THE STATE UNIVERSITY
OF MEDICINE AND PHARMACY
„NICOLAE TESTEMITANU"

TATIANA GLOBA

CYTOLOGY, EMBRYOLOGY AND HISTOLOGY

(courses for medical students)

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This book is dedicated with appreciation and love to my parients Aurica and Gheorghe, and my family.

To my grand teacher and tutor Nicolae Eşanu.

PREFACE TO THE STUDENT

This book is written for you. Human histology is more than just interesting – it is fascinating. To help get you involved in this study of this exciting subject, a number of special features are incorporated throughout the book.

This book is meant to be a guide to the understanding of your own body. I have tried to be selective about the information included.

The illustrations and tables are designed with your learning needs in mind. The tables are summaries of important information in the text. The figures are referenced where their viewing would be most advantageous to help in understanding the text. Special topic box, called *Clinical Correlations* challenges you to apply your learning to clinical situations.

I hope that you enjoy *Cytology, Embryology and Histology (courses for medical students)* and that this book makes learning about the body's structures an exciting and rewarding process. Perhaps the best bit of advice I can give you is that memory depends on understanding. Thus, if you strive to archive understanding instead of rote memorization, your memory will not fail you very often.

I would appreciate hearing from you about your experiences with this textbook or suggestions for improvements in future editions.

Author

TATIANA GLOBA

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Part III. GENERAL HISTOLOGY

Part IV. SPECIAL HISTOLOGY

TISSUE PREPARATION FOR MICROSCOPIC IMAGING

Medical histology applies microscopy to the human body, seeking to discover the nature of its smaller structures, how they relate to each other, and what they do. The thinking of histology runs along these lines. How does one prepare living and dead tissues for microscopy to preserve their images authentic to their true nature? What kinds of microscopy can be applied? How does one analyze and describe the images yielded at different orders of magnification by the various microscopes? Does the microscopic appearance of the tissue or cell suggest of how it works, its chemical nature, and what may go wrong in case of a disease? What experiments can one do to test concepts of how the structure relates to function?

The answers contain a large body of knowledge express in several ways. First, histology is colourful. Secondly, almost everything seen is actually there; which is not to say that what is not seen is absent. Third, one handles and views actual slides – the source material for most of histology, not just someone else's selected images. Fourth, the structures can be interpreted as parts in developmental and functional sequences, and can be grouped together by the following accounts, for example, how cells defend the body. So much is now known about the roles of cells and structures that histology is both descriptive microanatomy, and an introduction to function for the whole body.

The most common procedure used in the study of tissues is the preparation of histological sections that can be studied with the aid of the light microscope. Under the light microscope, tissues are examined through a light beam that is transmitted through the tissue. Because tissues and organs are usually too thick for light to pass through them, they must be sectioned to obtain thin, translucent sections. However, living cells, very thin layers of tissues, or transparent membranes of living animals (eg, the mesentery, the tail of a tadpole, the wall of a hamster's cheek pouch) can be observed directly in the microscope without first being sectioned. It is then possible to study these structures for long periods and under varying physiological or experimental conditions. In most cases, however, tissues must be sliced into thin sections and attached on glass slides before they can be examined. These sections are precisely cut from tissues previously prepared for sectioning using fine cutting instruments called **microtomes.**

Acquisition of the material

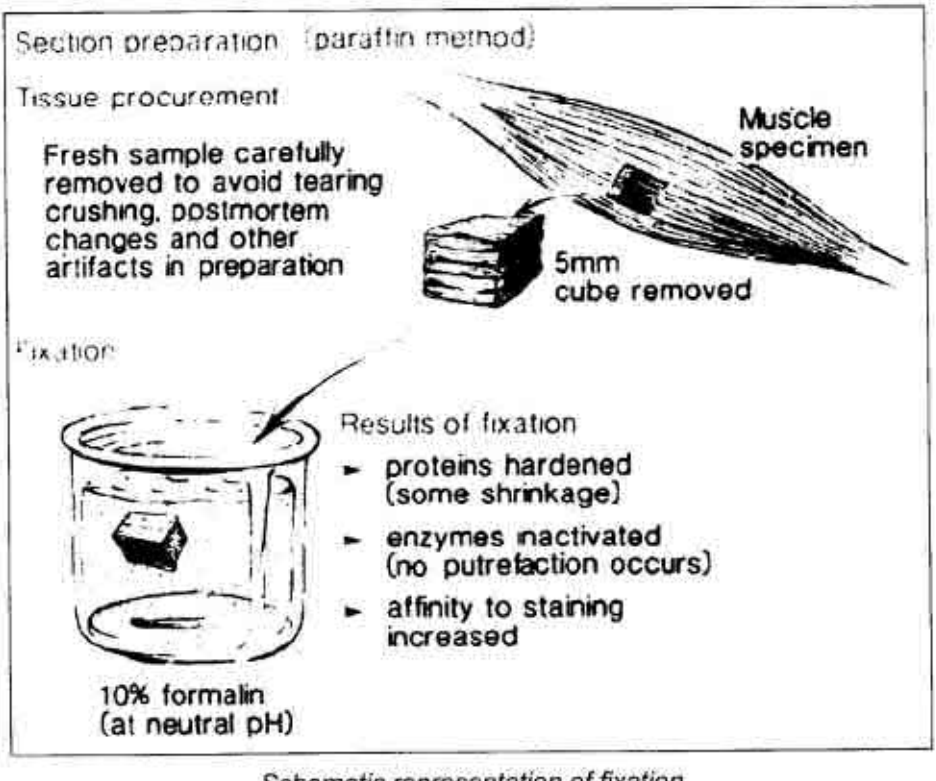
Tissues obtained from biopsy samples, post-mortem dissections or from tissue cultures in the lab must be cut down to an appropriate size, usually up to 1.5 cm for Light Microscopy and up to 0.5 cm for Electron Microscopy. A major distinction can be drawn between dead and living preparations

Steps needed to make and study a histological section

1. The first step in preparation of a tissue is **FIXATION**.

Fixation can be done by chemical or, less frequently, physical methods. In chemical fixation, the tissues are usually immersed in solutions of stabilizing or cross-linking agents called **fixatives.** The fixative should accomplish several objectives:

- Penetrate quickly, preventing postmortem changes (autolytic, bacterial).
- Render the protoplasm insoluble and harden it.
- Increase the affinity of protoplasm for future stains.



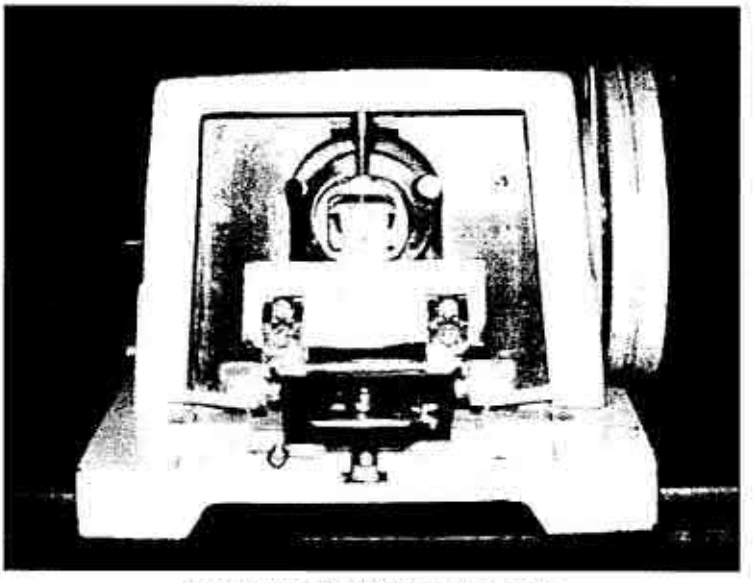
Schematic representation of fixation

Typical reagents are alcohol, formalin, osmic acid (for electron microscopy), etc. One of the best fixatives for routine light microscopy is 10% formalin. This substance cross-links proteins, thus maintaining a life-like image of the tissue.

Because the fixative needs some time to fully diffuse into the tissues, the tissues are usually cut into small fragments before fixation to facilitate the penetration of the fixative and to guarantee preservation of the tissue. Intravascular perfusion of fixatives can be used. Because the fixative in this case rapidly reaches the tissues through the blood vessels, fixation is greatly improved. The fixation takes from 2 to 24 hours.

2. In the second step the specimen is prepared for **EMBEDDING** in paraffin to permit sectioning. This is accomplished by:
   - Washing the specimen after fixation.
   - Because a large fraction of the tissue is composed of water is necessary to dehydrate the tissue. Dehydrating it in a series of alcohol solutions of ascending concentrations (70%, 80%, 90%, and 96% up to 100%). The alcohol helps remove the water.
   - Organic solvents such as xylol or toluol, chloroform, which are miscible in both alcohol and paraffin, are then used to remove the alcohol to permit to infiltration of the specimen with melted paraffin. Paraffin is common used for light microscopy; resins are used for both light and electron microscopy.
   - As the tissues are infiltrated with the solvent, they generally become transparent. Once the tissue is impregnated with the solvent, it is placed in melted paraffin in the oven, typically at 58–60°C. The heat causes the solvent to evaporate, and the spaces within the tissues become filled with paraffin. When the melted paraffin is cooled and hardened it is trimmed into small-sized paraffin blocks, which contain the tissue.
   - Then these blocks are cut with a steel knife in a special machine – called MICROTOME. For light microscopy the thickness of each section is about 5 to 10 μm. The resulting sections are then mounted on glass slides with albumin used as an adhesive.

A completely different way to prepare tissue sections is to submit the tissues to **rapid freezing.** In this process, the tissue is fixed by freezing (physically, not chemically) and it becomes hard and thus ready to be easily sectioned. A freezing microtome–the **cryostat** (Gr. *kryos,* cold, + *statos,* standing)–has been devised to section the frozen tissues. Because this method allows stained sections to be prepared rapidly (within a few minutes), it is routinely used in hospitals to study

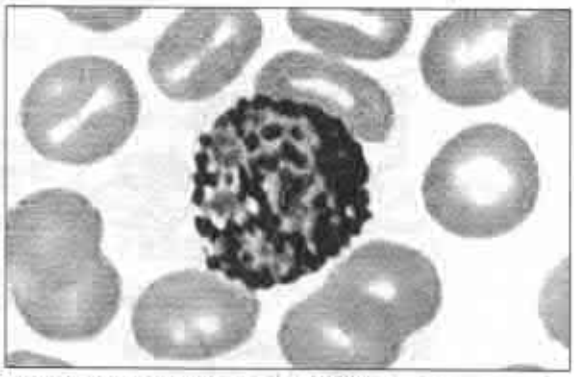


Microtome for light microscopy

specimens during surgical procedures. Freezing of tissues is also effective in the histochemical study of very fragile enzymes or small molecules, since freezing does not inactivate most enzymes. Because immersion of tissues in solvents such as xylene dissolves the tissue lipids, the use of frozen sections is advised when these compounds are to be studied.

