juxtaglomerular cells are stimulated to secrete renin.

JUXTAGLOMERULAR CELLS

Adjacent to the macula densa, the tunica media of the afferent arteriole of the glomerulus contains modified smooth muscle cells which are called juxtaglomerular (JG) cells. The JG cells have a large size and their nuclei are spherical. The cytoplasm of JG cells contains elaborate RER, many Golgi complexes, and a large number of zymogen granules containing the enzyme *renin*. Internal

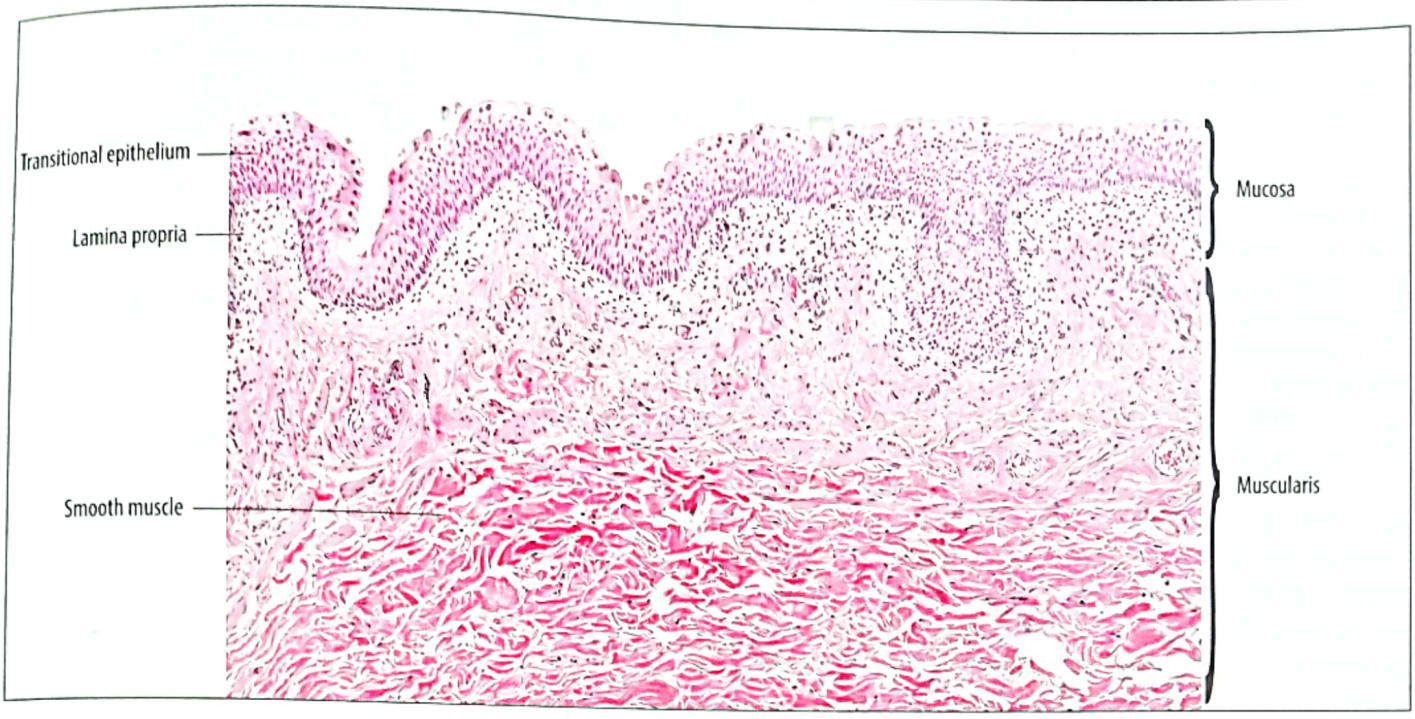

Fig. 20.10 A section through the wall of contracted (empty) urinary bladder.

URINARY BLADDER

The urinary bladder is a distensible reservoir that stores the urine till it is voided from the body through the urethra. The wall of the urinary bladder also consists of three coats: mucosa, muscularis, and adventitia/serosa.

MUCOSA

The mucosa of the empty (contracted) urinary bladder shows numerous folds that disappear when the bladder becomes distended with urine. The mucosa is lined by transitional epithelium (urothelium). The cells of this epithelium have a remarkable capability to stretch, shift on each other, and flatten, thus enabling this epithelium to rearrange its cell layers according to the state of distension of the bladder.

In the empty bladder the transitional epithelium appears to consist of 6 or more layers of cells (Fig. 20.10). There is a basal layer of cuboidal cells, over which are present several layers of polygonal cells. The most superficial layer consists of very large dome-shaped cells, called umbrella cells, whose highly convex apices bulge into the bladder cavity. As the bladder becomes distended due to accumulation of urine, the number of cell layers is reduced until, in the fully distended bladder, the epithelium is seen to consist only of two or three layers. The superficial cells also change their shape and become squamous. Consequently, the lining epithelium of the distended urinary bladder consists of a basal layer of cuboidal cells covered by one or two layers of squamous cells, and thus, has a close resemblance to the stratified squamous epithelium.

EM reveals that the plasmalemma covering the apical (i.e., luminal) surface of the most superficial cells of the transitional epithelium shows unusually thick and rigid areas called **plaques,** between which are present *interplaque regions* of normal, flexible cell membrane. In the relaxed (undistended) urinary bladder, the luminal plasmalemma of the superficial cells folds inwards and the plaque regions invaginate into the cell cytoplasm. Under EM, the stored plasmalemma is seen as fusiform vesicles in the cytoplasm of the cells of the most superficial layer of the urothelium. As the bladder fills, its wall undergoes distension and stretching and the stored plaques re-emerge onto the cell surface to increase the surface area of the epithelium.

The transitional epithelium of the urinary bladder forms an impermeable barrier that prevents the contents of the urine from passing into the epithelium itself or into the underlying tissues. Two special features of the urothelium are responsible for the creation of barrier between the toxic urine and tissues of the bladder wall:

i. The luminal plasmalemma of the most superficial cells of the urothelium is impermeable to salts and water.

ii. The surface cells are firmly bound to each other by continuous tight junctions and many desmosomes, so that leakage of urine via a paracellular route is not possible.

The urothelium of the urinary bladder is supported by a thick *lamina propria* composed of fine connective tissue. A few small mucus-secreting glands are found in the lamina propria near the ureteric orifices and the internal urethral orifice. Because of the absence of the muscularis mucosae, a well-defined submucosa is not present in the urinary bladder. However, some histologists call the loose connective tissue adjacent to the muscularis of the urinary bladder as submucosa.

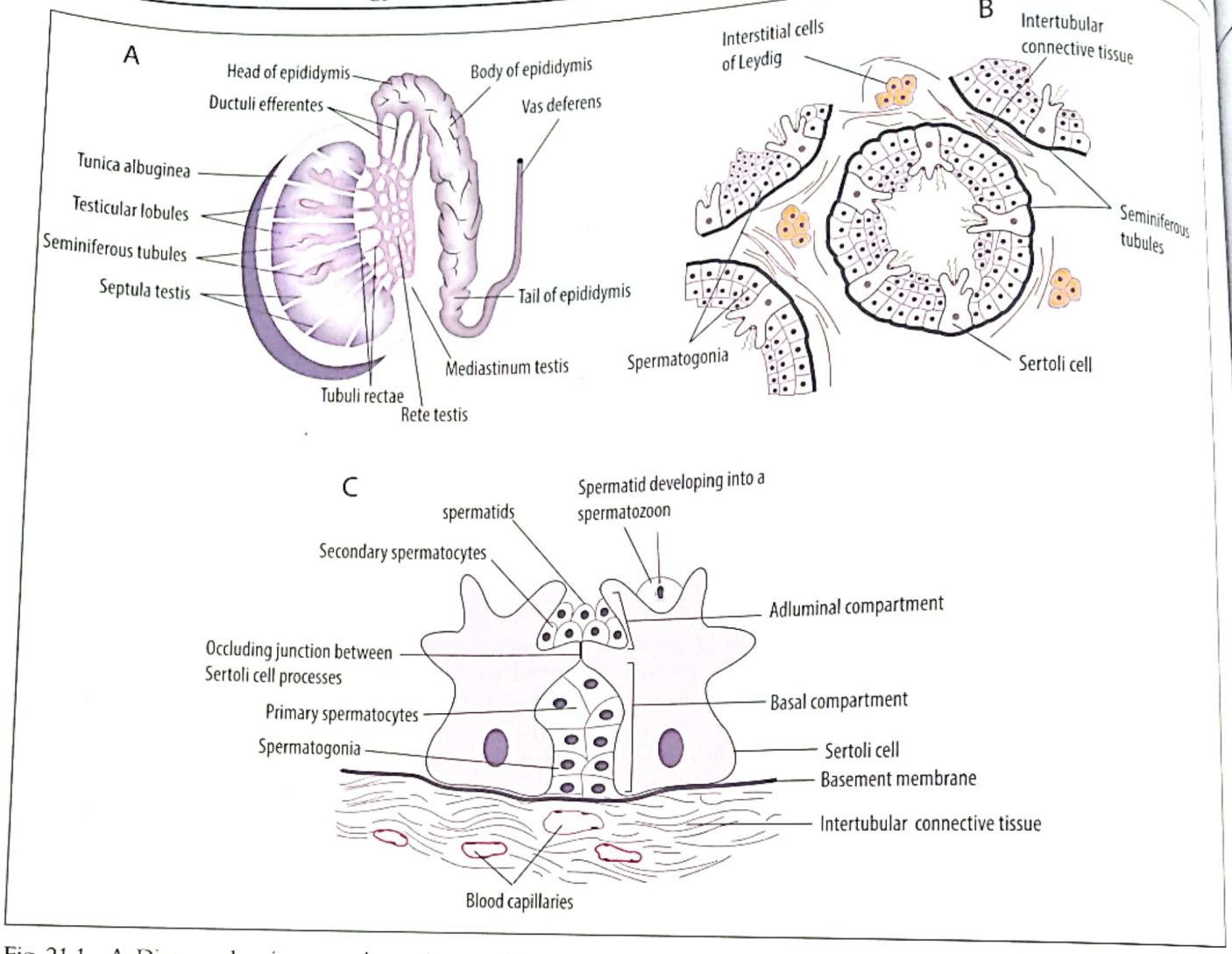

Fig. 21.1 A. Diagram showing general organization of the testis and genital ducts, B. Diagrammatic representation of the microscopic picture of a section of testis, C. Diagram of a portion of the seminiferous epithelium showing the division into the basal and adluminal compartments (due to the presence of occluding junction between the processes of neighboring Sertoli cells).

close to the nuclear membrane. They are the reserve cells and divide occasionally to maintain their own number and to give rise to type A pale spermatogonia.

ii. The ***type A pale spermatogonia*** contain a light-staining, vesicular nucleus. The nucleolus is located close to the nuclear envelope. These cells undergo regular mitotic division to produce other type A pale spermatogonia as well as type B spermatogonia.

The ***type B spermatogonia*** have a spherical nucleus that shows darkly staining clumps of chromatin located adjacent to the nuclear envelope. The nucleolus is situated in the center of the nucleus. Mitotic division of the type B spermatogonia produces daughter cells, all of which differentiate into primary spermatocytes.

The **primary spermatocytes** lie next to the spermatogonia. They are large cells having vesicular nuclei.

The **secondary spermatocytes** are smaller cells that arise from a primary spermatocyte as a result of the first meiotic division.

The **spermatids** are produced from the secondary spermatocytes by the second meiotic division. They lie adjacent to the lumen of the seminiferous tubule, closely applied to the surface of the Sertoli cells. A spermatid does not divide further but is transformed into a spermatozoon by a series of morphological changes which are collectively known as *spermiogenesis*.

Sertoli Cells

The Sertoli cells are tall columnar supporting cells that extend from the basal lamina to the lumen of the seminiferous tubule, interposed between the developing spermatogenic cells.

Each Sertoli cell measures about 80 µm in length and 30 µm in diameter. Due to their close association with the spermatogenic cells, the lateral margins of Sertoli cells are irregular and cannot easily be distinguished under LM. Each Sertoli cell contains a large, oval, vesicular nucleus which is located in the basal portion of the cell and contains a prominent, dark-staining nucleolus. The cytoplasm of the Sertoli cells contains numerous mitochondria, many

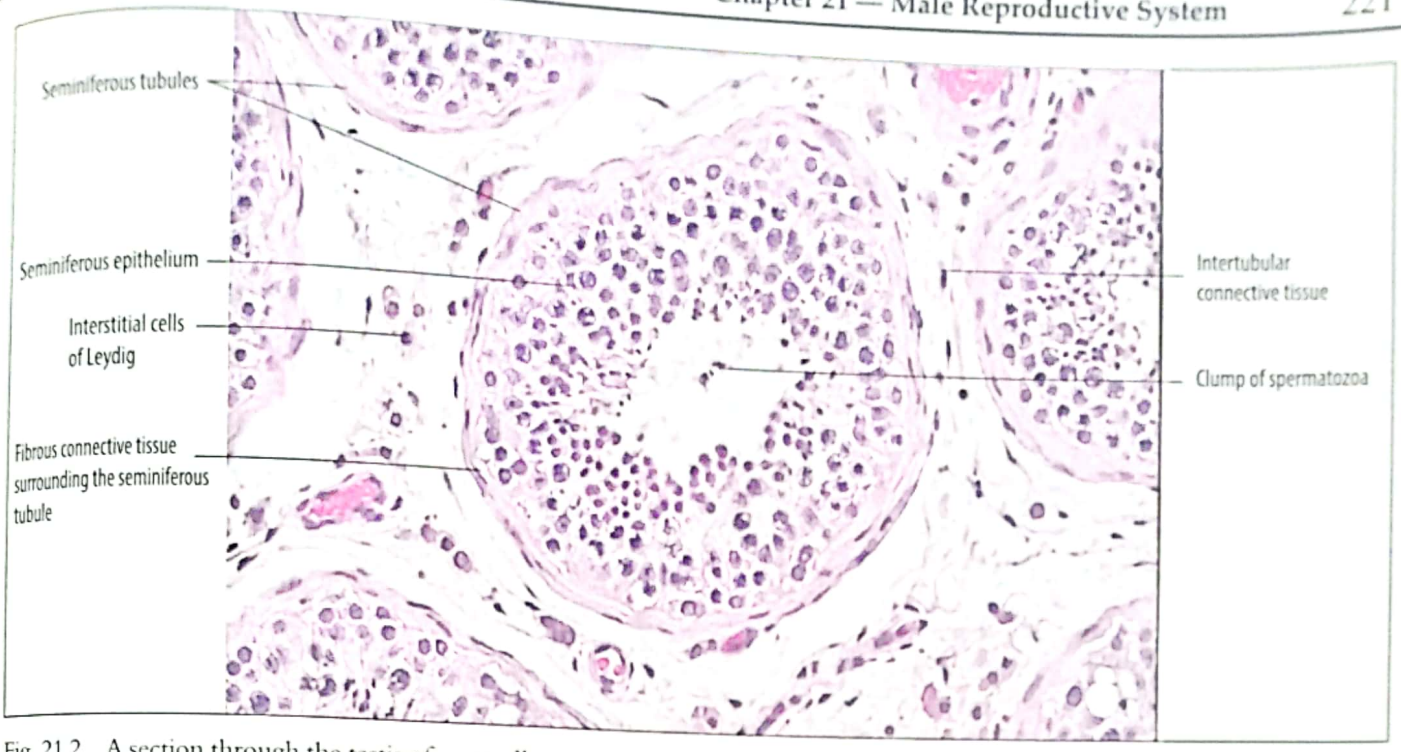

Fig. 21.2 A section through the testis of a sexually mature male.

Golgi complexes, abundant SER, some profiles of RER, and many lysosomes. Luminal surface of a Sertoli cell shows many grooves in which heads of the maturing spermatozoa are embedded.

The lateral walls of each Sertoli cells are irregular and exhibit cytoplasmic extensions which make contact with similar extensions from the neighboring Sertoli cells. Tight junctions (zonulae occludentes) are present where the lateral cytoplasmic extensions of a Sertoli cell meet with those of the adjoining Sertoli cells. These tight junctions establish two compartments in the seminiferous epithelium: a basal compartment and an adluminal compartment (Fig. 21.1 C). The **basal compartment** contains premeiotic germ cells, i.e., spermatogonia and early primary spermatocytes. As the primary spermatocytes become mature and are ready to undergo the first meiotic division, the occluding junctions between the Sertoli cell processes are temporarily disassembled and the mature primary spermatocytes move into the adluminal comapartment of the seminiferous tubule. The **adluminal compartment** contains meiotic populations of the developing germ cells which include the dividing primary spermatocytes, secondary spermatocytes, spermatids, and the developing spermatozoa. It is important to note that the adluminal compartment is occupied mainly by those germ cells whose nuclei contain haploid number of chromosomes.

BLOOD-TESTIS BARRIER

The blood-testis barrier is a physical barrier between the blood (circulating in the capillaries present in the intertubular connective tissue) and the germ cells present in the adluminal compartments of the seminiferous tubules.

As described above, tight junctions are present between the cytoplasmic processes of the neighboring Sertoli cells. These occluding junctions establish a permeability barrier, called blood-testis barrier, between the basal and adluminal compartments of each seminiferous tubule. The blood in the capillaries lying in the capillaries lying in the intertubular connective tissue has a direct access to the basal parts of the Sertoli cells and the germ cells lying in the basal compartments of the seminiferous tubules (spermatogonia and early spermatocytes). Due to the presence of occluding junctions between the lateral processes of the neighboring Sertoli cells, a barrier is created between the blood and contents of the adluminal compartment, which include those germ cells which contain a haploid number of chromosomes (secondary spermatocytes, spermatids, and developing spermatozoa). Due to the presence of the blood-testis barrier, these cells receive their nutrition through the Sertoli cells.

The blood-testis barrier isolates haploid germ cells in the adluminal compartment and prevents antigenic products of germ cell maturation from entering the circulation and generating an autoimmune response. In addition, the blood-testis barrier also prevents the passage of blood-borne toxins to the adluminal compartment of the seminiferous tubules and thus protects the developing germ cells from being damaged by these toxins.

Functions of the Sertoli Cells

Previously, the Sertoli cells were supposed to play only a supportive role for the spermatogenic cells, but now it is known that the Sertoli cells perform a number of important functions which can be grouped into 4 categories: (i) supportive functions, (ii) protective functions, (iii) secretory functions, and (iv) phagocytic functions.

1. **Support and nutrition of the developing germ**

6 µm long and consists only of the axoneme surrounded by the plasma membrane.

INTERTUBULAR CONNECTIVE TISSUE

The intertubular connective tissue of the testis fills the spaces between the seminiferous tubules (Fig. 21.2). This tissue, also called interstitial tissue, contains collagen fibers, lymph vessels, blood vessels and nerves. Many fibroblasts and a few macrophages and mast cells are also present. Most important content of the intertubular connective tissue are clusters of endocrine cells, called *interstitial cells*, which secrete the male hormone testosterone.

INTERSTITIAL CELLS

The interstitial cells of testis, also called **cells of Leydig**, are large, ovoid cells that are seen to be arranged as small groups in the intertubular connective tissue (Fig. 21.1 B & 21.2). Blood capillaries are found in close association to these cells. Each Leydig cell has a central, darkly staining nucleus and an eosinophilic cytoplasm. The cytoplasm of these cells contains a large number of lipid droplets. As the lipid is dissolved out during routine histological procedures, the cytoplasm gives a vacuolated appearance in the routinely stained sections examined under the light microscope. Electron microscopic studies show that Leydig cells possess the structural features of steroid-producing cells. Their cytoplasm contains fat droplets, abundant smooth endoplasmic reticulum, a large Golgi apparatus, and numerous mitochondria, many of which have tubular cristae.

The interstitial cells of Leydig secrete testosterone. This hormone is released into the blood capillaries as soon as it is synthesized and, therefore, the Leydig cells do not exhibit any secretory vesicles.

MALE GENITAL DUCTS

The male genital duct system can be divided into two parts: (1) ***intratesticular genital ducts*** which include tubuli recti and rete testis, and (2) ***excretory genital ducts*** which include ductuli efferentes, ductus epididymis, ductus deferens, and the ejaculatory duct.

TUBULI RECTI

At the apex of testicular lobule, each seminiferous tubules continues as a short, straight tubule (tubulus rectus). The **straight tubules** (tubuli recti) have an average diameter of 25 µm. The proximal (initial) part of a straight tubule is lined by Sertoli cells, whereas its distal part is lined by a single layer of cuboidal epithelial cells.

RETE TESTIS

This is an anastomosing network of delicate interconnecting channels lying in the connective tissue of the mediastinum testis (Fig. 21.1A). The channels of the rete testis are lined by simple cuboidal epithelium.

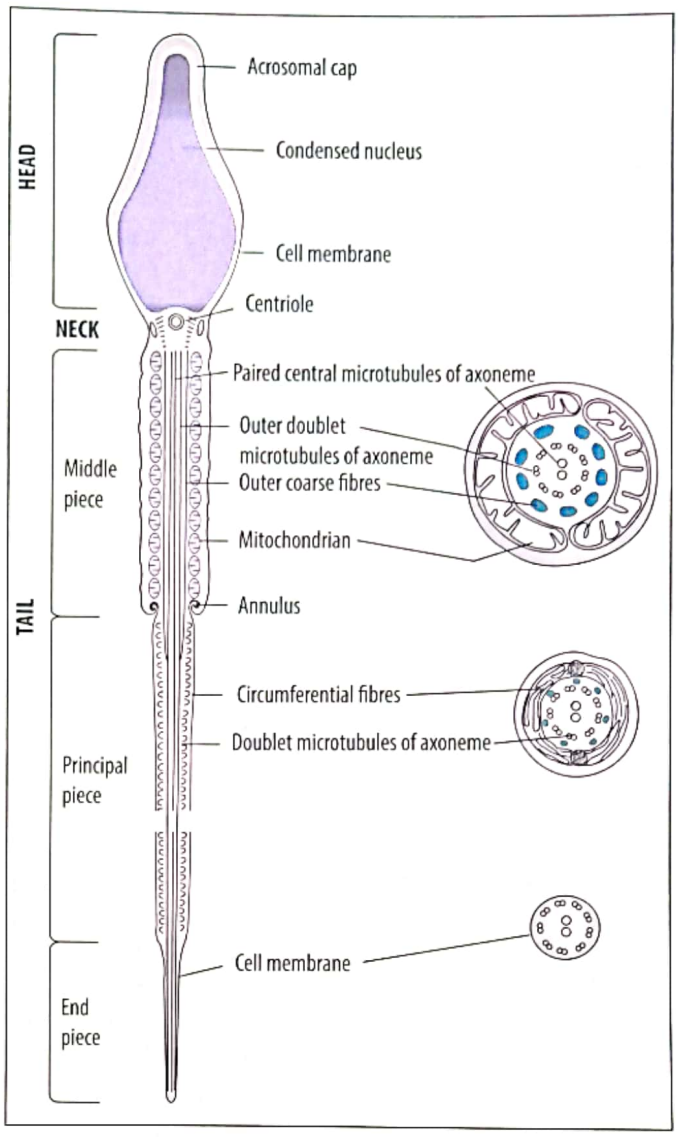

Fig. 21.3 Diagrammatic representation of the structure of a mature spermatozoon. At the left, a longitudinal section; at the right, transverse sections of the tail at the level of middle piece, principal piece, and end piece, respectively.

DUCTULI EFFERENTES

The rete testis is drained by 15-20 ductules which emerge from the superior part of the posterior border of testis. These ductules are titled ductuli efferentes testis (i.e., efferent ductules of the testis), but more commonly these ductules are just referred to as *ductuli efferentes* or *vasa efferentia*. Each efferent ductule is about 7 cm in length and 50 µm in diameter. After following a highly tortuous course, the ductuli efferentes pass into the head of the epididymis to open into the duct of the epididymis.

The ductuli efferentes are lined by a simple columnar epithelium which consists of groups of tall columnar *ciliated cells* alternating with groups of nonciliated, cuboidal cells which are called *principal cells. Due to the presence of alternating groups of columnar and cuboidal cells, the lumen of an efferent ductile exhibits a characteristic wavy outline.*

The cubodal principal cells of the efferent ductules are absorptive in function and bear microvilli on their luminal

consisting of tall columnar cells and basal cells. Many of the tall columnar cells, but not all, bear stereocilia on their free surface. The basal cells rest on the basement membrane and serve as stem cells for the ductal epithelium. Beneath the epithelium lies the ***lamina propria***, which consists of a thin layer of connective tissue rich in elastic fibers.

The **muscularis** of the ductus deferens is very thick. It is composed of three layers of smooth muscle: a thin inner longitudinal layer, a thick middle circular layer, and a thick outer longitudinal layer. At the time of ejaculation, strong peristaltic contractions of the thick muscularis of the vas deferens propel the spermatozoa into the ejaculatory duct.

The **adventitia** surrounds the muscularis and consists of a thin layer of connective tissue containing blood vessels and nerves.

The ampulla of the vas deferens is characterized by the presence of tall, highly branched mucosal folds and a thinner muscularis than rest of the vas. The columnar cells of the lining pseudostratified epithelium of the ampulla are supposed to perform a secretory function similar to that of the principal cells of the seminal vesicle.

Functions of the Ductus Deferens

The primary function of the ductus deferens is to transport spermatozoa from the duct of epididymis to the ejaculatory duct during the process of ejaculation. During this process, the smooth muscle of the vas deferens contracts forcefully to push the spermatozoa and testicular fluid into the urethra through the ejaculatory duct. Presence of stereocilia on some columnar cells of the lining epithelium indicates that absorption of water from the seminal fluid also occurs in the ductus deferens to make the semen more viscid.

EJACULATORY DUCT

At its termination, each ductus deferens joins the duct of the seminal vesicle of its own side. By the union of these two ducts, the ejaculatory duct is formed which pierces the prostate gland to open into the prostatic urethra. The ejaculatory ducts are lined by pseudostratified columnar epithelium.

ACCESSORY GLANDS OF THE MALE REPRODUCTIVE SYSTEM

The spermatozoa and testicular fluid constitute only 2-5% of the total volume of the semen released as a result of an ejaculation. The remaining volume of the ejaculate consists of secretory products of the accessory glands of the male reproductive system which are: the paired seminal vesicles, the prostate gland, and the paired bulbourethral glands. The products of these glands carry out several important functions concerning the nutrition, protection, and transport of the spermatozoa in the female genital tract. The secretory activity of all of the male accessory sex glands is under the control of the testosterone.

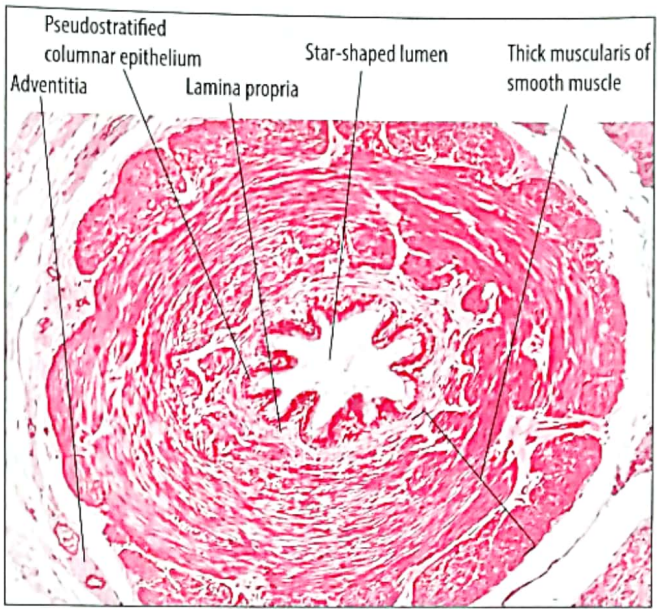

Fig. 21.5 A transverse section through the ductus deferens.

SEMINAL VESICLES

The seminal vesicles, also called ***seminal glands***, are paired, elongate, and highly tortuous tubular glands lying on the posterior surface of the urinary bladder. The total tube length of a seminal vesicle is 15 cm but, due to its highly convoluted nature, its forms a body which is about 5 cm long. The short duct of the seminal vesicle joins the ampulla of the vas deferens to form the ejaculatory duct which pierces the prostate gland to open into the prostatic urethra.

In histological sections, the convoluted tube of the seminal vesicle is seen to be cut in different orientations. The wall of each seminal vesicle is seen to be composed of three layers: mucosa, muscularis, and adventitia.

The **mucosa** of the seminal vesicle is thrown into complicated folds. The primary folds of the mucosa branch into secondary and tertiary folds that project far into the lumen and fuse with each other frequently (Fig. 21.6). These highly elaborate mucosal folds tremendously increase the secretory surface area of the gland. In sections, the lumen appears as irregular spaces between the extensive mucosal folds. The lining epithelium is of pseudostratified columnar variety. Beneath the epithelium is present a lamina propria of loose vascular connective tissue which is rich in elastic fibers.

The **pseudostratified columnar epithelium** of seminal vesicle consists of principal cells and basal cells. The ***principal cells*** are tall columnar and their cytoplasm exhibits the features of protein-secreting cells, i.e., prominent RER, well-developed Golgi complex, numerous mitochondria, and abundant secretory granules. The short ***basal cells*** serve as stem cells for the lining epithelium.

The **muscularis** of the seminal vesicle consists of an inner circular and an outer longitudinal layer of smooth muscle.

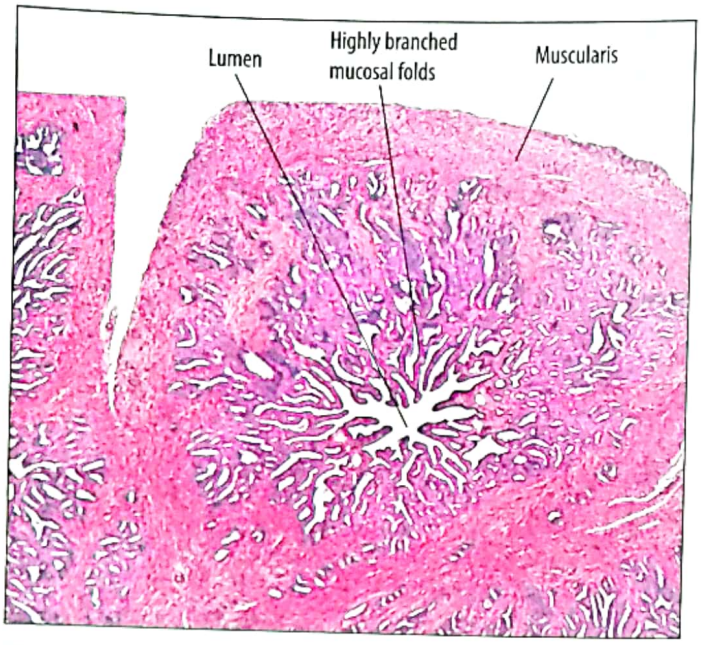

Fig. 21.6 A section through the seminal vesicle.

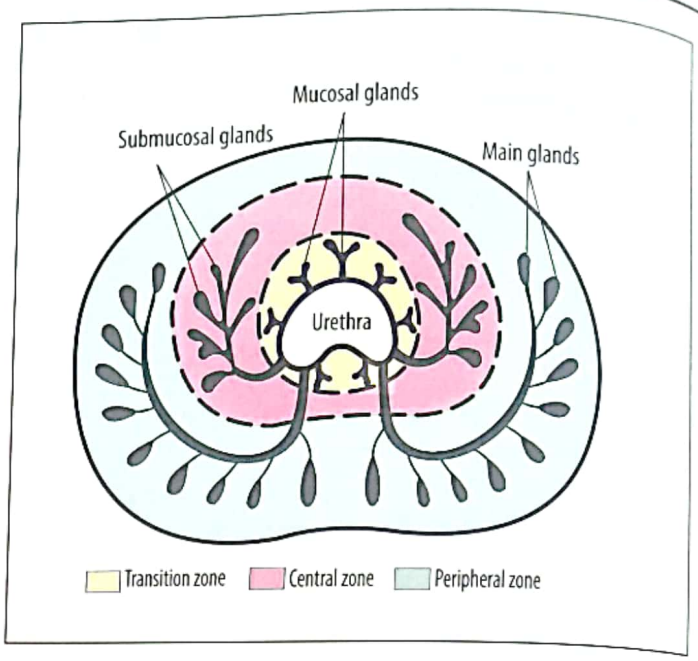

Fig. 21.7 Diagram illustrating the zones of the prostate gland.

Contraction of this smooth muscle forces the seminal vesicles to empty during ejaculation.

The **adventitia** consists of a thin layer of fibroelastic connective tissue.

The seminal vesicles produce a yellowish, alkaline, viscous fluid that makes up about 65-70% of the seminal fluid. The main contents of the secretory product of the seminal vesicles are: fructose, ascorbic acid, citric acid, fibrinogen, and prostaglandins.

The alkaline nature of the secretory product of seminal vesicles helps to neutralize the acidic pH of vagina. The fructose serves as the chief source of energy for the spermatozoa after their release from the body of the male. The fibrinogen causes the semen to become clotted immediately after being released from the urethra. The functional role of the prostaglandins in the semen is not clear. The research indicates that the seminal prostaglandins stimulate the smooth muscle in the wall of the female genital tract to undergo rhythmic contractions which facilitate the movement of spermatozoa toward the ovum.

PROSTATE GLAND

The prostate surrounds the initial part of the male urethra and is the largest accessory sex gland of the male reproductive system. It is composed of **multiple tubuloacinar glands** embedded in a **fibromuscular stroma** (Fig. 21.8). The ducts of the prostatic glands open into the prostatic part of the urethra, which passes through the center of this gland. The entire prostate is covered by a *capsule* of fibrocollagenous connective tissue from which septa extend into the gland substance and divide it into ill-defined lobes.

The **parenchyma** of the prostate consists of 30 to 50 ***compound tubuloacinar glands***, ducts of which open into the prostatic urethra. The prostatic glands lie in three concentric zones around the prostatic urethra (Fig. 21.7). The innermost zone, known as **transition zone,** makes up about 5% of the prostatic volume in the young men. The glands located in this zone are called *mucosal glands*; ducts of these glands open directly into the prostatic urethra. The middle zone, called **central zone,** constitutes nearly 25% of the prostatic volume. The glands present in this zone are called *submucosal glands*. Ducts of these glands pass through the transition zone to open into the prostatic urethra. The outermost zone, called **peripheral zone**, is the largest zone and constitutes almost 70% of the glandular tissue in young males. The glands present in this zone are referred to as *main glands*. The main prostatic glands have long ducts which pass through the central zone and transition zone to pour their secretions into the prostatic urethra.

The prostatic glands consist of irregularly-shaped secretory acini and tubules which are lined mainly by simple columnar epithelium but patches of simple cuboidal epithelium and pseudostratified culumnar epithelium are also present. The prostatic glands secretes the prostatic fluid which is kept in store within the lumina of the glands to be expelled during ejaculation.

The dense **stroma** of the prostate is fibromuscular in character. It consists of collagen fibers, elastic fibers, and a large number of smooth muscle fibers (Fig. 21.8). The stroma also lodges the blood vessels, lymphatics, and nerves of the prostate.

The smooth muscle of the fibromuscular prostatic stroma contracts forcefully at the time of ejaculation. Consequently, the secretions stored within the prostatic glands are pushed and released into the urethra to become a part of the seminal fluid.

In stained histological sections, small bodies called *corpora*

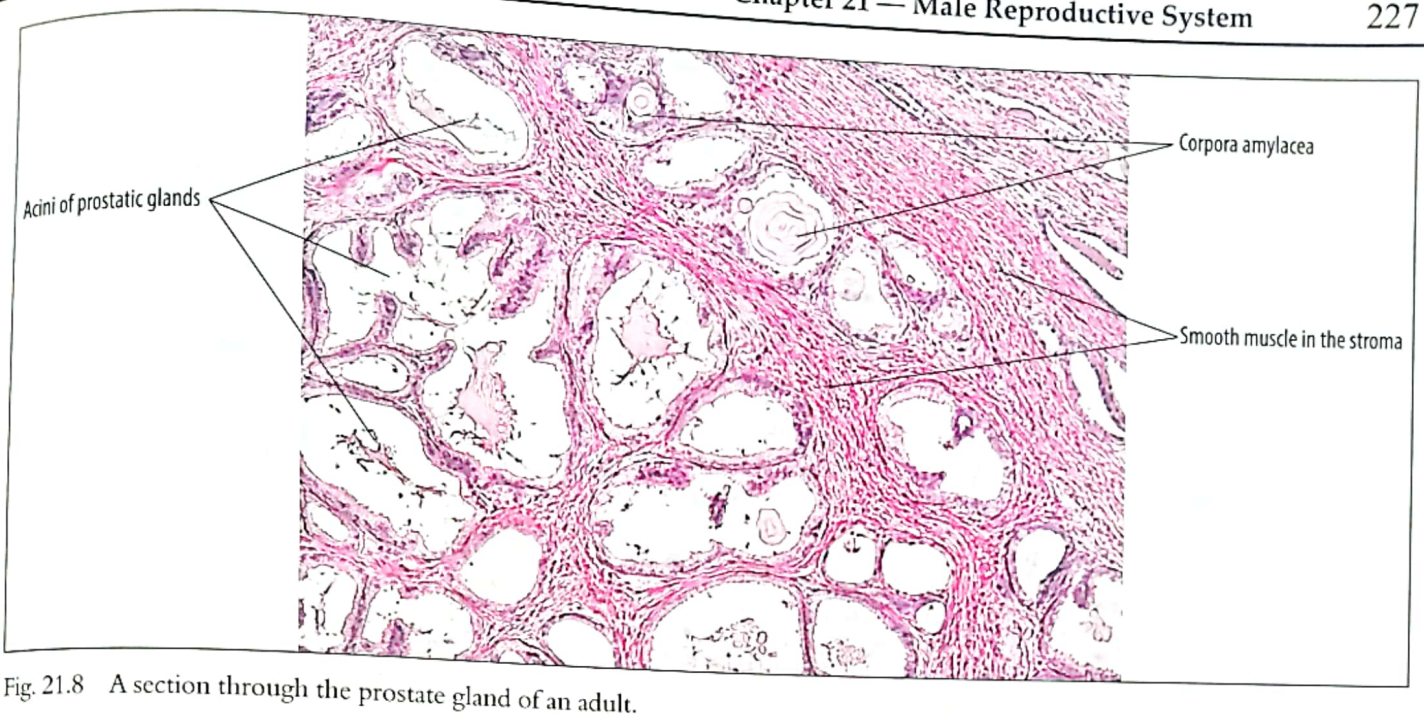

Fig. 21.8 A section through the prostate gland of an adult.

amylacea are often observed in the lumina of the acini of prostatic glands. These bodies, also called *prostatic concretions*, vary in shape and size considerably, varying in diameter from 250 µm to 2 mm. In sections, they take an acidophilic stain and appear to be made up of concentric lamellae (Fig. 21.8). The prostatic concretions are believed to represent condensation of the secretory materials, mainly glycoproteins, around the cell fragments. Their number increases with age and they may become partially calcified. No functional or clinical significance has as yet been ascribed to corpora amylacea.