3. The next step – the specimen is **STAINED** to permit examination.
   - With few exceptions, most tissues are colorless, so observing them unstained in the light microscope is useless. Methods of staining tissues have therefore been devised that not only makes the various tissue components more visible but also permit distinctions to be made between them. The dyes stain tissue components more or less selectively. Most of these dyes behave like acidic or basic compounds and have a tendency to form electrostatic (salt) linkages with ionizable radicals of the tissues. Tissue components that stain more readily with basic dyes are termed **basophilic** (nuclear chromatin, nucleolus); those with an affinity for acid dyes are termed **acidophilic** (cytoplasm, collagen fibers, etc). Some constituents may attract both dyes, such constituents are said to be **neutrophilic.**
   - Most of these dyes are water – soluble staining agents. Before the staining the paraffin from the section must be removed, after – the tissue is rehydrated and then stained. After staining the section is again dehydrated.

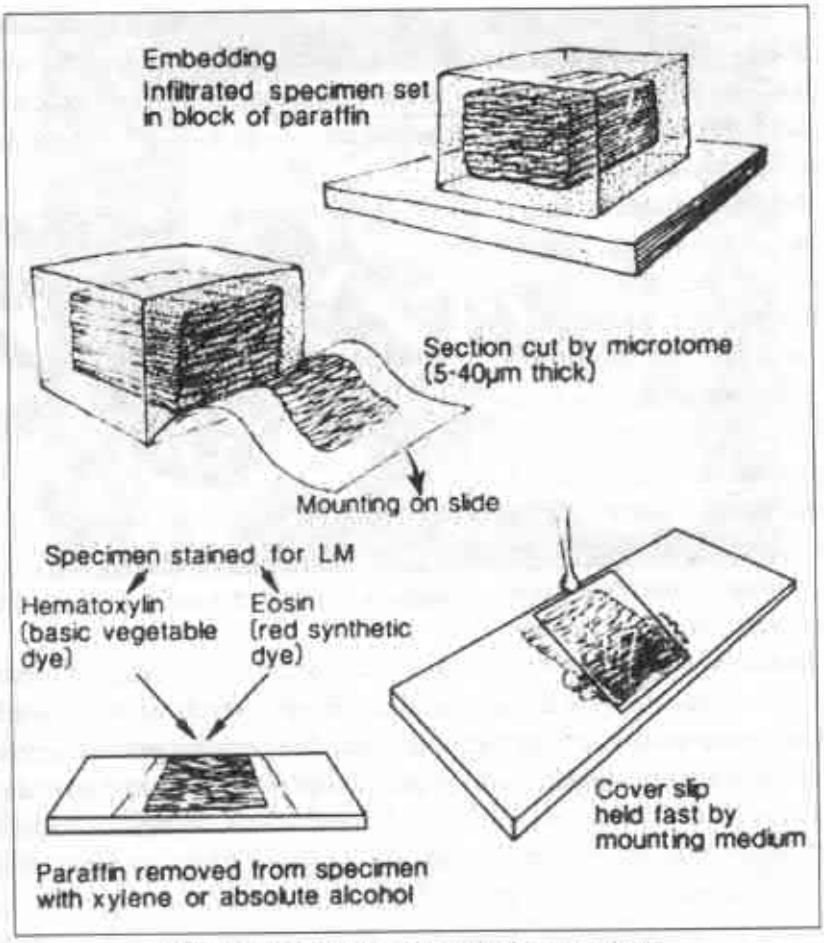
- The most common staining combination is **hematoxylin and eosin (H&E).** Hematoxylin stains the cell nucleus and other acidic structures (such as RNA-rich portions of the cytoplasm and the matrix of hyaline cartilage) with a blue hue. In contrast, eosin stains the cytoplasm and collagen pink.
- **Metachromasia** – some basic dyes, e.g., toluidine blue, can change their color depending on their concentration. Toluidine blue is blue in diluted solutions but purple in concentrated solutions. Some tissue components, e.g. the cartilage matrix, have polyanions which cause the dye molecules to clump up (even though it is a diluted solution) and those components will stain purple (exhibit **metachromasia**) while other structures such as cell nuclei will stain blue (exhibit **orthochromasia**).



Light micrograph of a cell with metachromasia

- There are many **special dyes** that are also used to stain particular tissue components such as elastic fibers (orsein stain), collagen fibers (Masson trichrome stain, Alcian blue stain), reticular fibers and nervous tissue (silver impregnation), carbohydrates (periodic acid – Schiff stain), etc. These are *histochemical* stains that show certain molecular components in the tissue section. *Immunohistochemistry* uses antibodies for specific molecules; the process allows those molecules to be detected in the tissue sections.

4. The last step in preparations of slides is **MOUNTING**. Stained sections are placed on a slide in a sticky medium that eventually hardens. This material has the same refractive index as glass. The preparation is than covered with a thin wafer of glass. The coverslip not only protects the tissue from damage but also is necessary for viewing the section with the microscope. For electron microscopy, sections are placed onto a thin plastic film.



The main steps in preparation of the slide

The histological sections can be studied with the aid of the microscopes. There are several kinds of microscopes which are available for revealing structural details.

LIGHT MICROSCOPY

The light microscope uses a visible light source with a system of condenser lenses to send the light through the object being examined. The image of this object is then magnified by two sets of lenses, the objective and the eyepiece.

Objective × Ocular = Magnification

Total magnification is then the product of these two lens systems, e.g., 40 X 10 = 400. Tissue sections can be magnified up to **1,000X**. The maximum resolu-

tion or resolving power – the smallest separation of two closely-placed structures which still permits them to be distinguished as separate – is a measure of the detail that can be seen, and for the light microscope is about 0.25 μm. This limit of resolution is limited by the wavelength of the light. However powerful the lens, 0.25 μm cannot be improved upon.

$$d_o = \frac{0.61\,\lambda}{\eta\,(\sin\alpha)} \quad \text{(optical microscope)}$$

η = refractive index $\sin\alpha$ = numerical aperture

λ (violet light) = 380 nm	d_o = 0.172 um	(50,000 X improved resolution)
λ (electrons) = 0.005 nm (at 60 kv)	d_o = 0.003 nm	

Objects smaller than 0.25 μm (such as a membrane or a filament of actin) cannot be distinguished with this instrument. Likewise, two objects, such as two mitochondria or two lysosomes, will be seen as only one object if they are separated by less than 0.25 μm. The **quality of the image** – its clarity and richness of detail – depends on the microscope's resolving power. Magnification is of value only when accompanied by high resolution. The resolving power of a microscope depends mostly on the quality of its objective lens. The eyepiece lens enlarges only the image obtained by the objective; it does not improve resolution. For this reason, when comparing objectives of different magnifications, those that provide higher magnification also have higher resolving power.

The only way to improve resolving power is to reduce substantially the wavelength of the light. This is achieved by the electromagnetic beam of the electron microscope.

ELECTRON MICROSCOPY

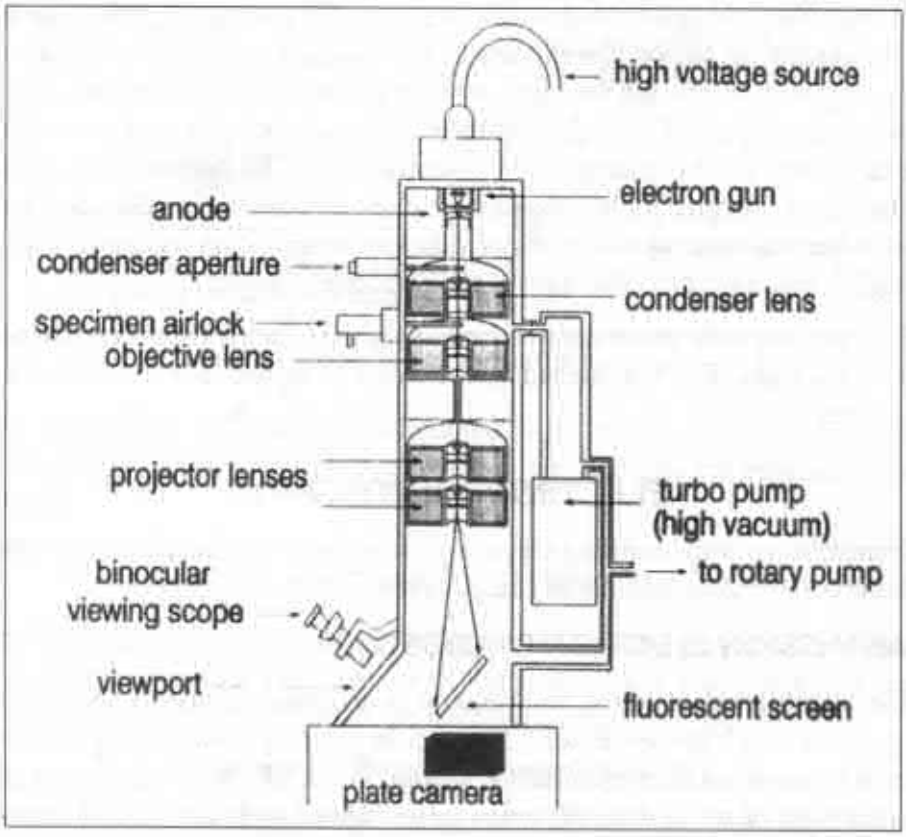
Transmission and scanning electron microscopes are based on the interaction between electrons and tissue components.

TRANSMISSION ELECTRON MICROSCOPY

The transmission electron microscope is an imaging system that theoretically permits very high resolution (0.1 nm). In practice, however, the resolution obtained by most good instruments is around 3 nm. This high resolution allows magnifications of up to 400,000 times to be viewed with detail. Unfortunately, this level of magnification applies only to isolated molecules or particles. Very

thin tissue sections can be observed with detail at magnifications of up to about 120,000 times.

The transmission electron microscope functions on the principle that a beam of electrons can be deflected by electromagnetic fields in a manner similar to light deflection in glass lenses. In the electron microscope, electrons are released by heating a very thin metallic (usually tungsten) filament (the cathode) in a vacuum. The electrons released are then submitted to a voltage difference of 60–120 kV between the cathode and the anode, which is a metallic plate with a hole in its center. Electrons are thus attracted to the anode and accelerated to high speeds. They pass through the central opening in the anode, forming a constant stream (or beam) of electrons that penetrates the tube of the microscope. The beam passes inside electric coils and is deflected in a way roughly analogous to what occurs in optical lenses, because electrons change their path when submitted to electromagnetic fields. For this reason, the electric coils of electron microscopes are called electromagnetic lenses.

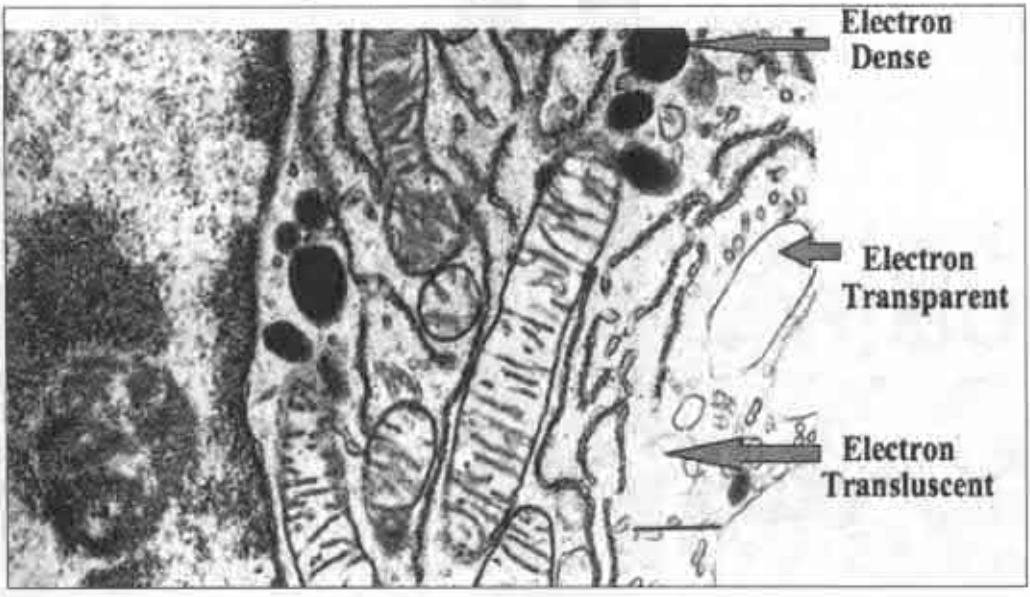


Schematic view of transmission electron microscope

The configuration of the electron microscope is very similar to that of the optical microscope, although the optics of the electron microscope are usually placed upside down. The first lens is a condenser that focuses the beam of electrons on the section. Some electrons interact with atoms of the section and continue their course, whereas others simply cross the specimen without interacting. Most electrons reach the objective lens, which forms a magnified image that is then projected through other magnifying lenses. Because the human eye is not sensitive to electrons, the image is finally projected on a fluorescent screen or is registered by photographic plates or a charged coupled device camera.

Because most of the image in the transmission electron microscope is produced by the balance between the electrons that hit the fluorescent screen (or the photographic plate) and the electrons that are retained in the tube of the microscope, the resulting image is always in black and white. Dark areas of an electron micrograph are usually called electron dense, whereas light areas are called electron lucent.

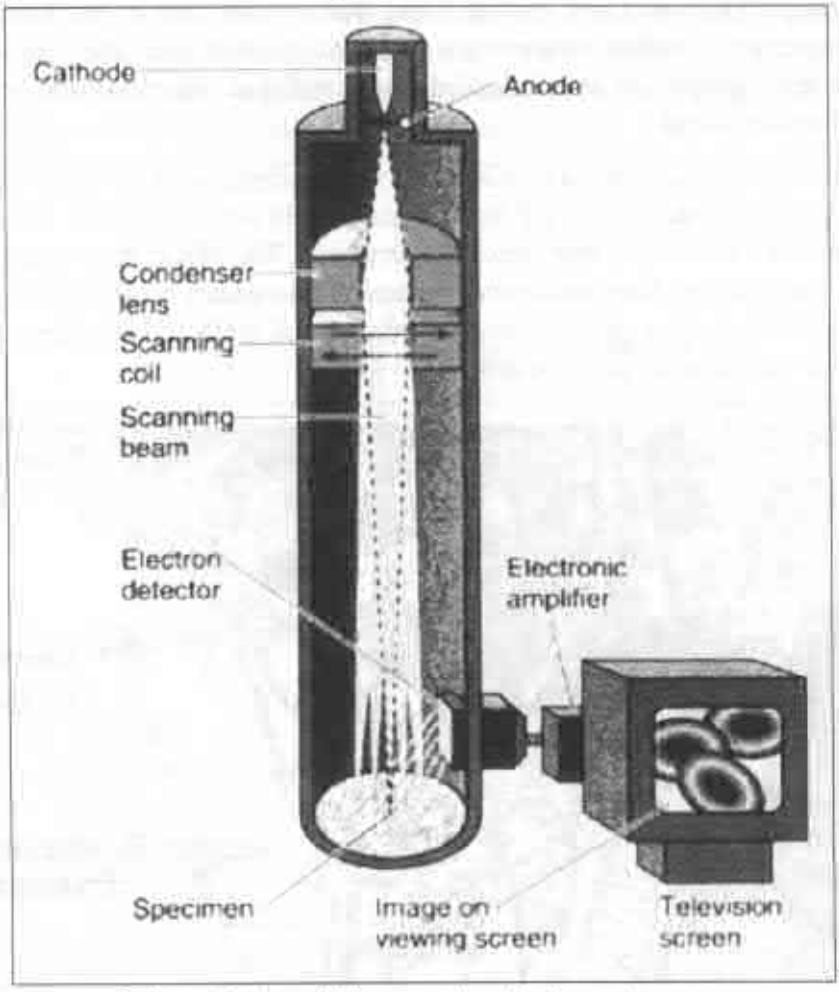
To provide a good interaction between the specimen and the electrons, electron microscopy requires very thin sections (40–90 nm); therefore, embedding is performed with a resin that becomes very hard. The blocks thus obtained are so hard that glass or diamond knives are usually necessary to section them. The extremely thin sections are collected on small metal grids and transferred to the interior of the microscope to be analyzed.



TEM electron micrograph

SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy permits pseudo-three-dimensional views of the surfaces of cells, tissues, and organs. This electron microscope produces a very narrow electron beam that is moved sequentially (scanned) from point to point across the specimen. Unlike the electrons in the transmission electron microscope, those in the scanning electron microscope do not pass through the specimen. The electron beam interacts with a very thin metal coating previously applied to the specimen and produces reflected or emitted electrons. These electrons are captured by a detector that transmits them to amplifiers and other devices so that in the end the signal is projected into a cathode ray tube (a monitor), resulting in a black-and-white image.



Schematic view of the scanning electron microscope

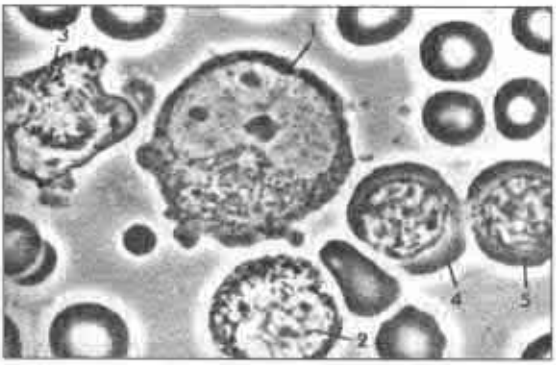
The resulting photographs are easily understood, since they present a view that appears to be illuminated from above, just as our ordinary macroscopic world is filled with highlights and shadows caused by illumination from above. The scanning electron microscope shows only surface views. The inside of organs can be analyzed by freezing the organs and fracturing them to expose their internal surfaces.

Scanning electron images of epithelium of the trachea and glomerulus of the kidney

PHASE-CONTRAST MICROSCOPY and DIFFERENTIAL INTERFERENCE MICROSCOPY

Some optical arrangements allow the observation of unstained cells and tissue sections. Unstained biological specimens are usually transparent and difficult to view in detail, since all parts of the specimen have almost the same optical density. Phase-contrast microscopy, however, uses a lens system that produces visible images from transparent objects.

Phase-contrast microscopy is based on the principle that light changes speed when passing through cellular and extracellular structures with different refractive indices. These changes are used by the phase-contrast system to cause the structures to appear lighter or darker relative to each other, which makes this kind of microscopy a powerful tool with



Phase-contrast image of bone marrow cells

which to observe living cells. Another way to observe unstained cells or tissue sections is differential interference microscopy, which produces an apparently three-dimensional image.

POLARIZING MICROSCOPY

Polarizing microscopy allows structures made of highly organized molecules to be recognized. When normal light passes through a **polarizing filter**, it exits vibrating in only one direction. If a second filter is placed in the microscope above the first one, with its main axis perpendicular to the first filter, no light passes through. If, however, tissue structures containing oriented molecules (such as cellulose, collagen, microtubules, and microfilaments) are located between the two polarizing filters, their repetitive, oriented molecular structure rotates the axis of the light emerging from the polarizer. Consequently, they appear as bright structures against a dark background. The ability to rotate the direction of vibration of polarized light is called **birefringence** and is a feature of crystalline substances or substances containing highly oriented molecules.

CONFOCAL MICROSCOPY

The depth of focus in the regular light microscope is relatively long, especially when small magnification objectives are used. This means that a rather wide extent of the specimen is seen in focus simultaneously, causing superimposition of the image of a three-dimensional object. With confocal microscopy, on the other hand, only a very thin plane of the specimen is seen in focus at one time. There are two principles on which this is based:

1. the specimen is illuminated by a very small beam of light (whereas in the common light microscope, a large beam of light floods the specimen)
2. the image collected from the specimen must pass through a small pinhole. The result is that only the image originating from the focused plane reaches the detector whereas the images in front of and behind this plane are blocked. The harmful glare of the out-of-focus objects is lost, and the definition of the focused object becomes better and allows the localization of any specimen component with much greater precision than in the common light microscope.

For practical reasons, the following arrangement is used in most confocal microscopes: (1) the illumination is provided by a laser source; (2) because it is a very small point, it must be moved over the specimen (scanned) to allow the observation of a larger area of the specimen; (3) the component of the specimen

that is of interest must be labeled with a fluorescent molecule (meaning that a routine section cannot be studied); (4) the light that is reflected by the specimen is used to form an image; (5) because the reflected light is captured by a detector, the signal can be electronically enhanced to be seen in a monitor. Because only a very thin focal plane (also called an optical section) is focused at a time, it is possible to reunite several focused planes of one specimen and reconstruct them into a three-dimensional image. To accomplish the reconstruction and many of its other features, the confocal microscope depends on heavy computing capacity.