The secretory product of the prostate, called prostatic fluid, constitutes nearly 20-30% of the total volume of the seminal fluid. Its main components include acid phosphatase, fibrinolysin, serine protease, prostaglandins and zinc. The fibrinolysin and serine protease cause slow liquefaction of the coagulated semen after it has been deposited in the vagina.

It is important to know that small quantities of the enzyme serine protease normally leak into the blood capillaries of the prostatic stroma and thus enter the bloodstream. In clinical terms, the serine protease circulating in the blood is called **prostate-specific antigen (PSA)**. The normal serum level of PSA in a healthy adult male is 4 nanograms per mL or less. An elevated serum PSA level is a strong indication of prostatic disease, especially cancer.

Cancer of prostate gland is the commonest type of cancer in the adult males. Nearly 80% of the prostatic cancers originate in the glands of the peripheral zone of this gland.

After the age of 40 years, the prostatic glands as well as the fibromuscular stroma begin to undergo hyperplasia. This hyperplasia, called **benign prostatic hyperplasia (BPH)**, mainly affects the transition zone of the prostate and leads to partial or complete obstruction of the urethra. The BPH causes enlargement of the prostate and results in the formation of nodules of various sizes in the prostatic tissue; therefore, the BPH is also called *nodular hyperplasia* of the prostate.

Bulbourethral Glands

The bulbourethral glands, also called *Cowper's glands*, are two pea-sized bodies located posterolateral to the membranous urethra. The secretions of the gland pass into the proximal part of the penile urethra by a short duct. A thin connective tissue capsule covers each gland. External to the capsule is present a layer of skeletal muscle fibers derived from the muscles which lie around the gland. The gland substance is divided into lobules by connective tissue septa which contain smooth muscle fibers.

The lobules of the bulbourethral gland contain the secretory units which are of tubuloacinar variety and are lined by a mucus-secreting simple columnar epithelium. During erotic stimulation, the bulbourethral glands secrete a thin, mucoid fluid which serves to lubricate the lumen of the penile urethra, so that the semen can pass through the urethra smoothly.

Semen

The normal semen is a slightly alkaline, viscous, pale-yellow fluid which is released from the penile urethra at the height of sexual stimulation. The semen, also called *seminal fluid*, consists of spermatozoa and seminal plasma. The spermatozoa constitute only 2-5% of the volume of the ejaculate and the seminal plasma makes up remaining

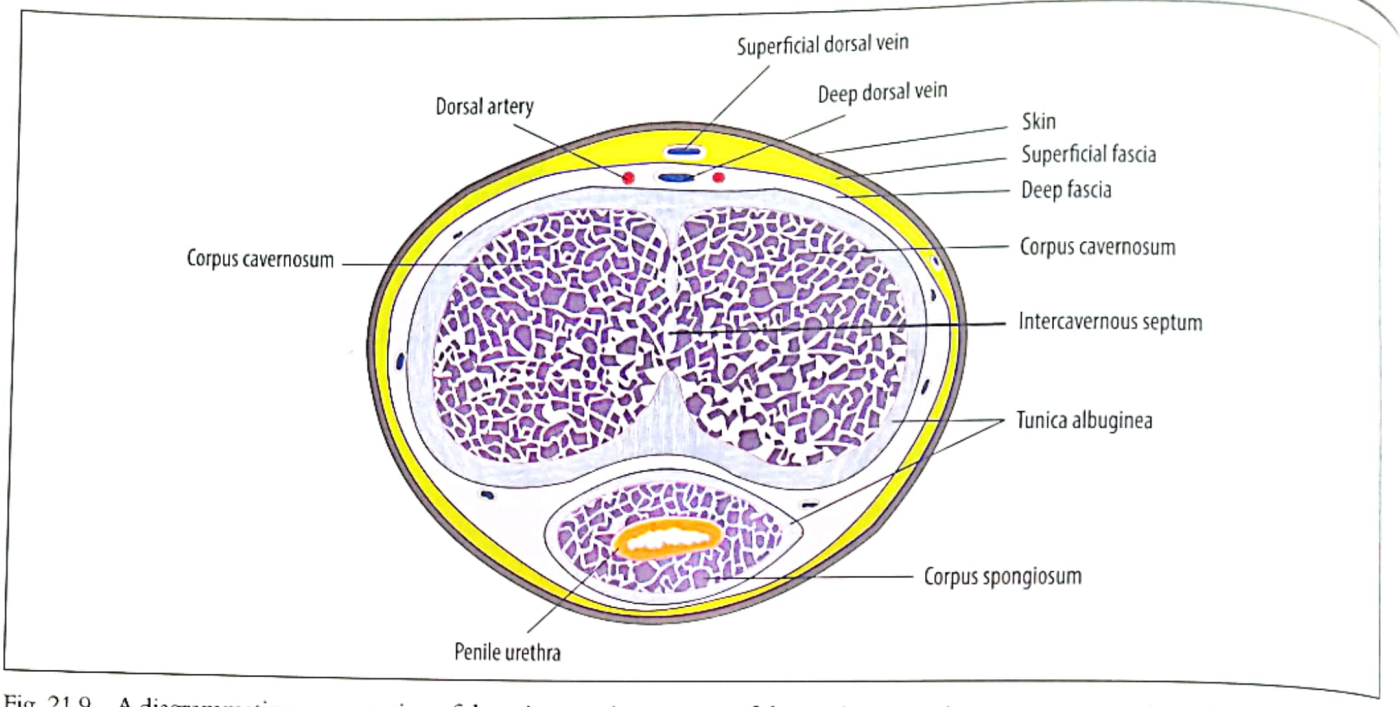

Fig. 21.9 A diagrammatic representation of the microscopic structure of the penis as seen in a transverse section of the organ.

95% or more of the semen. The seminal plasma consists of secretory products of the seminal vesicles, prostate, and bulbourethral glands. The density of spermatozoa in the semen is highly variable, a count of 20-150 million sperms per mL is considered to be normal. The normal volume of semen in a single ejaculation varies from 1.5 to 5 mL.

PENIS

The penis is the copulatory organ of the male which also contains major part of the urethra. It is composed chiefly of three cylindrical masses of erectile tissue which are enveloped by the skin. The three columns of the erectile tissue, which make the bulk of the penis, are: two *corpora cavernosa* and one *corpus spongiosum*. The two corpora cavernosa lie next to each other on the dorsal side, while the smaller, single corpus spongiosum lies between them on the ventral side (Fig. 21.9). The penile urethra passes through the center of the corpus spongiosum. The two corpora cavernosa are fused to each other in the body of the penis, but posteriorly they diverge away from each other to form the crura of the penis. The proximal end of the corpus spongiosum is dilated to form the bulb of the penis, while its distal end is expanded to form the glans penis.

Each cylinder of the erectile tissue is surrounded by a sheath of dense fibrous connective tissue called *tunica albuginea*. The two corpora cavernosa share a common tunica albuginea of considerable thickness. A median septum of fibrous tissue, called *intercavernous septum*, separates the corpora cavernosa from each other. In the distal half of the penis this septum is incomplete and thus permits cross-circulation blood between the two corpora cavernosa. The corpus spongiosum is surrounded by a much thinner tunica albuginea.

The skin of the penis is thin and hairless. It contains numerous sweat gland. In addition, sebaceous glands, not associated with hair follicles, are also present. The superficial fascia of the penis consists of loose connective tissue which is devoid of fat. It contains smooth muscle fibers which are continuous posteriorly with the dartos muscle of the scrotum (therefore, the superficial fascia of the penis is also called *dartos fascia*). The deep fascia of the penis, called Buck's fascia, is a thick and tough layer of connective tissue which is present outer to the tunica albuginea. It surrounds the fused corpora cavernosa and splits to enclose the corpus spongiosum.

The **erectile tissue** of the corpora cavernosa and corpus spongiosum consists of intercommunicating cavernous vascular spaces (sinusoids) which are lined by endothelium. As already mentioned, these spaces are separated from each other by connective tissue trabeculae which arise from the tunica albuginea. These trabeculae contain a large number of smooth muscle fibers. The sinusoids of the erectile tissue of the corpora remain empty when the penis is in the flaccid state but, during sexual arousal, they become engorged to make the penis erect and rigid.

The cavernous tissue of the corpora cavernosa receives blood through the deep arteries of the penis (also called *cavernosal arteries*), one of which runs in the center of each corpus cavernosum. These arteries give off branches (arterioles) which run in the trabeculae between the sinusoids. When the penis is in a flaccid state, these arterioles pursue a helical (i.e., spiral) course and, therefore, are called *helicine arteries*. During erection, these arterioles straighten and dilate to fill the cavernous tissue with blood. The blood from the cavernous spaces is drained into a venous plexus which lies under the tunica albuginea and finally drains into the deep dorsal vein of the penis. The corpus spongiosum

receives its blood supply through the artery of the bulb and becomes engorged with blood during erection, although its contribution to the rigidity of penis is minimal. Because of its thinner fibrous coats, the corpus spongiosum permits expansion of the penile urethra to allow the viscid semen to be easily discharged through the urethra at the time of ejaculation.

The nerve supply of the penis is derived from somatic, sympathetic and parasympathetic nerves. The skin and internal tissues of the penis are richly supplied by a variety of sensory nerve endings. The smooth muscle of the penile cavernous tissue and penile blood vessels is supplied by the autonomic (sympathetic and parasympathetic nerves).

Mechanism of Erection

When the penis is in the flaccid state, the smooth muscle present in the trabeculae of the cavernous tissue and in the walls of the penile arteries and arterioles remains in a state of tonic contraction, allowing only a small amount of arterial flow for the nutritional purposes. Sexual stimulation triggers release of neurotransmitters from the parasympathetic nerve terminals which results in relaxation of the arterial as well as trabecular smooth muscle, resulting in an increased arterial blood flow and expansion and engorgement of the cavernous vascular spaces. Compression of the venous plexuses lying between the tunica albuginea and the dilating sinusoids of the corpora decreases the venous outflow to a minimum. Trapping of the blood in the sinusoids leads to an increase in the diameter and length of the penis and makes it rigid and erect. When the erection is over, sympathetic stimulation causes contraction of the trabecular and arterial smooth muscle and, thus, flaccid state of the penis is restored.

PREPUCE

In the uncircumcised males, the glans penis is covered by a fold of skin called **prepuce**. The outer surface of the prepuce is covered by the ordinary epidermis consisting of stratified squamous keratinized epithelium. However, its inner surface is lined by stratified squamous non-keratinized epithelium. The skin of the inner surface also contains a large number of sebaceous glands, secretions of which keep this surface lubricated.

Female Reproductive System 22

The female reproductive system consists of: (1) a pair of ovaries, (2) a system of genital ducts (the uterine tubes, uterus and vagina), and (3) external genitalia. The mammary glands are also included in this chapter because their development and functional state are directly related to the hormonal activity of the female reproductive system.

During the reproductive period of the life of a female, cyclic changes in the blood levels of the female sex hormones, governed by the hypothalamic-pituitary system, lead to rhythmic, cyclic events in the female reproductive organs, especially in the ovary and the mucosal lining (endometrium) of the uterus. The cyclic events in the ovary are collectively known as the ovarian cycle, while the cyclic changes in the endometrium are called menstrual cycle.

OVARIES

The ovaries are the primary sex glands of the female and their chief function is to produce the female gametes (ova). However, like the testes, the ovaries also have endocrine function which is to produce the female sex hormones (estrogens and progestogens).

The ovary is covered by a layer of simple cuboidal epithelium called surface epithelium, which is also known as *germinal epithelium* (Fig. 22.1). In stained sections examined under LM, it is seen that, beneath the surface epithelium, the ovarian substance is surrounded by a capsule of dense, fibrous connective tissue known as tunica albuginea. The main substance of the ovary is seen to consist of two zones: an inner *medulla* and an outer *cortex*, which merge with each other without any distinct line of demarcation.

The **medulla** of the ovary lies in the center of the organ and consists of loose connective tissue. This connective tissue contains blood vessels, lymphatic vessels, and nerves of the ovary.

The ovarian **cortex** is the broader, peripheral zone of the organ. It consists of: (i) a compact, *richly cellular stroma*, and (ii) spherical bodies called *ovarian follicles*, which are irregularly scattered in the stroma.

The **stroma of the ovarian cortex** is composed chiefly of spindle-shaped cells called *stromal cells*. Collagen fibers, reticular fibers, fibroblasts, smooth muscle cells, and macrophages are present between the stromal cells. The cortical stroma also contains a large number of blood vessels (capillaries).

The fusiform ***stromal cells*** of the ovarian cortex are arranged in a haphazard manner or in ill-defined whorls. These cells have the capability to transform into endocrine cells and give rise to a sheath around each of the growing ovarian follicle; this sheath is called theca folliculi. The cells of the theca folliculi actively secrete female sex hormones.

With the increase in the age of the women, the collagen fibers in the cortical stroma gradually increase and stromal cells decrease in number. By the end of the reproductive age, the cortical stroma consists almost entirely of collagenous tissue.

THE OVARIAN CYCLE

During the reproductive period of the life (which begins at puberty and ends at menopause), the ovaries of every female normally undergo cyclic changes in structure. These changes constitute a rhythmically recurring cycle which is called ovarian cycle. The ovarian cycle is about 28 days long and is divided further into two phases: (i) follicular phase, and (ii) luteal phase.

The Follicular Phase

During this phase, which is also called preovulatory phase, many ovarian follicles undergo a growth process in both ovaries. Normally, this process results in the release of a single ovum (oocyte) from one of the two ovaries. The process of release of the ovum is called ovulation and, in this process, the two ovaries alternate with each other in an irregular manner. The growth of the ovarian follicles occurs under the influence of the follicle stimulating hormone (FSH) which is produced by the anterior pituitary. The growing ovarian follicles themselves secrete estrogens (mainly estradiol). Normal length of the follicular phase is 14 days, but it may vary considerably.

The Luteal Phase

This phase begins after ovulation and, therefore, is also called postovulatory phase. As this phase begins, a temporary endocrine structure, called corpus luteum, is formed in the ovary which ovulated. The corpus luteum is formed from the remains of the ovulated follicle and secretes large quantities of progesterone. The length of the luteal phase is relatively constant (14 days) because, if the ovum is not fertilized, the corpus luteum stops secreting progesterone after about 10 days of its formation and then rapidly degenerates.

OVARIAN FOLLICLES

An ovarian follicle is a spherical structure containing an immature ovum (oocyte) which is surrounded by epithelial cells which form a covering around it. In the growing follicles, a connective tissue covering is also present outer to the epithelial coat. The ovaries of a female child contain

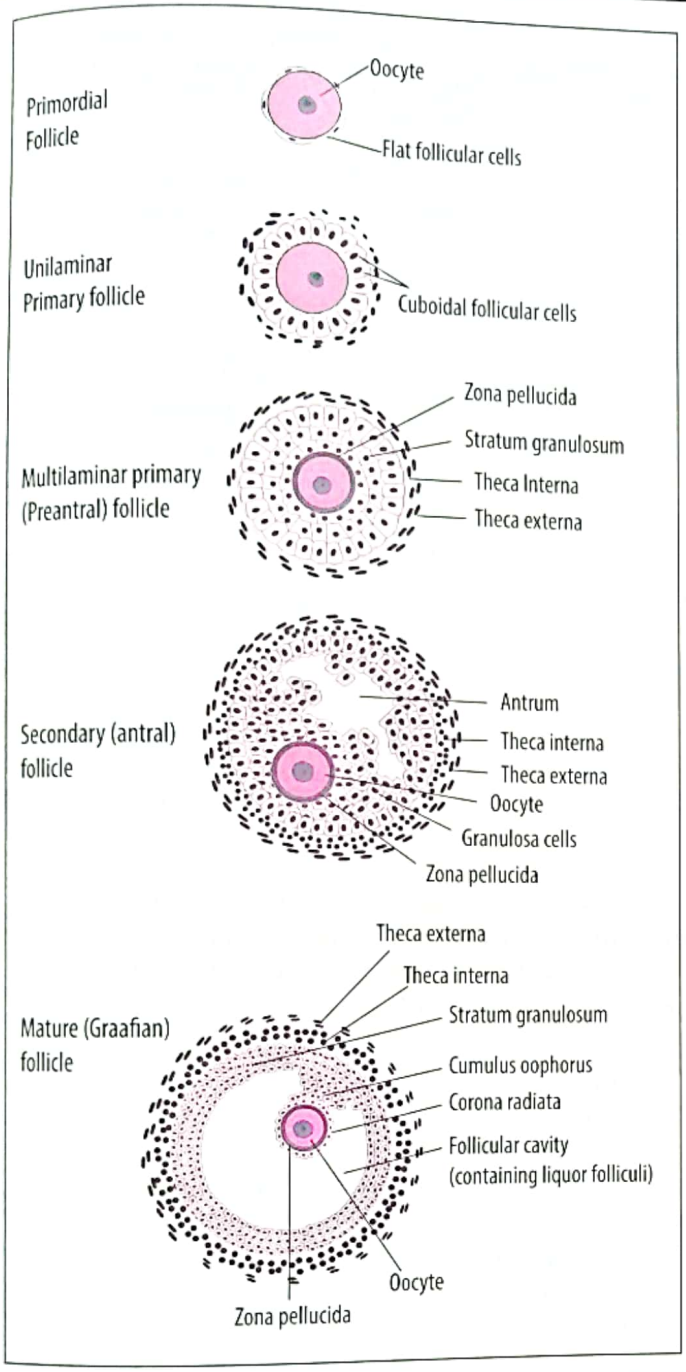

Fig. 22.2 Diagrammatic representation of structure of the ovarian follicles in various stages of development.

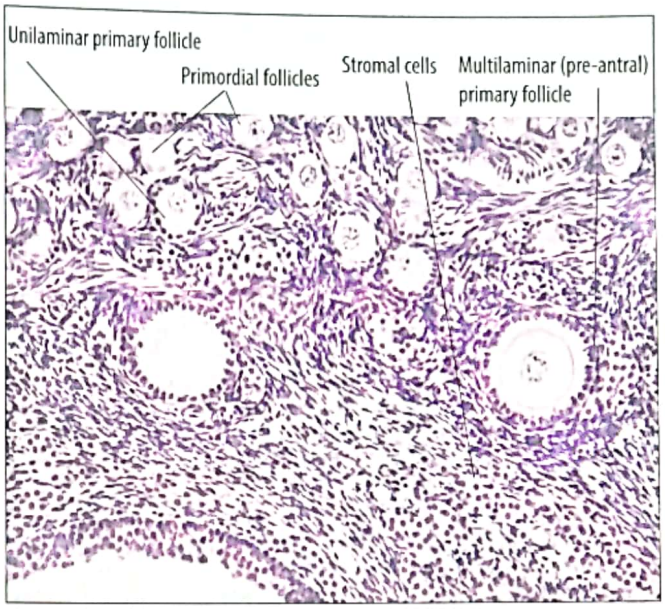

Fig. 22.3 A section through the ovarion cortex.

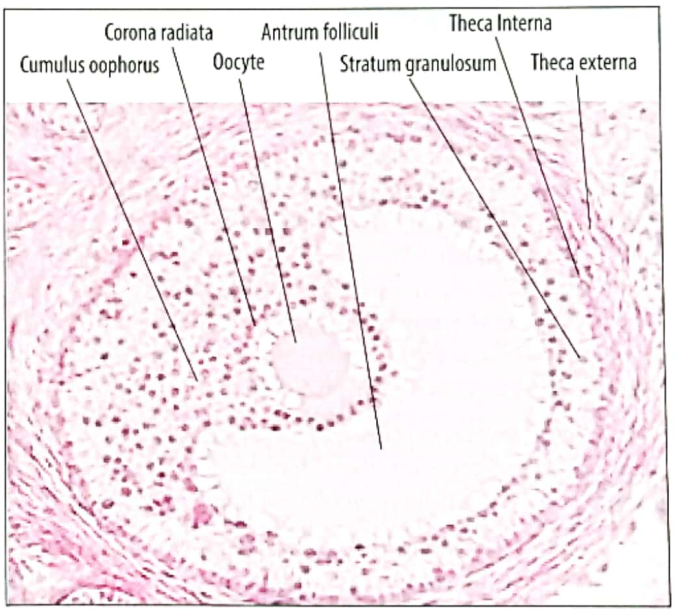

Fig. 22.4 A section through the ovarian cortex showing a secondary (antral) follicle.

are joined to each other by maculae communicantes (gap junctions).

A layer of amorphous, homogeneous material appears between the oocyte and granulosa cells. This layer, called **zona pellucida**, is 5-10 µm thick and takes a deep acidophilic stain in ordinary histological preparations. The zona pellucida also contains four glycoproteins titled ZP1, ZP2, ZP3, and ZP4, which are secreted by the oocyte. During the process of fertilization, some of these proteins serve as receptors that bind to the proteins present on the surface of the sperm. Thin tapering processes (filopodia) from the follicular cells and microvilli from the oolemma penetrate the zona pellucida and communicate with each other by gap junctions.

Multilaminar Primary Follicle

As growth of the ovarian follicle continues, the granulosa cells proliferate further and give rise to several layers of cells around the primary oocyte. Therefore, the growing ovarian follicle now comes to be known as multilaminar primary follicle. The multiple layers of granulosa cells are collectively known as *stratum granulosum*. As the follicular growth progresses further, the stroma of the ovarian cortex begins to be form a sheath around the ovarian follicle which is called *theca folliculi*. The stratum granulosum is separated from the theca folliculi by a well-defined basement membrane. The theca folliculi soon becomes differentiated into two layers: an inner layer called theca interna, and an outer layer named theca externa.

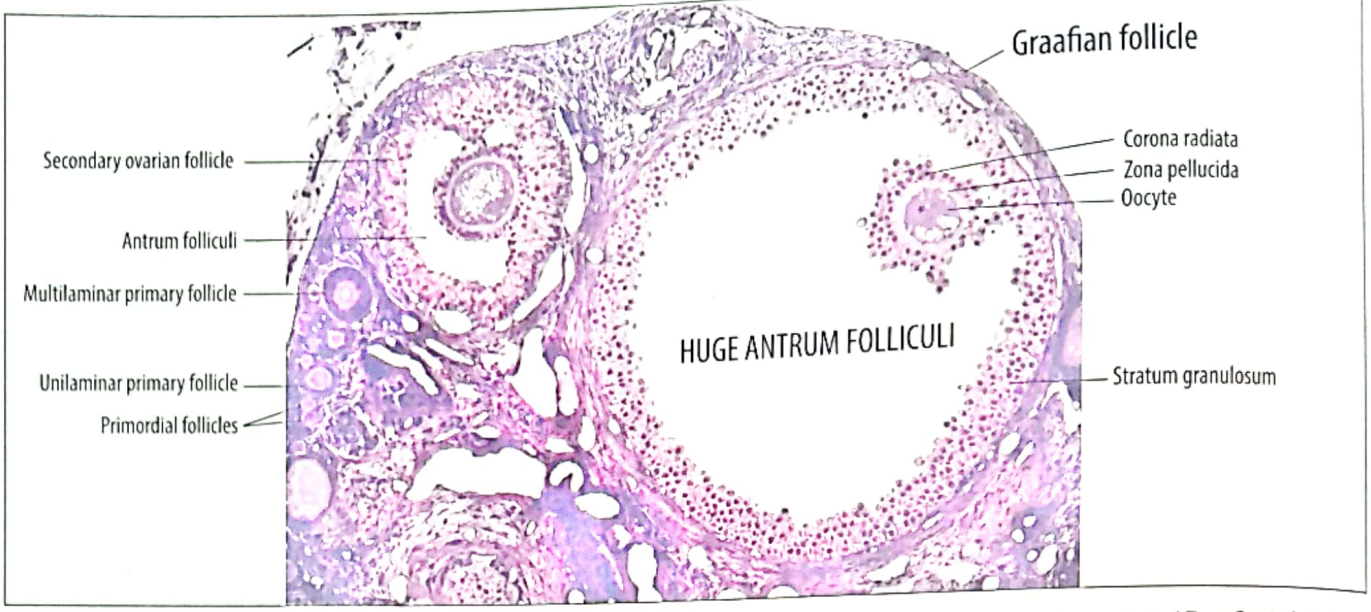

Fig. 22.5 A section of the ovarian cortex showing ovarian follicles in various stages of development and a mature (Graafian) follicle.

The **theca interna** consists mainly of roughly cuboidal secretory cells called *theca cells*. In addition, this layer contains fibroblasts, collagen fibers, and a rich network of fenestrated blood capillaries. The theca cells, which are actually transformed stromal cells of the ovarian cortex, exhibit the characteristics of steroid-producing cells (numerous lipid droplets, mitochondria with tubular cristae, and abundant SER). These cells synthesize and secrete a steroid called *androstenedione*, which passes to the granulosa cells where it is converted into 17 β *estradiol*, which is the most potent estrogen. The estradiol returns to theca interna and enters the blood stream through the capillaries present in this layer.

The **theca externa** consists of smooth muscle cells, fibroblasts, and collagen fibers. Contraction of the smooth muscle cells at the time of ovulation helps in ovum release.

A multilaminar primary follicle is also known as a *preantral follicle* because its stratum granulosum does not contain any cavity (antrum). The multilaminar primary follicles develop into secondary follicles (which contain cavities). It is also to be noted that as the ovarian follicles grow, they move to the deeper areas of the ovarian cortex.

Secondary Follicle

A secondary ovarian follicle is characterized by the presence of a cavity called *antrum folliculi* (Fig. 22.4). Therefore, the secondary ovarian follicles are also known as *antral follicles*. The antrum folliculi begins its development as small intercellular clefts that appear between the cells of stratum granulosum. These clefts are filled by a fluid called *liquor folliculi*. Later, these fluid-filled intercellular clefts coalesce to form a single, crescent-shaped antrum folliculi.

The cells of the stratum granulosum reorganize themselves around the developing cavity, so that the antrum folliculi is lined by a relatively uniform stratum granulosum (which consists further of 3 to 5 layers of granulosa cells). The antrum folliculi gradually enlarges in size and the fluid contained in it increases in amount. Consequently, the oocytes and the granulosa cells surrounding it are pushed toward the wall of the antrum. The oocyte now lies within a heap of granulosa cells that projects out from the follicular wall into the fluid-filled antrum folliculi. This heap of granulosa cells is called *cumulus oophorus*. Within the cumulus oophorus, a layer of radiating granulosa cells surrounds the oocyte outer to the zona pellucida; this layer is called corona radiata.

The **liquor folliculi** is an exudate of plasma but also contains the products secreted by the granulosa cells. Its components include hyaluronic acid, heparan sulfate, fibrinogen, plasminogen, and high concentrations of progesterone, estradiol, and androstenedione.

Most of the secondary follicles undergo atresia but at least one of them continues to develop further to give rise to the mature ovarian follicle.

Mature Follicle

Continued proliferation of the granulosa cells and a constant increase in the amount of the liquor folliculi results in the formation of a mature ovarian follicle which is known as a tertiary follicle or **Graafian follicle** (Fig. 22.5). A Graafian follicle is a large, cystic structure, measuring 2-3 cm in diameter. It extends through the full breadth of the ovarian cortex and produces a visible bulge on the surface of the ovary. As more and more fluid accumulates, the follicular cavity becomes very large. The granulosa cells cease to divide and the stratum granulosum becomes relatively thinner. Ultimately, the Graafian follicle ruptures and releases its oocyte. The process of release of the oocyte from the ovary is called *ovulation* and it takes place roughly in the middle of the ovarian cycle.

CORPUS LUTEUM

After ovulation, the ruptured follicle does not degenerate

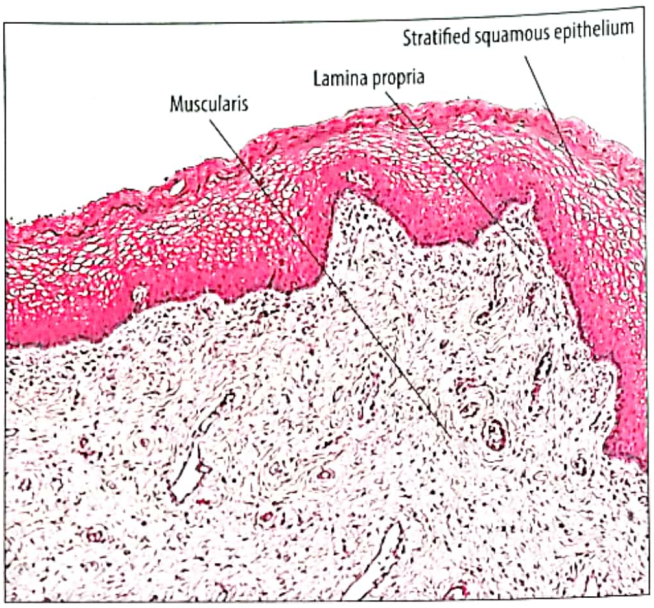

Fig. 22.11 A section through the vaginal wall.

vagina is an important factor in preventing the infection of vagina by the fungi and pathogenic bacteria.

The ***lamina propria*** of the vaginal mucosa consists of a layer of loose connective tissue which is rich in elastic fibers. Papillary projections from the lamina propria protrude into the epithelial layer. The lamina propria lodges a large number of neutrophils and lymphocytes, some of which may be seen to invade the epithelium. The lamina propria also contains a plexus of small veins.

The **muscularis** of vagina consists of smooth muscle fibers which are arranged into two intermingling layers. The inner layer is composed mainly of circularly arranged muscle fibers. The thick outer layer is composed of bundles of longitudinally running smooth muscle fibers, which are continuous above with the myometrium of the uterus. At the inferior end, the vaginal wall contains some skeletal muscle fibers. These fibers, which belong to the bulbospongiosus muscle, constitute a sphincter at the vaginal introitus.

The **adventitia** of vagina consists of an inner layer of dense connective tissue that contains a large number of elastic fibers, and an outer layer of loose connective tissue that binds the vagina to the surrounding structures. The outer layer also contains vaginal blood vessels, lymphatic vessels, and nerves.

The vaginal wall contains no glands. The mucosal surface of the vagina is lubricated by mucus derived chiefly from glands of the uterine cervix. Secretion of the major vestibular glands also helps to lubricate the vagina.

EXTERNAL GENITALIA

The female external genitalia (vulva) comprise the labia majora, labia minora, clitoris, and vestibule.

The **labia majora** are two longitudinal folds of skin which form the lateral boundaries of the urogenital cleft. The outer surface of a labium majus bears coarse curly hair, whereas its inner surface is smooth and hairless. Sebaceous and sweat glands are abundant on both surfaces. The core of each labium majus is formed by a large amount of adipose tissue and a few smooth muscle fibers.

The **labia minora** are paired, hairless folds of skin which form lateral walls of the vestibule. The core of each labium minus is formed by connective tissue that is rich in elastic fibers. This connective tissue also contains a large number of blood vessels. Although hairless, the labia minora contain a large number of sebaceous glands that open directly onto the surface of each labium minus.

The **clitoris** consists of two cavernous erectile bodies (the corpora cavernosa) which end in a small tubercle called *glans clitoridis*. The skin over the clitoris is very thin and hairless but is richly supplied by nerve fibers and lodges numerous sensory nerve endings.

The **vestibule** is the space into which urethra and vagina open. It is lined by stratified squamous nonkeratinized epithelium. Numerous small mucus-secreting glands are located in the wall of the vestibule. These glands, called *minor vestibular glands* (also known as *Skene's glands* or *periurethral glands),* occur mainly around the urethral opening and near the clitoris. Two pea-sized, tubuloacinar glands called *major vestibular glands* or *Bartholin's glands* are located in the lateral wall of the vestibule, one on each side of the vaginal opening. As a result of sexual arousal, the Bartholin's glands produce a mucoid secretion that serves to lubricate the vestibule and vagina.

The external genitalia are abundantly supplied with sensory nerve endings, including the Pacinian corpuscles and Meissner corpuscles. The sensations conveyed by these nerve endings play an important role in the physiological response during sexual arousal and act.

THE MAMMARY GLANDS

The mammary glands are specialized cutaneous glands lying within the subcutaneous tissue. Their function is to secrete milk for the newborn.

Mammary glands are present in both sexes but develop only slightly during childhood. At the onset of puberty in the males, the interstitial cells of the testes begin to produce testosterone which inhibits further growth of the mammary glands. In the females, the mammary glands undergo rapid development at puberty under the influence of estrogens. This growth and enlargement of the female mammary glands occurs due to: (i) deposition of large amounts of adipose tissue, and (ii) elongation and branching of the duct system of the gland.

In the adult female, each mammary gland comprises 15-25 lobes, each of which actually is an independent gland of compound tubuloacinar variety. Each lobe has its own duct, called *lactiferous duct,* which opens at the nipple. Adjacent lobes are separated from each other by dense

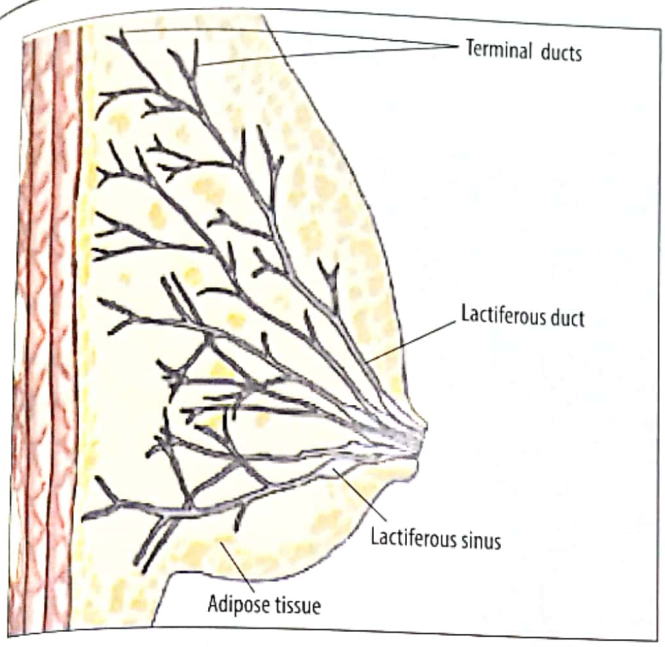

Fig. 22.12 Diagrammatic representation of the microscopic structure of an inactive mammary gland.

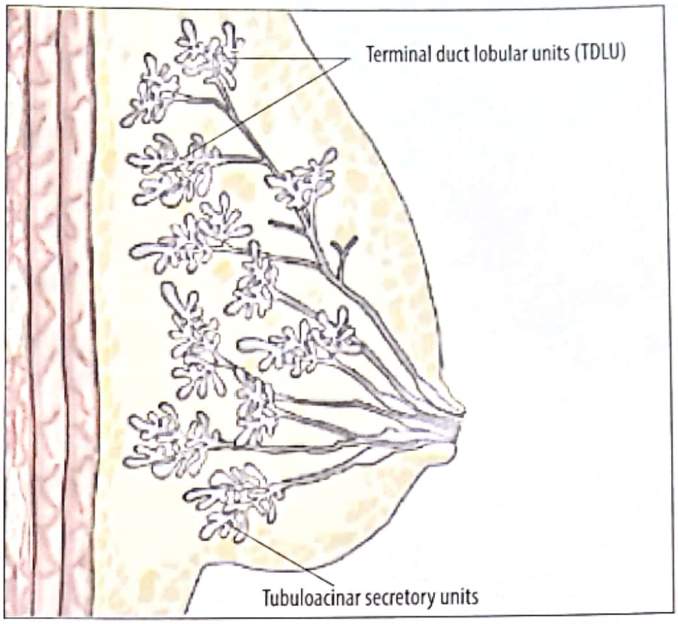

Fig. 22.13 Diagrammatic representation of the microscopic structure of a mammary gland in the second trimester of pregnancy.

collagenous connective tissue and adipose tissue. The lobes of mammary gland are further divided into lobules by loose and delicate intralobar connective tissue.

NIPPLE AND AREOLA

In an adult female, the epidermis of nipple and areola is highly pigmented. The underlying dermis shows tall papillae and has many smooth muscle fibers. Contraction of this muscle hardens and elevates the nipple. The skin of areola bears a variable number of coarse hairs and contains sebaceous and sweat glands. In addition, it lodges special glands called *areolar glands* which produce small elevations on the surface of areola. These glands are considered to be modified mammary glands that have a structure intermediate between the mammary glands and sweat glands. The areolar glands secrete a fatty product which protects the areola during lactation. The skin of the nipple is richly supplied by sensory nerve endings.