FLUORESCENCE MICROSCOPY

When certain substances are irradiated by light of a certain wavelength, they emit light with a longer wavelength. This phenomenon is called fluorescence. In fluorescence microscopy, tissue sections are irradiated with either ultraviolet (UV) light or laser, and the emission is in the visible portion of the spectrum.

The fluorescent substances appear brilliant or colored on a dark background. Fluorescent compounds that have an affinity for cell macromolecules may be used as fluorescent stains. Acridine orange, which can combine with DNA and RNA, is an example. When observed in the fluorescence microscope, the DNA–acridine orange complex emits a yellowish-green light, and the RNA–acridine orange complex emits a reddish-orange light. It is thus possible to identify and localize nucleic acids in the cells *(see fig. 1, plate I)*.

Fluorescence microscope

Another important application of fluorescence microscopy is achieved by coupling fluorescent substances to molecules that will specifically bind to components of the tissues and will thus allow the identification of these components under the microscope.

HISTOCHEMISTRY and CYTOCHEMISTRY

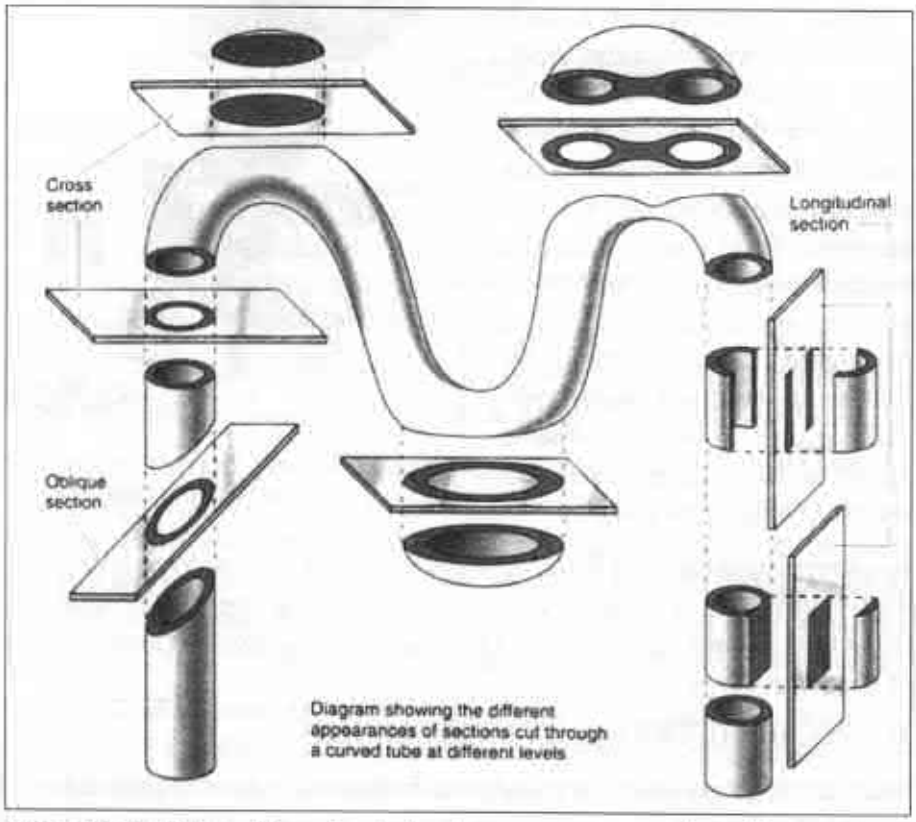
The terms **histochemistry** and **cytochemistry** are used to indicate methods for localizing substances in tissue sections. Several procedures are used to obtain

this type of information, most of them based on specific chemical reactions or on high-affinity interactions between macromolecules. These methods usually produce insoluble colored or electron-dense compounds that enable the localization of specific substances by means of light or electron microscopy.

PROBLEMS IN THE INTERPRETATION OF TISSUE SECTIONS

3-dimensional interpretation of 2-dimensional tissue sections:

When a three-dimensional volume is cut into very thin sections, the sections seem to have only two dimensions: length and width. This often leads observers to err if they do not realize that a sectioned ball looks like a circle and that a sectioned tube looks like a ring. When a section is observed under the microscope, the student must always imagine that something may be missing in front of or behind that section, because many structures are thicker than the section. It must also be remembered that the structures within a tissue are usually sectioned randomly.



How different three-dimensional structures may appear when thin sectioned

Artefacts (appearances not due to the original nature of the material as obtained from the body) can arise at all stages in the treatment of the section. Gross examples arise from: (l) clumsy excision from the body; (2) poor or inappropriate fixation; (3) shrinkage and, worse, uneven shrinkage, leading to artificial spaces and distorted relations; (4) cutting scores from a bad microtome knife; (5) the section not flat on the slide; (6) water, dirt or bubbles on or in the section; (7) dirt on the microscope lenses; (8) patchy or faded staining; unbalanced staining when more than one stain has been applied; (9) precipitate from fixative or stain; (l0) tears and folds in the section.

PART I

CYTOLOGY

CELL THEORY

People have been studying cells for the last 300 years. Discoveries made have only been possible with the development of new technologies. One of which came in the form of improved microscopic design.

In the mid 1600s, **Anton van Leewenhoek** created the first microscope.

Robert Hooke discovered cells. When observing cork with a simple microscope, he named the tiny rectangular boxes he saw, CELLS.

In the next 200 years, higher quality lenses were developed improving magnification and clarity. In 1838, Matthias Scheiden after studying plant structure concluded that all plants are composed of cells. In 1839, Theodor Schwann found that animal tissue looked similar to plant tissue; therefore concluded it too was also made up of cells. Some years later Robert Brown discovered an object near the center of many cells, now called the nucleus. In 1858, Rudolf Virchow stated that all cells come from other living cells.

All of the above scientists helped create what is called the CELL THEORY

FORMULATION OF THE CELL THEORY

CLASSICAL interpretation:

- All organisms are made up of one or more cells.
- Cells are the fundamental functional and structural unit of life.
- All cells come from pre-existing cells.
- The cell is the unit of structure, physiology, and organization in living things.
- The cell retains a dual existence as a distinct entity and a building block in the construction of organisms.

Ultrastructural research and modern molecular biology have added many tenets to the cell theory, but it remains as the preeminent theory of biology. The Cell Theory is to Biology as Atomic Theory is to Physics.

MODERN interpretation:

The generally accepted parts of modern cell theory include:

- The cell is the fundamental unit of structure and function in living things.
- All cells come from pre-existing cells by division.

- Energy flow (metabolism and biochemistry) occurs within cells.
- Cells contain hereditary information (DNA) which is passed from cell to cell during cell division
- All cells are basically the same in chemical composition.
- All known living things are made up of cells.
- Some organisms are unicellular, i.e., made up of only one cell.
- Others are multicellular, composed of a number of cells.
- The activity of an organism depends on the total activity of independent cells.

Cells are the structural units of all living organisms. In the human body there are about 200 types of cells, but they differ from one another by:

- shape
- size
- function

Because the body experiences considerable environmental diversity (eg, normal and pathological conditions), the same cell type can exhibit different characteristics and behaviors in different regions and circumstances. Thus, macrophages and neutrophils (both of which are phagocytic defense cells) will shift from oxidative metabolism to glycolysis in an anoxic, inflammatory environment.

Cells that appear to be structurally similar may react in different ways because they have different families of receptors for signaling molecules (such as hormones and extracellular matrix macromolecules). For example, because of their diverse library of receptors, breast fibroblasts and uterine smooth muscle cells are exceptionally sensitive to female sex hormones.

Each cell is composed of next major compartments:

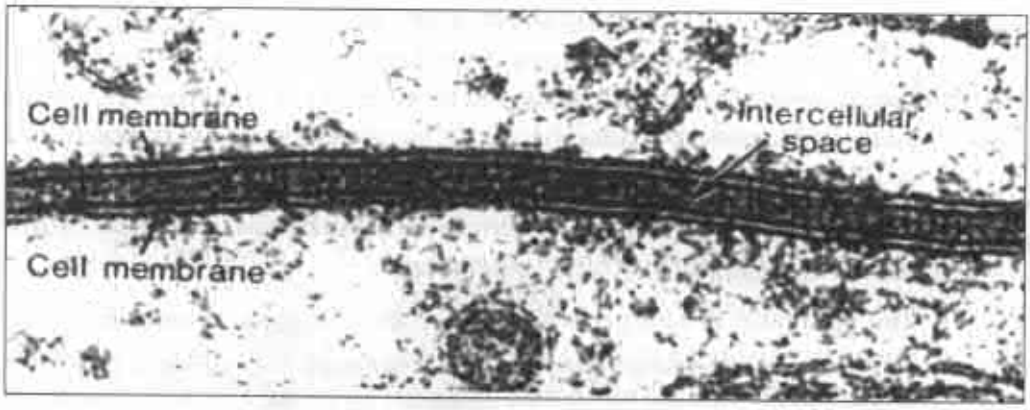
- **Cell membrane** (cytolemma)
- **Cytoplasm**
- **Nucleus**

CHAPTER I

CELL MEMBRANE

Cell membrane:

- is not visible with the light microscope.
- in electron microscope it appears as a trilaminar structure:
  - Two thin, dense lines with an intermediate light area.



The electron micrograph of the cell membrane

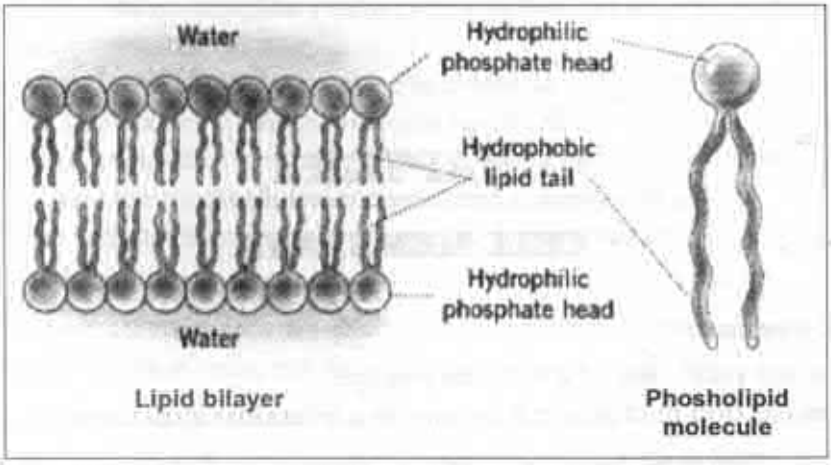
The basis of the cell membrane is formed by the **ELEMENTARY BIOLOGICAL MEMBRANE.** There are several types of elementary biological membranes.

- **PLASMALEMMA** – is the skeleton of the cell membrane.
- **ENDOMEMBRANES** – are membranes of cell organelles.
- **SPECIAL MEMBRANES** – are membranes of nerve fibers and photoreceptor cells.

ELEMENTARY BIOLOGICAL MEMBRANE is composed of:

1. **phospholipids bilayer**
2. **associated proteins**

The phospholipid bilayer is the fluid portion of the membrane.



Scheme of the phospholipids bilayer showing the structure of phospholipids molecule

- Each phospholipid molecule of the lipid bilayer is composed of a polar head (hydrophilic portion)ƒ located at the surface of the membrane, and two long highly hydrophobic and non-polar fatty acyl tails.
- The tail of the phospholipids orient towards each other creating a hydrophobic environment within the membrane.
- The lipid layer is semipermeable, meaning that some molecules are allowed to pass freely through the membrane. The lipid bilayer is virtually impermeable to large molecules, relatively impermeable to molecules as small as charged ions, and quite permeable to lipid soluble low molecular weight molecules.

Most plasma membranes consist of about 50% lipid and 50% protein.

– Principal membrane lipids are:
  - Glycolipids
  - Cholesterol
  - Phospholipids

<u>Cholesterol</u> – a rigid ring structure, which is inserted into the phospholipid bilayer. Functions:

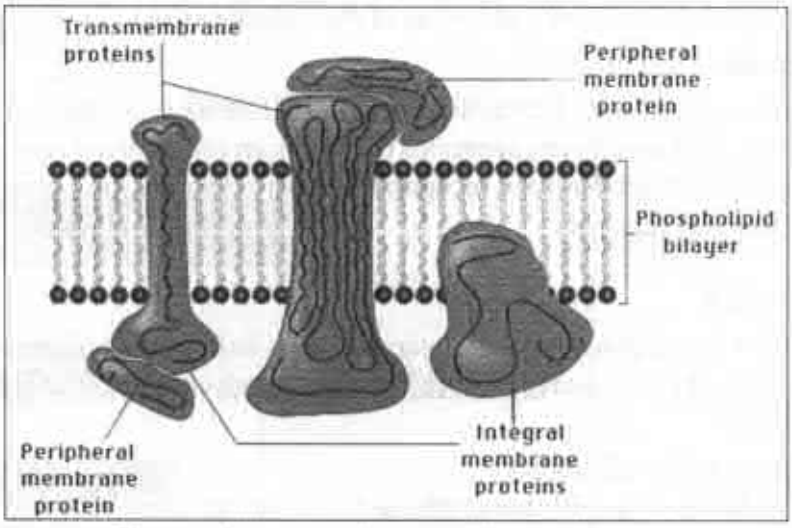
- To modulate membrane fluidity.
- Adds strength.

- Adds flexibility.
- Affects fluidity.

Two major classes of membrane associated **proteins** are recognized (according with their **position**):

1. **Integral proteins** (transmembrane and partial-integral)
2. **Peripheral proteins** (outer and inner)

- Proteins which are attached to the surface of the cell membrane are called **PERIPHERAL PROTEINS**, located on both the Internal and External Surface.
- The Proteins that are embedded in the lipid bilayer are called **INTEGRAL PROTEINS**.
- Some integral proteins extend over the entire cell membrane and are exposed to both the inside of the cell and the exterior environment (**transmembrane proteins**). Others extend only to the inside or only to the exterior surface (**integral membrane proteins**).
- The extracellular portion of integral and peripheral membrane proteins are generally glycosylated. The intracellular portion of membrane proteins are bound to cytoskeletal components.



The membrane proteins according their position

Membrane associated proteins can be classified by their **function** into:

- **Structural**
- **Protein-enzymes**

- **Receptor** proteins – bind molecules and trigger cellular responses
- **Transport** proteins – for passage of materials through the plasma membrane (channel vs. carrier proteins).

Fluid-mosaic model of membrane structure

1. Membranes are FLUID and have the consistency of vegetable oil.
2. The Lipids and Proteins of the Cell Membrane are always in motion.
3. Phospholipids are able to drift across the membrane, changing places with their neighbour.
4. Proteins in and on the membrane form PATTERNS, or MOSAICS.
5. Because the membrane is FLUID with a MOSAIC of proteins, scientists call the modern view of membrane structure THE FLUID MOSAIC MODEL.
6. The pattern or "Mosaic" of lipids and proteins in the cell membrane is constantly changing.

CELL MEMBRANE components are:

1. cell surface markers – **glycocalyx** (proteoglycans, glycolipids, glycoproteins).
2. Elementary biological membrane type **plasmalemma**, that consists of phospholipid bilayer and transmembrane (integral) and peripheral proteins.
3. interior protein network – elements of the **cytoskeleton.**

Functions of cell membrane:

- Act as selective barrier between the cytoplasm and the external environment. Cell membrane separates the cell from the external environment.
- Receptor functions (can recognize and connect with special substance).
- Transport function.

GLYCOCALYX

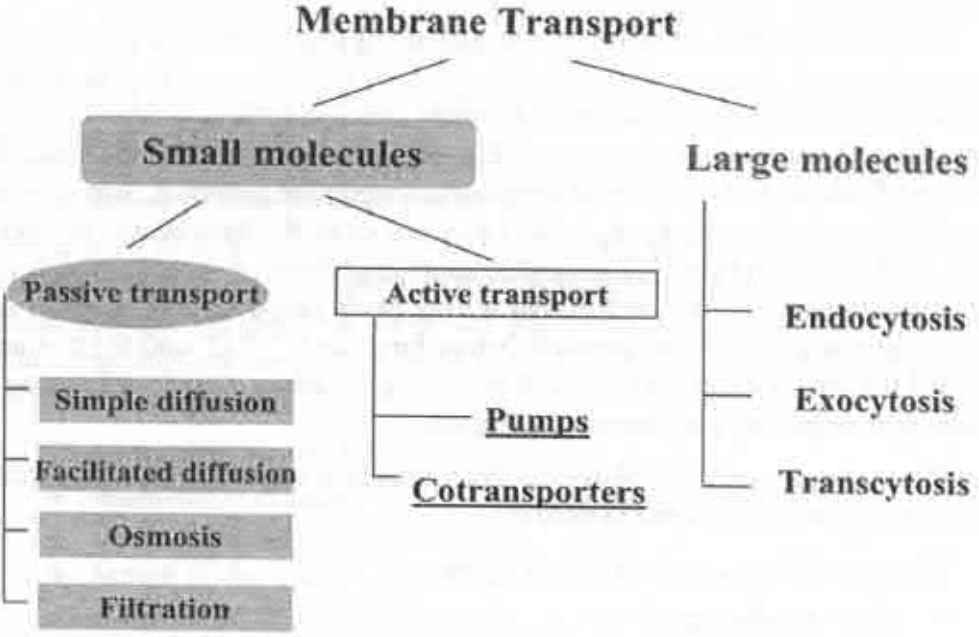
This layer is composed of carbohydrate chains linked to membrane proteins and lipids and of cell – secreted glycoproteins and glycolipids. The glycocalyx's role in the cell:

- Protects the cell surface.
- Facilitates cell-cell interaction, cell-cell recognition, receptor sites for hormones.
- Contributes to the establishment of extracellular microenvironments at the membrane surface that have specific functions in metabolism.
- Provides absolute individuality to the cell.
- Provides the formation of the **cytoreceptors**.

The carbohydrate moieties of glycoproteins and glycolipids project from the external surface of the plasma membrane; they are important components of specific molecules called **receptors** that participate in important interactions such as cell adhesion, recognition, and response to protein hormones. There are 2 groups **of cytoreceptors:**

- **For endogenous substances**
  - Neurotransmitters
  - Hormones
  - Complement
  - Immunoglobulins (antibodies)
  - Self antigens
- **For exogenous substances**
  - Viruses
  - Bacteria
  - Toxins
  - Drugs
  - Narcotics
  - Non-self antigens

MEMBRANE TRANSPORT is the transport of materials across the plasma membrane.



PASSIVE and ACTIVE transport

Passive transport is characterized:

- Movement of molecules down their concentration gradients.
- Requires no net energy expenditure.
- The gradients themselves provide energy

Active transport is characterized:

- Movement of molecules against their concentration gradients (molecules are transported from an area of low concentration to an area of high concentration)
- Requires energy! Energy is obtained from the enzymatic hydrolysis of one phosphate bond from an adenosine triphosphate molecule (ATP).

PASSIVE TRANSPORT. There are few types of passive transport:

1. Simple diffusion
2. Facilitated diffusion
3. Osmosis
4. Filtration

✶ Remember that no energy is required, and molecules move down their concentration gradients!

1. SIMPLE DIFFUSION

Simple diffusion is the random movement of particles from an area of high concentration to an area of low concentration to establish equilibrium. **Equilibrium** is the state of balance where the same concentration of solutes is found throughout the solution. In the case of cells, equilibrium means that the concentrations of solutes on both sides of the plasma membrane are equal. This type of transport is possible only for molecules that can cross the lipid bilayer on their own. As the cell membrane is composed mainly of a bilayer of phospholipid, lipid soluble molecules can diffuse through it very easily (ethyl alcohol, vitamin A, steroid hormones, etc). This is especially true for gases (O_2, CO_2 and N_2) that are all lipid soluble. In the cell, oxygen diffuses in while carbon dioxide diffuses out, down their respective concentration gradients.

Water, though not lipid soluble can diffuse easily as it is a very small molecule and literally cuts through the membrane.

The rate of this transport depends upon:

- Concentration gradient

- Size
- Lipid solubility

2. FACILITATED DIFFUSION

Some molecules diffuse through the cell membrane utilizing a protein carrier. In this situation, the protein carrier has a special affinity to the molecule. Once the molecule is bound to the protein, a conformational change (a change in shape) occurs in the protein carrier. Through this change, the bound molecule is released into the cytoplasm.

Special characteristics of these carriers include:

- **Specificity** (interact with specific molecules). The carrier molecules interact with specific molecules e.g. glucose carriers interact only with glucose, some amino acid carriers interact with one specific amino acid.
- **Competition** (can interact with more than one molecule). When a carrier molecule can interact with more than one molecule (e.g. a carrier molecule that can carry two different amino acids) the rate of transport of the amino acids is less when both are present than when one is present. This is because the amino acid molecules compete with each other for the special sits on the protein.
- **Saturation** (maximum occurs when all the interaction sites are fully occupied). As the concentration of the transported molecule is increased, so is its rate of diffusion but this up to a certain limit. Beyond this limit no further increase is observed.