DUCT SYSTEM AND PARENCHYMA OF THE MAMMARY GLAND

Each lobe of the mammary gland has its own main duct, called lactiferous duct, that opens at the nipple. Before opening onto the surface, each lactiferous duct forms a small dilatation which is called *lactiferous sinus.*

The main duct of each lobe of the mammary gland branches repeatedly to form a number of terminal ducts. The terminal ducts lead to lobules within each lobe. Inside each lobule, the terminal duct gives rise to several branches which end in dilatations called acini (alveoli). Each terminal duct, its branches and the associated alveoli are collectively known as a **terminal duct lobular unit (TDLU).**

The lactiferous duct, terminal ducts and alveoli ate lined by simple cuboidal epithelium. The epithelium of the alveoli and terminal ducts are associated with myoepithelial cells which are located between the cuboidal epithelial cells and their basement membrane. Contraction of these myoepithelial cells, which is controlled by the hormone oxytocin, results in the expulsion of the secretory product from the alveoli and secretory ducts.

STROMA OF THE MAMMARY GLAND

Within each lobule of the mammary gland lobe, the branches of the terminal duct and the acini associated with them are surrounded by intralobular connective tissue, which is loose connective tissue containing a large number of collagen fibers. The lobules of a lobe are surrounded and separated from each other by interlobular connective tissue, while interlobar connective tissue separates the lobes of the mammary gland from each other. The interlobular and interlobar connective tissue consists of dense irregular connective tissue and adipose tissue.

VARIATIONS IN THE STRUCTURE OF THE MAMMARY GLANDS

Parenchyma and stroma of the mammary glands show profound structural variations according to the functional state of these glands.

INACTIVE MAMMARY GLAND

In a non-pregnant woman, the glandular tissue is sparse and consists of branches of the terminal ducts which are lined by a simple cuboidal epithelium. Acini are either rudimentary or entirely absent (Fig. 22.12 & 22.14). The intralobular, interlobular and interlobar connective tissue is abundant.

DURING PREGNANCY

The mammary glands undergo dramatic development during pregnancy in preparation for lactation. This

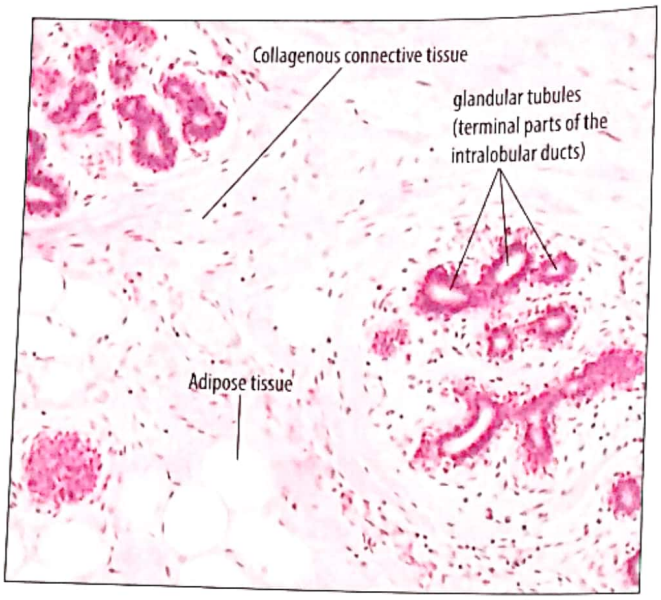

Fig. 22.14 A section through the inactive mammary gland.

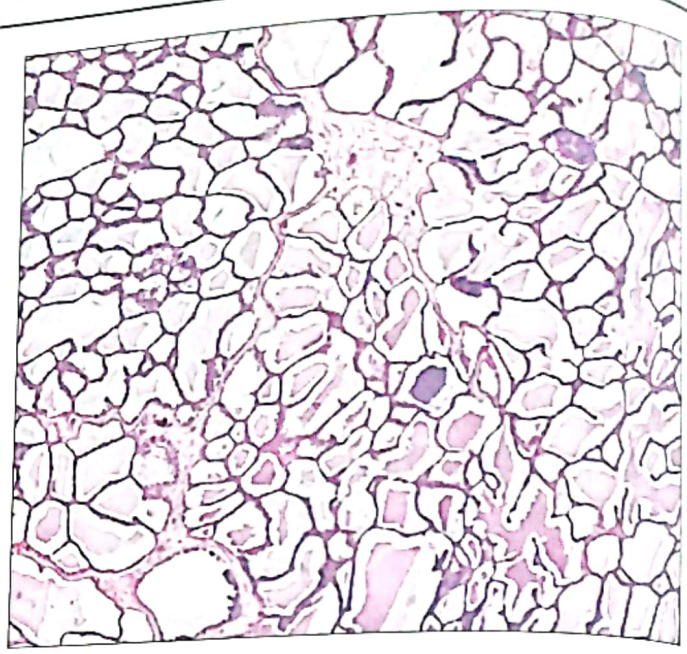

Fig. 22.15 A section through the lactating mammary gland. Note the innumerable acini filled with secretion (milk).

development occurs under the influence of estrogens, progesterone and prolactin. In the first trimester of pregnancy, the cells of the terminal ducts proliferate rapidly by mitotic division, so ducts the ducts elongate and branch extensively. In the second trimester, acini develop at the blind ends of the terminal ducts (Fig. 22.13). Like the ducts, the acini are also lined by simple cuboidal epithelium. Lymphocytes and plasma cells migrate in large numbers from the blood capillaries into the intralobular connective tissue (the plasma cells secrete antibodies into the milk when lactation begins). In the third trimester of pregnancy, the secretory units of the gland (terminal ducts and acini) become mature. The epithelial cells lining the secretory units enlarge in size. The cytoplasm of these cells develops an extensive rough endoplasmic reticulum and a large Golgi apparatus. Lipid droplets and secretory granules appear in the cytoplasm. The number myoepithelial cells associated with the secretory units also increases. The increase in the number of secretory units occurs at the expense of the intralobular, interlobular and interlobar connective tissue which becomes highly reduced in quantity.

DURING LACTATION

Soon after childbirth, the mammary glands begin active secretion of milk under the effect of the anterior pituitary hormone prolactin. The secretion accumulates in the lumen of many acini, due to which they become distended and appear as saccules (Fig. 22.15). The epithelial lining of the dilated acini becomes low cuboidal or even squamous. The cytoplasm of the acinar epithelial cells contains numerous large fat droplets and small, dense granules of milk proteins.

The intralobular ducts of the mammary glands appear structurally similar to acini. Functionally, these ducts are true secretory ducts; therefore, the mammary glands are included in the tubuloacinar variety of glands.

The **milk** is an opaque, white fluid which contains water, lipids (mainly triglycerides), proteins (mainly casein and α-lactalbumin), carbohydrates (chiefly lactose), minerals (mainly calcium), vitamins, lactoferrin, lysozyme and secretory IgA, etc. The secretion of milk employs apocrine as well as merocrine modes of secretion. The lipids are secreted by an apocrine method, so that each fat globule secreted into the milk is surrounded by a very small amount of the cytoplasm and is enveloped within a membrane derived from the apical plasmalemma of the secretory cell. The proteins and other components of the milk are secreted by a merocrine method of secretion.

AFTER LACTATION

After the cessation of lactation, the mammary glands undergo regressive changes and return to the state of an inactive gland. The acini gradually decrease in size and disappear, so that in a histological section only tubules cab seen in the gland lobules and no acini are visible. The intralobular, interlobular and interlobar fibrous connective tissue and adipose tissue become abundant once again.

AFTER MENOPAUSE

After the menopause the mammary glands undergo progressive atrophy. The secretory epithelium atrophies and, ultimately, only a few remnants of the duct system persist. The connective tissue also undergoes degenerative changes and there is a marked decrease in the number of fibroblasts, collagen fibers, and elastic fibers.

Cancer of the breast is a commonly occurring condition in women. Most of the malignant tumors of the breast are derived from the lining epithelium of the ducts of the mammary glands; such tumors are called ductal carcinomas. In the adult females, the incidence of breast cancer is reported to be nearly 10%. It has also been observed that the risk of developing a breast cancer increases with age.

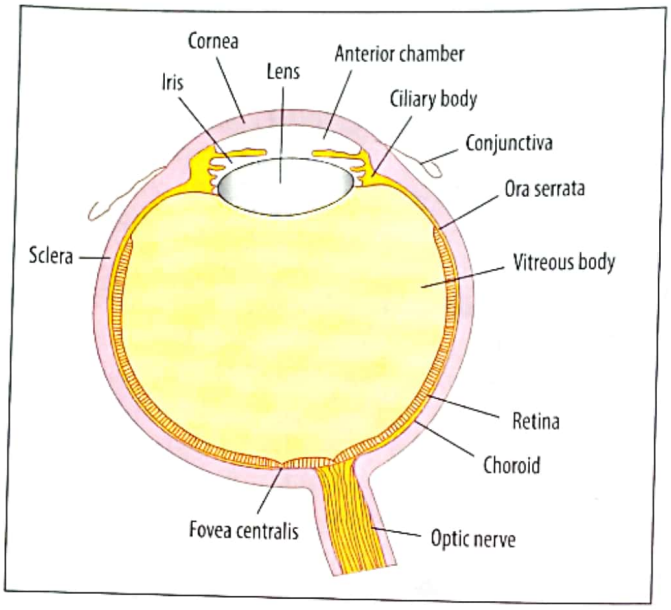

Fig. 23.1 A diagrammatic representation of a horizontal section through the eyeball showing its general structure.

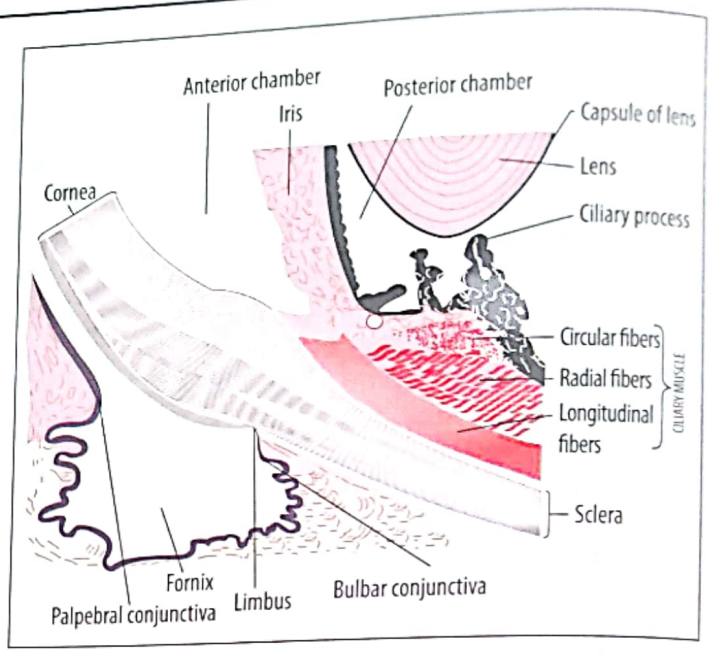

Fig. 23.2 A diagrammatic representation of a section through anterior part of the eyeball showing various structures in the region of corneal limbus.

numerous pinocytotic vesicles. Nutrients and oxygen present in the aqueous humor pass into the corneal tissues through the corneal endothelium and its basement membrane. The most important function of the corneal endothelium is to maintain corneal transparency by regulating stromal hydration. The corneal endothelium performs this critically important function by transporting excess water from the corneal stroma into the aqueous humor.

Functions of the Cornea

The major function of the cornea is to refract the incoming light onto the lens of eye. Due to its sharp curvature, the cornea contributes 65-70% of the eye's total focusing power. The cornea also helps to protect rest of the eye from dust, microorganisms, and other harmful matter.

CORNEAL LIMBUS

The corneal limbus, generally called simply as limbus, is about 1.5 mm wide between cornea on one side and the sclera and conjunctiva on the other (Fig. 23.2). At the limbus, the corneal epithelium becomes continuous with the epithelium of the conjunctiva covering the anterior part of the sclera, the Bowman's membrane disappears, and the corneal stroma becomes continuous with the connective tissue of the sclera proper. The Descemet's membrane and corneal endothelium are replaced by a system of endothelium-lined, irregular channels known as trabecular meshwork. This porous meshwork allows continuous but slow drainage of the aqueous humor from the anterior chamber of the eye into the scleral venous sinus (also called canal of Schlemm). The canal of Schlemm is a ring-like, vascular channel around the outer angle of the anterior chamber in front of the iris. From the Schlemm's canal, the fluid enters the veins of the sclera. As mentioned earlier, the corneoscleral limbus also serves as a reservoir of stem cells for the corneal epithelium.

SCLERA

The sclera forms the posterior five-sixths of the fibrous coat of the eyeball. It is opaque white in color and has an average thickness of about 0.5 mm. It is composed of dense fibrous tissue and helps to maintain the shape and size of the eyeball. Tendons of the extraocular muscles are inserted into the anterior region of the sclera. In a stained section examined under LM, the sclera is seen to be composed of three distinct layers. From without inwards, these layers are: episclera, sclera proper and suprachoroidal lamina.

1. The **episclera** is a thin layer of loose connective tissue that connects the sclera proper to the fascial sheath of the eyeball.
2. The **sclera Proper** is composed of flat bundles of type I collagen fibers which lie parallel to the surface but intersect each other in an irregular and complicated manner; the irregular arrangement of these fibers is responsible for the opaque nature of the sclera. Between the bundles of collagen fibers are present some elastic fibers, a moderate amount of ground substance, and a few fibroblasts.
3. The **suprachoroidal lamina**, also called *lamina fusca*, represent a zone of transition between the sclera and the underlying choroid. It is made up of delicate collagenous and elastic fibers, and contains many fibroblasts, macrophages, and melanocytes.

THE VASCULAR COAT

This coat of the eyeball lies between the fibrous coat and the neural coat. It is also called tunica vasculosa, uveal layer, or *uvea*. The uvea comprises choroid, ciliary body, and iris.

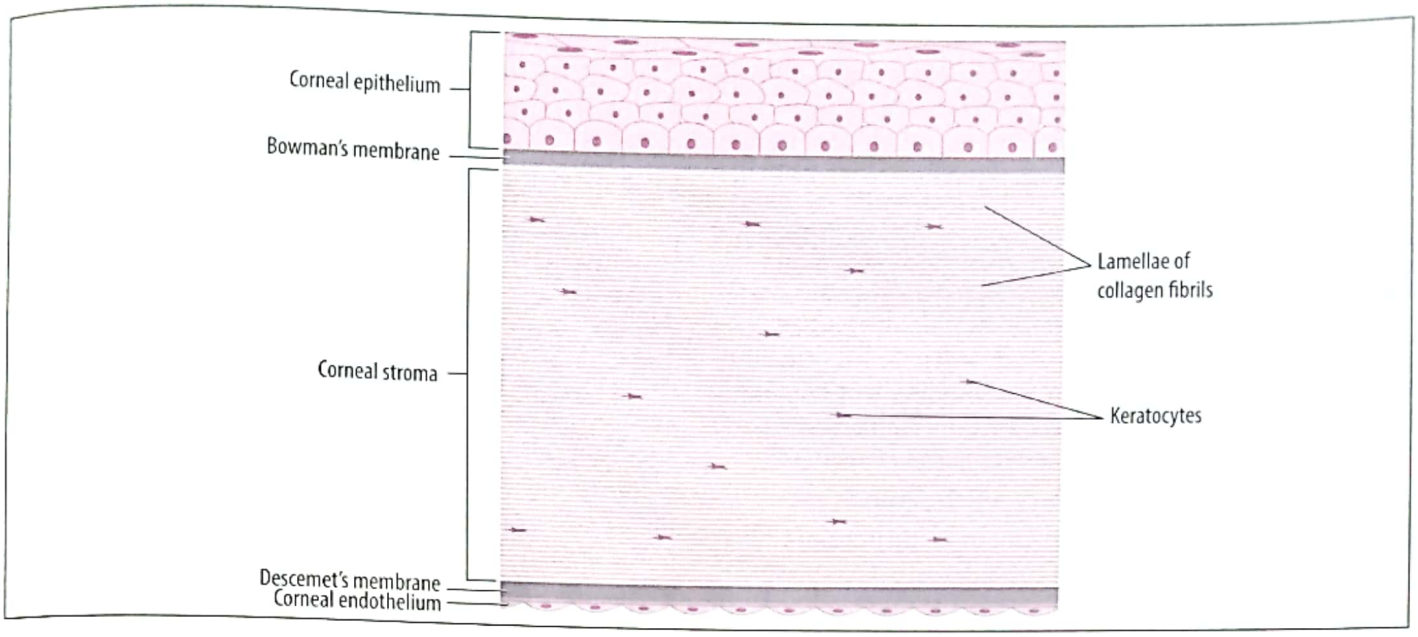

Fig. 23.3 A diagrammatic representation of the microscopic structure of the cornea.

CHOROID

The choroid forms the posterior part of the uvea. It is a thin, highly vascular, dark brown sheet that lies between the sclera and retina. The choroid is about 0.25 mm thick posteriorly but thins out anteriorly where its thickness is just 0.1 mm. Externally it is separated from the sclera by a potential space called *perichoroidal space*. In histological sections, the choroid is seen to consist of three layers: choroid stroma, choriocapillary layer, and Bruch's membrane.

1. **Choroid Stroma**. This layer lies just next to the sclera and consists of a loose meshwork of type I collagen fibers and elastic fibers. In the interstices of the meshwork are present a few fibroblasts, many macrophages, and numerous melanocytes. The main arteries and veins of the choroid also run in this layer.
2. **Choriocapillary Layer.** This layer consists of a plexus of fenestrated capillaries of unusually large size which arise from the choroidal arteries. This capillary plexus has an important role in the nutrition of the outer layers of retina. The spaces between the choriocapillaries are occupied by a network of delicate collagenous and elastic fibers. A few fibroblasts and some melanocytes are also present.
3. **Bruch's Membrane.** Under LM, this layer, which is also known as *lamina vitrea*, appears as a thin shiny, homogeneous layer that borders directly on the pigment epithelium of retina. EM shows that the lamina vitrea is 2-4 µm thick and is composed further of five layers: (i) basal lamina of the endothelium of choriocapillaries, (ii) an external layer of collagen fibers, (iii) a meshwork of elastic fibers, (iv) an internal layer of collagen fibers, and (v) basal lamina of the retinal pigment epithelium.

CILIARY BODY

The choroid extends anteriorly as far as the ora serrata (which is the anterior margin of the sensory portion of the retina). Anterior to the ora serrata, the uveal layer is thickened to form the ciliary body, which is a 6 mm wide ring of smooth muscle and vascular tissue. In transverse sections the ciliary body appears triangular in shape. Its anterior surface (base) projects toward the lens and faces the posterior chamber of the eye. The outer surface of the ciliary body blends with the sclera, while its inner surface lies in contact with the vitreous body. When examined by naked eye, the inner surface of ciliary body exhibits two zones. The posterior two-thirds, called *pars plana,* are relatively smooth but darkly pigmented. The anterior one-third, called *pars plicata,* appears pale and bears about 70 to 80 radially arranged ridges called *ciliary processes.*

A series of delicate fibers, called **zonular fibers or ciliary zonule,** radiate from the ciliary processes to insert into the lens capsule. The zonular fibers constitute the suspensory ligament of the lens which holds the lens in place.

A section through the ciliary body shows that, from without inwards, it is composed of: (i) ciliary muscle, (ii) ciliary stroma, and (iii) ciliary epithelium.

CILIARY MUSCLE

The bulk of ciliary body is formed by the ciliary muscle. It consists of smooth muscle fibers which are divided into three groups: longitudinal (meridional), radial, and circular. As the lens is held in place by the ciliary zonule, contraction and relaxation of the ciliary muscle results in changes in the shape of lens that are needed for the process of visual accommodation.

CILIARY STROMA

This layer, also called *vessel layer*, contains loose connective

tissue and blood vessels. The connective tissue consists mainly of loosely arranged bundles of collagen fibers. Within the connective tissue is present a network of fenestrated capillaries. These capillaries lie adjacent to the ciliary epithelium and are especially concentrated in the ciliary processes.

CILIARY EPITHELIUM

The inner surface of the ciliary body and ciliary processes are covered by two layers of columnar epithelial cells; the cells of the outer layer contain melanin pigment granules, while the cells of the inner layer are non-pigmented.

Pigmented layer of the ciliary epithelium

This is a continuation of the pigment epithelium of retina and consists of a single layer of cuboidal cells. Cytoplasm of these cells is heavily filled with brownish-black granules of melanin pigment. Therefore, it is difficult to distinguish the boundaries of individual cells in the ordinary histological sections. However, on the summit of the ciliary processes the epithelium is much less pigmented; this accounts for the characteristic whitish appearance of these processes.

Nonpigmented layer of the ciliary epithelium

This epithelium represents the forward continuation of the sensory portion of the retina. It consists of a single layer of columnar unpigmented cells. These cells show lateral interdigitations and infoldings of basal plasmalemma. Numerous sodium pumps are located in the plasmalemma of these cells. The cytoplasm of these cells contains numerous mitochondria, a well-developed Golgi apparatus, and extensive SER. The principal function of the ciliary epithelium is to secrete the aqueous humor. In addition, the ciliary epithelium performs the function of synthesis and secretion of the zonular fibers (which are made up of the protein fibrillin).

Aqueous Humor

The aqueous humor is a filtrate of plasma that is secreted by the ciliary epithelium. Fluid from the capillaries of the ciliary stroma moves across the ciliary epithelium as aqueous humor, which is a clear fluid that has nearly the same ionic composition as that of the plasma but is almost free of protein.

The aqueous humor flows from the posterior chamber of the eye into the anterior chamber through the pupil (which is a circular opening in the center of the iris). In the anterior chamber of the eye, the aqueous humor flows to the angle between the iris and cornea (iridocorneal angle) and enters the trabecular meshwork of the corneal limbus. From the labyrinthine spaces of the trabecular meshwork, the aqueous humor drains into the canal of Schlemm (scleral venous sinus). This canal communicates with the scleral veins, and thus, the aqueous humor finally enters the bloodstream. The aqueous humor serves as a source of provision of nutrients and oxygen to the cornea and lens.

Any obstruction to the drainage of aqueous humor increases the intraocular pressure and produces the clinical condition called **glaucoma.** If left untreated, glaucoma may lead to loss of vision.

IRIS

The iris is the most anterior part of the uvea. It forms a contractile diaphragm that divides the aqueous compartment into an anterior chamber and a posterior chamber. In the center of the pupil is present a circular aperture called *pupil*. To control the amount of light entering the eye, the diameter of this aperture can be increased or decreased by the activity of the musculature of the iris.

The periphery of the iris, called *ciliary margin*, is attached to the anterior surface of the ciliary body. The *pupillary margin* of the iris surrounds the pupil. The anterior surface of the iris is devoid of epithelium and is rough and irregular. It also shows radially arranged ridges and grooves.

Microscopically, the iris consists of two layers: (1) the iris stroma which is situated anteriorly and is derived, embryologically, from the mesenchyme, and (2) two epithelial layers located posteriorly, which are derived from the neuroectoderm.

IRIS STROMA

The stroma of the iris contains collagen fibers, fibroblasts, melanocytes, blood vessels, nerve fibers, smooth muscle of the sphincter pupillae, and myoepithelial cells of the dilator pupillae. At the anterior surface of the iris the collagen fibers, fibroblasts and melanocytes are compactly arranged to form an ill-defined layer which is called *anterior border layer.*

The **sphincter pupillae** muscle forms a ring of smooth muscle fibers around the pupil; this muscular ring measures about 1 mm in width. Contraction of the sphincter pupillae decreases the pupillary diameter. This muscle is supplied by postganglionic nerve fibers from the ciliary ganglion via the short ciliary nerves.

The **dilator pupillae** muscle is a thin sheet of myoepithelium which extends from the iris root as far as the sphincter pupillae. The radially arranged fibers of the dilator pupillae represent the myoepithelial processes of the cells forming the anterior tier of the two posterior epithelial layers of the iris. The muscle processes are fusiform in shape and contain abundant myofilaments. When the dilator pupillae contracts, the diameter of the pupil becomes larger. The dilator pupillae receives its nerve supply from the sympathetic postganglionic nerve fibers reaching via the long ciliary nerves.

THE TWO EPITHELIAL LAYERS OF THE IRIS

The posterior surface of the iris is smooth and is covered by two specialized epithelial layers; these layers are: (i)

posterior pigment epithelium of the iris, and (ii) anterior pigment myoepithelium of the iris.

The ***posterior pigment epithelium of the iris*** faces the posterior chamber. The cells of this layer are larger in size and their cytoplasm is very heavily pigmented, being almost packed with melanin granules.

The ***anterior pigment myoepithelium of the iris*** consists of myoepithelial cells, each of which has an apical and a basal part. The apical (posterior) parts of these cells contain a large number of melanin granules, so that their boundaries with the cells of the posterior pigment epithelium of the iris are obscured. The basal (anterior) portions of these cells contain contractile apparatus (myofilaments) and extend radially into the iris stroma as myoepithelial processes; collectively, the myoepithelial processes of these cells constitute the *dilator pupillae* muscle.

The melanin pigment in the epithelial layers of the iris and melanocytes of the iris stroma blocks the entry of light into the eyeball, except through the pupil.

The *color of the iris* depends on number of mela-nocytes in its stroma. If the number of melanocytes in the stroma is small, the light reflected from the pigmented epithelial layers gives the iris a blue appearance. If the stroma contains a moderate number of melanocytes, the iris appears to be gray, and if the number of melanocytes is very high, the iris appears brown or dark brown in color.

RETINA

The retina constitutes the innermost layer (neural layer) of the eyeball. It transduces the stimulus of light into nerve impulses, resulting in the sensation of vision.

The retina develops as an evagination of the forebrain, called *optic vesicle*, which is connected to the brain by a narrow *optic stalk* (which becomes the optic nerve in later development). The optic vesicle becomes transformed into the double-walled *optic cup*. The outer wall of the optic cup gives rise to pigment epithelium of retina, while its inner wall develops into the functional part of retina, called *neural retina*. The pigment epithelium is attached rather loosely to the neural retina, but is attached firmly to the Bruch's membrane of the choroid. In histological sections the neural retina is often seen to be detached from the pigment epithelium because the two layers become easily separated during processing of the ocular tissues for microscopy.

As a result of injury or disease (e.g., diabetes mellitus), the neural retina may become partially or completely detached from the pigment epithelium of retina. This condition, called retinal detachment, results in partial or complete loss of vision in the affected eye.

LAYERS OF RETINA

On the basis of structural features which are readily evident under the light microscope, the retina is conventionally divided into ten layers (Fig. 23.4 & 23.5). These layers, from without inwards, are:

1. Retinal pigment epithelium.
2. Layer of rods and cones.
3. Outer limiting layer.
4. Outer nuclear layer.
5. Outer plexiform layer.
6. Inner nuclear layer.
7. Inner plexiform layer.
8. Ganglion cell layer.
9. Optic nerve fiber layer.
10. Inner limiting layer.

The apparently complex structure of retina can easily be understood by appreciation of the fact that the neural retina is only three neurons deep. The photoreceptor cells (rod and cone cells) represent the first order neurons. Each rod or cone cell has three parts: (i) a photosensitive process lying against the pigment epithelium, (ii) a cell body (perikaryon) that contains the nucleus, and (iii) an axonal process. The nuclei (and cell bodies) of these cells lie in the outer nuclear layer. This layer comprises several rows of nuclei. Out of these, the single outer row (i.e., the one immediately next to the outer limiting layer) is composed of cone nuclei, while the remaining rows consist of rod nuclei. The outer plexiform layer is the region where rod and cone cells make synaptic junctions with the dendrites of bipolar neurons. The bipolar cells constitute the second order neurons and their nuclei lie in the inner nuclear layer. Axons of the bipolar neurons pass into the inner plexiform layer, where they synapse with dendrites of the ganglion cells. The ganglion cells represent the third neuron of the retina and their relatively large cell bodies lie in the ganglion cell layer. The long axons of the ganglion cells course in the optic nerve fiber layer to the optic disc, where all of these axons converge to form the optic nerve.

It must be noted that the true photoreceptor elements, i.e., the rods and cones, constitute the outermost layer of the neural retina, lying away from the light stimulus. The light has to pass through the thickness of retina to reach the photoreceptors. This seemingly inverted retina is a characteristic feature of all vertebrates.

Retinal Pigment Epithelium (RPE)

The retinal pigment epithelium is a single layer of low columnar cells with basal nuclei. The basal regions of RPE cells adhere firmly to the Bruch's membrane of the choroid. Adjacent RPE cells are bound together by tight junctions of zonula occludens type. The apical part of each pigment cell has many cylindrical processes that surround and interdigitate with the outer segments of the rod and cone cells. As explained earlier, the cytoplasmic processes of the RPE cells and the photoreceptors are not anatomically joined to each other and may become easily separated due to injury or disease, leading to the clinical condition called *retinal detachment*.

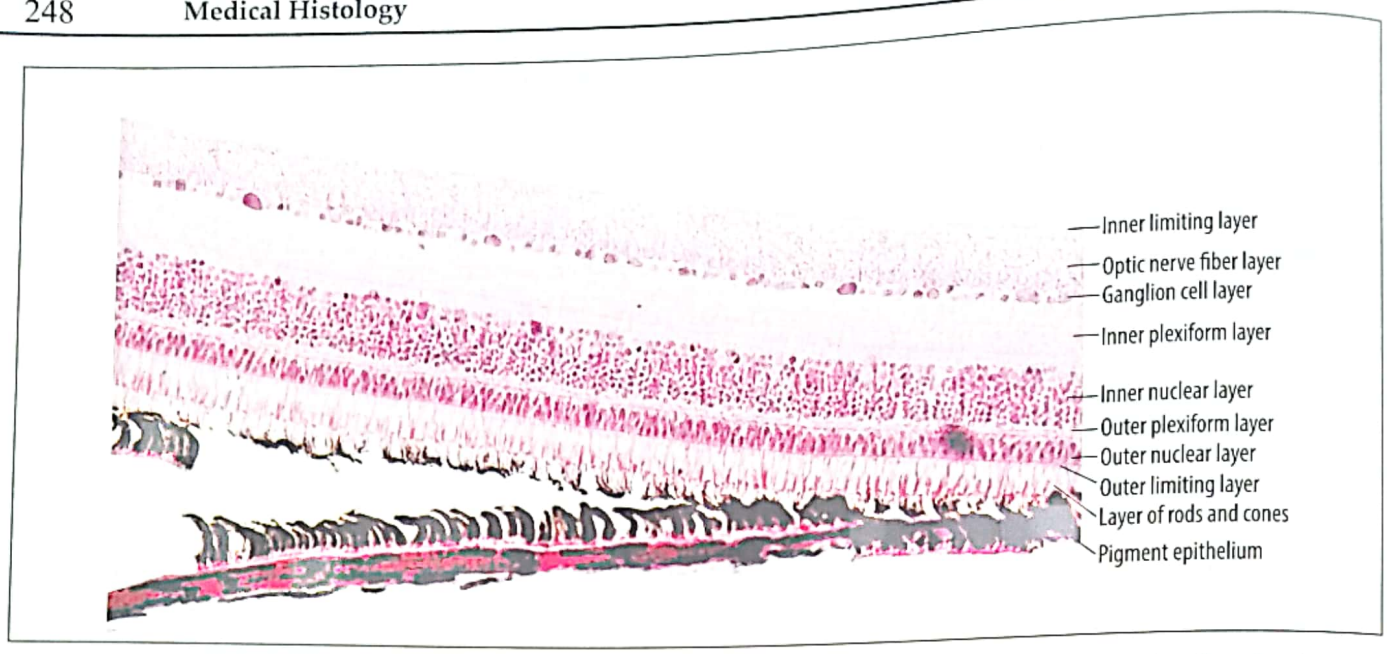

Fig. 23.4 A section through the eyeball showing the microscopic structure retina. Notice that the pigment epithelium has become separated from the neural retina during tissue processing.

The cytoplasm of the RPE cells is characterized by the presence of a large number of brownish-black melanin granules which are especially abundant in the apical region as well as processes. The cytoplasm of these cells also contains many mitochondria, abundant smooth endoplasmic reticulum, a Golgi apparatus, many lysosomes, and numerous phagosomes.

Functions of the Retinal Pigment Epithelium

1. *Absorption of light* after it has passed through and stimulated the photoreceptors. This prevents reflection of light from the deeper tunics of the eyeball and, thus, creates a dark chamber effect in the eye.
2. *Storage of vitamin A and its supply to the photoreceptors.* The pigment epithelium stores large quantities of vitamin A, which is exchanged back and forth through the plasma membranes of the pigment epithelial cells and outer segments of the photoreceptor (rod and cone) cells. This interchange of vitamin A is important for the adjustment of light sensitivity of rod and cone cells, because vitamin A is an essential precursor of the photosensitive pigments of these cells (see below).
3. *Phagocytosis of the membranous lamellae shed by the photoreceptors.* The membranous lamellae of the outer segments of the rod and cone cells of retina are constantly shed to be replaced by new lamellae synthesized by the cell. The RPE cells serve as scavenger cells and phagocytose the cast off lamellae (this is the reason why the RPE cells contain so many phagosomes).
4. *Contribution to the blood-retina barrier.* Presence of tight junctions between the RPE cells enables these cells to contribute to the formation of blood-retina barrier (described in detail later). The tight junctions seal the intercellular space, so that exchange of all types of materials (gases, nutrients, metabolites, etc) between the choroid capillaries and the photoreceptor cells of retina occurs through the RPE cells. This arrangement prevents the passage of any type of toxic macromolecules from the blood in choroid capillaries to the photoreceptor cells of retina.
5. The RPE cells also support the neural retina by secreting ATP and many important growth factors.

ELEMENTS OF THE NEURAL RETINA

Four cell groups are present in the neural retina:

1. Photoreceptors (rod cells and cone cells).
2. Direct conducting neurons (bipolar neurons and ganglion cells).
3. Association neurons (horizontal cells, amacrine cells, and interplexiform cells).
4. Supporting cells (Muller cells and neuroglia).

Photoreceptors

The photoreceptor cells of the retina are modified neurons, each having a photosensitive process (actually a dendrite), a cell body, and an axonal process (Fig. 23.6). The photosensitive processes of these cells are either rod-shaped or cone-shaped and, accordingly, the photoreceptor cells are classified into two types: (i) rod cells, and (ii) cone cells. The rod cells and cone cells are also known simply as rods and cones, respectively.

The photosensitive processes of the rod and cone cells lie in the second layer of retina (which is accordingly known as the layer of rods and cones). The nuclei and cell bodies of the rod and cone cells lie in the outer nuclear layer. The axonal processes of these cells pass into the outer plexiform layer to synapse with the dendrites of the bipolar neurons.

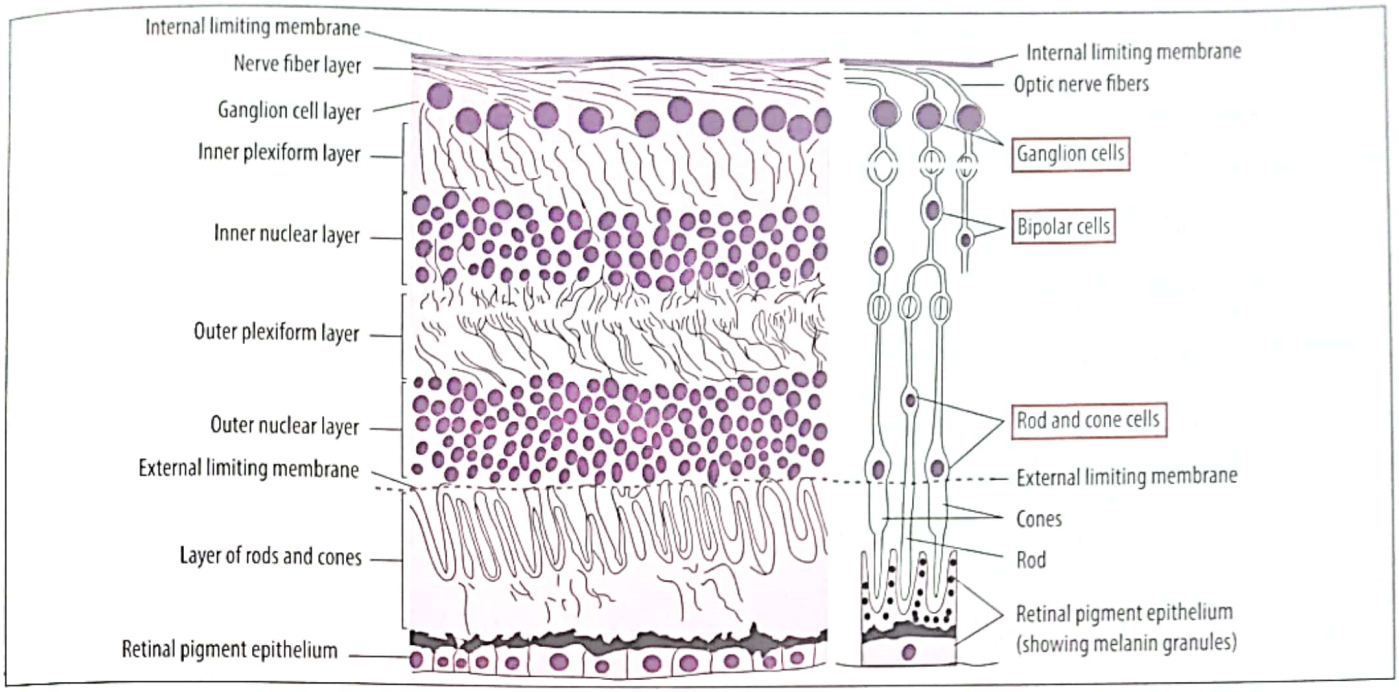

Fig. 23.5 A diagrammatic representation of structural organization of the retina.

ROD CELLS

These are thin, elongated cells measuring about 2 µm in diameter and having an average length of 60 µm. They are oriented parallel to each other but perpendicular to the retina. The rod-shaped photosensitive process (dendrite) of a rod cell (also called *rod proper*) consists of an inner segment and an outer segment which are connected to each other by a narrow connecting stalk (Fig. 23.6). The tip of the outer segment is embedded in the pigment epithelium and exhibits transverse striations due to the presence of transversely arranged membranous discs (described below). The number of rod cells in the human retina is about 120 million. The rods are extremely sensitive and can be stimulated at very low light levels and, therefore, are of paramount importance for the night vision.