Examples of facilitated diffusion are the intracellular diffusion of **glucose** and **amino acids** *(see fig.2, plate I)*.

Diffusion of Ions through channels

Due to their large size (all ions in solution are hydrated) as well as their charge, ions cannot diffuse through the cell membrane. Ions enter the cell membrane through ion channels composed of proteins that span the cell membrane. These channels are usually highly selective i.e. they will let through only one type of ion or a small number of ions.

This selectively is due the:

- **diameter** of channel
- internal **charge**
- **length** of channel
- **shape** of internal channel

Certain channels are gated. By gated, one means that the channels are either open or closed. These gates are either controlled through chemical signals (ligand gated) or by electrical changes (voltage gated). Though utilizing protein channels, the ions would still be diffusing down their concentration gradient and hence there is no energy expenditure *(see fig. 3, plate I)*.

3. **FILTRATION** – movement of water through a membrane from a region of higher hydrostatic pressure to region of lower hydrostatic pressure.

4. **OSMOSIS**

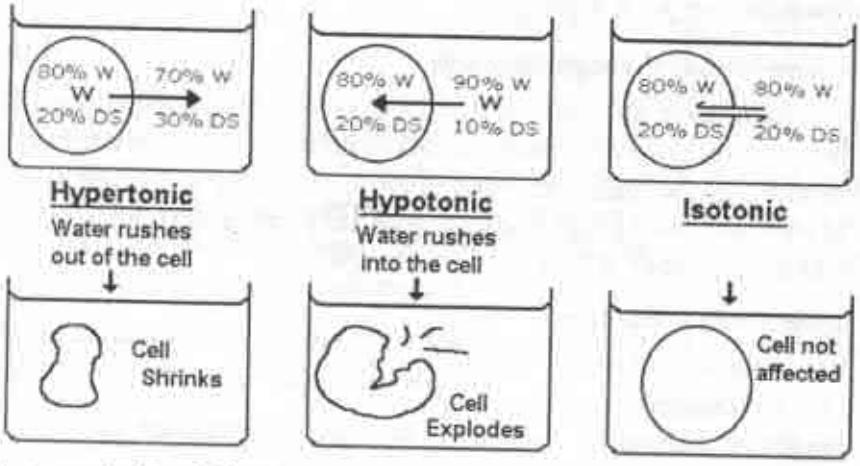
The osmosis is the diffusion of water through a selectively permeable membrane. Water diffuses faster than any other particles through the membrane. Thus, water tries to establish equilibrium across the membrane. Water will always diffuse across a membrane to where there is a higher concentration of solutes. Water attempts to dissolve them and establish equilibrium on both sides of the membrane.

Note:

- Osmotic pressure is directly proportional to total solute concentration. It is the number of particles of solute that is important – not the kind of solutes.
- Cells cannot stop the diffusion of water through membranes. As a result, the osmotic pressure puts stress on the membranes of the cell. This pressure on cell membranes can have disastrous effects.

So what happens to the cell in hypertonic, hypotonic, and isotonic solutions?

Remember: Water diffuses faster than dissolved substances, thus water will move to where there is a higher concentration of dissolved substances / solutes.



DS = % concentration of Dissolved Substances/solutes **W** = % concentration of Water

ACTIVE TRANSPORT

Active transport is the process of moving particles across a selectively permeable membrane from an area of low concentration to an area of high concentration. This is like pushing a bolder up-hill; it takes a lot of energy. It usually requires transport proteins to pump needed particles across the membrane.

SODIUM-POTASSIUM PUMP

Cells need to maintain lower Na+ concentration inside cell and higher K+ inside the cell. This phenomenon is crucial for transmission of nerve impulses; maintains osmotic conditions in other cells.

One very common pump and found in all the cells of the body is the sodium-potassium pump. This pump is made up of three protein molecules that span the cell membrane. It has three intracellular sites that have a high affinity to sodium and two extracellular sites that have a high affinity to potassium. It has also got ATPase activity i.e. it can act as an enzyme to hydrolyse one phosphate bond from an ATP molecule.

When both the sodium and potassium sites are full, the ATPase is activated and splits an ATP molecule into ADP, Phosphate group and energy. The energy is utilized to bring about a conformational change in the proteins whereas the sodium sites project into the extracellular space and the potassium sites are presented into the intracellular space. At the same time the energy releases these ions. Thus this pump removes three sodium ions from the intracellular space and exchanges them to two potassium ions.

This pump serves at least four purposes:

1. Due to the large number of sodium/potassium pumps in the body, it serves to regulate the metabolic rate of the individual especially through the thyroid hormone.
2. Produces a steep sodium gradient used to provide energy for the "coupled transport" of other molecules.
3. The sodium/potassium gradient across the membrane is partially responsible for the electrochemical potential across nerve and muscle membranes.
4. The extrusion of sodium is important to reduce the osmotic inflow of water into the cell.

Secondary Active Transport

Much of the active transport accomplished by a cell isn't directly powered by ATP. Instead, much active transport is powered by membrane potentials (i.e.,

electrochemical gradients). Such electrochemical-gradient-driven active transport is called **COTRANSPORT**. In cotransport, one substance, such as a sugar, is driven up its concentration gradient while a second substance, e.g., sodium ions or protons, are allowed to fall down their electrochemical gradient; the energy gained from the latter is employed to power the former (i.e., energy coupling).

How to explain cotransport?

Active transport involves the expenditure of energy to pump something across a membrane up its concentration gradient. That energy may be derived from ATP but that is not the only possible source. Another source is membrane potentials. That is, by pumping ions, a cell can set it up so that (typically) the interior of the cell has a net negative charge while the exterior has a net positive charge. This arrangement essentially represents a battery, i.e., it is a storage of potential energy. Allowing ions to cross the membrane by heading toward the side containing the net opposite charge allows the system to return to equilibrium.

Movement toward equilibrium is exergonic, i.e., energy is liberated. This energy can be used to do work, such as the transport of other substances up their concentration gradient. The coupling of these two reactions is termed cotransport. Another way of looking at this is that the ions waiting to cross the membrane are equivalent to water found at the top of a waterfall. As they cross the membrane they are equivalent to water going over a waterfall. When they reach the other side of the membrane they are equivalent to water found at the bottom of the waterfall. During movement over the waterfall, potential energy is converted to kinetic energy (by gravity in the waterfall; with membrane potentials this occurs via the attraction between opposite charges), which may be harnessed to do work. In the case of cotransport the work done is the movement of the cotransported substance across the membrane against its concentration gradient.

There are two types of cotransport:

- **antiport** – substances transported in opposite directions.
  - Example: Na+ – K+ pump
- **symport** – substances transported in the same direction.
  - Example: Na+ – glucose uptake by digestive tract cells.

UNIPORT – a single molecule moving in one direction *(see fig. 6, plate I)*.

BULK (vesicular) TRANSPORT – is a transport of large molecules

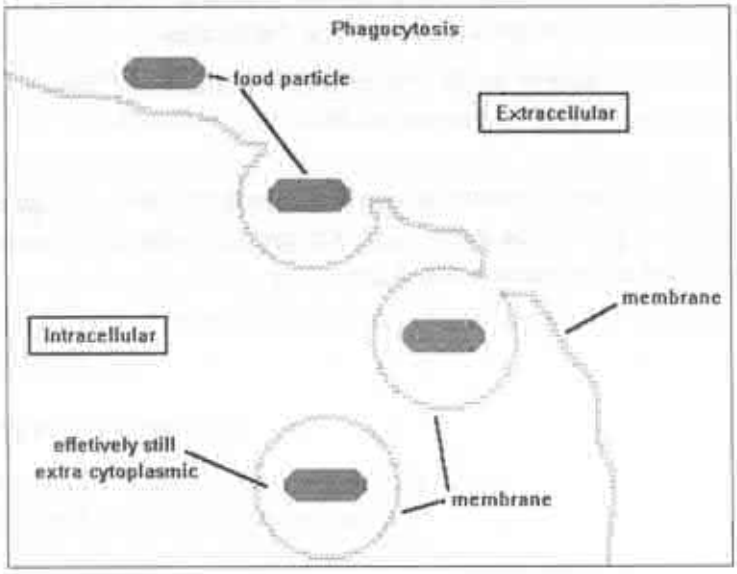
I. **ENDOCYTOSIS** – infolding of the plasma membrane to bring large materials into the cell. Types of endocytosis:

- **Pinocytosis**, "cell drinking" – extracellular fluid and materials suspended in it (water and solutes) are enclosed in invaginating vesicle. Frequently is used in digestive tract *(see fig. 4, plate I).*
- **Receptor-mediated endocytosis**

Receptors for many substances, such as low-density lipoproteins and protein hormones, are located at the cell surface. The receptors are either originally widely dispersed over the surface or aggregated in special regions called **coated pits.** Binding of the ligand (a molecule with high affinity for a receptor) to its receptor causes widely dispersed receptors to accumulate in coated pits. The coating on the cytoplasmic surface of the membrane is composed of several polypeptides, the major one being clathrin. These proteins form a lattice composed of pentagons and hexagons very similar in arrangement to the struts in a geodesic dome. The coated pit invaginates and pinches off from the cell membrane, forming a coated vesicle that carries the ligand and its receptor into the cell *(see fig. 5, plate I).*

The coated vesicles soon lose their clathrin coat and fuse with **endosomes.** The membrane of all endosomes contains ATP-driven H^+ pumps that acidify their interior. The clathrin molecules separated from the coated vesicles are moved back to the cell membrane to participate in the formation of new coated pits.

- **Phagocytosis** "cell eating" – brings large materials into a cell by wrapping extensions of the plasma membrane (pseudopodia "false feet") around the materials and fusing the extension together.



Schematic representation of phagocytosis

Certain cell types, such as macrophages and polymorphonuclear leukocytes, are specialized for incorporating and removing foreign bacteria, protozoa, fungi, damaged cells, and unneeded extracellular constituents. For example, after a bacterium becomes bound to the surface of a macrophage, cytoplasmic processes of the macrophage are extended and ultimately surround the bacterium. The edges of these processes fuse, enclosing the bacterium in an intracellular **phagosome.**

II. **EXOCYTOSIS** -is the term used to describe the fusion of a membrane-limited structure with the plasma membrane, resulting in the release of its contents into the extracellular space without compromising the integrity of the plasma membrane. A typical example is the release of stored products from secretory cells, such as those of the exocrine pancreas and the salivary glands.

The fusion of membranes in exocytosis is a complex process. Because cell membranes exhibit a high density of negative charges (phosphate residues of the phospholipids), membrane-covered structures coming close to each other will not fuse but will rather repel each other, unless specific interactions facilitate the fusion process. Consequently, exocytosis is mediated by a number of specific fusogenic proteins. Usually, Ca^{2+} regulates the process. An increase in cytosolic Ca^{2+} often triggers exocytosis.

III. **TRANSCYTOSIS** represents transport of macromolecules through the cell without stopping in it. This transport takes place in both directions. Pinocytotic vesicles can cross the cell in about 2-3 minutes.

- Can see this phenomenon in continuous capillaries of muscle, connective tissue, exocrine glands and nervous tissue, etc.

During endocytosis, portions of the cell membrane become an endocytotic vesicle; during exocytosis, the membrane is returned to the cell surface. This phenomenon is called **membrane trafficking.**

INTERCELLULAR JUNCTIONS

Cell junctions are symmetrical structures formed between two adjacent cells. In the intercellular junctions take part the glycocalyx and plasmalemma. **Intercellular junctions** are responsible for the adherence of adjoining cells; provide a barrier for the diffusion of substances between the cells; and allow for communication between cells.

These junctions can be classified into:

1. **SIMPLE**
   - linear-shaped
   - digital-shaped
2. **COMPOUND** – there are three major classes of compound cell junctions:
   - Occluding junctions
   - Anchoring junctions
   - Communicating junctions

OCCLUDING JUNCTIONS – also called tight junction. They are located at the most apical portion of the lateral cell surface. **Tight Junction** defines cell polarity.

Occluding junctions consist of a narrow band that completely encircles the cell (**zonula = belt**). The outer leaflets of the plasma membranes of adjoined cells appear to converge and fuse at multiple sites via transmembrane proteins (**occludins**). These fused areas are impermeable to water and solutes and serve to separate the apical from the basolateral environment of the cell. They act as seals to prevent the flow of substances between cells *(see fig. 7, plate I)*.

The "tightness" of these junctions varies with the epithelium, with those having the most complex strands of fused areas being the most impermeable (e.g. intestinal and urinary bladder epithelium). **ZO-1, ZO-2** and **ZO-3** proteins bind the cytoplasmic portion of the occludin protein; ZO-1 binds occludin to the actin cytoskeleton. Pathogenic agents such as **cholera toxin** act on ZO-1 and ZO-2 to cause junctional permeability.

ANCHORING JUNCTIONS

There are three classes of anchoring junctions:

- zonula adherens (belt desmosome)
- macula adherens (spot desmosome)
- hemidesmosome

Zonula adherens (pl. ***zonulae adherentes***) – an adhesive device that is located just beneath the zonula occludens. It, too, forms a circumferential belt around each epithelial cell. On the cytoplasmic side of the junction, a dense plaque is formed by the protein **catenin** and actin-binding proteins (**α-actinin and vinculin**) which bind to the ***actin filaments*** of the terminal web in the apical cytoplasm. There is an intercellular space of 15 – 20 nm that is filled with a transmembrane adhesion molecule (**E-cadherin**) that binds to **Ca^{++}**, which is essential to maintain junctional integrity *(see fig. 8, plate I).*

Macula adherens (pl. ***maculae adherentes***) is an adhesive device that is also referred to as a **desmosome**. It is a disc-shaped structure **(macula = spot)** which is typically located just below the zonula adherens, although several of these attachments may be found along the lateral surface, forming rows of "spot welds" between adjacent cells. On the cytoplasmic side of the plasma membrane is a dense **plaque** consisting of specialized proteins **(desmoplakins and plakoglobins)** into which 10 nm ***intermediate filaments*** ***(tonofilaments)*** are anchored. A desmosomal plaque on one cell will line up with a desmosomal plaque on its neighboring cell; the 20-30 nm intercellular space between the two desmosomes is filled by transmembrane adhesive glycoproteins called **desmocollins** and **desmogleins**. Since tonofilaments are important components of the epithelial cytoskeleton, desmosomes effectively link the cytoskeletons of neighboring cells, enabling an epithelial sheet to distribute mechanical stress without losing its shape or its functional integrity *(see fig. 9, plate I).*

They are commonly present in epithelial tissue because it undergoes the mechanical stress and abrasion, e.g. epidermis of the skin. Is a spot like junction associated with intermediate filaments extending from the one spot to another on the lateral and basal surface.

Hemidesmosomes are half desmosomes. They are asymmetrical structures anchoring the basal domain of an epithelial cell to the underlying basal lamina via integrins. Hemidesmosomes contributes to overall stability of epithelia. They consist of:

- **a cytoplasmic plate associated with intermediate filaments (tonofilaments).**
- **membrane plaque linking hemidesmosome to basal lamina via anchoring filaments.**

*NOTE: The **zonula occludens, zonula adherens and macula adherens** are collectively called the* **JUNCTIONAL COMPLEX.**

GAP JUNCTION allow for **communication** between neighboring epithelial cells. They can occur anywhere along the lateral membranes of epithelial cells. In a gap junction, the adjacent cell membranes are separated by only 2 nm, and this gap is bridged at intervals by functional units known as **connexons** *(see fig. 10, plate I)*.

Each connexon is formed by a hexagonal group of 6 individual **connexin protein** units in each of the apposing cell membranes. A hydrophilic pore, 1.5 nm in diameter, is located in the center of each connexon. Connexons of adjacent cells align with each other to allow the exchange of ions, cyclic AMP or GMP and some hormones (<1500 molecular weight) between cells, which allows them to function in a coordinated manner. High intracellular Ca^{++} concentrations cause closure of gap junctions – the mechanism unknown!

Gap junction, also, transmits electrical activity between cardiac and smooth muscle cells; allows chemical messengers to cross from one cell to another, in such way coordinates activities between cells.

CHAPTER II

CYTOPLASM

Cytoplasm is the cellular region between cell membrane and the membrane surrounding the nucleus. The cytoplasm is necessary for maintaining cellular shape and consistency and for providing suspension to organelles. The cytoplasm has three main functions: energy, storage and manufacturing. It's also a storage area for life-supporting chemicals. The cytoplasm contains:

- **Cytosol** – intracellular fluid containing dissolved nutrients, ions, soluble, and insoluble proteins, and waste products.
- **Organelles** – specialized structures that perform specific functions related to the cell structure, growth, maintenance, and metabolism (except the nucleus).
- **Cytoskeleton** – network of filaments and fibers.
- **Inclusions** (storage substances – for example, fat droplets or glycogen).

CYTOSOL (cytoplasmic matrix)

The part of the cytoplasm that is not held within organelles is called the cytosol. It is a jelly-like material that is eighty percent water and usually clear in color. The fluid of the cytosol is a thick soup of proteins, carbohydrates, salts, sugars, lipids, nucleotides, and amino acids.

- It behaves like a gel sometimes, depending on the activity phase of the cell; in this state, it is called cytogel. When instead it behaves like a liquid, it's called cytosol. Most cells have cytoplasm behaving both ways, with the gel-like portions on the outer rims of the cell.

The cytosol represents the site where metabolic reactions occur.

ORGANELLES are small specialized structures for particular functions.

They are divided into:

- **Organelles of GENERAL importance** – are organelles that are obligatory contents of all cells (mitochondrion, ribosome, apparatus Golgi, etc).

- **Organelles of SPECIAL importance** – are organelles that are present in some cells (for example, **microvilli** on the apical surface of cells that are responsible for absorption, **cilia** on the apical surface of columnar ciliated cells in the respiratory system, **flagellum** and **acrosome** of spermatozoon).

Other classifications:

I. **Structural classification.**
   - **Nonmembranous organelles** are not enclosed by a membrane and are always in touch with the cytosol. Nonmembranous organelles include: cytoskeleton (microtubules, microfilaments, intermediate filaments), microvilli, centrioles, cilia, ribosomes, flagella.
   - **Membranous organelles** are surrounded by endomembranes. They include: endoplasmic reticulum, Golgi apparatus, lysosomes, peroxisomes, mitochondria.

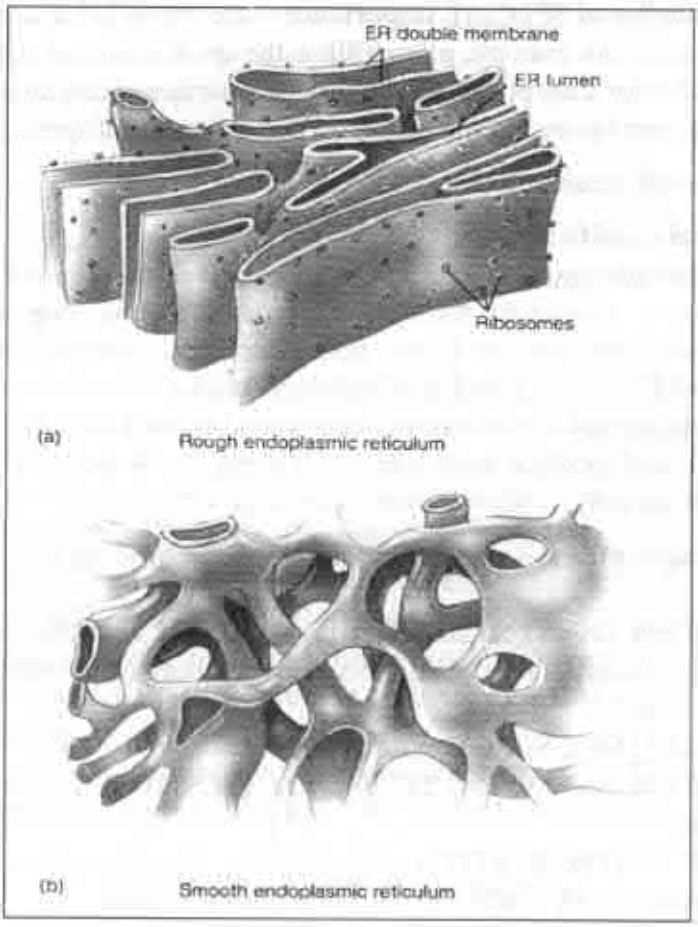
II. **According to the modern world view** the cytoplasmic organelles are divided into:
   - **SYSTEM OF SYNTHESIS**: rough-surfaced endoplasmic reticulum (rER), smooth-surfaced endoplasmic reticulum (sER), ribosomes, Golgi apparatus.
   - **SYSTEM OF ENERGY**: mitocondria.
   - **SYSTEM OF INTRACELLULAR DIGESTION**: lysosomes, peroxisomes.
   - **THE CYTOSKELETON**: microtubules, microfilaments, intermediate filaments, centrioles.

ENDOPLASMIC RETICULUM (ER) = *(Endoplasmic=within the cytoplasm; reticulum = network)*

The endoplasmic reticulum is an extensive membranous network of tubules and sacs (cisternae) which sequesters its internal lumen (cisternal space) from the cytosol. Continuous with the outer membrane of the nuclear envelope; therefore, the space between the membranes of the nuclear envelope is continuous with cisternal space.