The **outer segment** of the photosensitive process of a rod cell contains about 600 to 1000 flattened membranous discs of uniform diameter which are stacked like a pile of coins. Each disc is about 2 µm in diameter and is composed of two membranes separated from each other by a space that measures about 8 nm in width. The discs are produced by repetitive infolding of the plasmalemma of the innermost part of the outer segment. These membranous discs lose their continuity with the plasmalemma and constantly move in a distal direction along the length of the rod-shaped outer segment. At the outermost region of the outer segment, the membranous discs are shed continuously and are phagocytosed by the RPE cells.

The membranous discs of the rod cells contain the photosensitive pigment rhodopsin, also called *visual purple*. This substance is a combination of vitamin A aldehyde *retinal* (also known as *retinaldehyde*) with a protein called *scotopsin*. The globular molecules of rhodopsin are located on the outer surface of the lipid bilayer of the membranous discs. The outer segment is the site of photosensitivity. The transverse orientation of the membranous discs allows the rhodopsin to interact with the path of the incident light. Exposure to light breaks the bond between retinal and scotopsin, resulting in the generation of a nerve impulse.

The narrow **connecting stalk** joins the outer and inner segments. It is placed eccentrically and contains a modified cilium consisting only of nine peripheral doublet microtubules. These microtubules originate from a basal body located in the outer end of the inner segment.

The **inner segment** of the rod is the site of metabolic activity, where proteins and phospholipids are synthesized and energy is produced. It contains numerous mitochondria, a Golgi complex, SER, RER, free ribosomes, and glycogen granules. The proteins and phospholipids synthesized in the inner segment pass through the connecting stalk to the outer segment for incorporation into the new membranous discs.

The inner segment of the rod proper is connected to its perikaryon (in the outer nuclear layer) by a narrow region called *outer rod fiber*. The axonal process of the rod cell consists of a slender *inner rod fiber* that ends in a knob-like dilatation called *rod spherule*. The rod spherules make synaptic contact with the dendrites of the bipolar neurons and axons of horizontal cells in the outer plexiform layer.

CONE CELLS

The cone cells are considerably lesser in number than the rod cells and the human retina contains only 6 million cone cells. The cones respond to bright light and are also responsible for color perception. The structure of cone cells resembles that of the rod cells, but there are certain marked differences as well. The cones are also elongated cells measuring about 3.5 µm in diameter and 40 µm in

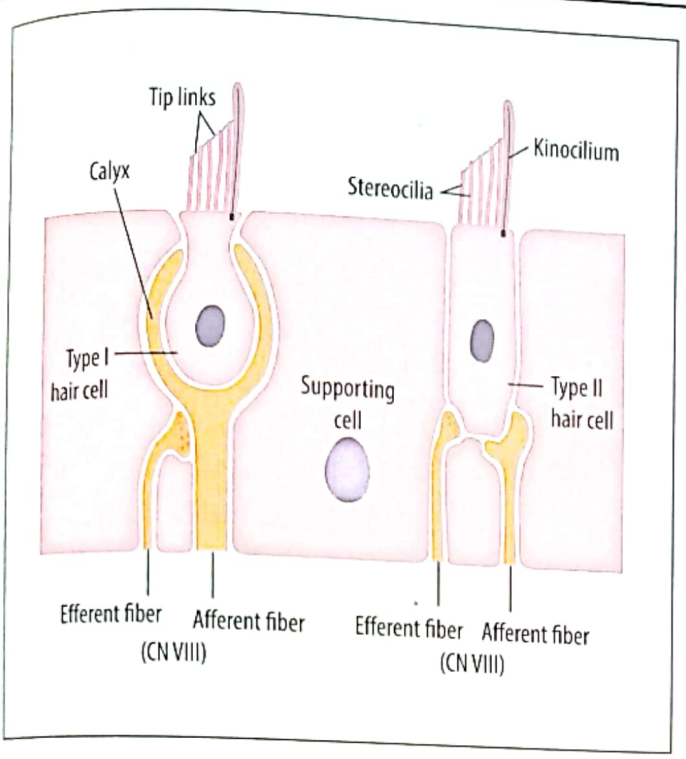

Fig. 24.2 Diagrammatic representation of type I and type II hair cells and supporting cells of the cristae and maculae.

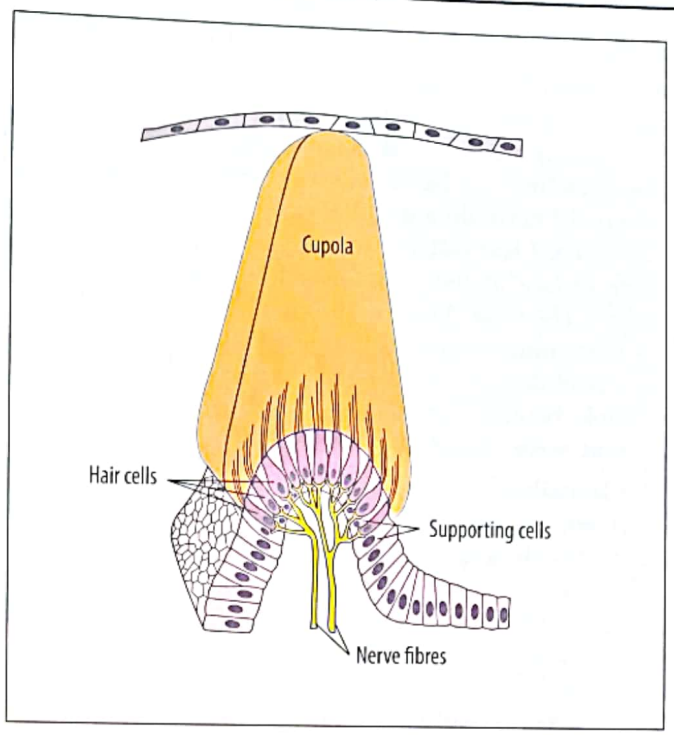

Fig. 24.3 Diagrammatic representation of the structure of a crista ampullaris.

semicircular canal, (2) *maculae*, which are two in number; one is present in the utricle (macula utriculi) and the other in the saccule (macula sacculi), and (3) a single *organ of Corti*, which is located in the cochlear duct.

The semicircular canals, utricle, and saccule collectively constitute the vestibular apparatus which plays an important role in the maintenance of equilibrium (postural balance) of the body. The vestibular apparatus receives and conducts to the brain those stimuli which are concerned with the perception of position of the body in space, in the resting state as well as during movement. The equilibrium of the body is maintained chiefly by the activation of antigravity muscles by the stimuli sent from the vestibular nuclei through the medial and lateral vestibulospinal tracts.

The organ of Corti, also known as the *spiral organ*, is a specialized sensory area in the cochlear duct which functions as a sound receptor. It detects sound vibrations of various frequencies, generates electrical stimuli and conveys these to the brain for perception of the sounds.

CRISTAE

The **cristae ampullares**, three in number, are located in the ampullae of the semicircular canals. Their function is to detect rotary movement (angular acceleration) of the head.

In the region where a crista ampullaris is present, the membranous wall of the semicircular canal is thickened to form a ridge which is placed transversely in relation to the long axis of the canal. This ridge consists of connective tissue which contains many blood vessels and nerve fibers and is surmounted by a specialized columnar epithelium.

Each crista ampullaris consists of two major varieties of cells: (i) hair cells, and (ii) supporting cells.

HAIR CELLS

The hair cells are neuroepithelial cells that serve as mechanoelectric transducers, i.e., they convert mechanical energy into electrical energy, which is transmitted to the brain via the 8th cranial nerve. The hair cells are characterized by the presence of a **hair bundle** at their apical surface. This so-called hair bundle consists of 40-50 stereocilia arranged in rows of increasing length. Behind the row of the longest stereocilia is present a longer, single kinocilium which is about 40 µm long.

The stereocilia are relatively rigid structures containing a core of tightly packed actin filaments. The kinocilium contains the typical nine peripheral doublet microtubules and two central singlets. Studies by very high resolution electron microscopes have revealed that very thin proteinaceous filaments, called *tip links*, connect the tip of each stereocilium to the next longer stereocilium. Finally, similar filaments attach the longest stereocilia to the kinocilium. This arrangement ensures that when the kinocilium moves in an outward direction with regard to the cell body, the stereocilia also move in the same direction. This results in the opening of numerous fluid channels in the plasmalemma of the hair cells around the bases of the stereocilia. As these channels are opened, the endolymph containing a large number of cations (mainly K^+) enters into the hair cells resulting in the depolarization of the plasma membrane of these cells and, thus, initiation of a nerve impulse.

Two types of hair cells, titled type I hair cells and type II hair cells,

can be identified in the cristae (Fig. 24.2). **The type I hair cells** are flask-shaped cells, having a broad,

rounded basal part and a narrow apical part. The rounded basal part of each type I hair cell is almost completely surrounded by a cup-shaped ending of an afferent nerve fiber; this nerve ending is called calyx. The efferent never fibers reaching the hair cells via the vestibular part of the 8th cranial nerve do not end directly on the plasmalemma of the type I hair cell, but make synaptic contact with the outer surface of the *calyx* formed by the afferent nerve ending. The **type II hair cells** are cylindrical in shape and are more numerous than the type I hair cells. The basal regions of these cells make direct synaptic junctions with multiple bouton type nerve endings of the afferent and efferent nerve fibers*.

The kinocilium and stereocilia of the hair cells of each crista ampullaris are embedded in a vertical, dome-shaped mass of a thick gelatinous proteoglycan material called **cupola or** *cupula* (Fig. 24.3). The cupola is conical in shape and can sway to and fro by the flow of endolymph within the semicircular canal. Rotation of the head causes a deflection of cupolas in two or more semicircular canals. This deflection results in the movement of hairs embedded in these cupolas. Consequently, the afferent nerve endings associated with the hair cells are stimulated and send signals to brain, which provide information regarding the speed and direction of the head rotation.

SUPPORTING CELLS

The supporting cells are columnar in shape and are interposed between the hair cells. Their bases rest on a basal lamina and their apices show microvilli. The supporting cells are bound to each other and to the hair cells by junctional complexes.

MACULAE

As mentioned before, there are two maculae, one is located in the utricle and the other in the saccule. The function of the maculae is to detect linear movement of the head.

Histologically, the maculae also consist of hair cells and supporting cells which have the same structure as described for the cristae ampullares. However, the free surface of the hair cells of each macula is covered by a layer of gelatinous proteoglycan mass called **otolithic membrane** (also called *statoconial membrane*). This layer shows surface deposits of small calcium carbonate crystals called ***otoliths***, which are also called *otoconia* or *statoconia* (Fig. 24.4).

During the forward or backward or side-to-side movement of the head, the maculae detect the direction of gravity by sensing the direction of the pull of the otolithic membrane and its otoliths on the stereocilia of the hair cells. Pressure or tension on the otolithic membrane results in the stimulation of the hair cells of the maculae with consequent stimulation of afferent nerve endings associated with these cells. The nerve impulses so generated go to the brain to provide information regarding the static position and linear acceleration of the head.

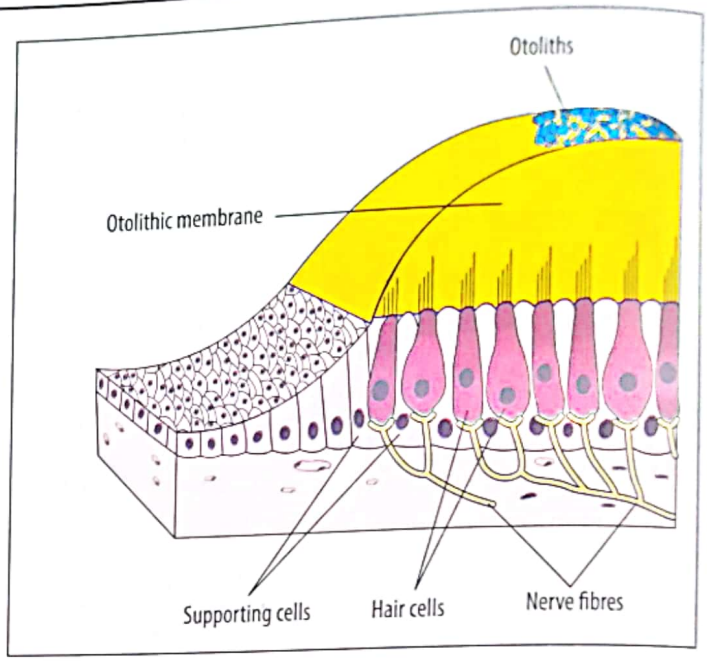

Fig. 24.4 Diagrammatic representation of the structure of a macula.

COCHLEA

The cochlea is an osseous canal that spirals around the *modiolus*, which is a cone-shaped structure composed of spongy bone. *Osseous spiral lamina* is a shelf of bone which projects from the modiolus, pursuing a spiral course around it (like the thread of a screw which spirals around its stem).

Transverse sections of the coils of the cochlea show that its membranous labyrinth, called ***cochlear duct*** (also known as ***scala media***), is triangular in shape and is attached to the bony labyrinth at places. The apex of the triangle rests on the osseous spiral lamina, whereas its base is in contact with the lateral bony wall of the osseous labyrinth. Thus perilymphatic space of the cochlea becomes divided into two canals, one above and one below the cochlear duct. The upper canal is called *scala vestibuli* and the lower one is known as *scala tympani* (Fig. 24.5).

The membrane that separates scala media from the scala tympani is called *basilar membrane*. This membrane is composed of collagen fibers and extends from the osseous spiral lamina to the *spiral crest* (which is a thickening of periosteum on the outer wall of the cochlear canal). Inferior surface of the basilar membrane (i.e., the one that faces the scala tympani) is covered by simple squamous epithelium. On the upper surface of the basilar membrane lies the spiral organ of Corti.

The boundary between the scala media and the scala vestibuli is formed by the *vestibular membrane*, also called *Reissner's membrane*, which consists of a very thin layer of connective tissue covered on both surfaces by simple squamous epithelium.

* The efferent nerve fibers serve to inhibit the activity of the afferent nerve fibers either directly (as in type I hair cells) or indirectly (as in type II hair cells).

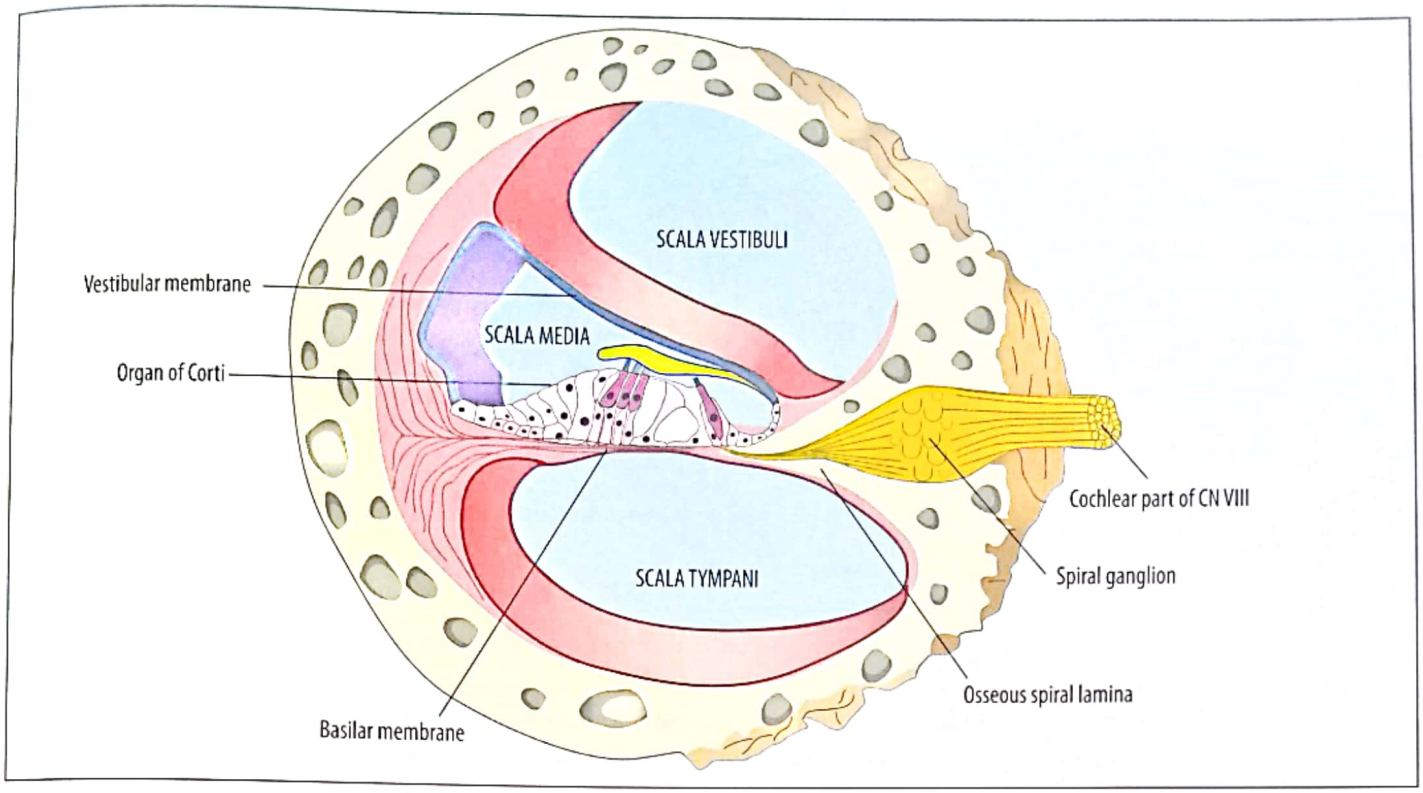

Fig. 24.5 Diagram showing the three scalae of the cochlear duct and the organ of Corti located in the scala media.

The scala vestibuli and scala tympani contain perilymph and their walls consist of connective tissue covered by simple squamous epithelium.

At the base of the scala vestibuli is present the *oval window* which is sealed by the footplate of stapes. The scala tympani extends laterally to the *round window* where the *secondary tympanic membrane* separates it from the tympanic cavity. At the apex of the cochlea, the scala vestibuli and scala tympani communicate with each other through a slit-like opening called *helicotrema*. The scala tympani is connected to the subarachnoid space via a narrow duct called *cochlear aqueduct*.

The scala media (cochlear duct) communicates with the saccule through the ductus reuniens, but terminates blindly near the helicotrema. The epithelium lining the scala media varies with location. The epithelium over the vestibular membrane is of simple squamous variety. Over the limbus it varies in height from low columnar to cuboidal. Laterally the epithelium is pseudostratified columnar and is underlain by vascular connective tissue. This region, known as *stria vascularis*, secretes the endolymph (and, therefore, the endolymph has a different composition than that of the perilymph). The basal cells of the pseudostratified columnar epithelium of the stria vascularis continue over the spiral crest as cuboidal cells. These cells also extend over the outermost part of the basilar membrane as *cells of Claudius*. In the basal turns of the cochlea, the cells of Claudius rest on smaller polyhedral cells that are called *cells of Bottcher*. However, most of the basilar membrane is covered by the specialized epithelium which forms the organ of Corti, which is the receptor organ for hearing.

ORGAN OF CORTI

The spiral organ of Corti extends over the entire length of the basilar membrane. It is composed of neuroepithelial hair cells and many types of supporting cells.

Neuroepithelial Cells of the Organ of Corti

The neuroepithelial cells of the organ of Corti occur as inner hair cells (situated close to the limbus spiralis) and outer hair cells (located farther from the limbus spiralis). The hair cells of the organ of Corti also bear stereocilia on their free (apical) surface. However, unlike the hair cells of the cristae and maculae, these hair cells do not bear any kinocilium. The stereocilia of the hair cells of organ of Corti are embedded in a gelatinous mass called tectorial membrane (Fig. 24.6). The basal regions of the hair cells synapse with afferent nerve fibers of the cochlear division of the 8th cranial nerve.

The **inner hair cells** are short, pear-shaped cells which form a single row of cells along the entire length of the basilar membrane. These cells do not reach the basilar membrane directly but, instead, each cell is surrounded by a supporting cell called inner phalangeal cell. The apical surface of an inner hair cell bears 50 to 60 stereocilia. The **outer hair cells** are long cylindrical cells present as three to five rows of cells along the entire length of the basilar membrane. An outer hair cell bears about 100 stereocilia on it apical surface. As in the hair cells of the maculae and cristae, the stereocilia of hair cells of the organ of Corti are arranged in several rows of progressively ascending height and the tips of the shorter stereocilia are connected to the adjacent taller sterocilia by filaments called tip links.

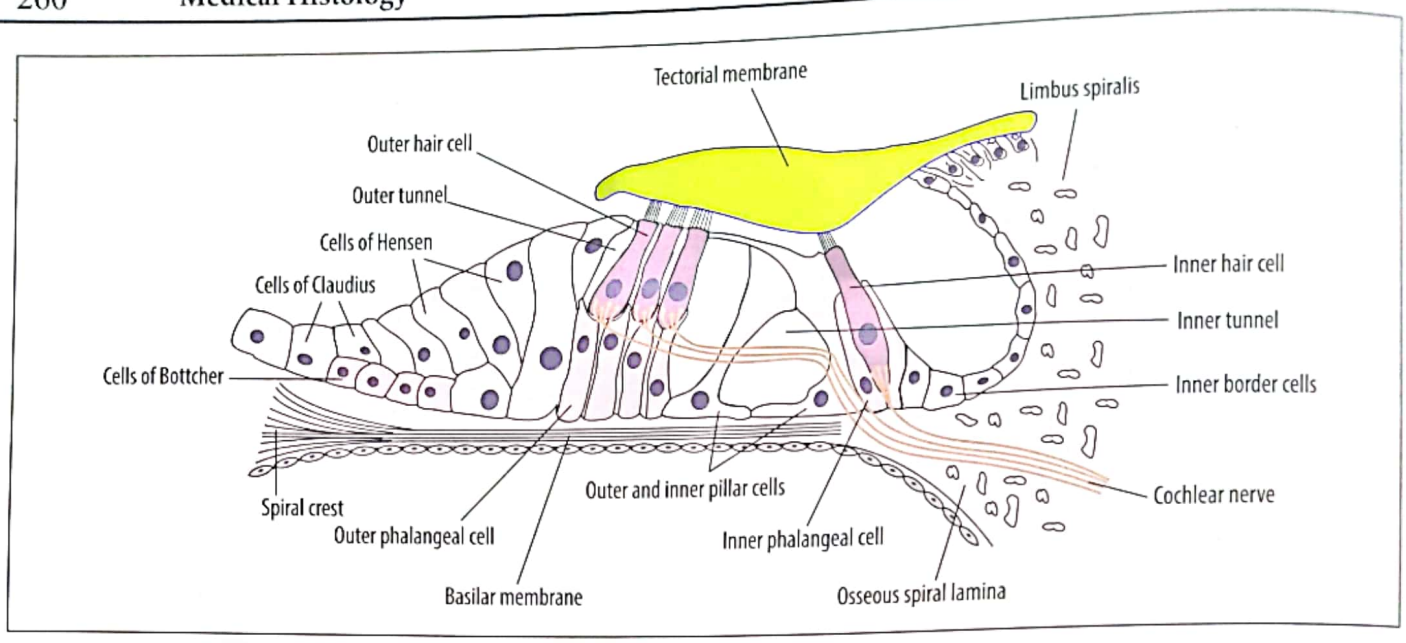

Fig. 24.6 A diagrammatic representation of electron microscopic structure of the spiral organ of Corti.

Supporting Cells of the Organ of Corti

The organ of Corti contains many varieties of supporting cells, which include the inner and outer pillar cells, inner and outer phalangeal cells, cells of Hensen, and inner border cells. The supporting cells are characterized by the presence of abundant microtubules and microfilaments in their cytoplasm, which impart a certain degree of rigidity and stiffness to these cells.

The **inner pillar cells** and **outer pillar cells** are tall columnar cells that lie next to the inner hair cells. Each pillar cell has an expanded base (that contains the nucleus) and an elongated body or *pillar.* The apical end of each pillar cell is also expanded and is known as head. The head of inner pillar cell shows a concavity into which fits a corresponding convexity of the head of outer pillar cell. The triangular space between the inner and outer pillar cells is called *tunnel of Corti.*

The **inner phalangeal cells** support the inner hair cells. Each *inner phalangeal cell* is a flask-shaped cell whose basal part contains the nucleus and rests on the basilar membrane. Apical part of each inner phalangeal cell completely surrounds an inner hair cell.

The **outer phalangeal cells,** also called *Deiters' cells,* are tall columnar cells that lie between the rows of the outer hair cells. The expanded bases of the Deiters' cells lie on the basilar membrane, while their apical ends partially envelop the bases of the outer hair cells. Each outer phalangeal cell has a finger-like process, called phalangeal process, which extends upwards between the outer hair cells and terminates in a plate-like expansion that fills the gap between the basal ends of the hair cells.

The **cells of Hensen** are tall columnar cells that are located adjacent to the outermost row of the outer phalangeal cells. The narrow space between these cells and outer phalangeal cells is known as the *outer tunnel.* The cells of Hensen constitute the outer border of the organ of Corti and, therefore, are also known as *outer border cells.*

The **inner border cells** are cuboidal cells that form three or four rows of cells at the inner border of the organ of Corti.

Tectorial Membrane

The tectorial membrane is an acellular, gelatinous layer that overlies the organ of Corti. It is composed of fibrillar collagens of the type II and type V, embedded in a ground substance consisting of glycosaminoglycans and glycoproteins. Medially, the tectorial membrane is attached to the modiolus, whereas its lateral free edge extends over the organ of Corti, so that the stereocilia of the inner and outer hair cells lie embedded in it.

Vibrations in the endolymph are transmitted to the tectorial membrane. These vibrations cause deflection of the hair cell stereocilia which are embedded in the tectorial membrane. Deflection of the stereocilia causes depolarization of the plasmalemma of the hair cells, resulting in the initiation of an impulse in the afferent nerve fibers which are in synaptic contact with the basal regions of the hair cells. This impulse is transmitted to the brain via the cochlear part of the vestibulocochlear nerve.

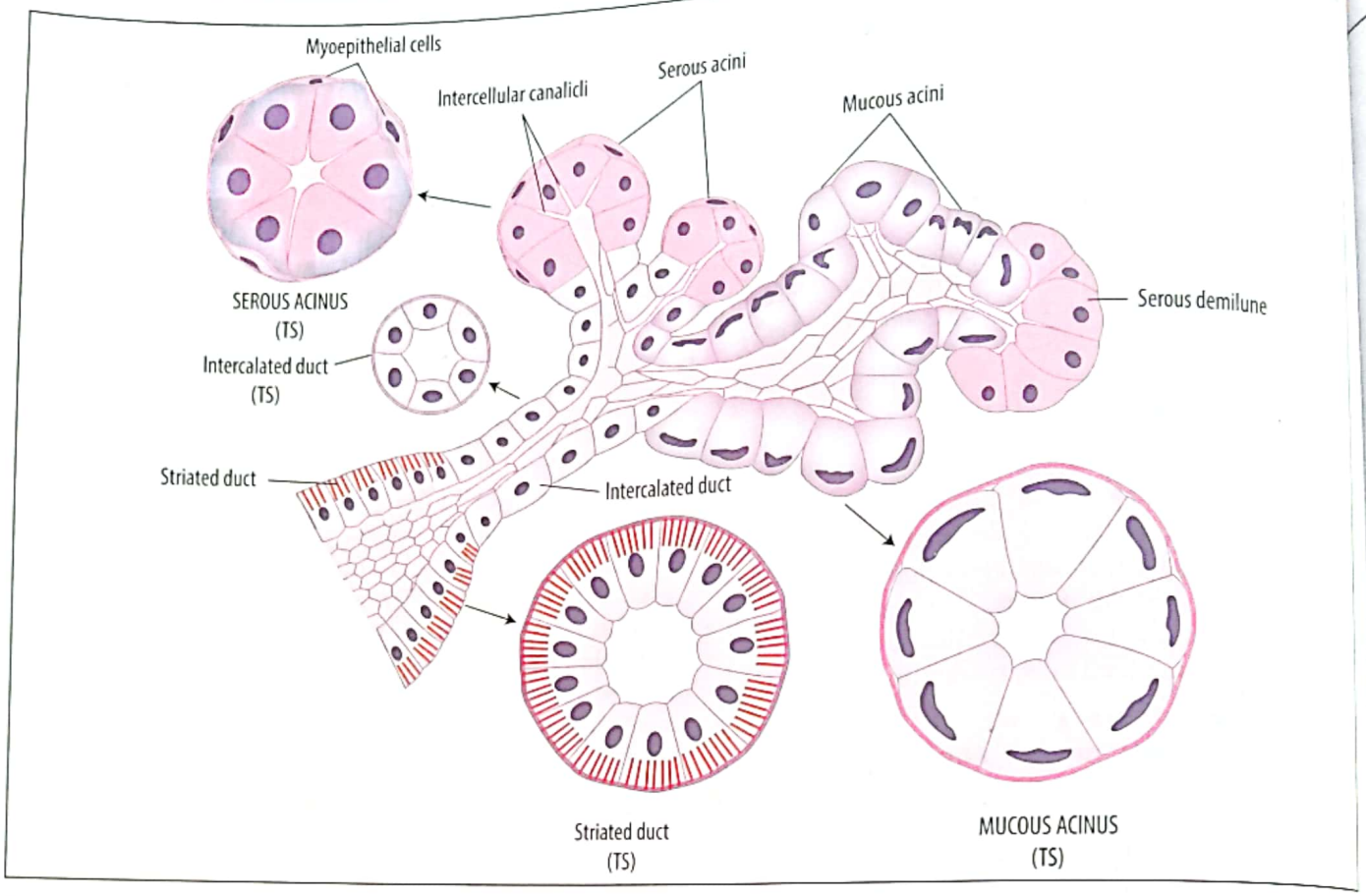

Fig. 19.1 Diagrammatic representation of the structure of a mixed (seromucous) submandibular salivary gland. (TS= Transverse Section).

MUCOUS CELLS

The mucous cells are also pyramidal in shape but their nuclei are oval (or flattened) and pressed against the basal plasmalemma (Fig. 19.1). The apical region of each mucous cell is occupied by numerous mucin granules, which are washed out during histological procedures. Therefore, in the ordinary H&E stained sections examined under LM, the apical regions of the mucous cells stain poorly and appear to be empty. EM reveals that the mucous cells contain mitochondria, a moderate amount of RER, but a very large Golgi apparatus. The large Golgi complex performs the important function of addition of carbohydrates to the proteins (which are synthesized by the RER) to produce the glycoproteins of the mucin.

The adjacent cells of the mucous acini are bound to each other by apical junctional complexes and do not exhibit intercellular canaliculi.

The mucous cells secrete **mucin** which is a strongly hydrophilic glycoprotein materiel. Upon contact with water, the mucin becomes converted into **mucus** which is a gel-like lubricant that serves to protect the delicate mucous membranes.

MYOEPITHELIAL CELLS

These cells are found to be located between the basement membrane and basal plasmalemma of the serous/mucous cells forming the secretory end pieces, i.e., acini or tubules (Fig. 19.1). Each myoepithelial cells has a body and many long processes that envelop the secretory cells. The cell body contains the nucleus and a small number of organelles. The cell processes contain actin and myosin. The myoepithelial cells are contractile cells and their contractions push the secretory product of the acinar cells toward the excretory duct. These cells stain poorly and cannot be easily identified in the H&E stained sections.

DUCT SYSTEM OF THE SALIVARY GLANDS

The salivary glands are compound glands having a highly branched duct system. The salivary ducts are generally classified into the following three types: (1) intercalated ducts, (2) striated ducts, and (3) excretory ducts.

INTERCALATED DUCTS

The secretory end pieces (acini or tubules) open into intercalated ducts that have a small diameter and are lined by simple cuboidal epithelium (Fig. 19.1). The lining cells of the intercalated ducts absorb chloride ions from the acinar secretory product and secrete bicarbonate ions into it and thus help in increasing the pH of the saliva. These cells also serve as stem cells because they have the capability to divide and differentiate into secretory acinar cells or ductal cells.

The intercalated ducts are considerably long in the parotid

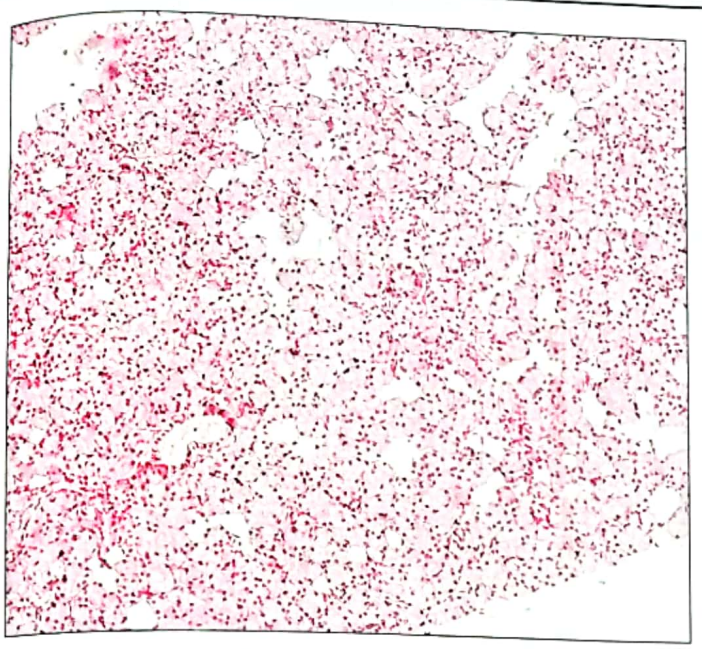

Fig. 19.2 A section of the parotid gland as seen under the low power magnification of the light microscope.

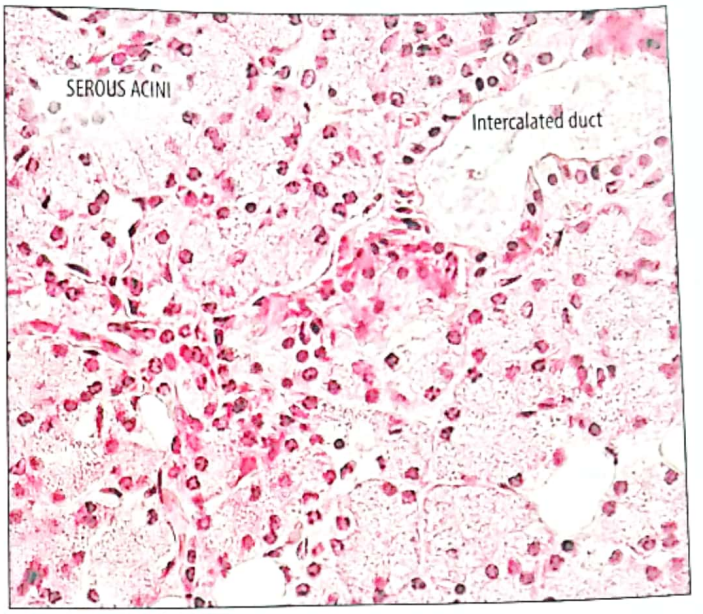

Fig. 19.3 A section of the parotid gland as seen under the high power magnification of the light microscope.

and submandibular glands but are very short in the sublingual gland.

STRIATED DUCTS

Several intercalated ducts open into a striated duct, which has a much larger caliber and is lined by a single layer of low columnar epithelial cells. The cytoplasm of these cells stains intensely with eosin and shows basal striations in the ordinary (H&E) sections (Fig. 19.1). The nucleus of a striated duct cell typically occupies a central (rather than basal) position within the cell. It is due to the presence of prominent eosinophilic basal striations that these ducts are called striated ducts.

Under EM, a striated duct cell is seen to possess numerous deep infoldings of the basal plasmalemma, between which elongated mitochondria are oriented in rows. These structural features, which are characteristic of ion-transporting cells, account for the presence of basal striations as observed with the light microscope.

The striated ducts reabsorb sodium and chloride ions from the acinar secretion passing through the duct and add potassium ions to it. Reabsorption of sodium from the primary acinar secretion is an important factor in reducing the tonicity of saliva.

The intercalated and striated ducts lie within the lobules of the glands and, therefore, are regarded to be *intralobular ducts.*

EXCRETORY DUCTS

The striated ducts from each gland lobule converge and drain into *interlobular ducts* that lie in the connective tissue septa which separate the adjacent lobules. The interlobular ducts join to form *interlobar ducts* that ultimately drain into the main duct of the gland, which conveys the secretory product of the gland into the oral cavity. The interlobular, interlobar, and the main ducts are purely excretory ducts, i.e., they serve as simple conduits and do not modify the secretory product flowing through them.

The interlobular ducts are lined by simple cuboidal epithelium, whereas the interlobar ducts are lined by pseudostratified columnar epithelium. The main duct of a salivary gland is lined by stratified columnar epithelium that changes into stratified squamous nonkeratinized variety near its opening into the oral cavity.

After this general description, characteristic features of the three major salivary glands will be discussed.

PAROTID GLAND

The parotid is the largest salivary gland but produces only 30% of the total output of saliva. It is a compound tubuloacinar gland of purely serous variety. It is covered by a well-developed connective tissue capsule from which numerous septa pass into the gland substance to divide it into lobes and lobules. The septa carry ducts, nerves, blood vessels, and lymph vessels.

The parenchyma of parotid gland consists only of serous acini (Fig. 19.2 & 19.3). The intercalated ducts are long and, therefore, prominent in histological sections. The striated ducts are also large and conspicuous. Adipocytes (fat cells) are abundant in the connective tissue between the gland lobules. The number of fat cells increases considerably after the age of 40 years.

Being a purely serous gland, the secretory product of the parotid gland is thin and watery, and consists mainly of α-amylase, which initiates the breakdown of the ingested carbohydrates in the mouth. In addition, the parotid secretion also contains proline-rich proteins which possess antimicrobial properties. The proline-rich proteins also

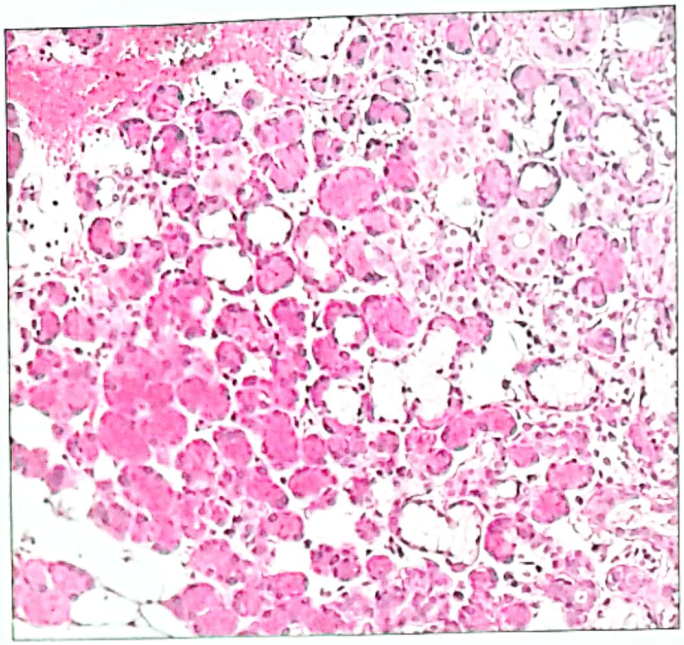

Fig. 19.4 A section of the submandibular gland as seen under the low power magnification of the light microscope.

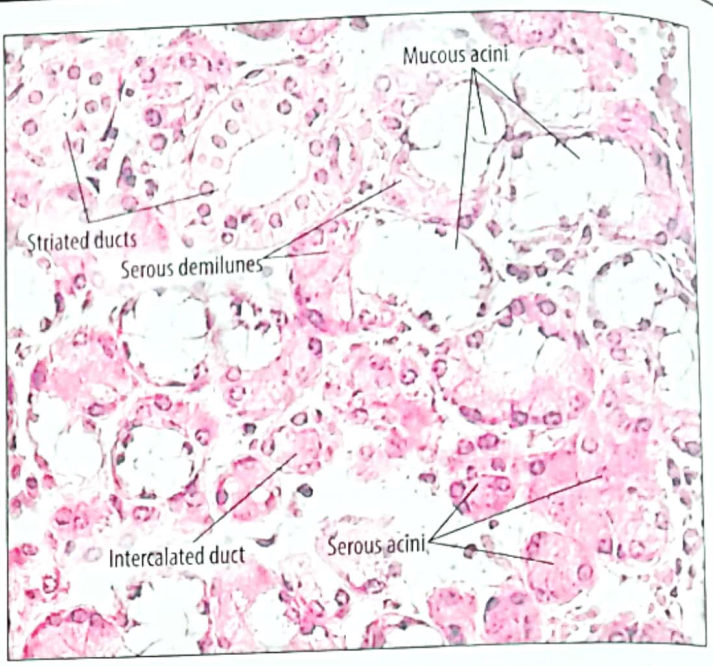

Fig. 19.5 A section of the submandibular gland as seen under the high power magnification of the light microscope.

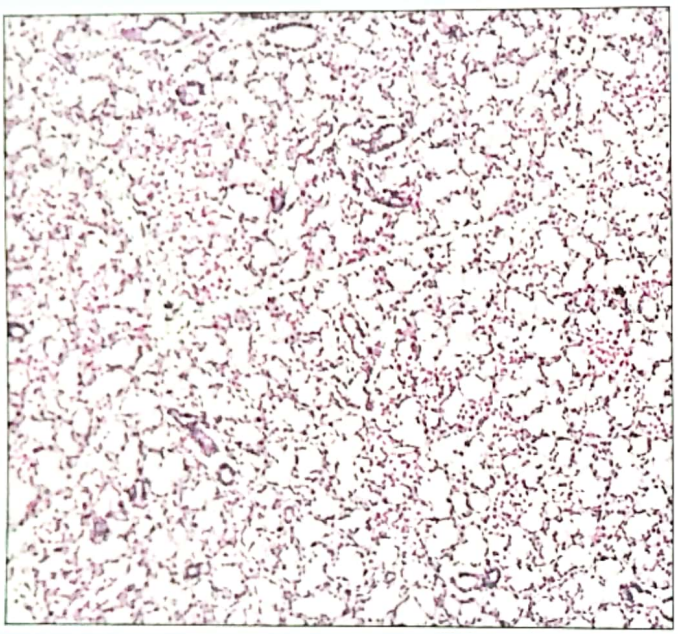

Fig. 19.6 A section of the sublingual gland as seen under the low power magnification of the light microscope.

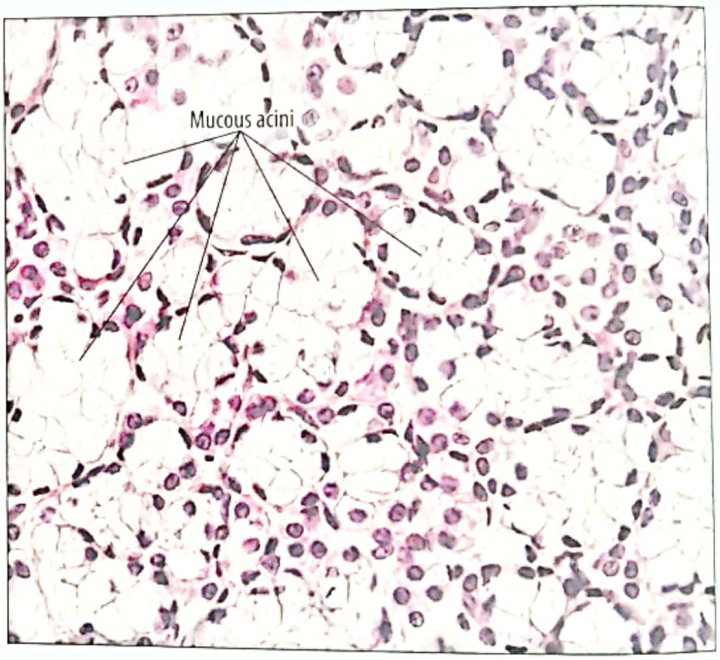

Fig. 19.7 A section of the sublingual gland as seen under the high power magnification of the light microscope.

have calcium-binding ability which is supposed to play an important role in keeping the enamel of teeth in healthy condition.

SUBMANDIBULAR GLAND

The submandibular gland is a compound tubuloacinar gland of mixed (seromucous) variety (Fig. 19.4 & 19.5). However, its secretion is predominantly serous in nature and 90% of its parenchyma consists of serous acini. Remaining 10% parenchyma consists of mucous end pieces in the form of tubules lined by mucous cells. Some of the mucous end pieces bear caps of serous cells called ***serous demilunes*** that exhibit a crescent-shaped appearance in histological sections.

In the submandibular salivary gland, the intercalated ducts are narrow and short and, therefore, are not conspicuous in sections. The striated ducts of this gland are much longer than those of the parotid and sublingual glands and, therefore, are very prominent in histological sections.

The connective tissue capsule of submandibular gland is well-developed and sends in septa that divide the gland substance into lobes and lobules.

The submandibular gland produces about 60% of the total salivary output. The secretory product of this gland contains mucin, α-amylase, and lysozyme.

- **Secretion of bile.** The liver synthesizes and secrets a fluid called *bile*. The bile mainly contains the conjugated and degraded waste products which are delivered to the intestine to be eliminated from the body in fecal excretion. In addition, the bile contains substances which aid in the digestion of lipids in the small intestine.
- **Excretion of bilirubin.** The bilirubin, an orange-yellow pigment, is produced chiefly in the spleen where the effete erythrocytes are removed from circulation and the heme portion of the hemoglobin is broken down to form bilirubin. The portal venous blood carries the bilirubin to the liver where it is conjugated with glucronic acid and the conjugate is excreted in the bile.

LIVER STROMA

The liver is covered by a thin connective tissue capsule called Glisson's capsule. This capsule is thicker at the hilum where the blood vessels, lymphatic vessels, and bile ducts enter or leave the organ. The human liver contains a relatively smaller amount of connective tissue (as compared to other mammals). Extensions of connective tissue from the capsule follow the blood vessels and ducts throughout their course in the liver and finally form a delicate network of reticular fibers that constitutes the supporting framework of the liver lobules.

BLOOD SUPPLY OF THE LIVER

The liver has a unique, dual blood supply which consists of a venous supply (via the hepatic portal vein) and an arterial supply (via the hepatic artery). The major part (75%) of the blood supply is received through the portal vein which brings oxygen-poor blood that comes from the digestive tubes and some major abdominal organs like the spleen and pancreas. This blood contains nutrients (as well as toxins) absorbed from the digestive tract and break down products of the red blood cells from the spleen. The hepatic artery brings oxygen-rich blood to the liver but its contribution to the total blood reaching the liver is only 25 percent.

In the liver, the branches of the portal vein and hepatic artery, along with a draining branch of the biliary duct system, run in portal tracts (described later). The liver cells (hepatocytes) get their blood supply from sinusoidal capillaries, called hepatic sinusoids, which lie between the plates of the hepatocytes. The hepatic sinusoids receive blood from the distributing branches arising from the branches of the portal vein and hepatic artery running in the portal canals (Fig. 19.14 & 19.16). The distributing branches from the portal vein and hepatic artery anastomose with each other before supplying the sinusoidal capillaries and, thus, the venous arterial bloods mix with each other before supplying the hepatocytes. Therefore, the liver cells are never exposed to fully oxygenated blood.

The hepatic sinusoids of a hepatic lobule drain into a terminal portal venule which is more commonly called

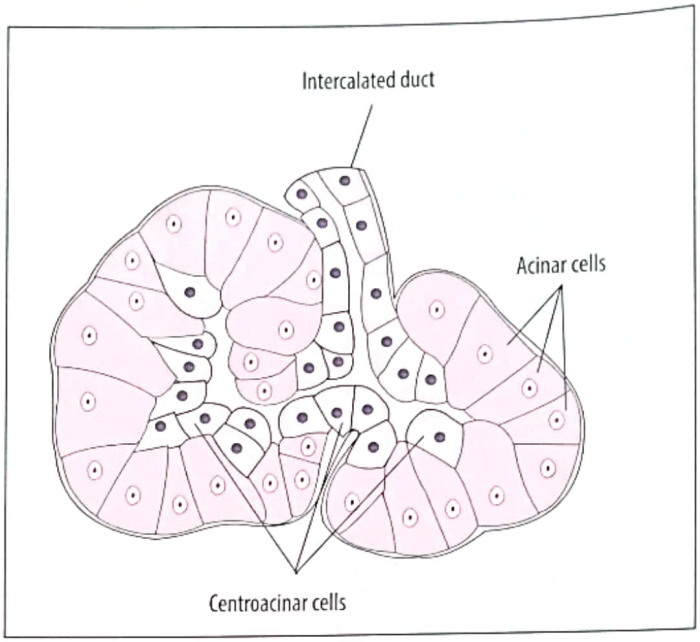

Fig. 19.9 Diagram showing a pancreatic acinus, centroacinar cells and intercalated duct.

central vein. The central veins from the hepatic lobules drain into sublobular veins which deliver their blood to a pair of hepatic veins that drain into the inferior vena cava.

LIVER PARENCHYMA AND GENERAL STRUCTURAL PLAN OF THE LIVER

The parenchyma of the liver consists of masses of epithelial cells called **hepatocytes** that are arranged as anastomosing and branching plates. Between the plates are present sinusoidal capillaries, which are about 10 µm wide and are called ***hepatic sinusoids***.

Three interpretations of the liver structure have been proposed. These include: the classical hepatic lobule, the portal lobule, and the hepatic acinus (Fig. 19.13).

THE CLASSICAL HEPATIC LOBULE

According to the traditional concept of the liver structure, the liver substance is described to consist of innumerable classical hepatic lobules (Fig. 19.10, 19.11 & 19.12). Each classical liver lobule is shaped roughly like a polygonal prism, measuring about 0.7 mm in width and 2 mm in length. The central structure of the lobule, traversing its long axis, is a relatively large venule called **central vein**, which is a tributary of the hepatic vein. Irregular and anastomosing **plates of hepatocytes** radiate from the central vein to the perimeter of lobule. The plates are one cell thick and are separated from each other by **hepatic sinusoids** which are also radially arranged around the central vein.

In sections examined under LM, the cross sections of hepatic lobules usually appear hexagonal in shape. Also, the radiating plates of the hepatocytes are seen as cords which are called ***hepatic cords***. Because of scarcity of connective tissue in the human liver, boundaries between adjacent hepatic lobules cannot be clearly distinguished, and the

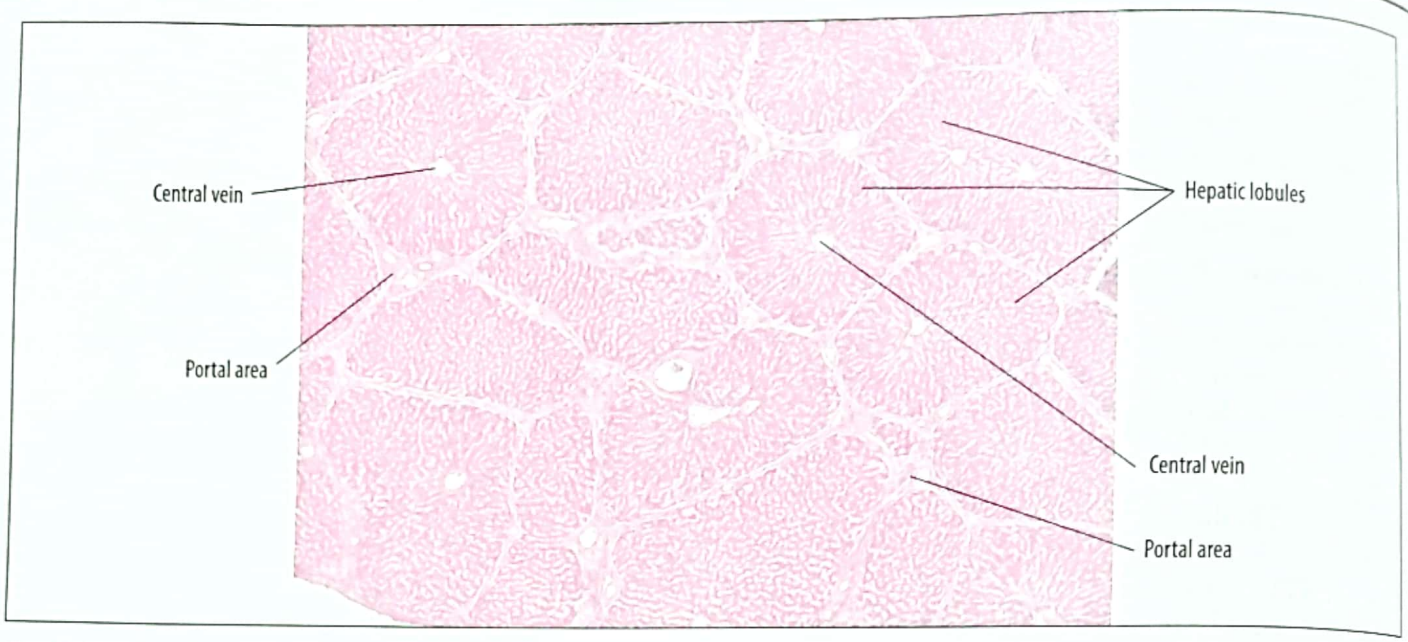

Fig. 19.10 A section of the liver as seen under the low power magnification of the light microscope.

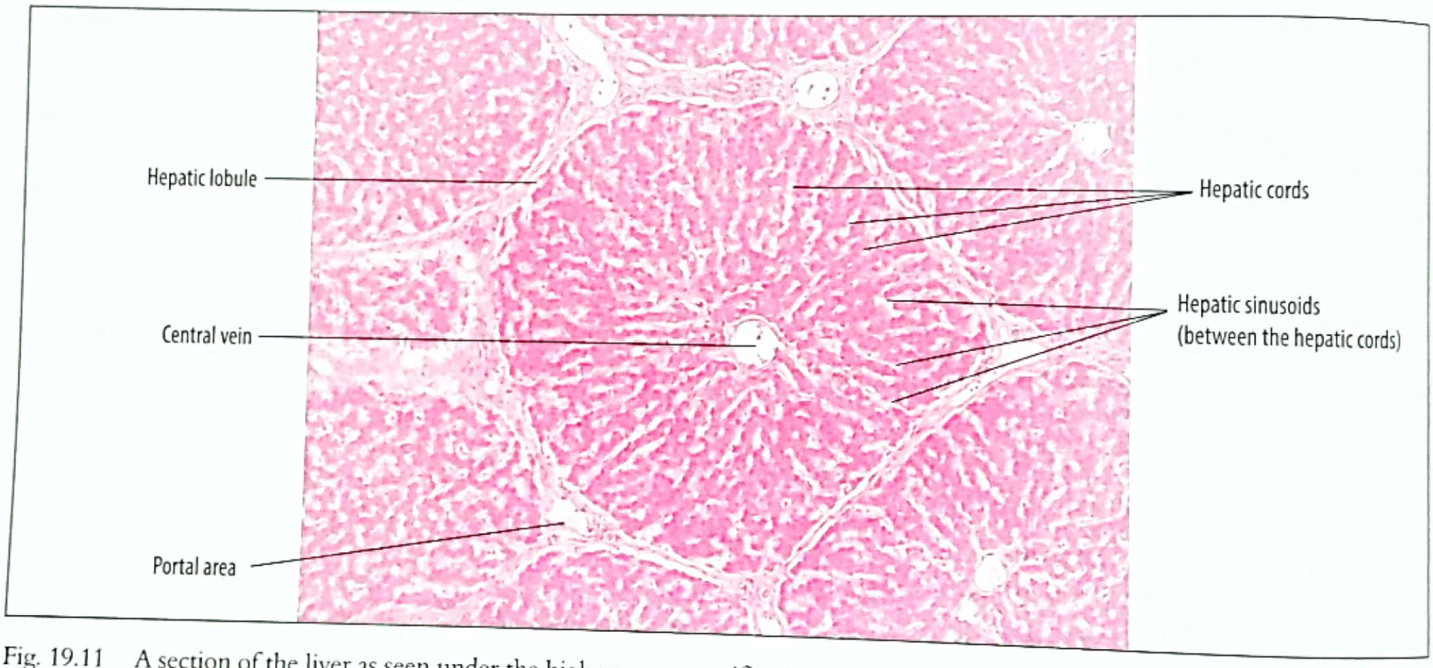

Fig. 19.11 A section of the liver as seen under the high power magnification of the light microscope.

cellular cords of one lobule appear to be continuous with those of the neighboring lobules.

At the angles of the hexagonal hepatic lobules are present roughly triangular areas called **portal areas** or **portal tracts.** Each portal area is seen to contain three distinct tubular structures lying in a small amount of connective tissue. These tubular structures, collectively known as a portal triad, include: (i) a bile ductule, (ii) a venule, which is a branch of the portal vein, and (iii) an arteriole, which is a branch of hepatic artery. However, it should also be noted that the lymphatics of the liver also run in the portal tracts.

With branches of afferent blood vessels (portal vein and hepatic artery) situated at the periphery of hepatic lobule and the efferent vessel (the central vein) at the center of the lobule, it is clear that the blood flows from the periphery through the hepatic sinusoids to the central vein. On the other hand, bile passes from the liver cells to the bile ducts lying at the periphery of the lobule.

The bile flows in narrow channels called **bile canaliculi** which are located between the adjacent cells in the plates of the liver cells. It is to be specially understood that the bile canaliculi are simply tubular spaces between adjoining liver cells, so that their walls are formed by the plasmalemmas of the liver cell (and the canaliculi do not have any specific lining epithelium of their own).

THE PORTAL LOBULE

This interpretation of the liver structure is based exclusively on the exocrine function of the liver, i.e., production of bile. A portal lobule has at its center a portal tract and

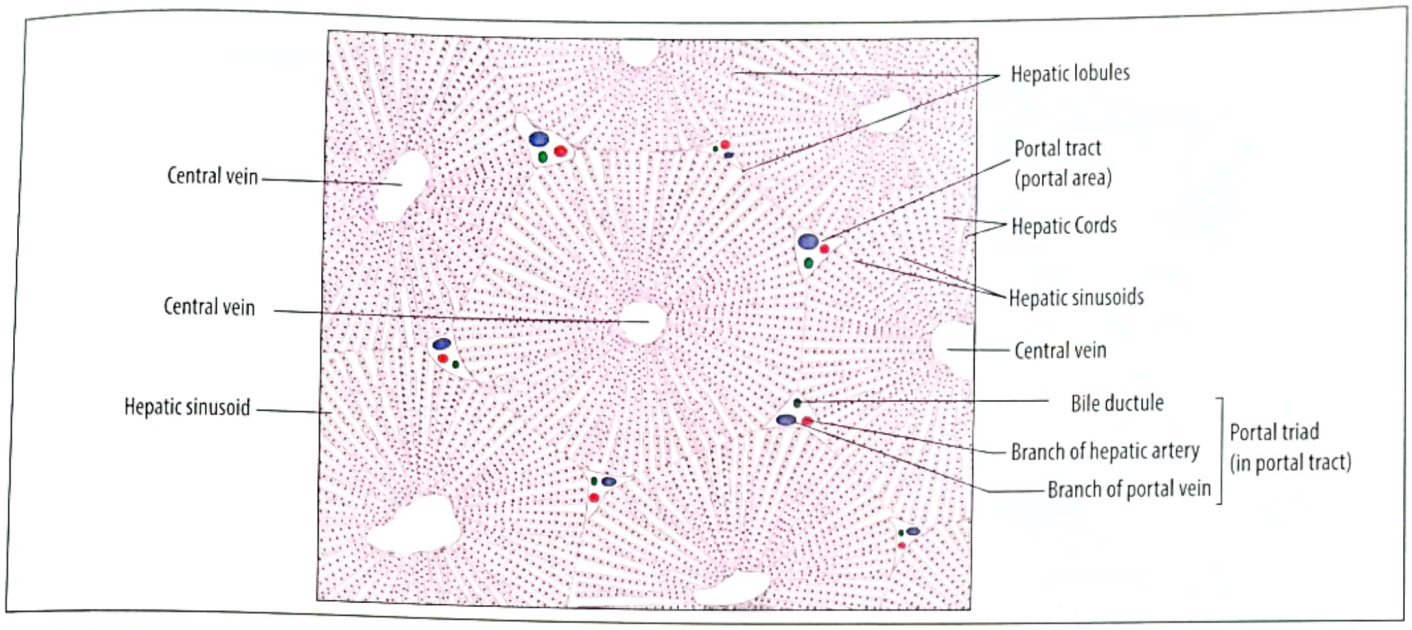

Fig. 19.12 Diagrammatic representation of microscopic structure of the liver.

consists of liver tissue which drains bile into the bile duct of that portal tract (Fig. 19.13). Such a lobule is triangular in cross section, contains parts of three adjoining hepatic lobules, and has a central vein at each of its three corners.

THE HEPATIC ACINUS

This is another way of subdividing the liver tissue into structural units. This structural unit provides the best correlation between the blood perfusion and metabolic activity of the hepatocytes, and also explains the response of these cells to the blood-borne toxins.

Each hepatic acinus is an ovoid area of the liver parenchyma (Fig. 19.13). The long axis of this ovoid (or elliptical) area corresponds to a line drawn between two neighboring central veins. The short axis of the ovoid hepatic acinus corresponds to a line drawn between the two adjacent portal triads. The distributing branches of the portal vein (portal venules) and those of the hepatic artery (hepatic arterioles) run in the short axis of the hepatic acinus.

Each hepatic acinus includes adjacent areas of two neighboring hepatic lobules. Taking into account the proximity of the hepatocytes to the blood vessels and bile ductile running in the central axis, each hepatic acinus is divided into three zones.

The innermost zone (Zone 1). The hepatocytes of this zone lie closest to the penetrating branches of the hepatic artery and portal vein and, therefore, these cells are first to receive oxygen, and nutrients. Consequently, these cells are last to die if the blood supply of the liver is impaired and first to regenerate when the blood supply is restored. The cells of the zone 1 are also the first to show pathological changes if the blood entering the liver contains toxins absorbed from the small intestine.

The intermediate zone (Zone 2). This zone consists of the hepatocytes lying between the innermost and outermost zones. Their response to ischemia and blood-borne toxins is of intermediate extent as compared to the hepatocytes of the innermost and outermost zones.

The outermost zone (Zone 3). The hepatocytes of this zone are located farthest from the branches of the hepatic artery and portal vein. In case of liver ischemia, the cells of the zone 3 die first of all and they are last to regenerate when the blood supply is restored. Understandably, the hepatocytes of the zone 3 are last to be affected by the blood-borne toxins.

HEPATOCYTES

The hepatocytes are large, polygonal cells having six or more surfaces. Average diameter of a hepatocyte is 20-30 µm. The hepatocytes are closely packed together to form anastomosing plates of cells that are arranged radially around the central vein. The plates of hepatocytes are generally one cell thick and are separated from one another by hepatic sinusoids.

Most of the hepatocytes have a single, spherical nucleus but nearly 25% of them contain two nuclei. The nuclei of liver cells vary in size and ploidy. The small nuclei are diploid, while the large nuclei are polyploidy. The conspicuous feature of hepatocyte nuclei is the presence of scattered clumps of heterochromatin. Each nucleus also contains one or two prominent nucleoli.

Under EM, the cytoplasm of a hepatocyte is seen to contain the usual cell organelles. Mitochondria are numerous and scattered throughout the cytoplasm. Multiple small Golgi complexes are present in the peripheral part of a hepatocyte near the bile canaliculi (Fig. 19.15). The smooth endoplasmic reticulum(SER) is very abundant in the cytoplasm of a hepatocyte, but a considerable amount of the rough endoplasmic reticulum (RER) is also present. The peroxisomes and lysosomes are also present in large numbers.

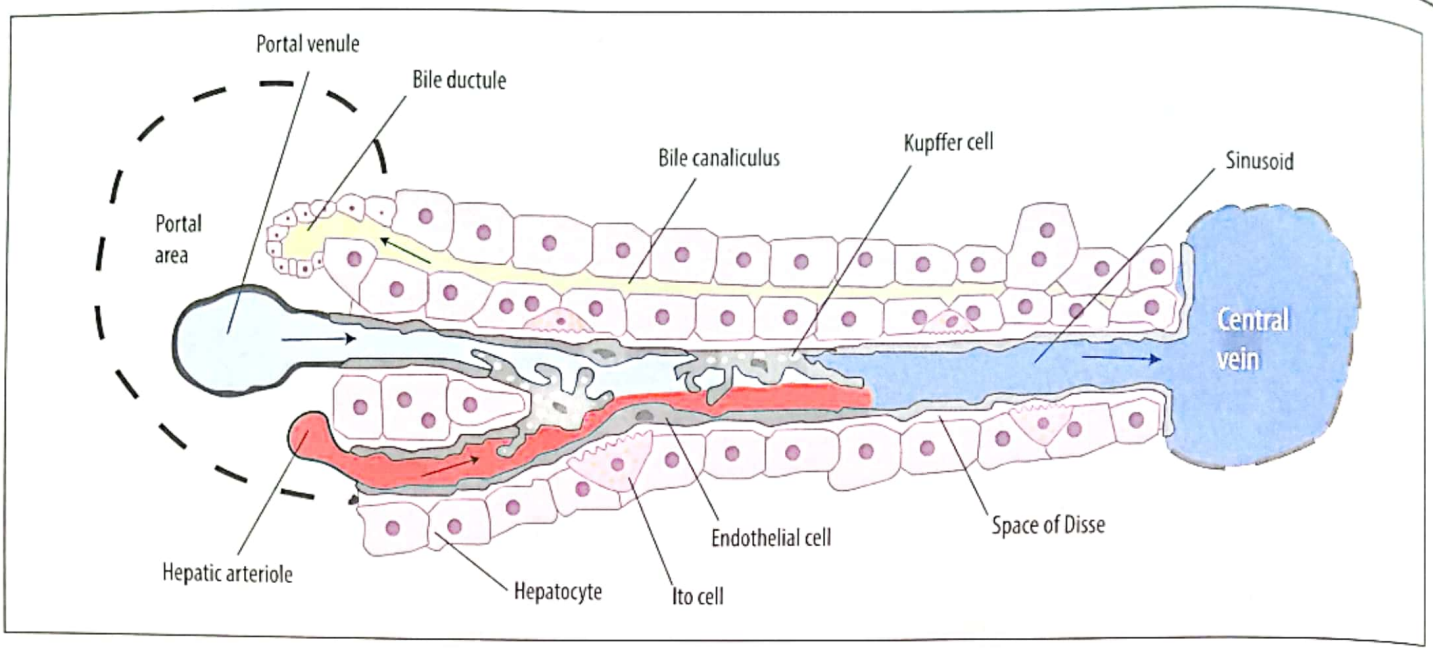

Fig. 19.16 Schematic diagram showing the direction of flow of blood and bile in the hepatic lobule.

increases as the duct diameter becomes greater and in the larger ducts the cholangiocytes are columnar in shape. Adjacent cholangiocytes are firmly bound to each other by tight junctions. The cholangiocytes bear many microvilli and a single 'primary cilium' on their luminal surface. The cilia of the cholangiocytes project into the lumen of the biliary ducts and monitor the components of the bile flowing in the duct lumen. Signals generated by the primary cilia result in alterations in the functional activity of the cholangiocytes.

Functions of the Cholangiocytes

The cholangiocytes have secretory as well as absorptive functions. They secrete water, chloride ions and bicarbonate ions into the bile, and reabsorb bile acids, amino acids and glucose from the bile.

BILE CANALICULI

The biliary tree begins as very small channels (0.5 to 2.5 µm in diameter) which are called *bile canaliculi*. As explained earlier, each bile canaliculus is a narrow tubular passage formed by the perfect opposition of grooves on the canalicular surface of the adjacent hepatocytes. Therefore, the walls of the bile canaliculi are formed by the cell membrane of the hepatocytes, and they are not lined by cholangiocytes or any type of epithelium. A bile canaliculus exhibits an irregular or stellate lumen in sections examined under EM because microvilli project from the hepatocytes into the cavity of the canaliculus. The microvilli serve to increase the surface area through which the bile can be secreted from the hepatocytes. The lumen of each bile canaliculus is sealed off from the intercellular compartment by occluding junctions which are part of the junctional complexes by which the canalicular surfaces of the adjacent hepatocytes are bound together.

CANALS OF HERING

The bile canaliculi pour the bile into short and narrow channels which are called *canals of Hering*. These canals convey the bile into bile ductules and are lined mainly by cuboidal cholangiocytes. A special feature of the epithelial lining of these canals is the presence of **hepatic stem cells** among the cholangiocytes. These cells are oval in shape and are also called *oval hepatic cells* or simply ***oval cells***. The oval cells are capable of producing progenitor cells for cholangiocytes as well as hepatocytes and, therefore, play an extremely important role in regeneration of liver tissue after trauma or disease.

BILE DUCTULES

These channels range in diameter from 15-40 µm and lie in the connective tissue of the portal tracts, forming one of the three components of the portal triad. The bile ductules are lined by cuboidal cholangiocytes. Outer to the basement membrane of the cholangiocytes is present a thin sheath of connective tissue.

THE INTERLOBULAR (TRABECULAR) DUCTS AND RIGHT AND LEFT HEPATIC DUCTS

These ducts are lined by columnar cholangiocytes. The connective tissue coat of these ducts becomes thicker with the increasing diameter and smooth muscle cells appear in the connective tissue as the ducts approach the hilum.

THE EXTRAHEPATIC BILIARY DUCTS

These ducts include the common hepatic duct, cystic duct, and the common bile duct. The wall of these ducts consists of mucosa, submucosa, muscularis and adventitia. The mucosa is the innermost layer and is lined by a single layer of columnar cholangiocytes. A thin layer of fine, loose connective tissue lying under the cholangiocytes constitutes the lamina propria. No muscularis mucosae

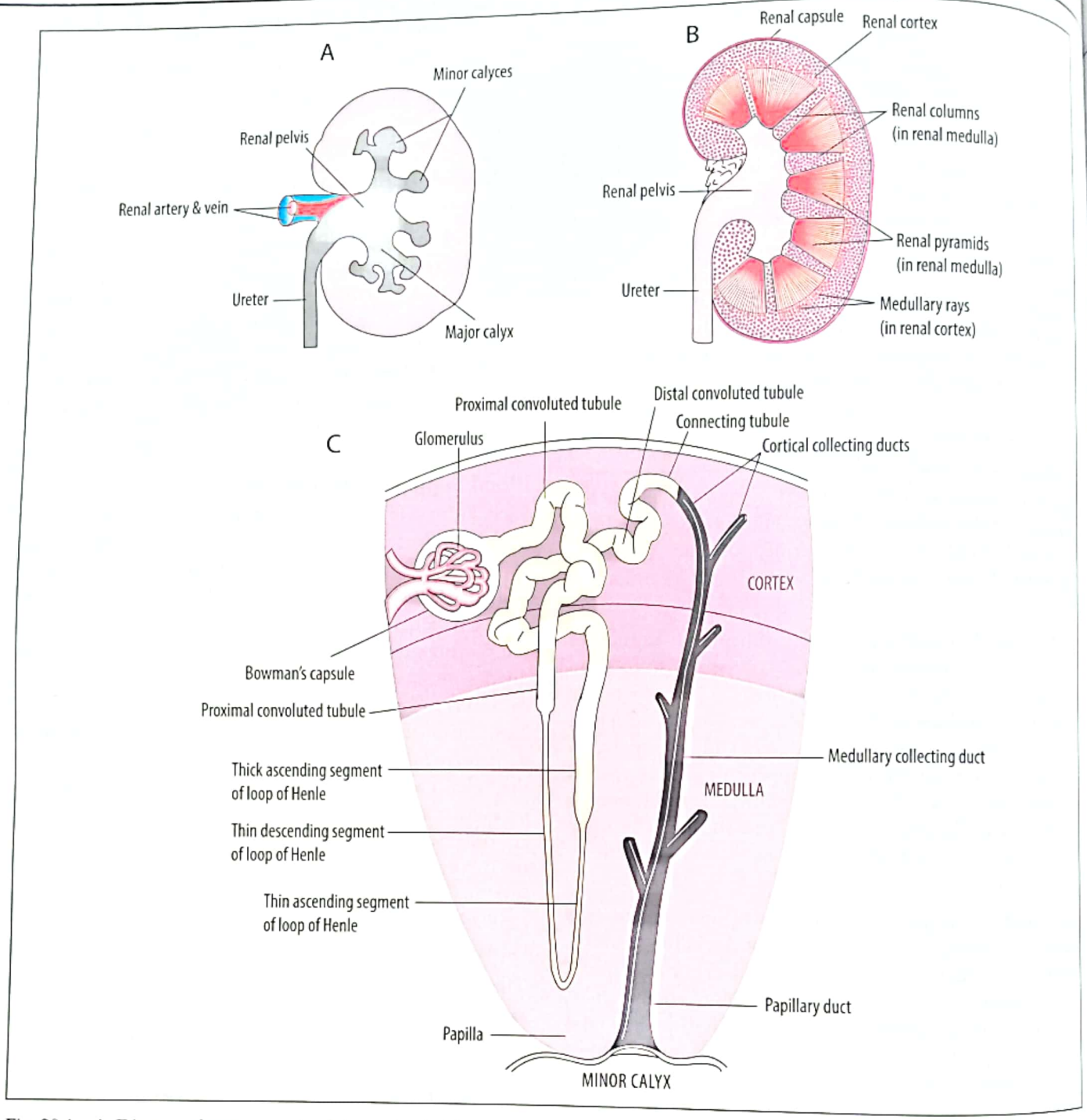

Fig. 20.1 A. Diagram showing renal pelvis and its divisions, B. Macroscopic appearance of kidney as seen in a coronal hemisection, C. The cortical and medullary localization of nephron segments and collecting ducts.

PARENCHYMA OF THE KIDNEY

The parenchyma of each kidney consists mainly of 1-4 million tubular structures called ***nephrons***, which are the chief structural and functional units of the kidney. The nephrons finally drain into ***collecting ducts***. The scanty space between the nephrons and collecting tubules is occupied by peritubular blood capillaries and interstitial connective tissue of the kidney.

THE NEPHRON

Each nephron is a long, epithelium-lined tube that begins blindly and ends by joining a collecting tubule. Each nephron is highly tortuous for the greater part of its course and consists of several segments having different structure and function. A nephron consists of 5 major segments: (1) **renal corpuscle**, (2) **proximal tubule**, (3) **loop of Henle**, (4) **distal tubule**, and (5) **connecting tubule** (also called **cortical collecting tubule**). Each of these segments is positioned in a definite location in the cortex or medulla of the kidney (Fig. 20.1 C & 20.2).

As already mentioned, the connecting tubules deliver the urinary filtrate to a system of collecting tubules and collecting ducts, which are not parts of the nephrons. However, a nephron and the collecting tubule into which

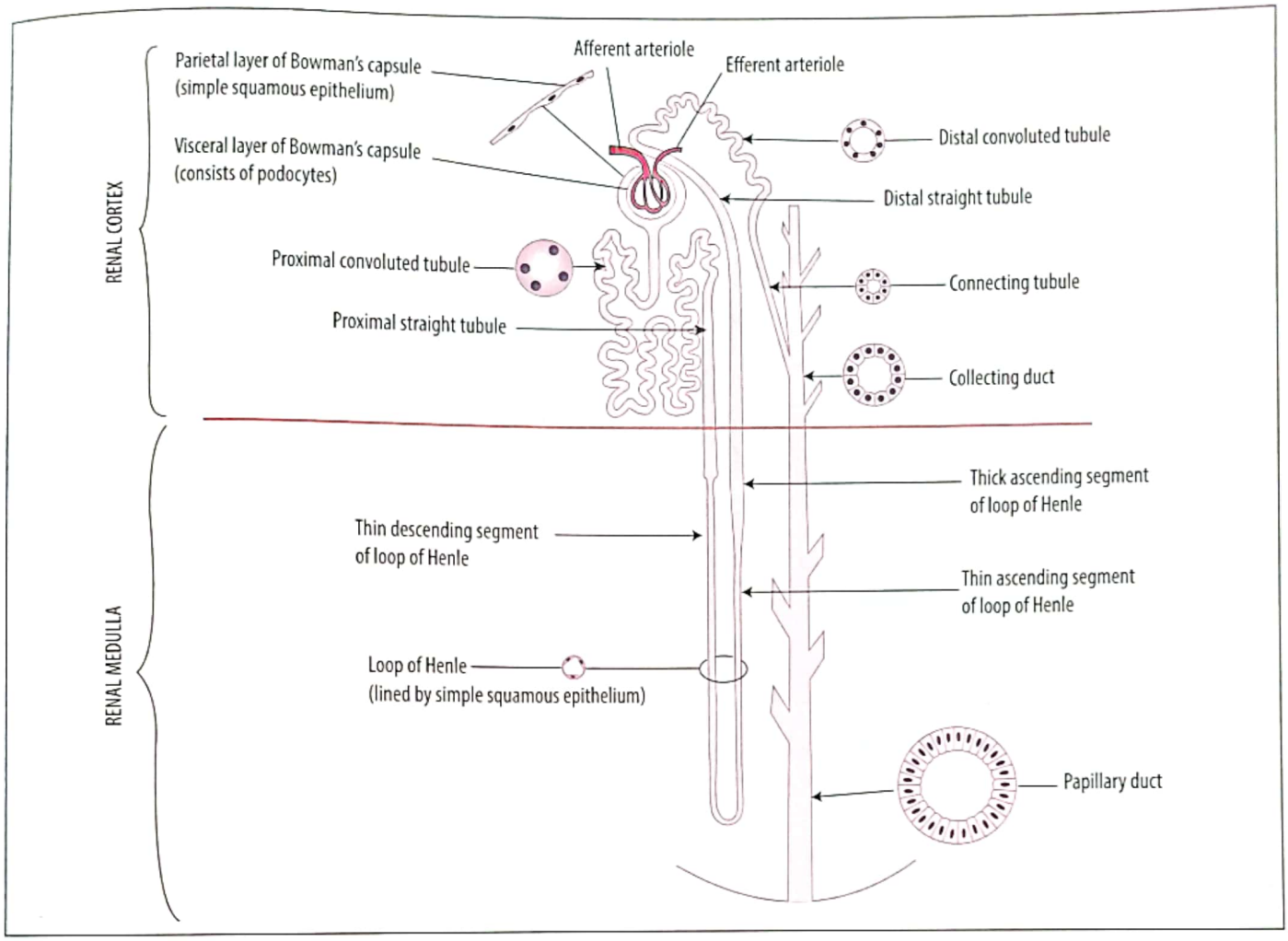

Fig. 20.2 Structural features of different nephron segments and collecting ducts.

it drains are sometimes collectively referred to as the uriniferous tubule.

PARTS OF THE NEPHRON

1. **The Renal Corpuscle**. Each nephron begins as a double-walled, cup-shaped dilatation known as ***Bowman's capsule***. A spherical tuft of capillaries, called ***glomerulus***, occupies the concavity of the capsule. The Bowman's capsule and glomerulus collectively known as a *renal corpuscle* (Fig. 20.3 & 20.4). All the renal corpuscles are located in the renal cortex.
2. **The Proximal Tubule**. This segment of the nephron consists further of two segments: (i) a very long but highly convoluted part called *proximal convoluted tubule*, which is located in the renal cortex, and (ii) a shorter, straight part called *proximal straight tubule*, which enters the renal medulla.
3. **The Loop of Henle**. This is a U-shaped segment of the nephron which lies between the proximal and distal tubules; it is also known as ***nephron loop***.
4. **The Distal Tubule**. This part of the nephron also consists of two parts: (i) a thick straight part, called *distal straight tubule*, which ascends from the loop of Henle back into the renal cortex, and (ii) a long, convoluted and tortuous part called *distal convoluted tubule*, which lies entirely in the cortex.
5. **The Connecting Tubule**. This terminal segment of the nephron, also called ***cortical collecting tubule,*** links the distal convoluted tubule to a collecting duct.

Depending on the location of their renal corpuscles, the nephrons are classified into three categories: (1) superficial cortical nephrons, (2) midcortical nephrons, and (3) juxtamedullary nephrons.

The **superficial cortical nephrons** have their renal corpuscles situated in the outer part of the renal cortex. They have short loops of Henle, which do not extend beyond the outer part of the renal medulla.

The **midcortical nephrons** are those nephrons whose renal corpuscles are situated in the middle part of the renal cortex. The Henle's loops of these nephrons are of intermediate length and extend almost to the middle of the medullary pyramids.

The **juxtamedullary nephrons** have their renal corpuscles located in the deepest part of the renal cortex in close proximity to the base of a medullary pyramid. These

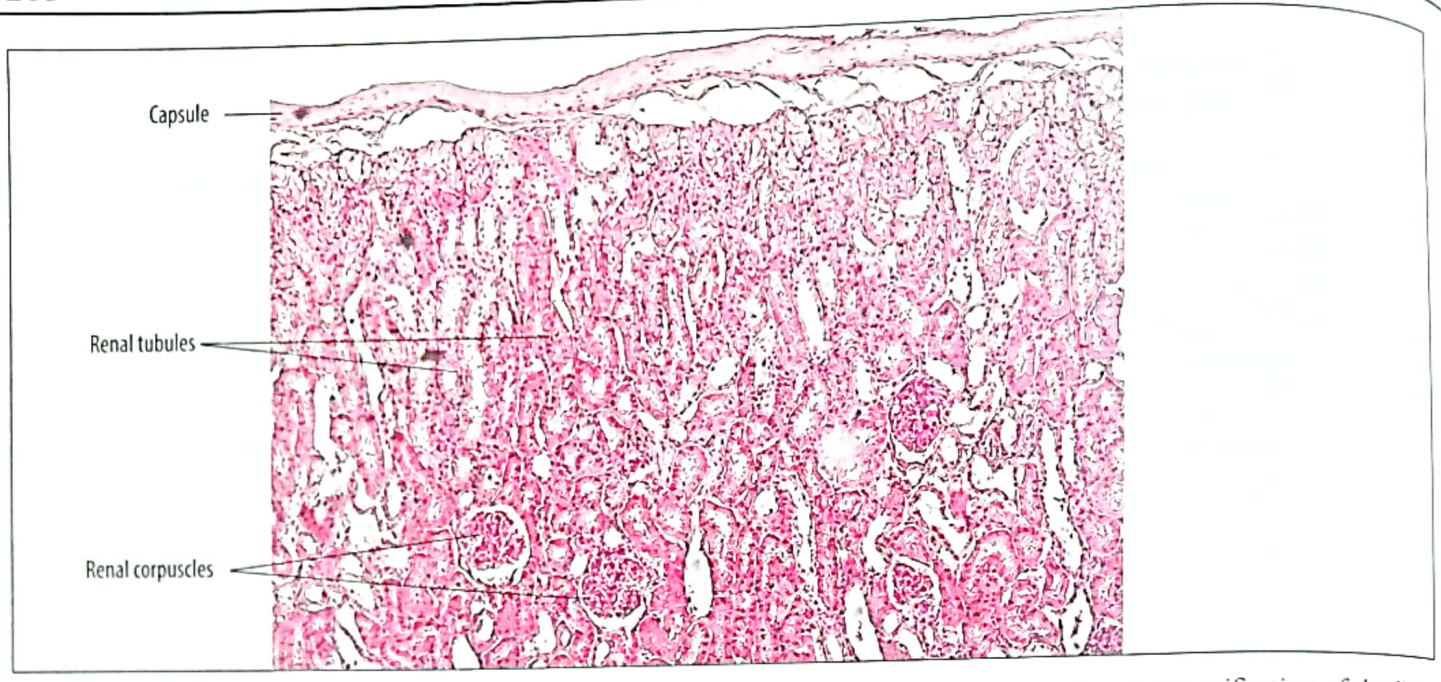

Fig. 20.3 A section showing the histological features of the cortical region of kidney under low power magnification of the light microscope.

nephrons have long loops of Henle that extend deep into the renal medulla.

Renal Corpuscle

The renal corpuscles are spherical bodies, ranging from 200 to 250 µm in diameter. Each of them has a *vascular pole* where the afferent arteriole enters and the efferent arteriole leaves, and a *urinary pole* where the proximal convoluted tubule begins (Fig. 20.5). As mentioned earlier, each renal corpuscle is composed of two components: (i) Bowman's capsule, and (ii) glomerulus.

The Bowman's Capsule

The Bowman's capsule, also called **glomerular capsule**, is a double-walled cup-like structure, having an outer wall called *parietal layer,* and an inner wall known as *visceral layer.* The narrow space between the parietal and visceral layers of the Bowman's capsule is referred to as the *capsular space* or *urinary space* (Fig. 20.5).

The **parietal layer** of Bowman's capsule consists of a simple squamous epithelium. The flat epithelial cells of this layer rest on a basement membrane which covers the outer surface of these cells. At the urinary pole, the simple squamous epithelium becomes continuous with the simple cuboidal epithelium of the proximal convoluted tubule.

The **visceral layer** of Bowman's capsule covers the glomerulus and invests it closely. It is composed of a single layer of specialized epithelial cells called *podocytes,* which are highly modified to perform a filtering function.

The Podocytes

The podocytes are large, irregularly star-shaped cells which share a common basement membrane with the endothelial cells of the glomerular capillaries (Fig. 20.5 & 20.6). Cell bodies of the podocytes are located in the capsular space, 1 to 2 µm away from the basement membrane. From the cell body arise several *primary processes* which resemble the tentacles of an octopus. Each of these processes extends toward one or more glomerular capillary loops and gives rise to numerous *secondary processes,* known as **pedicels**. The pedicels (also called *foot processes*) make a direct contact with the capsular surface of the common basal lamina.

EM reveals that a podocyte contains a roughly oval nucleus and its cytoplasm exhibits many mitochondria, a Golgi apparatus, a moderate amount of rough endoplasmic reticulum, and abundant free ribosomes. Numerous microtubules and actin filaments are found in the cell body and in the primary and secondary processes. Presence of actin filaments indicates that the podocytes have contractile capability.

The foot processes (pedicels) of each podocyte interdigitate with those of the neighboring podocytes. The interdigitating pedicels are separated from each other by very narrow gaps called **filtration slits, which** have an average width of only 40 nm.

The filtration slits are covered by very thin membranes, called *slit diaphragms* or *slit membranes*. Each slit diaphragm is a thin, porous sheet which is composed of special type of proteins called nephrins. In addition, other proteins like podocalyxin and P-cadherin are also present. The slit diaphragms allow the passage of small molecules, e.g., water, glucose, and various ionic salts, from the blood into the urinary space of the Bowman's capsule, but restrict the passage of macromolecules like serum albumin and gamma globulin. The slit diaphragms are an important component of the *filtration barrier* (described in detail later).

Glomerular Basement Membrane (GBM)

The basal lamina of the visceral layer of Bowman's capsule that lies between the podocytes and the endothelial cells

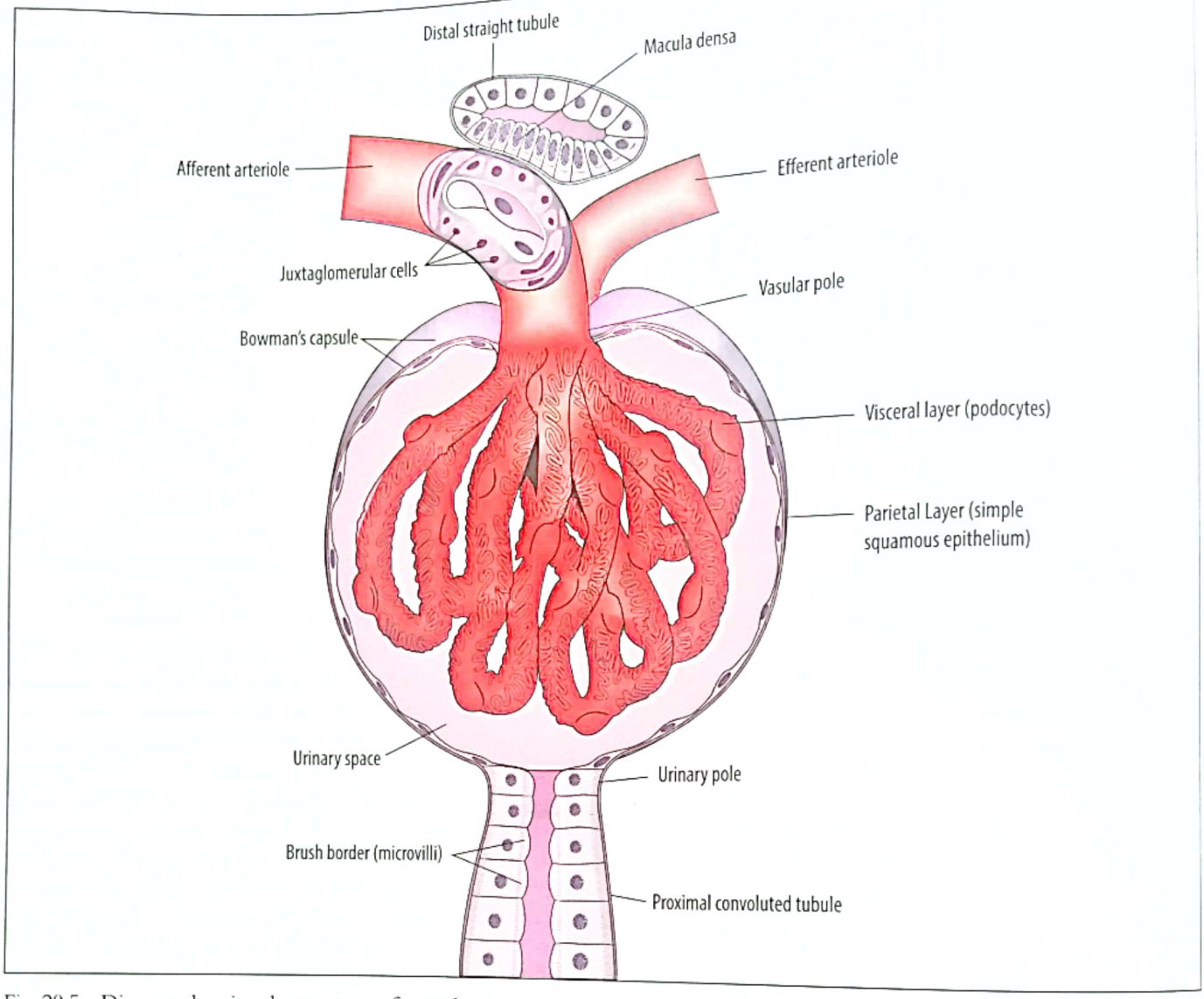

Fig. 20.5 Diagram showing the structure of a renal corpuscle. The upper part shows vascular pole with afferent and efferent arterioles. The lower part shows the PCT as it begins from the urinary pole of the renal corpuscle. To be specially noted are JG cells in the wall of the afferent arteriole and macula densa in the wall of DCT.

processes. The mesangial cells cannot be identified in routine sections. Electron microscopic studies show that the mesangial cells have an irregular shape and, like the pericytes of ordinary capillaries which are enclosed by the endothelial basal lamina, the mesangial cells are enclosed by the GBM. The cytoplasm of the mesangial cells contains bundles of the actin and myosin filaments, indicating that these cells have the ability to contract.

Functions of the Mesangial Cells

Research has shown that the mesangial cells perform several functions. The principal functions performed by these cells are:

i. **Phagocytosis of unwanted macromolecules.** The mesangial cells serve to keep the glomerular basement membrane clean and free of debris by phagocytizing the unwanted macromolecules adhering to this membrane. They engulf the protein aggregates which are trapped by the GBM during the filtration process and would clog this membrane if not regularly removed. The mesangial cells also phagocytize other macromolecules like antigen-antibody complexes which may accumulate in the proximity of the GBM in certain diseases.

ii. **Provision of structural support for the glomerular capillaries.** The mesangial cells and their extracellular matrix provide structural support for the glomerular capillaries in the areas where the GBM is thin or incomplete.

iii. **Secretion of cytokines and other substances.** The mesangial cells secrete cytokines (especially interleukin-1), prostaglandins, and platelet-derived growth factor, etc. These substances provide immune defense for the glomerular structures, and help in the repair of these structures after they are damaged due to some disease.

iv. **Regulation of blood flow through the glomerular**

long and has an outside diameter of about 60 µm. After commencing at the urinary pole, it follows a tortuous course and terminates by straightening out and passing into a nearest medullary ray to become continuous with the loop of Henle. Being the longest and widest part of the nephron, the proximal convoluted tubules occupy the major part of the cortex, appearing in sections as oblique and transverse profiles, which are usually located close to the renal corpuscles.

Examination of a cross section of the proximal convoluted tubule under LM reveals that this part of the nephron is lined by a simple cuboidal epithelium. Each lining cell contains a centrally-located, spherical nucleus and the usual cell organelles, being exceptionally rich in mitochondria. The lining cells are relatively large in size and a cross section of the PCT generally shows 3 to 5 nuclei. The luminal surface of the cells bears densely-packed, long microvilli which are covered by a thick layer of glycocalyx. Due to the presence of microvilli, the luminal surface of the PCT cells usually exhibits a prominent **brush border** in histological sections (Fig. 20.7 A). However, in such sections the transversely (or obliquely) cut sections of the PCT generally give a fuzz-filled appearance. This is because, during processing and sectioning of the tissue, the brush border is usually disorganized and the glycocalyx, along with the aggregates of small plasma proteins bound to it, appear as a fuzzy material in the lumen of the PCT.

EM shows that the PCT cells have numerous long invaginations of the basal plasmalemma. Mitochondria are aligned in rows between these invaginations and this arrangement is seen in the LM as eosinophilic **basal striations**. The abundant mitochondria supply the high quantity of energy which is needed by the PCT cells to carry out their normal functions, especially for the efficient working of the numerous sodium pumps located in the invaginations of the basal plasmalemma.

EM studies also reveal that the apical plasmalemma of the PCT cells shows small invaginations (pits) at multiple places. These pits, also called *apical canaliculi*, serve to increase the capability of the PCT to endocytose small proteins that manage to cross the filtration barrier. The cytoplasm of PCT cells contains pinocytotic vesicles, which form at the bases of the apical canaliculi and carry the absorbed macromolecules to the lysosomal system of the cell for digestion.

The lateral borders of a PCT cell are not smooth but show folds (evaginations) and grooves (invaginations) and interdigitate with those the adjoining cells. Therefore, the lateral cell boundaries of the PCT cells cannot be clearly distinguished in ordinary stained sections examined under the light microscope.

EM studies reveal that the apical parts of the adjacent PCT cells are bound to each other by junctional complexes consisting of a zonula occludens and a zonula adherens. But it is very important to know that these junctions are not very tight and water, along with the dissolved solutes, can pass between the PCT cells from the tubular lumen to the renal interstitial tissue.

Functions of the PCT

The chief function of the PCT is reabsorption of water, electrolytes and organic nutrients from the glomerular filtrate. All the materials absorbed by the PCT cells are passed to the interstitial tissue of the renal medulla and absorbed into the peritubular capillaries lying in the interstitial tissue. About 65% of the sodium and water filtered from the plasma in the renal corpuscles are reabsorbed when the filtrate passes through the PCTs. The sodium pumps located in the basal domain of the PCT cells pump sodium from the cytoplasm of these cells into the peritubular interstitial tissue of kidney and increase its tonicity. This results in reabsorption of water and dissolved solutes (mainly sodium, glucose, amino acids, and chloride) from the tubular lumen into the peritubular blood capillaries in the following way. Increased tonicity of the renal interstitial tissue attracts water present in the tubular lumen and forces it to pass through the paracellular route (in between the lining cells of the PCT) to reach the interstitial tissue. From the renal interstitium, the water, along with the dissolved solutes, is absorbed into the bloodstream through the peritubular capillaries. Reabsorption of a fraction of the bicarbonate, potassium, phosphate, and calcium also occurs in the PCT. Normally, all the organic nutrients present in the glomerular filtrate (such as glucose, amino acids, and vitamins) are completely reabsorbed in the PCT. In persons suffering from diabetes mellitus, the amount of glucose in the glomerular filtrate is greater than the absorbing capacity of the PCT. Therefore, glucose is passed in urine which also results in polyuria (excessive micturition).

Small proteins present in the glomerular filtrate are generally degraded by the peptidases present in the glycocalyx coat covering the luminal plasmalemma of the PCT cells and the amino acids so released are reabsorbed. Sometimes, the glomerular filtrate contains larger proteins which cannot be degraded in the glycocalyx. Such proteins are endocytosed by the PCT cells and transported in membrane-bound vesicles to the lysosomes of these cells where they are broken down to amino acids.

The PCT cells are also involved in secretion of certain substances into the filtrate passing through these tubules. These substances are taken up by the PCT cells from the peritubular capillaries and secreted into the glomerular filtrate for rapid elimination from the body. Such substances include bile salts, oxalate, urate, and many drugs.

The Proximal Straight Tubule

This part of the strait tubules is also lined by low cuboidal epithelial cells that have less well-developed brush border and fewer apical canaliculi than the PCT cells. The lining cells also have smaller and fewer mitochondria. The lateral cell processes and basal infoldings of the basal plasmalemma of these cells are also infrequent. In the renal

begins. As indicated by its name, this part of the nephron is highly twisted and convoluted. After pursuing a tortuous and winding course, each DCT joins a cortical collecting tubule.

The DCT is divided into two segments which differ in structure and function. These segments are: (i) early DCT which is the proximal, major part of the distal convoluted tubule, and (ii) late DCT which is the distal, smaller part of this tubule. The late distal convoluted tubule is similar in structure and function to the cortical collecting tubule (connecting tubule) and both of these will be discussed together.

THE EARLY DISTAL CONVOLUTED TUBULE

Like the PCTs, the early DCTs are also lined by simple cuboidal epithelium. However, these cells are smaller in size and, therefore, usually 6 to 8 spherical nuclei are visible in the cross section of a DCT when stained sections of renal tissue are examined under LM. It is to be noted that the overall diameter of the DCT (about 40 µm) is smaller than that of the PCT (which averages 60 µm in diameter). However, the caliber (luminal diameter) of a distal convoluted tubule is seen to be larger than that of a proximal convoluted tubule; this because of two factors: (1) the epithelial cells lining the DCT are smaller in size and, therefore, low in height, and (2) the DCT cells not possess very short and sparse microvilli on their luminal surface (therefore, no brush border can be seen on these cells). The average length of the DCT is 5 mm which is also much shorter than that of the PCT (which has an average length of 14 mm). As a result of their shorter length, the distal convoluted tubules appear less numerous (than the proximal convoluted tubules) in sections of the cortical region of the kidney.

The cuboidal epithelial cells lining the DCT also contain numerous mitochondria but stains relatively less acidophilic than the PCT cells. In their lateral domain, the DCT cells also interdigitate with the neighboring cells and, therefore, lateral boundaries of these cells cannot be clearly distinguished in stained sections. The basal plasmalemma of these cells also invaginates into the cytoplasm to form multiple infoldings. Mitochondria are aligned in rows between these plasmalemmal invaginations and, due to this arrangement, the DCT cells also show eosinophilic basal striations in stained histological sections (Fig. 20.7B).

Functions of the Early Distal Convoluted Tubule

The early DCTs reabsorb sodium, potassium, chloride, and bicarbonate ions from the glomerular filtrate and secrete H^+ and K^+ ions into it. The process of absorption of sodium with simultaneous secretion of potassium is controlled by the adrenal cortical hormone *aldosterone*. By acidifying the urine, the distal convoluted tubules contribute to the regulation of acid-base balance of the body. The DCTs are virtually impermeable to water

LATE DISTAL CONVOLUTED TUBULES AND CORTICAL COLLECTING TUBULES

Each distal convoluted tubule continues into a cortical collecting tubule, also called connecting tubule, which is the last part of the nephron. The cortical collecting tubules are not convoluted and ultimately join a collecting duct running in a medullary ray lying within the renal cortex. Like the late distal convoluted tubule, the cortical collecting tubule also measures about 40 µm in diameter and is lined by a simple cuboidal epithelium. The late DCTs and the cortical collecting ducts are lined by a simple columnar epithelium which contains two types of cells: principal cells and intercalated cells.

Principal Cells

These cells stain lightly and, therefore, the cell boundaries between the adjoining cells are distinct. The luminal surface of the principal cells exhibits a few short microvilli, while their basal plasmalemma shows numerous infoldings. The principal cells reabsorb water and sodium from the renal filtrate and secrete potassium ions into it.

Intercalated Cells

These cells are fewer in number than the principal cells. Their cytoplasm stains intensely eosinophilic due to abundance of mitochondria. Their apical surface shows microfolds but no microvilli are present. Functionally, there are two types of intercalated cells: type A cells and type B cells. The intercalated cells play a significant role in the regulation of the acid-base balance of the body. During acidosis, the type A intercalated cells secrete hydrogen ions into the urinary filtrate and absorb bicarbonate. In case of alkalosis, the type B intercalated cells secrete bicarbonate and reabsorb hydrogen ions from the urinary filtrate.

Role of ADH

As in the early DCTs, the process of reabsorption of sodium and secretion of potassium in the late DCTs and cortical collecting tubules is also controlled by the adrenal cortical hormone aldosterone. However, the process of reabsorption of water in these tubules is controlled by the antidiuretic hormone (ADH) which is secreted by the posterior pituitary. The principal cells of the late DCTs and cortical collecting tubules are rich in aquaporin-2 (AQP-2) which are cell membrane proteins that serve as channels for absorption of water molecules. Under normal conditions, when the serum levels of ADH are low, the aquaporins remain stored within membranous vesicles in the cytoplasm of the principal cells. Therefore, no water from the renal filtrate is normally absorbed in the late DCTs and cortical collecting tubules which allows diuresis (i.e., production of large amount of urine). However, when there is a deficiency of water in the body, large amounts of ADH are released from the posterior pituitary. This leads to insertion of AQP-2 molecules into the luminal plasmalemma of the principal cells which causes reabsorption of large quantities of water in the late DCTs

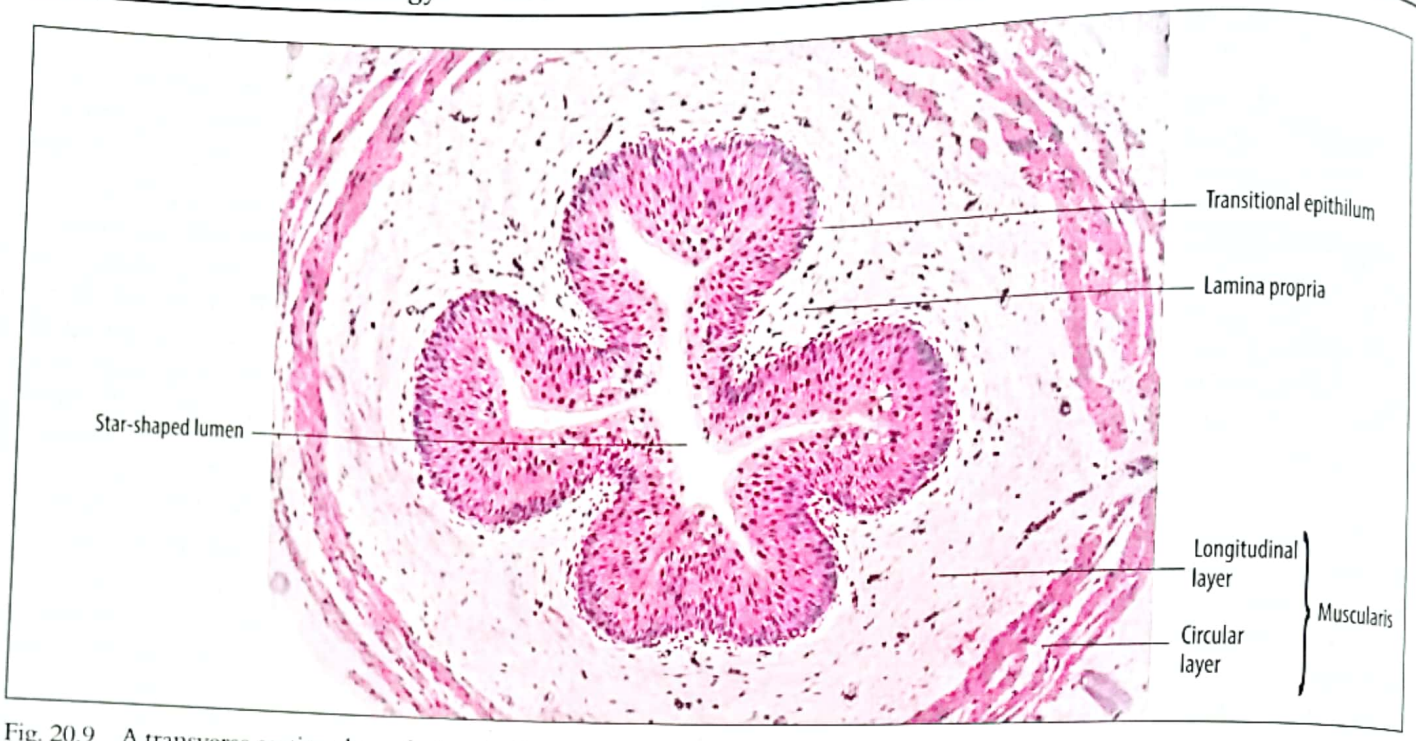

Fig. 20.9 A transverse section through the middle part of empty ureter.

elastic lamina is absent in this part of the afferent arteriole, so that the JG cells lie close to the endothelium (and thus to the blood in the lumen of the arteriole).

The JG cells secrete the enzyme ***renin***. The renin causes hydrolysis of the plasma protein angiotensinogen to produce angiotensin I. The angiotensin I is converted into angiotensin II by the action of the angiotensin converting enzyme (ACE) which is secreted by the endothelium of the lung capillaries. The angiotensin II is a potent vasoconstrictor which causes an increase in the systemic blood pressure.

EXTRAGLOMERULAR MESANGIAL CELLS

The extraglomerular mesangial cells (also called *lacis cells*) form a cushion of cells between the afferent and efferent arterioles of the renal corpuscle. Structurally these cells resemble the intraglomerular mesangial cells with which they are contiguous. Functional significance of the extraglomerular mesangial cells is not well understood.

RENAL INTERSTITIAL TISSUE

The space external to the basal laminae of the kidney tubules is occupied by the interstitial tissue of kidney (also called *renal interstitium*). This tissue is scanty in the renal cortex, but is relatively more abundant in the medulla. The renal interstitium consists mainly of two types of cells: (i) renal fibroblast (also called renal interstitial cells), and (ii) cells of the immune system (mainly antigen-presenting dendritic cells and a few lymphocytes). In addition to these cells, the renal interstitium contains collagen fibers lying in a small amount of extracellular matrix.

The renal fibroblasts produce two important hormones: (i) erythropoietin, which stimulates erythropoiesis in the bone marrow, and (ii) medullipin. The medullipin is converted to medullipin II in the liver. The medullipin to is a vasodilator which causes the blood pressure to fall.

URETER

The ureters conduct urine from the kidneys to the urinary bladder. The wall of each ureter is composed of the following three coats:

1. Mucosa.
2. Muscularis.
3. Adventitia.

The **mucosa** of the empty ureter seems to be thrown into folds, due to which the lumen appears star-shaped in transverse sections (Fig. 20.9). However, these folds disappear when the ureter is distended. The mucosa is lined by the *transitional epithelium* consisting of 3 to 5 cell layers. Beneath the epithelium is present *lamina propria*, which is rich in collagenous fibers.

The **muscularis** of the ureter is thick and is made up of smooth muscle which consists of two layers in the upper two-thirds of the organ: an inner longitudinal and an outer circular layer. In the lower third of the ureter, the smooth muscle is disposed into three layers: an inner longitudinal, a middle circular, and an outer longitudinal layer. The ureteric muscle undergoes regular peristaltic movements to help the flow of urine toward the bladder.

The **adventitia** is the outermost layer and consists of loose fibroelastic connective tissue. This layer also contains blood vessels, lymphatics, and nerves of the ureter.

MUSCULARIS

The muscularis of the urinary bladder consists of interlacing bundles of smooth muscle fibers and distinction into different layers is not possible (except in the region of the neck of the bladder) and, therefore, the ill-defined, intermingling layers of the smooth muscle in the bladder wall are collectively known as *detrusor muscle* (i.e., pushing muscle). In the neck of the urinary bladder, the smooth muscle fibers make three well-defined layers: a thin inner longitudinal layer, a thick middle circular layer, and a thin outer longitudinal layer. Contraction of the detrusor muscle pushes the urine through the urethra to the exterior.

ADVENTITIA/SEROSA

Most of the bladder wall is covered by an adventitia of fibroelastic connective tissue. Only the superior surface of the urinary bladder is covered by a serosa formed by the peritoneum covering this surface.

URETHRA

The urethra is a tubular structure that conveys urine from the urinary bladder to the exterior of body. In both sexes its wall consists of a lining epithelium and a connective tissue lamina propria. However, the male urethra and female urethra differ from each other not only in length but also in histological features.

FEMALE URETHRA

In females the urethra measures 4 to 5 cm in length and 5 to 6 mm in diameter. The initial part of female urethra is lined by transitional epithelium, while its distal part is lined by stratified squamous nonkeratinized epithelium. Outer to the epithelium is present a thick fibroelastic lamina propria containing a plexus of thin-walled veins. A thin muscular coat, consisting of inner longitudinal and outer circular layers of smooth muscle, surrounds the lamina propria.

MALE URETHRA

The male urethra is divided into three segments: prostatic urethra, membranous urethra, and penile urethra.

The **prostatic urethra** is about 4 cm long. It passes through the prostate gland and receives the openings of the paired ejaculatory ducts and many small prostatic ducts. It is lined by transitional epithelium.

The **membranous urethra** is only 1 cm long and passes through the skeletal musculature of the urogenital diaphragm. It is lined by stratified columnar epithelium interspersed with patches of pseudostratified columnar epithelium. The skeletal muscle surrounding the membranous urethra forms the powerful *external urethral sphincter* which is under the voluntary control.

The **penile urethra** is about 15 cm long and runs through the penis to open at the external urethral orifice at the tip of the glans penis. Before its termination, the penile urethra dilates to form the *fossa navicularis* which lies in the glans penis. The penile urethra is embedded in the corpus spongiosum of the penis and, therefore, is also known as *spongy urethra*. It is lined by pseudostratified columnar epithelium except at its distal dilated part (fossa navicularis) which is lined by stratified squamous nonkeratinized epithelium.

The lamina propria of all the three parts of male urethra consists of loose fibroelastic connective tissue. Mucus-secreting *urethral glands* are located in the lamina propria of the penile urethra which open into the urethral lumen by narrow ducts. Under erotic stimuli, these glands release their thin, mucoid secretions into the urethra. These secretions, along with the secretory product of the bulbourethral gland, serve to lubricate the urethra in anticipation of ejaculation of the semen.

Male Reproductive System | 21

The male reproductive system comprises the following four components:

1. A pair of testes.
2. A system of genital ducts.
3. Accessory sex glands.
4. A copulatory organ – the penis.

TESTES

The testes produce the male gametes called spermatozoa. In addition, the testes perform an endocrine function and secrete testosterone, which is the chief male sex hormone.

Each testis is covered by a thick capsule of dense, fibrous connective tissue called ***tunica albuginea***. This coat is thickened along the posterior wall of the testis and projects inward as ***mediastinum testis***. Blood vessels, lymphatic vessels, and the channels carrying the spermatozoa pass through the connective tissue of the mediastinum testis. Fibrous septa, called ***septula testis***, arise from the mediastinum testis and penetrate the substance of testis to divide it into 250-350 pyramidal compartments called ***testicular lobules*** (Fig. 21.1 A). Beneath the tunica albuginea there is a layer of loose connective tissue containing networks of blood vessels. This layer, known as *tunica vasculosa*, also lines the septula testis. Each testicular lobule contains 1-4 highly coiled tubules called ***seminiferous tubules***. These tubules are the site of production of the male gametes. The seminiferous tubules are surrounded and supported by loose connective tissue called *intertubular connective tissue*.

SEMINIFEROUS TUBULES

The seminiferous tubules produce and transport sperm cells inside the testes. A seminiferous tubule measures about 200 µm (0.2 mm) in diameter and approximately 50 cm in length. To accommodate themselves in the small space available within the testicular lobules, the seminiferous tubules are highly coiled and convoluted. Each seminiferous tubule commences as a free blind end near the apex of a testicular lobule and, after making a highly tortuous loop, it continues into a short and narrow straight tubule (tubulus rectus). The tubuli recti lie in the apical part of the testicular lobules and constitute the initial segment of the male genital duct system (described later).

When a stained section from the testis of a sexually mature male is examined under LM, the seminiferous tubules are seen as numerous cross and oblique sections. These tubules are lined by a complex ***seminiferous epithelium*** which is a modified stratified cuboidal epithelium (Fig. 21.2). The epithelium rests on a basement membrane which surrounds the seminiferous tubule. Outer to basement membrane is present a thin layer of fibrous connective tissue which contains flat smooth muscle cells called myoid cells. Contraction of the myoid cells helps to push the spermatozoa through the seminiferous tubules into the tubuli recti. Clumps of spermatozoa are commonly seen in the lumen of the seminiferous tubules.

SEMINIFEROUS EPITHELIUM

The seminiferous epithelium consists of two types of cells: (i) **spermatogenic cells**, from which the spermatozoa are formed, and (ii) **Sertoli cells**, which are the supporting cells for the spermatogenic cells (Fig. 21.1 B).

Spermatogenic Cells

These cells lie between the Sertoli cells and form a stratified epithelium consisting of several layers of cells which occupy the space between the basal lamina and lumen of the seminiferous tubule. In the testis of a child, only the primitive germ cells, called *spermatogonia*, are present. With the onset of sexual maturity, the process of *spermatogenesis* begins under the influence of the male hormone testosterone (which is secreted by the interstitial cells of the testes).

In the histological sections of the testis of an adult male, the spermatogenic cells are seen in various stages of differentiation, arranged in an orderly manner. The most immature cells (spermatogonia) are located near the basal lamina of the seminiferous epithelium. As the cells proliferate and undergo differentiation, they gradually move toward the lumen of the tubule. Finally, the cells come to lie at the luminal surface of the seminiferous tubule, where they transform into spermatozoa which become free to lie within the lumen of the tubule.

Usually 4 to 5 concentric layers of morphologically distinct spermatogenic cells, representing generations of cells at various stages of development, can be identified in the adult seminiferous epithelium. Starting with a spermatogonium, these stages include; primary spermatocyte, secondary spermatocyte, and spermatid.

The **spermatogonia** are located in the most peripheral part of the seminiferous tubule, just next to the basement membrane of the seminiferous epithelium. They are roughly spherical cells, each containing a centrally-located, rounded nucleus. Depending on the nuclear structure, the spermatogonia can be classified into three types: (i) type A dark spermatogonia, (ii) type A pale spermatogonia, and (iii) type B spermatogonia.

i. The ***type A dark spermatogonia*** contain an oval, dark-staining nucleus, in which the nucleolus is located

cells. As already explained, the germ cells in the adluminal compartments of the seminiferous tubules are isolated from the blood circulation by the blood-testis barrier, created by the occluding junctions between the lateral cytoplasmic projections of the neighboring Sertoli cells. Blood capillaries, coursing in the intertubular connective tissue, lie in close relation to the basal regions of the Sertoli cells. The oxygen and all nutrients picked from these capillaries are transported through the cytoplasm of the Sertoli cells to the spermatogenic cells lying in the adluminal compartment.

2. **Protection of the germ cells.** By creating the blood-testis barrier, the Sertoli cells make the adluminal compartment of the seminiferous tubules an immune-privileged site. Thus, those germ cells which contain haploid nuclei are protected from a reaction against them by the immune system of the body. In addition, the blood-testis barrier protects the developing germ cells from the toxic agents carried in the blood by not permitting any of such agents to reach the germ cells.
3. **Secretory Function.** The Sertoli cells synthesize many substances, some of which are secreted in an exocrine way, while others are released in an endocrine manner. These cells secrete large amounts of a watery ***testicular fluid*** into the lumen of the seminiferous tubules. This fluid is plasma-like in its ionic content and serves as a transport medium for the newly-shed, immature, non-motile spermatozoa. The Sertoli cells also synthesize and secrete a protein called ***androgen-binding protein*** which concentrates testosterone in the adluminal compartment of the seminiferous tubules; this concentration of testosterone is necessary for the normal spermatogenesis to take place. Secretion of the androgen-binding protein by the Sertoli cells is stimulated by the hormone FSH which is secreted by the pituitary gland. The Sertoli cells also secrete a glycoprotein called ***inhibin*** which is released into the blood in an endocrine manner. Inhibin is involved in the feedback loop which inhibits FSH secretion from the anterior pituitary. During the fetal life, the Sertoli cells secrete a glycoprotein called ***mullerian-inhibiting substance (MIS)*** which acts in a paracrine manner to inhibit and suppress the development of the paramesonephric ducts (Mullerian ducts) in the male fetus during the 9th and 10th weeks of the intrauterine life.
4. **Phagocytic Function.** The Sertoli cells act as scavengers and phagocytize the excess cytoplasm of the spermatids, which is shed during the process of spermiogenesis as *residual bodies*. These bodies are engulfed by the Sertoli cells and finally broken down and digested by the lysosomal system of these cells.

SPERMATOZOA

The spermatozoa are the male gametes (i.e., haploid reproductive cells). They are slender, motile, flagellate bodies, each having an average length of 60 µm. A mature spermatozoon (sperm) consists of two main parts: a head and a tail. The head as the tail are covered externally by the cell membrane of the spermatozoon (Fig. 21.3).

Head of the Spermatozoon

The sperm head is a flattened, pear-shaped body surrounded externally by the plasmalemma. It measures about 5 µm in length, 3 µm in width, and 1µm in thickness. The head is occupied chiefly by the *condensed nucleus*, present as a compact mass of chromatin enclosed in the nuclear envelope. The anterior two-thirds of the condensed nucleus are covered by a flattened, membrane-bound sac called *acrosomal cap* or *acrosome*. The acrosome contains various enzymes which include hyaluronidase, neuraminidase, acid phosphatase, and a trypsin-like protease called *acrosin*. During fertilization, these enzymes facilitate the entry of the sperm into the ovum by penetrating through the barriers surrounding the ovum (i.e., the corona radiata and zona pellucida).

Tail of the Spermatozoon

The sperm tail is about 55 µm long and can be subdivided into four parts: neck, middle piece, principal piece, and end piece. All parts of the tail are covered externally by a plasmalemma which is continuous with that covering the sperm head. The core structure of the tail is the axial filament or *axoneme*. The axoneme has the typical structure of a flagellum and consists of two central singlet microtubules surrounded by nine doublets.

The **neck** is a short region that contains the centrioles and is the site of origin of the coarse fibers that continue into the middle piece of the tail. The axoneme also begins in the neck.

The **middle piece** of the sperm tail measures about 8 µm in length and 1µm in diameter. In the center of the middle piece is present axoneme, which is surrounded by a ring of nine longitudinally oriented *outer coarse fibers* (which originate in the neck). Outer to the coarse fibers is present a sheath of helically arranged mitochondria. These mitochondria provide the energy required for the movement of the tail. The middle piece terminates at the *annulus*, which is a dense, ring-like structure adherent to the plasmalemma of the tail. The annulus prevents the mitochondria from slipping into the principal piece during rapid movements of the tail.

The **principal piece** of the tail is the longest segment of the sperm. It measures about 40 µm in length and is slightly narrower than the middle piece. In this segment the axoneme is surrounded by seven longitudinally running outer coarse fibers. External to the coarse fibers is present a fibrous sheath composed of rib-like circumferential fibers. These fibers pass half-way round the axoneme to fuse with two longitudinal columns of fibers.

The **end piece** is the terminal segment of the tail. It is about

surface. These cells reabsorb a large part of the testicular fluid which is secreted by the Sertoli cells of the testis. The ciliated cells facilitate the absorptive process by stirring the luminal fluid by rapid movement of their cilia.

The lining epithelium of the ductuli efferentes is surrounded by a thin layer of smooth muscle in which the fibers are mostly circularly arranged. Contraction of the smooth muscle and movement of the cilia propel the spermatozoa (which are suspended in the testicular fluid) toward the duct of the epididymis.

EPIDIDYMIS

Over the posterosuperior surface of each testis lies a comma-shaped body which is called epididymis. Grossly, the epididymis is divided into 3 parts: head, body and tail (Fig. 21.1A).

In fact, the epididymis consists almost entirely of a single, complexly convoluted duct called **ductus epididymis**. This duct is 6 to 7 meters long and begins in the head of the epididymis (where it receives the ductuli efferentes) and winds in a most intricate manner through the body and tail of the epididymis. The coils of the duct are held together by means of connective tissue. At the tail of the epididymis, the ductus epididymis passes without any definite demarcation into the ductus deferens.

A section of the epididymis shows numerous transversely or obliquely cut sections of its extremely tortuous duct. These sections show that the lumen of the ductus epididymis has a smooth and even contour (Fig. 21.4). Large clumps of spermatozoa are also seen to be present in the lumen of the duct.

Examination of a cross section reveals that the ductus epididymis is lined by a pseudostratified columnar epithelium surrounded by a muscularis consisting of smooth muscle fibers.

The pseudostratified epithelium of the epididymis consists of tall columnar cells and basal cells. The tall cells, also called ***principal cells***, bear numerous stereocilia which greatly increase their luminal surface area. The principal cells absorb testicular fluid, secrete glycolipids, and phagocytize cellular debris. The ***basal cells*** are small round cells which rest on the basal lamina. These cells serve as reserve cells for the lining epithelium of the ductus epididymis.

In the head and body of the epididymis, the muscularis consists only of a thin layer of smooth muscle fibers. However, in the tail the muscular coat is thick and consists of 3 layers: an inner longitudinal, a middle circular, and an outer longitudinal layer.

Functions of the Epididymis

1. Temporary storage of spermatozoa.
2. Transportation of spermatozoa to the ductus deferens.
3. Reabsorption of most of the fluid that leaves the testis with the spermatozoa. Reabsorption of this fluid is essential to make the semen thick and viscid.

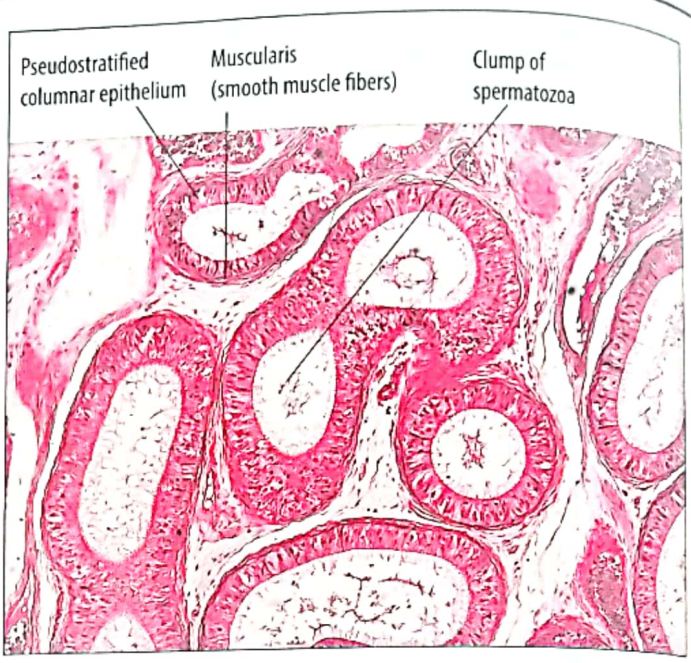

Fig. 21.4 A section through the epididymus showing multiple transverse and oblique sections of the duct of empdidymus. Note that the duct exhibits smooth and even luminal contour.

4. While in the duct of epididymis, the spermatozoa mature further and become motile.
5. The fluid in the duct of epididymis contains certain glycolipids which are secreted by the principal cells of the epithelium lining the duct. These glycolipids serve as *decapacitation factors* for the spermatozoa. The cell membrane covering the head region of each spermatozoon receives a thin coat of these decapacitation factors which temporarily inhibit the fertilizing capability of the spermatozoa. When the spermatozoa have been deposited into the female genital tract, the decapacitation factors are removed from the surface of the spermatozoa by enzymes present in the uterine fluid; this process is called *capacitation*.

DUCTUS DEFERENS

The ductus deferens, also called **vas deferens**, is the direct continuation of the ductus epididymis. It terminates by joining the duct of seminal vesicle to form the ejaculatory duct, which opens into the prostatic urethra. Just before its termination, the vas deferens has a dilated segment which is called *ampulla*.

A section of the ductus deferens shows that it is has a very thick, muscular wall and a relatively narrow, star-shaped lumen (Fig. 21.5). From within outwards, the wall of the vas deferens consists of three coats: mucosa, muscularis, and adventitia.

The **mucosa** is seen to be thrown into longitudinal folds (due to which the lumen of the duct appears star-shaped in cross sections). It consists of epithelium and lamina propria. Like the ductus epididymis, the ductus deferens is also lined by ***pseudostratified columnar epithelium***.

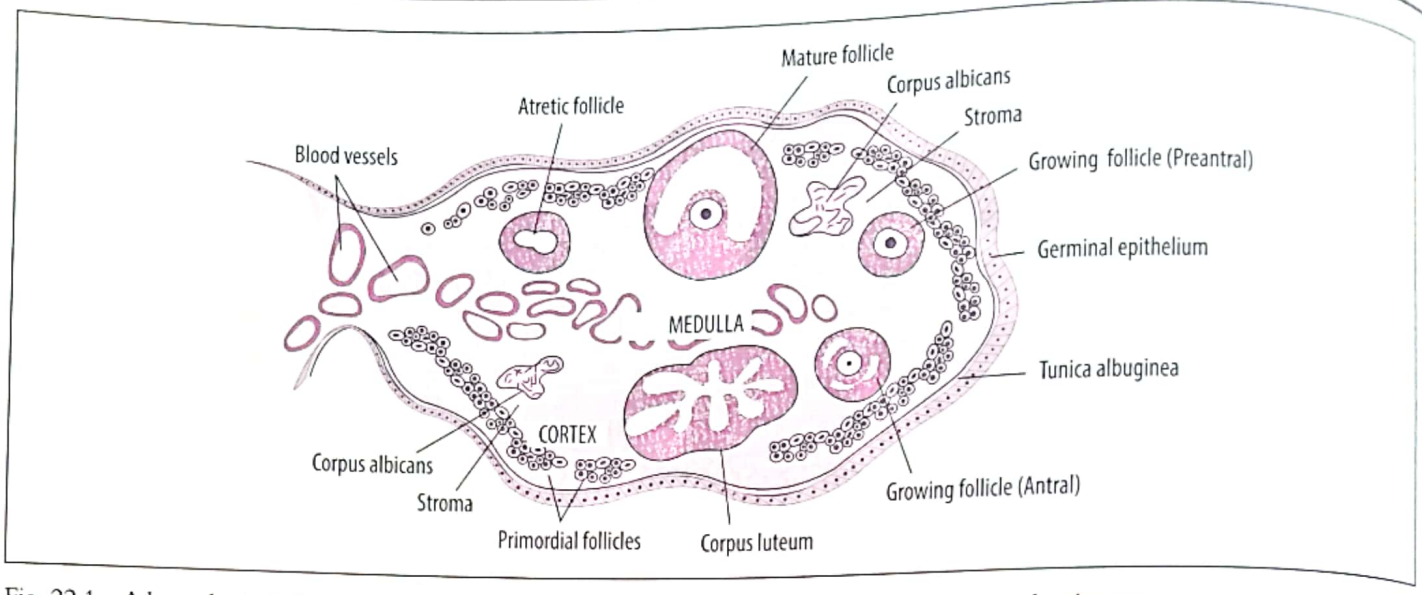

Fig. 22.1 A hypothetical diagram showing the chief structural features of the ovary in the reproductive age.

only one type of ovarian follicles which are called *primordial follicles*. However, during the reproductive period of the life of an adult female, three varieties of follicles can be seen in the ovaries; each of these three types represents a different stage in the follicular development.

During the first few days of each ovarian cycle, a number of primordial follicles begin to grow in both ovaries; these follicles are titled *growing follicles*. Normally, only one growing follicle becomes fully mature and ovulates approximately in the middle of each ovarian cycle. All other growing follicles, in both ovaries, undergo a degenerative process called *follicular atresia*. As a result of this process, the follicular cells die by apoptosis, the ovum undergoes autolysis, and the zona pellucida collapses. The remnants of the degenerated follicles are engulfed and disposed of by the macrophages. As already explained, the remains of the ovulated follicle do not degenerate, but are converted into corpus luteum which is a temporary endocrine glandular structure.

PRIMORDIAL FOLLICLES

A primordial follicle contains an immature ovum (a primary oocyte arrested in the prophase of meiosis-I) surrounded by a single layer of flattened (squamous) epithelial cells called *follicular cells* (Fig. 22.2 & 22.3).

The **oocyte** of a primordial ovarian follicle measures 25-30 µm in diameter and contains a large vesicular nucleus with a prominent nucleolus. The vesicular appearance of the nucleus is due to the finely dispersed chromatin of the primary oocyte arrested in meiosis-I. The cytoplasm of the oocyte contains abundant mitochondria, many Golgi complexes, extensive RER, and numerous lysosomes. The cell membrane of the oocyte is also known as *oolemma*.

The **follicular cells**, also called *granulosa cells*, are squamous epithelial cells that completely surround the oocyte and are attached to each other by desmosomes. They rest on a basal lamina which separates them from the stroma of the ovarian cortex.

Growing Follicles

Growth of the primordial follicles is stimulated by the follicle-stimulating hormone (FSH) which begins to be secreted from the anterior pituitary as the female reaches the age of puberty. The follicular growth is characterized by alterations in the oocyte as well as granulosa cells. In addition, the stromal cells surrounding the ovarian follicle also become modified.

As a primordial follicle grows, it passes through the following developmental stages:

1. Unilaminar primary follicle.
2. Multilaminar primary follicle.
3. Secondary follicle.

Although several ovarian follicles in each ovary pass through these stages of growth, normally only a single follicle in one of the two ovaries becomes fully mature and ovulates in the middle of the ovarian cycle. Regarding the event of ovulation, the two ovaries alternate with each other in an irregular sequence.

Unilaminar Primary Follicle (Fig. 22.2 & 22.3)

Growth of the primordial follicle leads to changes in the oocyte as well as follicular cells.

The oocyte enlarges in size and soon attains a diameter of approximately 120 µm. The nucleus becomes bigger, mitochondria increase in number, and RER becomes more extensive. The Golgi complexes also increase in number and migrate to the peripheral region of the cell. Special secretory granules, called cortical granules appear in the most peripheral part of the cytoplasm of the oocyte. During the process of fertilization, the cortical granules are released by the oocyte into the zona pellucida to prevent polyspermy.

The follicular (granulosa) cells increase in number by mitotic division and also change their shape from squamous to cuboidal. Thus, a unilaminar primary ovarian follicle is surrounded by a single layer of cuboidal epithelial cells resting on a basement membrane. The granulosa cells

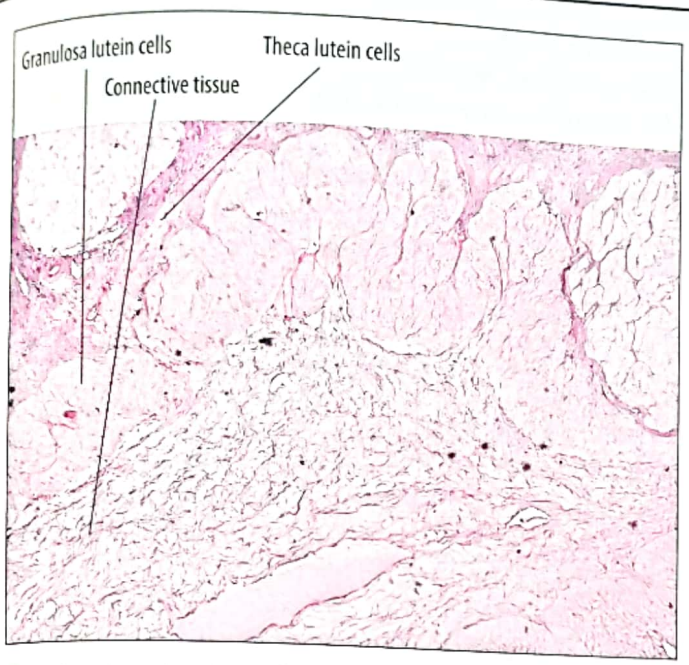

Fig. 22.6 A section through the ovarian cortex showing a corpus luteum.

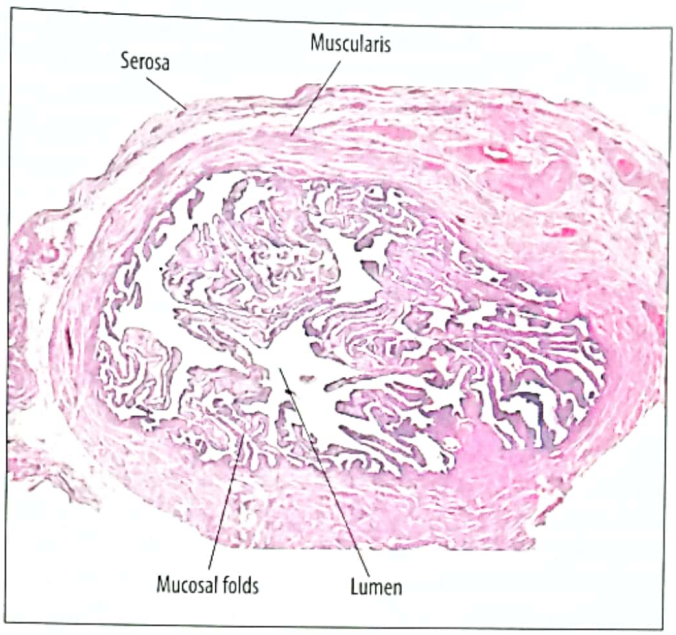

Fig. 22.7 A transverse section through the uterine tube.

at once but is transformed into a temporary endocrine organ called corpus luteum. When the follicular fluid is released under pressure, the follicle collapses and its wall is thrown into deep folds. Bleeding from the capillaries of the theca interna into the follicular lumen usually leads to the formation of a central blood clot. The follicular cavity and blood clot are later invaded by stromal connective tissue and blood capillaries. Under the influence of continued high levels of the luteinizing hormone (LH), significant morphological changes occur in the cells of the stratum granulosum and theca interna, which give rise to granulosa lutein cells and theca lutein cells, respectively (Fig. 22.6).

The **granulosa lutein cells** are pale-staining, large cells that have an average diameter of 30 µm. They constitute about 80% of the total cell population of the corpus luteum. These cells exhibit structural features of the steroid-producing cells, i.e., lipid droplets, abundant smooth endoplasmic reticulum, and abundant mitochondria, many of which have tubular cristae. The SER of these cells is rich in the enzyme aromatase. The cytoplasm of granulosa lutein cells also contains granules of the yellowish lipochrome pigment *lutein*, which is responsible for the characteristic yellow color of the corpus luteum observed when this body is examined in the fresh, unstained condition (in Greek, *corpus luteum* means yellow body). The principal function of the granulosa lutein cells is to secrete progesterone. In addition, they produce estradiol from androstenedione (which is secreted by the theca lutein cells and passed on to the granulosa cells).

The **theca lutein cells are smaller than the granulosa lutein cells and their average diameter is** about 15 µm. The cytoplasm of these cells also contains fat droplets, mitochondria with tubular cristae, smooth endoplasmic reticulum, and lutein pigment. Although the theca lutein cells are much smaller than the granulosa cells, but they stain more intensely. The theca lutein cells are chiefly located at the periphery of the corpus luteum, but clusters of these cells are also found along the connective tissue septa that penetrate into the substance of the corpus luteum. The theca lutein cells secrete progesterone as well as androstenedione. The androstenedione passes to the granulosa lutein cells for conversion into estradiol.

Fate of the Corpus Luteum

If the discharged ovum is not fertilized, the corpus luteum attains its greatest development about 9 days after ovulation and then degenerates. If fertilization takes place, the corpus luteum increases in size, persists for about 6 months and then gradually declines over the remaining period of pregnancy. Degeneration of the corpus luteum (following menstruation or pregnancy) results in autolysis of lutein cells and an increase in connective tissue. Finally, the corpus luteum becomes completely replaced by a mass of white fibrous tissue which is called *corpus albicans* (meaning *white body*).

UTERINE TUBES

The uterine tubes, also called Fallopian tubes, are paired muscular tubes, each consisting of three parts: infundibulum, ampulla and isthmus. These tubes pick up the ova ovulated from the ovary, provide necessary environment required for fertilization and, if fertilization takes place, initial development of the zygote also occurs in the uterine tube. Finally, the zygote is passed on to the uterine lumen from the cavity of the Fallopian tube. The wall of each uterine tube is composed of three coats: mucosa, muscularis, and serosa (Fig. 22.7).

The **mucosa** is thrown into thin, branching folds which project into the lumen of the tube. The mucosal folds are most numerous, highly branched and complex in the ampulla of the Fallopian tube, so that the lumen appears

as a labyrinth of spaces between the mucosal folds. The mucosa of the uterine tube consists of epithelium and lamina propria.

The ***epithelium*** lining the uterine tubes is of simple columnar variety and contains two types of cells: secretory cells and ciliated cells. The *secretory cells* (also called *peg cells*) do not bear cilia and their free surface bulges into the lumen of the uterine tube. Their cytoplasm stains darker than the ciliated cells and shows the structural features of the protein-secreting cells (abundant RER and an extensive Golgi apparatus located in a supranuclear position). The secretory cells produce the *tubal fluid* which contains glucose, proteins and other substances that provide nourishment to the ovum, spermatozoa, and the zygote. The *ciliated cells* are more numerous than the secretory cell. They bear kinocilia on their free surface which beat toward the cavity of the uterus. Rhythmical beating of the kinocilia results in transportation of the ovum through the Fallopian tube toward the uterus.

The tubal fluid also contains substances called *capacitation factors*, which bring about capacitation of the spermatozoa by removing the glycolipids and seminal proteins from their plasma membrane. The process of capacitation, which is completed in about 7 hours, enables the spermatozoa to begin the penetration of zona pellucida.

The ***lamina propria*** of the uterine tubes consists of loose connective tissue containing fibroblasts, mast cells, lymphocytes, reticular fibers, and collagen fibers. The lamina propria also extends into the mucosal folds.

The **muscularis** of uterine tube consists of a thick inner circular and a thin outer longitudinal layer of smooth muscle fibers. Peristaltic contractions of the muscle coat also aid in the movement of the ovum down the Fallopian tube toward the uterine cavity.

The serosa forms the outermost covering of the uterine tube. It is actually the visceral peritoneum covering the tube, and consists of a thin layer of loose connective tissue covered by simple squamous epithelium (mesothelium).

UTERUS

The uterus is a muscular organ in which the fertilized ovum implants and, subsequently, all the prenatal development of the offspring occurs in this organ. The uterus consists of two main parts: (i) **body** which is the upper expanded portion of the organ, and (ii) **cervix** which is the cylindrical, narrow inferior part of the uterus that protrudes into the uppermost part of the vagina. The rounded upper end of the body is called *fundus*. The relatively constricted segment of the body just superior to the cervix is called *isthmus*.

The uterine wall is composed of the following three coats or layers:

1. The outer layer - **perimetrium**.
2. The middle layer - **myometrium**.
3. The innermost layer - **endometrium**.

PERIMETRIUM

The perimetrium is either serosa or adventitia. Most of the uterus is covered by pelvic peritoneum that forms a typical serosa consisting of a single layer of simple squamous epithelium (mesothelium) under which lies a thin layer of loose connective tissue. The lower half of the anterior surface of the uterus (which lies against the urinary bladder) is devoid of peritoneum and, therefore, its outermost covering consists of an *adventitia* of loose connective tissue.

MYOMETRIUM

In the child-bearing age, the myometrium is the thickest coat of the uterine wall, measuring about 10 to 15 mm in thickness. It consists of smooth muscle forming three ill-defined layers: a thin outer layer of longitudinally running muscle fibers, a middle layer of circularly disposed muscle fibers, and an inner layer of longitudinally arranged muscle fibers. The middle layer is the thickest layer and contains numerous large blood vessels, due to which this layer is also known as *stratum vasculare*. Between the bundles of the smooth muscle fibers of the myometrium are present small bundles of collagen fibers along with fibroblasts.

During pregnancy, the myometrium passes through a period of great growth under the influence of female hormones, especially progesterone. This growth occurs as a result of proliferation (increase in number) as well as hypertrophy (increase in size) of smooth muscle fibers. The increase in number occurs by the multiplication of the existing muscle fibers. As a result of hypertrophy, an individual smooth muscle fiber may reach a length of 500 µm (from the original size of 50 µm). To support the increasing bulk of the smooth muscle, the number of collagen fibers also increases considerably.

After the childbirth, the myometrium regresses to its original size due to the degeneration of some smooth muscle fibers and reduction in size of others. The increased amount of collagen is also brought to normal proportions by enzymatic degradation.

After menopause, the smooth muscle cells of the myometrium undergo atrophy and the uterus shrinks in size. As the amount of smooth muscle decreases, the collagen fibers becomes abundant in the myometrium.

Benign tumors of the smooth muscle of the myometrium, called fibroids, are common in the reproductive age of the female. The uterine fibroids, also known as leiomyomas, may become so large in size as to cause abnormal bleeding, pelvic pressure and pain.

ENDOMETRIUM

The endometrium is the mucosal lining of the uterus and, during the child-bearing age, its histological appearance is not constant and depends mainly on the following two factors: (i) whether the uterus is non-pregnant or pregnant,

and (ii) if the uterus is non-pregnant, what was the phase of the menstrual cycle when the endometrial tissue was obtained for histological examination.

Generally, the endometrium consists of two layers: (i) a simple columnar epithelium lining the luminal surface of the uterus, and (ii) a connective tissue lamina propria, also called endometrial stroma.

The lining **epithelium** of the uterine mucosa is of simple columnar variety and consists of two types of cells: secretory cells and ciliated cells. The epithelium invaginates into the underlying lamina propria to form the endometrial glands.

The **secretory cells** are the main cells of the endometrial epithelium. They produce a secretion rich in glycogen and glycoproteins. Their cytoplasm contains abundant mitochondria, extensive RER, a large Golgi apparatus, and secretory granules. No cilia are present on the secretory cells. The secretory activity of these cell is controlled by the female sex hormones (estrogens and progesterone).

The **ciliated cells** constitute about 20% of the lining epithelial cells. They are nonsecretory and bear kinocilia on their luminal surface. Functional role of the ciliated cells in the endometrial epithelium is not well-understood.

The lining epithelium of the endometrium invaginates into the underlying lamina propria to form uterine glands (described below).

The **lamina propria** of the endometrium, also called endometrial stroma, is a thick layer containing type III collagen fibrils, a large amount of ground substance, and specialized fibroblasts called endometrial stromal cells. The endometrial stroma lodges simple tubular glands, called **uterine glands or endometrial glands**, which are formed by invaginations of the lining epithelium into the underlying stroma. These glands are lined only by the secretory cells and penetrate the full thickness of the endometrial stroma. The length, width, and secretory activity of the uterine glands are also under the hormonal control and vary in different phases of the menstrual cycle (described later).

During the reproductive life, the endometrium can be divided into two zones: (1) stratum functionale, and (2) stratum basale.

The **stratum functionale,** also called, functional layer or *functionalis,* is the thick superficial zone that is sloughed off at the beginning of each menstrual cycle.

The **stratum basale,** also known as basal layer or *basalis,* is the deeper, narrow zone lying adjacent to the myometrim. It is not lost during the menstrual phase and serves a source for the regeneration of the functionalis in the next menstrual cycle.

Blood Supply of the Uterus

The uterus is supplied by two uterine arteries, one of which runs on either side of the organ. Each uterine artery gives rise to many branches that pass into the uterine wall and, on reaching the stratum vasculare of the myometrium, divide into anterior and posterior *arcuate arteries.* These arteries run circumferentially in the myometrium and give rise to terminal branches, which anastomose across the midline with similar vessels from the opposite side. This arterial arcade gives rise to two systems of branches. One system consists of peripheral branches which run outwards and supply the outer part of the myometrium. The other system consists of branches called *radial arteries* (Fig. 22.8). These arteries course centrally (i.e., in the direction of cavity of the uterus) and give rise to two sets of branches. One of these sets consists of small branches called *straight arteries* (also known as *basal arteries*) which supply the inner part of the myometrium and stratum basale of the endometrium. The second set consists of larger, highly coiled branches, which are referred to as *spiral arteries.* Running in a central direction, the spiral arteries give off numerous arterioles, which anastomose with one another and ultimately give rise to a rich capillary bed in the functionalis part of the endometrium. The endometrial veins are arranged upon a plan similar to that of the arteries.

THE MENSTRUAL CYCLE

Beginning with puberty and ending at the menopause, the endometrium of the body of uterus passes through cyclic structural modifications which constitute the menstrual cycles. The duration of a menstrual cycle is variable but averages 28 days. For descriptive purposes, this continuous cycle of events is divided into four phases, each of which gradually passes into the next. These four phases are: menstrual phase, proliferative phase, secretory phase, and premenstrual phase. For easy understanding of the structural modifications in the endometrium, the phases of the menstrual cycle will be described in the following order: proliferative phase, secretory phase, premenstrual phase, and menstrual phase.

PROLIFERATIVE PHASE

This phase follows the menstrual flow and is also known as *regenerative phase*. At the end of the menstrual phase, the endometrium is reduced to a thin band which is 0.5 mm or less in width. This band represents the stratum basale of the endometrium and contains basal parts of the uterine glands, basal arteries, and proximal portions of the spiral arteries. During the proliferative phase regeneration of endometrium occurs from this narrow band. This regeneration takes place under the influence of estrogens secreted by the growing ovarian follicles. Consequently, this phase is also known as *follicular phase or estrogenic phase*.

The length of the proliferative phase is generally 8-10 days. During this period the epithelial cells in the basal portions of the glands rapidly proliferate (i.e., increase in number by mitotic division). The newly produced cells not only reconstitute the uterine glands but also migrate to cover the denuded endometrial surface. The stromal fibroblasts also proliferate and secrete collagen type III and ground substance. The spiral arteries gradually lengthen but do not

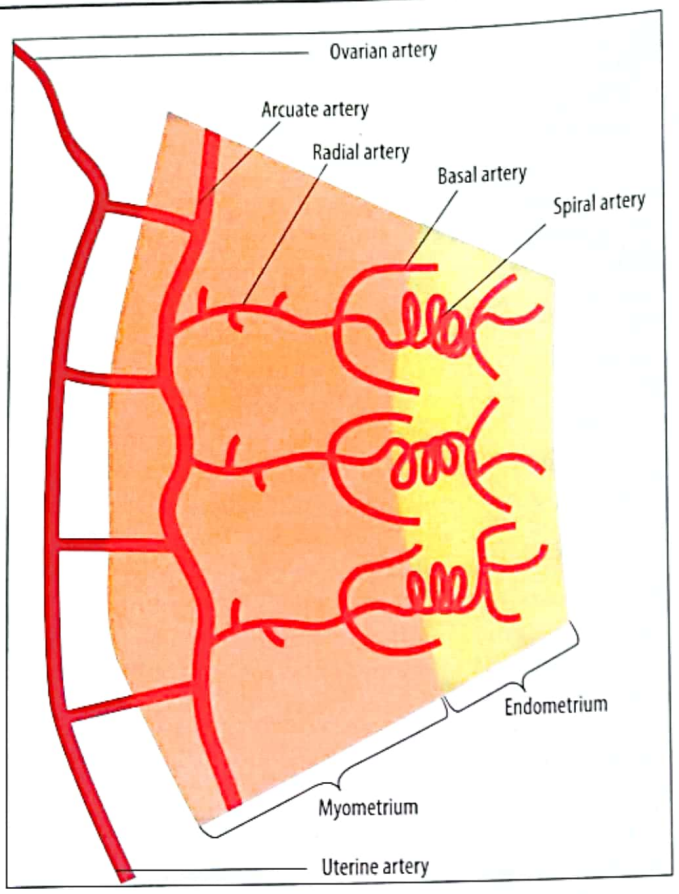

Fig. 22.8 A diagram illustrating the pattern of arterial blood supply of the uterine wall.

extend into the superficial one third of the endometrium.

At the end of proliferative phase, the endometrium attains a thickness of 3 mm. The uterine glands still appear narrow and straight in histological sections (Fig. 22.9). Glycogen granules accumulate in the basal regions of the gland cells.

SECRETORY PHASE

The secretory phase, also called *luteal phase*, commences after ovulation in response to secretion of progesterone by the corpus luteum. The endometrial glands become dilated and highly convoluted, assuming a corkscrew-shaped appearance (Fig. 22.10). The glycogen granules move to the apical region of the cells. In addition, secretory granules containing glycoproteins also appear in the apical cytoplasm of the cells. The secretory product of the glands is released into the uterine lumen. This product, also called *histotroph*, serves as a source of nutrition for the early embryo, if pregnancy occurs.

The endometrium thickens further and attains a thickness of 6 mm at the end of the secretory phase. The endometrial glands elongate further and become dilated and tortuous. In histological sections, these glands give corkscrew-shaped appearance and secretion can be seen within their lumen. Due to accumulation of large quantity of water, the endometrial stroma becomes highly edematous. The spiral arteries become highly coiled, grow nearly to the surface of the endometrium. This capillary bed of the stratum

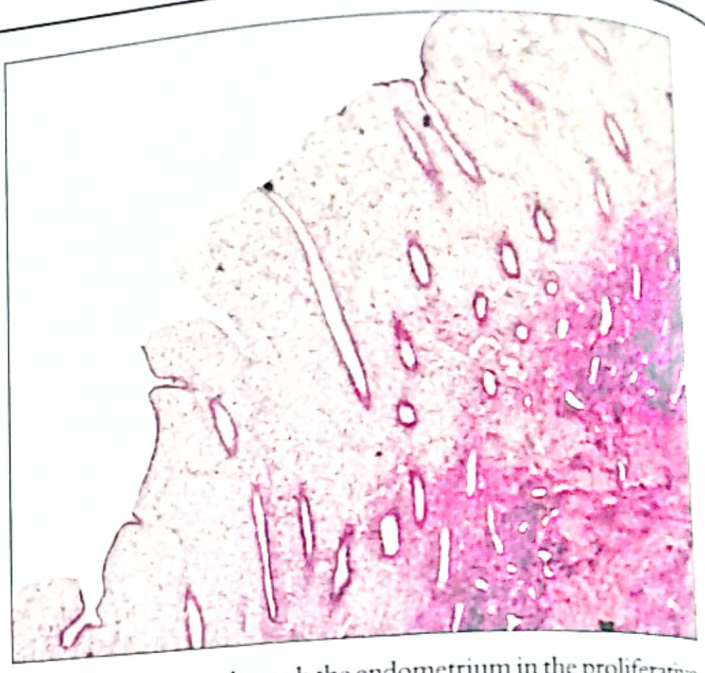

Fig. 22.9 A section through the endometrium in the proliferative phase of the menstrual cycle. Note the narrow and straight endometrial glands.

functionale is now characterized by the presence of small, thin-walled dilated spaces called vascular lacunae.

If fertilization of the ovulated oocyte does not take place, the secretory phase last for about 14 days and then endometrium passes into the premenstrual phase. However, if fertilization occurs, the corpus luteum does not degenerate and the endometrium remains in the secretory phase to receive the zygote.

PREMENSTRUAL PHASE

If fertilization does not occur, the corpus luteum degenerates and becomes nonfunctional about 12 days after ovulation. This leads to a rapid decrease in the blood levels of progesterone and estrogens. The declining hormone levels lead to dramatic changes in the blood supply of the stratum functionale part of the endometrium. Initially, the spiral arteries of the endometrium undergo periodic contractions lasting for several hours. This leads to ischemia (reduction in blood supply) of the stratum functionale and, therefore, this phase is also known as *ischemic phase*. Due to the ischemia, the uterine glands cease to secrete and the endometrium decreases in height because of a marked reduction in the edema of the endometrium. Finally, the spiral arteries become completely constricted leading to necrosis of the stratum functionale.

MENSTRUAL PHASE

After several hours of constriction, the spiral arteries dilate once again. As the distal portions of these arteries have been damaged due to prolonged ischemia, these vessels rupture, so that blood escapes into the endometrium and breaks out into the uterine lumen. The rapidly escaping blood causes sloughing of patches of the necrotic stratum functionale. Blood, uterine fluid, and sloughing stromal

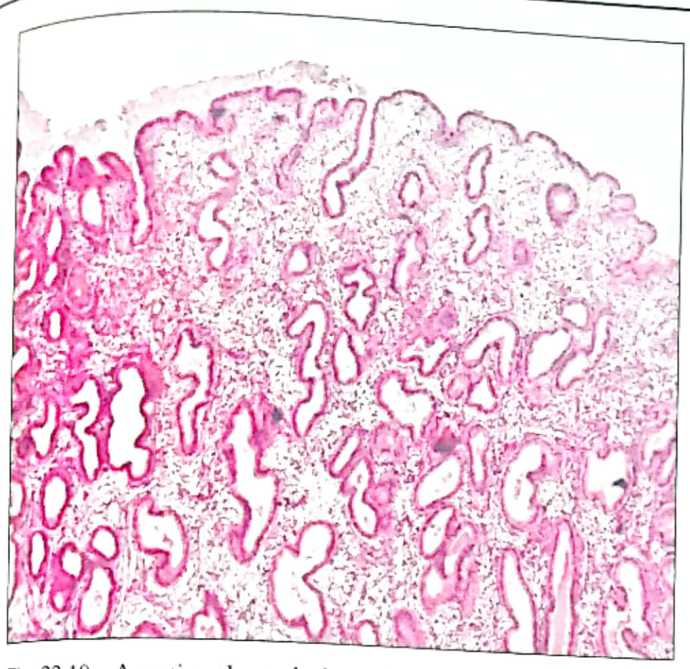

Fig. 22.10 A section through the endometrium in the secretory phase of the menstrual cycle. Note the dilated and tortuous endometrial glands.

and epithelial cells from the stratum functionale are discharged through the vagina as *menses*. Although the entire stratum functionale becomes necrosed, it is not completely sloughed from the uterine wall at once, but this process continues for many days. Average length of the menstrual phase is 3-4 days.

UTERINE CERVIX

The cervix is the lowermost, cylindrical part of the uterus which projects into the vagina. Through the center of the cervix runs a narrow passage called cervical canal which connects the uterine cavity to the lumen of the vagina. The opening of the cervical canal into the uterine cavity is called *internal os*, while its opening into the vagina is called *external os*. The cervical canal is lined by endocervical mucosa, while the outer surface of the vaginal part of the cervix is covered by exocervical mucosa.

The bulk of the wall of the cervix uteri consists chiefly of dense connective tissue containing type I collagen fibers and elastic fibers. Smooth muscle fibers are also present but their number is very small. Presence of abundant elastic fibers makes the cervix capable of stretching considerably during the childbirth

The **endocervical mucosa** is lined by simple columnar epithelium. A thick lamina propria of connective tissue is present under the epithelium. The lamina propria of the cervix contains numerous large, simple tubular, branched mucus-secreting glands called cervical glands. The endocervical mucosa does not show any remarkable change in thickness during the menstrual cycle and does not slough during menstruation.

The amount and nature of the secretion of the cervical glands varies during the menstrual cycle under the influence of the ovarian hormones. At the end of the follicular phase (i.e., in the midcycle), the amount of mucus produced by the cervical glands increases tremendously and its viscosity decreases considerably. The purpose of these changes is to facilitate the entry of spermatozoa from the vagina into the uterus. During the luteal phase the secretion of the cervical glands becomes very viscous. During pregnancy, the cervical glands produce a highly viscous mucus which forms a protective plug in the cervical canal; this mucus plug prevents the entry of spermatozoa and microorganisms into the uterine cavity.

The ectocervical mucosa, also called exocervical mucosa, covers the outer surface of the vaginal part of the cervix uteri and is lined by stratified squamous nonkeratinized epithelium.

The junction between the ectocervical stratified squamous and the endocervical simple columnar epithelia occurs in the *transformation zone* which, during the reproductive life, lies just outside the external os. Before puberty and after menopause, the transformation zone lies within the cervical canal.

The transformation zone of the uterine cervix is a common site where metaplastic changes in the endocervical epithelium begin, which lead to replacement of the simple columnar epithelium by the stratified squamous epithelium. Such a metaplastic change in the endocervical epithelium are considered to be a precancerous condition, which may ultimately lead to the development of carcinoma of the cervix.

The aperture of a cervical gland sometimes becomes occluded. This occlusion results in the formation of a cyst as the gland becomes dilated by the accumulation of mucous secretion. Such a cystic cervical gland is called a *Nabothian cyst*.

VAGINA

The vaginal wall consists of three coats: mucosa, muscularis and adventitia.

The vaginal **mucosa** shows transverse folds (rugae) which provide increased surface area for extension and stretching. The mucosa consists of two layers: epithelium and lamina propria.

The ***epithelium*** of the vaginal mucosa is of stratified squamous nonkeratinized variety (Fig. 22.11). A special feature of the vaginal epithelium during the reproductive age is its unusual thickness (150-200 µm). Under the influence of the estrogens, the cells of the vaginal epithelium synthesize and store a large number of glycogen granules in their cytoplasm. In the routine histological sections, the glycogen is washed out, due to which these cells give a vacuolated appearance.

As the vaginal epithelial cells desquamate, the glycogen is released into the vaginal lumen. The vaginal flora (i.e., the bacteria which normally reside in vagina) metabolize this glycogen to form lactic acid. This leads to the presence of an unusually low pH in the vagina. The acidic pH of

The eyes are a pair of complex photosensitive organs that permit a fairly accurate analysis of the form, light intensity, and color reflected from the objects. Each eye consists of a globe called eyeball. Each eyeball is located in a bony cavity within the skull which is called orbit.

The wall of each eyeball is composed of three concentric layers or coats: (i) an outer **fibrous coat** or *corneoscleral layer,* (ii) a middle **vascular coat** or *uveal layer,* and (iii) the innermost **neural coat** or *retinal layer.*

THE FIBROUS COAT

The outermost coat of the eyeball consists mainly of collagenous fibers. This coat comprises the cornea and sclera and, therefore, is also known as the *corneoscleral layer.* The corneoscleral junction is known as the *corneal limbus.*

CORNEA

The cornea forms the anterior one-sixth of the fibrous coat. Unlike sclera, which is opaque, the cornea is clear and transparent like glass. Because transparency is of prime importance, no blood vessels are present in the cornea. The corneal tissues receive oxygen, water, and nutrients by diffusion from the tear film covering the anterior surface of the cornea. In addition, the nutrients and oxygen also reach the deeper corneal layers by diffusion from the aqueous humor present in the anterior chamber of the eyeball.

The cornea is slightly thicker than sclera, having a thickness of about 0.8 mm in the center and 1.0 mm at the periphery. A section through the cornea shows that it is composed of the following five layers: corneal epithelium, Bowman's membrane, corneal stroma, Descemet's membrane, and corneal endothelium (Fig. 23.3).

1. The **corneal epithelium** forms the outermost layer of the cornea. It is a typical stratified squamous nonkeratinized epithelium and is composed of 5-7 layers of cells. Like other stratified squamous epithelia, the corneal epithelium also undergoes constant renewal throughout life. The cells in the basal layer of the epithelium continuously proliferate, while the most superficial cells constantly desquamate from the surface of the cornea. The corneal limbus serves as a reservoir of stem cells, from which new cells are constantly added to the basal layer of the corneal stratified squamous epithelium. EM reveals that the most superficial cells of the corneal epithelium have surface projections in the form of microvilli and microplicae. These projections serve to stabilize the normal tear film over the corneal surface. The corneal epithelium is very richly supplied by free nerve endings; these sensory nerve endings make the cornea extremely sensitive to pain.
2. The **Bowman's membrane**, also called *anterior limiting membrane*, is an acellular layer located between the corneal epithelium and corneal stroma. It serves as basement membrane for the corneal epithelium. It is 8-12 μm thick and appears homogeneous under the light microscope. EM shows that this layer consists of a network of fine type I collagen fibrils embedded in a ground substance. The Bowman's membrane contributes to the strength of the cornea and acts as a barrier to the spread of infections. This membrane does not regenerate and any damage to it leads to the formation of an opaque scar that may impair the vision.
3. The **corneal stroma**, also known as *substantia propria*, is the thickest layer and constitutes about 90 percent of the corneal thickness. It consists of about 60 lamellae composed of type I collagen fibers. The collagen fibers are embedded in a ground substance of proteoglycans like lumican, keratan sulfate, and chondroitin sulfate. To provide maximum mechanical strength, direction of collagen fibrils is different in different lamellae; generally, the adjacent lamellae are oriented at right angles to each other. Flattened fibroblast-like cells, called *keratocytes*, lie between the lamellae of the corneal stroma. Slender cytoplasmic processes of these cells extend between the lamellae of collagen fibers. The major factors responsible for the transparency of the cornea are: (i) complete absence of blood vessels in corneal tissues (ii) uniform, orthogonal array of the collagen fiber lamellae in the corneal stroma (i.e., alternating lamellae run at right angles to each other), and (iii) strict regulation of the water content of the corneal stroma (accumulation of excessive water in the stroma would make the cornea opaque).
4. The **Descemet's membrane**, also called *posterior limiting membrane*, is a thin, acellular layer which appears homogeneous and highly refractile under the light microscope. Actually, it is a modified basement membrane for the corneal endothelium. The Descemet's membrane is only 5 μm thick in the newborns, but slowly thickens with age and may attain a thickness of 17 μm in the old people.
5. The **corneal endothelium** is a layer of simple squamous epithelium that lines the posterior surface of the cornea. These cells contain many mitochondria, a Golgi apparatus, RER, and

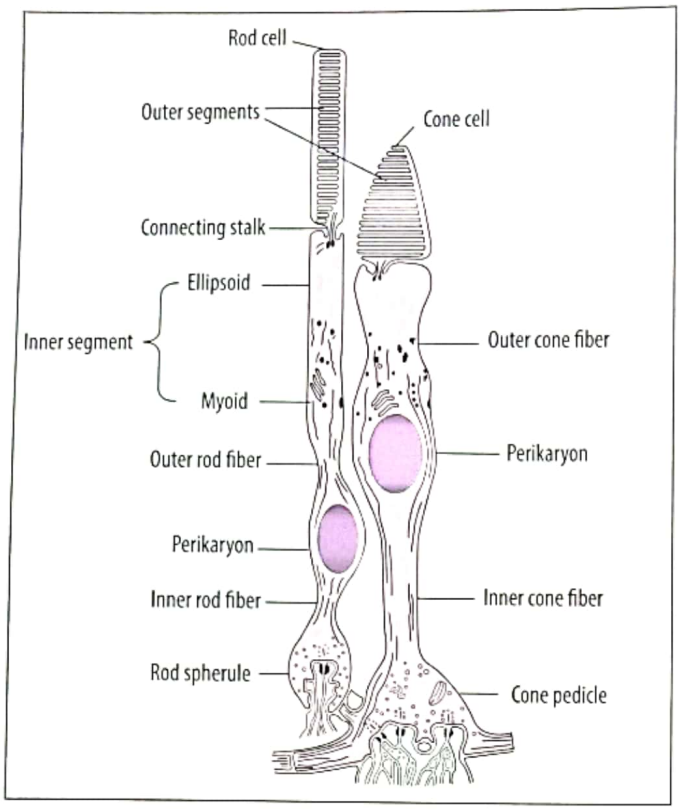

Fig. 23.6 Diagrammatic representation of electron microscopic structure of rod and cone cells of the retina.

length*. Like the rod cells, each of them consists of an inner segment and an outer segment which are connected to each other by the modified cilium which constitutes the connecting stalk. The outer segment of a cone cell is shorter than that of a rod cell, and is conical in shape.

The outer segment of a cone cell also shows transverse membranous discs, but these discs are not independent of the enveloping plasma membrane but remain attached to this membrane. The cone discs are also shed from their distal ends, but this shedding is not as continuous and as regular as that which occurs from the rod cells.

The inner segment of the cone joins the perikaryon almost directly and, therefore, the outer cone fiber is very short. The nuclei and perikarya of the cone cells are larger than those of the rods and lie just internal to the outer limiting layer. The axonal process of a cone cell consists of a relatively longer inner cone fiber that expands at its termination in the outer plexiform layer to form a *cone pedicle,* which synapses with the dendrites of the bipolar neurons in the outer plexiform layer.

The membranous lamellae of the outer segments of the cone cells contain the photosensitive pigment **iodopsin.** This substance consists of *retinal* and a variety of proteins called *photopsins.*

The cone cells are of three types, each type containing a different variety of iodopsin. Each variety of iodopsin has a maximum sensitivity for the red, green or blue color, depending on the type of photopsin present in the iodopsin. Here it is important to note that the three functional varieties of cones are morphologically similar and cannot be distinguished from each other under the microscope.

* It is to be noted that in the region of fovea centralis the length of cones as well as rods is much greater than their length in other parts of the retina.

DIRECT CONDUCTING NEURONS

1. **Bipolar Cells.** Perikarya of these neurons lie mostly in the central region of the inner nuclear layer. These cells are divided into two main groups:
   i. *Diffuse bipolar cells*. Dendrites of these cells make synapses with two or more photoreceptors and the axon contacts several ganglion cells.
   ii. *Monosynaptic bipolar cells* (also called *midget cells*). The dendrite of a midget cell makes contact with only one cone photoreceptor and its axon synapses with only one ganglion cell.
2. **Ganglion Cells.** The ganglion cells are multipolar, large neurons measuring up to 30 µm in diameter. Each of them has a big, vesicular nucleus and a large amount of Nissl substance (representing the rough endoplasmic reticulum of the cell). Their perikarya lie in the ganglion cell layer and their dendrites extend into the inner plexiform layer. The axons of the ganglion cells constitute the optic nerve fibers. Like the bipolar cells, the ganglion cells are also classified into two main types:
   i. *Diffuse type*, dendrites of which contact several bipolar cells.
   ii. *Monosynaptic type*, dendrites of which synapse with a single midget bipolar cell only.

ASSOCIATION NEURONS

1. **Horizontal Cells.** The bodies of horizontal cells lie in the outer part of the inner nuclear layer, while their dendrites and axons extend into and run in the outer plexiform layer. The dendrites of a horizontal cell terminate around numerous cone pedicles, whereas the single axon branches at its termination to synapse with rod spherules as well as cone pedicles. The horizontal cells serve to connect a group of cone cells in one area with a group of rod or cone cells in another area.
2. **Amacrine Cells.** These neurons have many dendrites but no axon. Perikarya of these cells lie in the inner part of the inner nuclear layer. The branching processes (dendrites) of these neurons pass into the inner plexiform layer, branch extensively, and synapse with the dendrites of the ganglion cells.
3. **Interplexiform Cells.** The perikarya of these cells lie in the inner nuclear layer. Their processes pass into the outer plexiform as well as inner plexiform layers of the retina. In the outer plexiform layer, the processes of the interplexiform cells make synapses with the processes of the rod and cone cells, while in the inner plexiform layer they synapse with the processes of the bipolar cells and ganglion cells.

SUPPORTING CELLS OF THE RETINA

1. **Muller Cells.** These cells, also called *retinal gliocytes*, have very large perikarya which lie in the inner nuclear layer. Thin cytoplasmic processes of these cells extend from their cell body to the external as well as internal limiting membranes. In the region of the outer limiting layer, the Muller cells form zonulae adherentes with the rod and cone cell. The internal limiting membrane is actually the basement membrane of the Muller cells. These cells are believed to be functionally analogous to neuroglia because they serve to support, nourish and insulate the retinal neurons and their processes.
2. **Neuroglial cells**, mainly **astrocytes** and **microglial cells**, are found in some layers of the retina.

Summary of the Structure of Various Layers of Retina

1. **Retinal Pigment Epithelium (RPE).** The pigment epithelium of retina consists of a single layer of closely apposed, low columnar epithelial cells. The apical surface of these cells bears finger-like processes. The characteristic feature of the RPE is the presence of a large number of brownish-black melanin granules in the cells as well as their processes.
2. **Layer of Rods and Cones.** This layer contains the outer and inner segments of the rod and cone cells.
3. **Outer Limiting Layer.** This layer, also called *external limiting membrane*, is not a true membrane. It represents a row of adhering and tight junctions that attach the outer ends of the Muller cells to each other and to the inner segments of the rod and cone cells. The external limiting membrane forms a metabolic barrier, which prevents the passage of large molecules into the inner layers of retina.
4. **Outer Nuclear Layer.** This layer lodges the cell bodies of rod and cone cells along with their nuclei. The nuclei of the cone cells are ovoid in shape and are limited to a single row close to the external limiting membrane. The rod nuclei are spherical in shape and are distributed in several (4 or 5) rows.
5. **Outer Plexiform Layer.** This layer, also called *outer synaptic layer*, represents the region where the processes of the rod and cone cells make synapses with the processes of bipolar cells, horizontal cells, and interplexiform cells.
6. **Inner Nuclear Layer.** This layer contains the cell bodies and nuclei of the bipolar neurons, horizontal cells, amacrine cells, interplexiform cells, and Muller cells.
7. **Inner Plexiform Layer.** This layer is also called *inner synaptic layer.* In this region the axons of bipolar neurons make synaptic connections with dendrites of the ganglion cells. In addition, the processes of amacrine cells and interplexiform neurons run in this layer and make synapses with the processes of the bipolar cells and ganglion cells.
8. **Ganglion Cell Layer.** This layer contains the perikarya of ganglion cells which are large multipolar neurons.
9. **Optic Nerve Fiber Layer.** This layer is made up of the unmyelinated axons of the ganglion cells. These axons run parallel to the retinal surface and converge to the posterior pole of the eyeball, where they form the optic disc and pierce the sclera to form the optic nerve. This layer also contains some neuroglial cells, especially the fibrous astrocytes.
10. **Inner Limiting layer.** This layer, also called *internal limiting membrane*, is the basement membrane of the Muller cells.

Blood Vessels of Retina

The outer one-third of the retina (comprising the pigment epithelium, the layer of rods and cones, the outer nuclear layer and the outer plexiform layer) receives its nutrition by diffusion from the fenestrated capillaries of the choroid. The inner two-thirds of the retina are supplied by a system of retinal vessels which are branches of the central retinal artery. The larger branches of this artery run in the nerve fiber layer. Smaller branches of these arteries pass into the ganglion cell layer and inner plexiform layer. Branches derived from these arteries form two capillary plexuses; one of these plexuses is located in the nerve fiber layer, while the other lies in the inner nuclear layer.

Fovea Centralis

This is a shallow, circular depression lying at the posterior pole of the optical axis of eye. The depression is caused by the absence of inner layers of retina in this area. All the visual cells in the floor of fovea are cones. The bipolar and ganglion cells accumulate in the periphery of this depression. There are no retinal vessels in the area of the fovea. The fovea centralis is the region where extremely precise visual acuity takes place.

BLOOD-RETINA BARRIER

The neural retina is protected from the circulating toxic macromolecules by a blood-retina barrier. The outer one-third of the retina, which receives its nutrition from the blood capillaries of the choroid, is protected by the presence of tight junctions between the cells of the retinal pigment epithelium. The inner two-thirds of the retina receive their blood supply from the branches of the central artery of retina. This part of the retina is protected from the toxic molecules circulating in the blood by the presence of tight junctions between the endothelial cells of the capillaries arising from the branches of the central artery of retina.

LENS

The lens of the eye is a transparent, biconvex body situated between the iris and vitreous body. In the young, the lens is elastic but becomes harder with age. Structurally, the lens consists of three components: lens capsule, subcapsular epithelium, and lens substance. The lens is suspended in place by the zonular fibers (ciliary zonule) which form the suspensory ligament of the lens.

LENS CAPSULE

The lens capsule is a thin, transparent, homogeneous, and elastic membrane that envelops the entire lens. Actually it is a basal lamina ranging from 10 to 20 µm in thickness. It is composed mainly of collagen type IV and sulfated glycosaminoglycans. Being very elastic, the lens capsule allows the lens to assume a more globular shape when the tension in the zonular fibers decreases.

SUBCAPSULAR EPITHELIUM

This is a single layer of cuboidal cells present only on the anterior surface of the lens just under the capsule. The apices of these cells are directed posteriorly toward the lens fibers with which they interdigitate. Toward the equator of the lens, these cells become elongated and transform into *lens fibers*. The lens grows throughout life by the addition of these fibers.

LENS SUBSTANCE

The lens substance consists of elongated prismatic **lens fibers**. These fibers are actually highly differentiated cells derived from the posterior wall of the embryonic lens vesicle. The lens fibers run lengthwise from the posterior pole to the anterior pole of the lens and, when examined in a horizontal section of the lens, these fiber appear to be arranged concentrically. New lens fibers are constantly added by mitotic division and transformation of the epithelial cells at the equator. The newly formed lens fibers become highly elongated, lose their nuclei and organelles, and their cytoplasm becomes filled with special proteins called ***crystallins***. Individual lens fibers can be identified more easily in the outer (superficial) part of the lens which is known as *cortex of the lens*. In the central region of the lens, called *nucleus of the lens*, the lens fibers are condensed and appear homogeneous.

The lens is normally transparent but may become opaque, due to which the vision becomes impaired. This condition, called **cataract**, normally occurs in the old age. However, cataract may occur in the children or young people due to injury, metabolic disorders (diabetes mellitus), or exposure to UV radiation.

VITREOUS BODY

The vitreous body is a transparent, jelly-like substance that fills the cavity of the eyeball behind the lens. It is loosely attached to the surrounding structures including the internal limiting membrane of retina.

The vitreous body, also called *vitreous humor*, has a volume of 4 mL and is composed mainly (99%) of water. The remaining 1% volume consists of electrolytes, collagen type XI fibrils, and glycosaminoglycans (principally hyaluronic acid). The vitreous body also contains a very small number of cells called *hyalocytes*. These cells are believed to secrete the hyaluronic acid and collagen fibrils.

ACCESSORY STRUCTURES OF THE EYE

CONJUNCTIVA

The conjunctiva is a thin transparent mucous membrane that lines the posterior surface of the eyelids from which it is reflected on to the anterior surface of the eyeball, covering it up to the corneal margin. The conjunctiva lining the posterior surface of the eyelids is called *palpebral conjunctiva*, while that which covers the eyeball is titled *bulbar conjunctiva*. The area where the palpebral and bulbar conjunctivae meet is known as *fornix*.

The conjunctiva consists of epithelium and lamina propria. The conjunctival epithelium is of stratified columnar variety containing many goblet cells. The lamina propria consists of a thin layer of loose connective tissue. Secretion of the goblet cells becomes a part of the tear film.

EYELIDS (Fig. 23.7)

The eyelids are movable folds which protect the eyes from injury and excessive light. They also serve to lubricate the anterior portion of the eyeballs.

The skin covering the eyelids is extremely thin. It contains small hairs, sebaceous glands, and sweat glands. The subcutaneous layer consists of loose connective tissue, which is rich in elastic fibers but contains no fat. At the lid margin, there are present three or four rows of long, stiff, curved hairs which are called eyelashes. They are associated with large sebaceous glands called *glands of Zeis*. Between the eyelashes are present large sweat glands called *glands of Moll* (also called *ciliary glands*).

Beneath the skin of the eyelids is present a layer of skeletal muscle fibers, which is the palpebral part of the orbicularis oculi muscle. In the upper eyelid there are also present fibers of insertion of the levator palpebrae superioris muscle, and slender slips of smooth muscle which constitute the superior tarsal muscle.

In each eyelid, next to the muscle layer lies a curved plate of dense fibrous connective tissue called **tarsal plate**. Embedded in the tarsal plate is a single row of very large sebaceous glands known as *tarsal glands* (also called *Meibomian glands*). Ducts of the tarsal glands open at the lid margin. Each tarsal gland consists of numerous acini which open into a central straight duct. Secretion of these glands creates an oily layer on the surface of the tear film. This helps to prevent rapid evaporation of the normal tear layer. The posterior (inner) surface of each eyelid is covered by the palpebral conjunctiva.

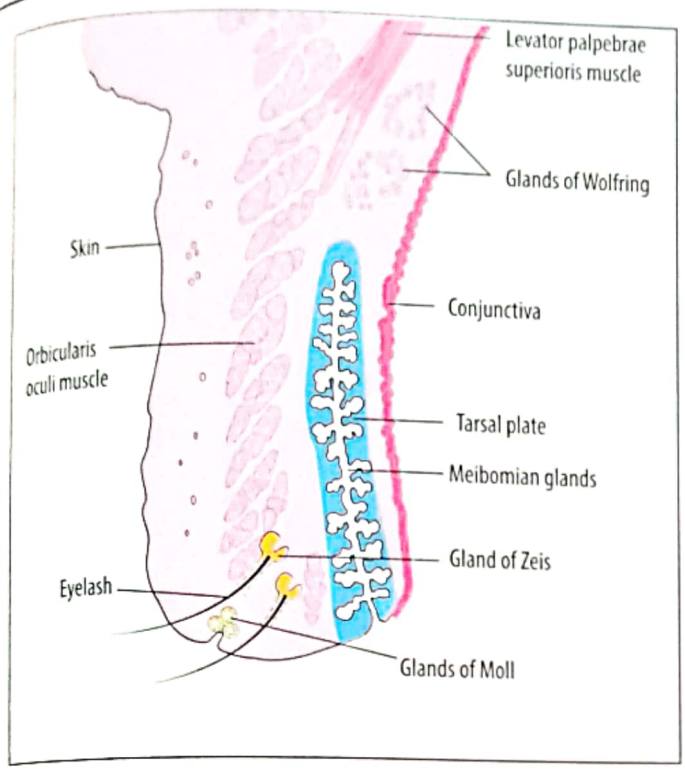

Fig. 23.7 Diagram illustrating the structure of the upper eyelid.

STY. A sty, also called *hordeolum*, is a small, red and painful swelling on the eyelid. It is caused by acute bacterial infection of one of the following: an eyelash follicle, a Meibomian gland, gland of Zeis, or gland of Moll. It usually subsides on its own but sometimes requires treatment by antibiotics.

CHALAZION. A localized, painless and progressive swelling on an eyelid is called a chalazion. It results from the chronic inflammation of a blocked Meibomian gland. Spontaneous regression of a chalazion is uncommon and it usually needs to be treated by surgical excision.

LACRIMAL GLANDS

The lacrimal glands are compound tubuloacinar glands of serous variety, which produce a very thin, watery secretion called lacrimal fluid or tears. One lacrimal gland is located in the lacrimal fossa in the roof of each orbit. Each lacrimal gland consists of several separate lobes which drain via 10 to 15 excretory ducts into the superior conjunctival fornix. The acini of gland are lined by pyramidal cells which show eosinophilic apical secretory granules. Myoepithelial cells occur between the acinar cells and their basal lamina. The stroma of lacrimal gland consists of loose connective tissue. The ducts of the gland are lined by simple cuboidal epithelium.

Accessory Lacrimal Glands

These are multiple, small compound tubuloacinar glands of serous variety. They are located in the fornix of the conjunctival sac (*glands of Krause*) and on the inner surface of the upper eyelid (*glands of Wolfring*).

Lacrimal Fluid

The lacrimal fluid represents a mixture of products secreted by the lacrimal glands, accessory lacrimal glands, tarsal glands of the eyelids, and goblet cells of the conjunctiva. It consists mostly of water but also contains proteins (tear albumins and lactoferrin), electrolytes, and the antibacterial enzyme *lysozyme*. Normal secretion of the lacrimal fluid creates a tear film that lubricates and protects the corneal and conjunctival epithelia. The tear film also supplies oxygen and nutrients to the corneal tissues. Excessive secretion of the tears (lacrimation) occurs in response to pain or strong emotions.

24 Ear

The ear is the organ of hearing, but it also plays an important role in the maintenance of equilibrium (balance) of the body. It consists of three parts: external ear, middle ear, and internal ear.

THE EXTERNAL EAR

This part of the ear collects and amplifies the sound waves. It consists further of three parts:

1. Auricle (pinna).
2. External auditory meatus.
3. Tympanic membrane.

AURICLE

The auricle of the ear, also called pinna, is composed of an irregular plate of elastic cartilage covered by thin skin that contains hair follicles, sebaceous glands, and sweat glands. The skin adheres tightly to the underlying elastic cartilage. The skin of pinna also contains adipose tissue along its posteromedial border and inside the lobule.

EXTERNAL AUDITORY MEATUS

The external auditory meatus is an air-filled canal that extends from the pinna to the tympanic membrane. It consists further of two parts: (i) the outer one-third, called cartilaginous part, has a wall of elastic cartilage which is continuous with the cartilage of pinna, and (ii) the inner two-thirds of the external auditory meatus are called bony part because this part of the meatus lies within the temporal bone.

The **cartilaginous part** of the external auditory meatus is lined by thick skin which bears many thick and stout hairs. The follicles of these hairs are associated with sebaceous glands. No eccrine sweat glands are present in the skin of this part of the external acoustic meatus, but it contains large, simple tubular, coiled apocrine sweat glands called *ceruminous glands*. The secretory products of the sebaceous and ceruminous glands mix with the keratinocytes shed from the epidermis and form a yellowish, waxy material called *cerumen* or *ear wax*. The major functions of the cerumen are: (i) it lubricates the meatal skin (so that it does not become dry and itchy), (ii) it creates a slightly acidic environment which prevents the growth of bacteria and fungi, and (iii) it traps dust and tiny bits of other foreign materials and moves this debris outward, away from the eardrum.

The longer **bony part** of the external auditory meatus is lined by thin skin that closely adheres to the periosteum of the underlying bone. This skin bears small and fine hairs which are confined to the roof of the meatus. No ceruminous glands are present in this skin.

TYMPANIC MEMBRANE

The tympanic membrane, or *eardrum*, separates the external auditory meatus from the middle ear. It vibrates when the sound waves reach it, and thus, the sound waves are converted into mechanical energy, which is transmitted to the auditory ossicles in the middle ear. The tympanic membrane is composed of three layers: an outer (cutaneous) layer, a middle (fibrous) layer and an inner (mucosal) layer. The **cutaneous layer** consists of thin skin consisting of stratified squamous keratinized epithelium under which lies a very thin layer of connective tissue without any dermal papillae; this skin is hairless. The **fibrous layer** is made up of collagenous and elastic fibers, which are arranged in two laminae. The superficial lamina contains radial fibers, whereas the deep lamina is composed of circularly arranged fibers. The **mucosal layer** is a part of the mucous membrane of the tympanic cavity. It consists of a single layer of low cuboidal epithelial cells that rest upon a very thin lamina propria.

THE MIDDLE EAR

The middle ear is an irregular cavity situated in the interior of the temporal bone. The middle ear cavity, also called *tympanic cavity*, communicates anteriorly with the auditory tube and posteriorly with the mastoid air cells. The tympanic cavity contains three small bones called *ear ossicles* (which include malleus, incus, and stapes) and two small muscles (tensor tympani and stapedius). The ear ossicles are composed of compact bone; none of them has a marrow cavity. The tensor tympani and stapedius are striated skeletal muscles.

The tympanic cavity is lined by a mucosa consisting of epithelium and lamina propria. The lining epithelium is mostly of simple squamous type, but over the inner surface of tympanic membrane it changes to low cuboidal variety. The lamina propria consists of a thin layer of connective tissue which adheres closely to the periosteum of the underlying bone.

The **auditory tube,** also called *Eustachian tube,* connects the middle ear cavity to the pharynx. It is lined by pseudostratified columnar ciliated epithelium which contains many goblet cells.

THE INTERNAL EAR

The internal ear consists of a series of fluid-filled membranous sacs and canals lodged within cavities present in the petrous part of the temporal bone. The membranous sacs and canals form the *membranous labyrinth*; the corresponding bony cavities constitute the *bony labyrinth*

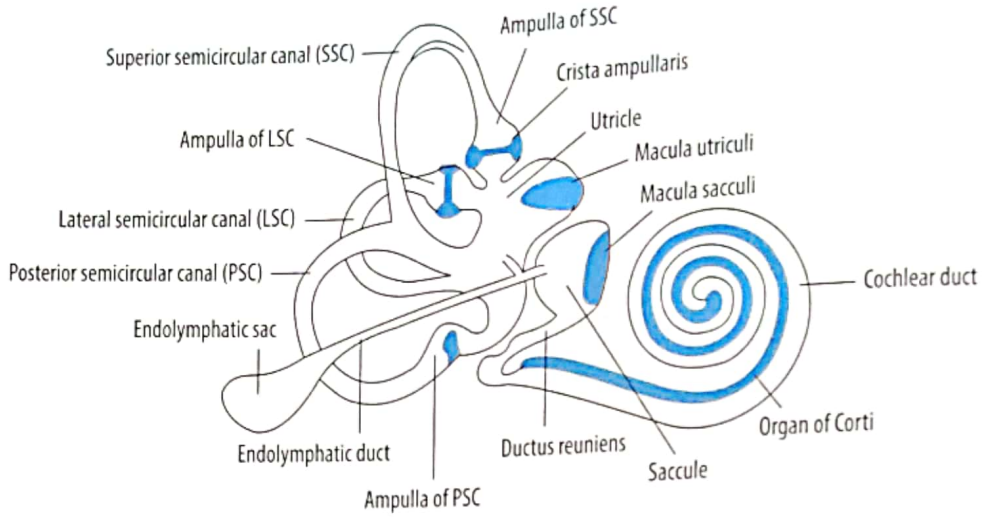

Fig. 24.1 Diagram showing the outline of cavities of the left membranous labyrinth. Receptor regions of the neuroepithelium are shown in blue color.

(osseous labyrinth). The space between the membranous labyrinth and the bony labyrinth is filled with a fluid called *perilymph*, while the membranous labyrinth contains a fluid called endolymph.

OSSEOUS LABYRINTH

The osseous labyrinth lies within the petrous part of the temporal bone and consists of three part: (i) **vestibule**, (ii) **semicircular canals**, and (iii) **cochlea**. The vestibule is the central chamber which lies between the semicircular canals and cochlear duct. The lateral wall of the vestibule, which faces the tympanic cavity, has an oval opening called **oval window** or *fenestra vestibuli*. The oval window is normally closed by the footplate of stapes and its associated annular ligament. The medial wall of the vestibule contains many perforated recesses which serve as passageways for the blood vessels, nerve rootlets, and the endolymphatic duct.

Posterosuperior to the bony chamber of the vestibule are present three loops of bony channels which are called *semicircular canals*. According to their orientation, these canals are named superior, inferior, and lateral semicircular canals. Each of them is about 0.8 mm in diameter and, also, each has a dilatation at one end which is known as *ampulla*.

Anterior to the vestibule, lies the snail-shaped *cochlea* which consists of a conical bony core, called *modiolus*, and a a spiral bony canal which runs around the modiolus, making approximately 2 ¾ turns. The cochlea also communicates with the tympanic cavity through a small circular opening called **round window** or *fenestra cochleae*. In a living person, the round window is closed by a thin membrane known as *secondary tympanic membrane*.

MEMBRANOUS LABYRINTH

The membranous labyrinth is separated from the bony labyrinth by a narrow space, called *perilymphatic space*, which contains the *perilymph*. Thin strands of connective tissue pass across the perilymphatic space to suspend the membranous labyrinth within the osseous labyrinth. The composition of the perilymph is similar to that of the CSF because the perilymphatic space communicates with the subarachnoid space of the brain (explained later).

The membranous labyrinth has the same form as that of the osseous labyrinth except that the vestibule contains not one but two membranous sacs which are named **utricle** and **saccule** (Fig. 24.1). The **semicircular canals** open into the utricle. Each semicircular canal presents a dilatation at one end which is called *ampulla*. The saccule communicates with the utricle and scala media of the cochlea, which is called **cochlear duct**, by means of narrow ducts. The duct connecting the saccule and cochlear duct is called *ductus reuniens*. The duct that connects the saccule and utricle assumes the form of a Y whose stalk terminates in a blind sac called *endolymphatic sac*.

The endolymphatic sac is lined by specialized simple columnar epithelial cells bearing microvilli on their free surface. These cells are absorptive in function and play an important role in the transfer of endolymph to the blood capillaries lying adjacent to the endolymphatic sac. This process of drainage of the endolymph (occurring parallel with its production) ensures constant renewal of this fluid.

The membranous labyrinth consists of a thin layer of connective tissue lined internally by an epithelial layer which is ectodermal in origin. The lining epithelium is generally of simple squamous variety, but in certain regions it is modified to form specialized sensory areas. There are six such areas in the membranous labyrinth of each ear. On the basis of structural characteristics, these sensory areas can be divided into three categories: (1) *cristae ampullares* which are three in number, one being present in the ampulla of each