There are two distinct regions of ER that differ in structure and function: **smooth ER** and **rough ER**.

- **Rough ER** – studded with ribosomes that are attached to cytosolic side of rER.
- ***Smooth ER***- has no ribosomes.



Schematic representations of a small portion of the rough endoplasmic reticulum (a) and smooth endoplasmic reticulum (b)

ROUGH ENDOPLASMIC RETICULUM

- Modifies proteins produced by the ribosomes (the ribosomes on the rough ER make the proteins with a specific amino acid sequence that allows it to enter the inner ER before it obtains its tertiary structure). Because the proteins cannot travel through the lumen in its 3-D shape, it is pushed out into the smooth ER. Areas of the ER membrane break off to form a sac around the protein called a vesicle. A vesicle is a transport vehicle for the protein. Because most proteins are not mature when they leave the lumen, the vesicles take them to a Golgi complex or other cellular organelle.

- Synthesis of proteins for export from the cell, integral proteins of cell membrane, enzymes of lysosomes.

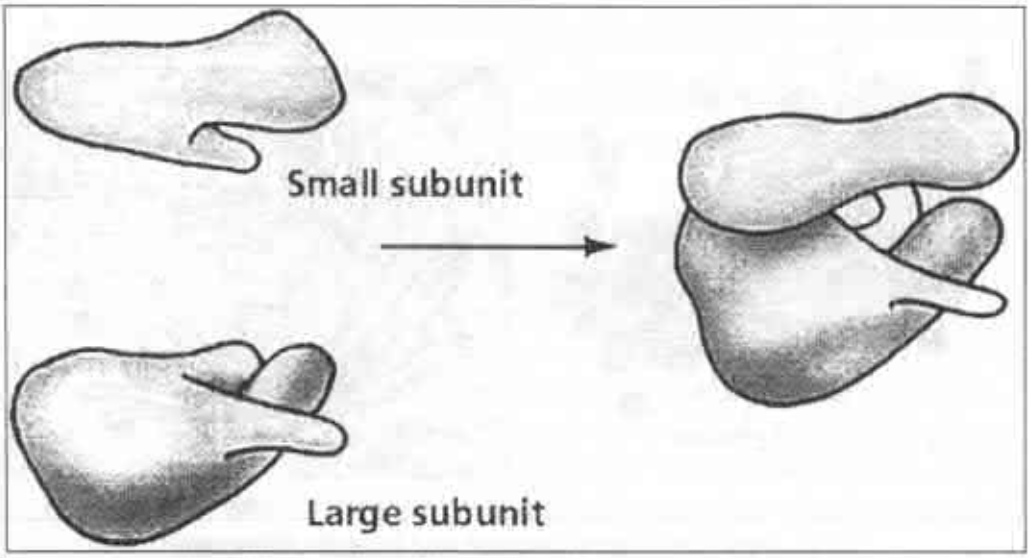
SMOOTH ENDOPLASMIC RETICULUM

- Synthesizes lipids, phospholipids and steroids. For example: sex hormones and steroids secreted by the adrenal gland.
- Participates in carbohydrate metabolism (site for hydrolysis of glycogen).
- Detoxifies drugs and poisons (contains enzymes that help detoxify).
- Stores calcium ions necessary for muscle contraction. In a muscle cell, the ER membrane pumps Ca++ from the cytosol into the cisternal space.

RIBOSOMES

Ribosomes are small, dark-staining structures composed of ribosomal RNA (rRNA) and proteins. They are produced in the nucleolus. Ribosomal proteins, made in the cytoplasm, enter the nucleus, join with the rRNA, and then move through the nuclear pores to the cytoplasm where they are assembled into ribosomes.

Each ribosome contains a small and large subunit that fit together like the body and cap of an acorn. These subunits are joined in the cytoplasm only when they attach to mRNA. Several ribosomes are connected to mRNA are called **POLYSOMES.**



Schematic drawing of small and large subunits of the ribosome

Function of ribosomes is to produce proteins by assembling amino acids according to instructions from RNA (they are the site where DNA code is translated into proteins).

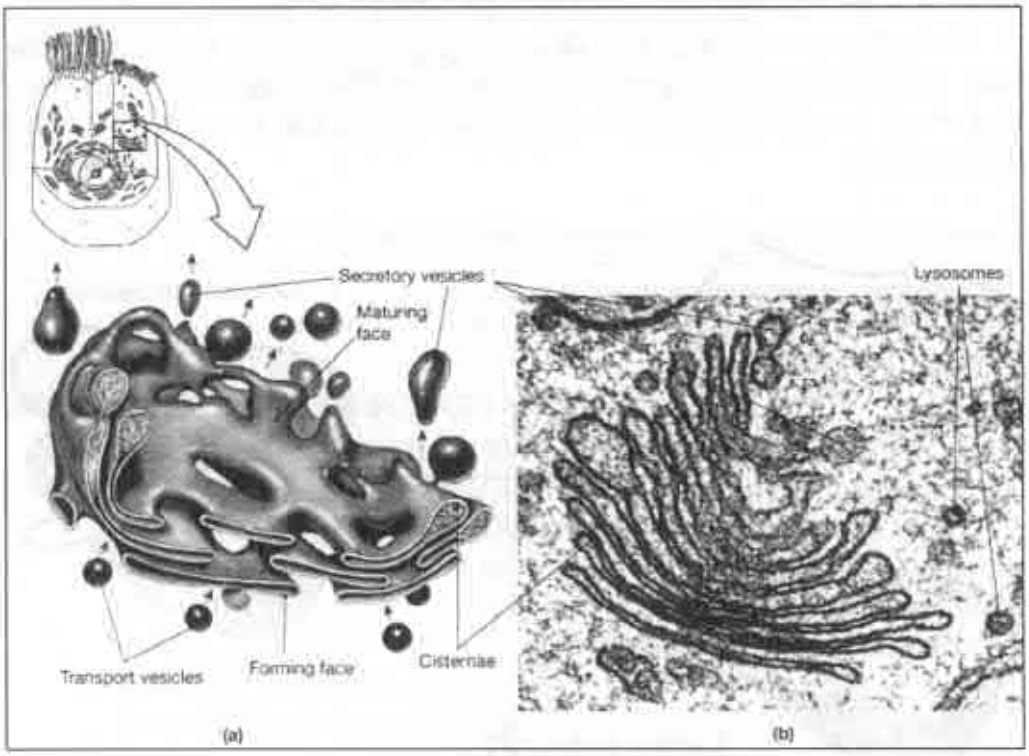
- There are few types of ribosomes:
  - **Free** – move through the cytoplasm. They are unbound in the cytoplasm; produce proteins for usage inside the cell.
  - **Fixed (bound)** – attached to rough endoplasmic reticulum; produce proteins for export and for the plasma membrane.

THE GOLGI APPARATUS

The Golgi apparatus sometimes called the Golgi body or Golgi complex is the principal "traffic director" for cellular proteins.

Structure:

- series of sacs with internal spaces like rER, look like a set of membranous flattend sacs shaped like hollow "dinner plates".



Three-dimensional representation and electron micrograph of a Golgi complex.

- two faces: **cis-face** (outer) it's convex – the "receiving" or "forming"side of Golgi Apparatus; **trans-face** (inner) it's concave – the "shipping"or "maturing" side of Golgi Apparatus.

Functions:

- Site of protein processing.
  - modifies, sorts and packs proteins and lipids for shipment to appropriate location (for secretion from the cell, enzymes for lysosomes, for the cell membrane).
  - transport vesicles from rER fuse with Golgi complex
- Forms secretory vesicles.
  - Discharged by exocytosis
- Forms new membrane components.
- Packs lysosomes.
- Modifies the oligosaccharide portion of glycoproteins.

MITOCHONDRION/A

Mitochondria are the largest organelles in the cytoplasm. They are oval or round shaped. The number of mitochondria per cell varies and directly correlates with the cell's metabolic activity. Found in nearly all eukaryotic cells.

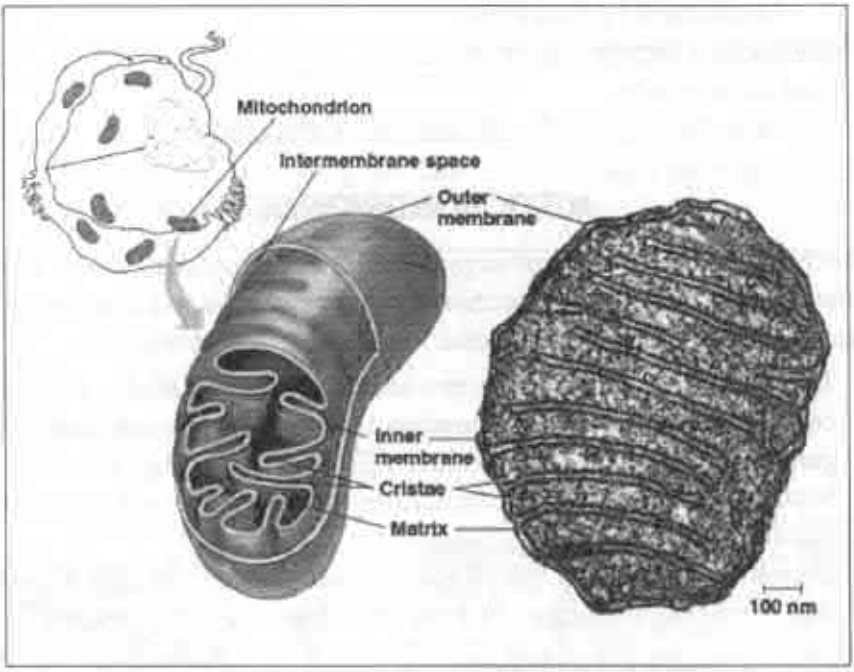
- They are organelles which represent the sites of cellular respiration, a catabolic oxygen-requiring process that uses energy extracted from organic macromolecules to produce ATP (provide energy for cell).
- Mitochondria are dynamic structures that move, change their shape and divide.
- They have their own DNA, RNA, ribosomes enzymes for lipid and protein synthesis and Krebs cycle enzymes (about 200 types of enzymes).

Structure of the Mitochondrion:

- Enclosed by two membranes that have their own unique combination of proteins embedded in phospholipid bilayers.
- Smooth **outer membrane** is highly permeable to small solutes, but it blocks passage of proteins and other macromolecules.
- Convoluted **inner membrane** contains embedded enzymes that are involved in cellular respiration. The membrane's multitudes of infoldings or cristae increase the surface area available for these reactions to occur.
- The inner and outer membranes divide the mitochondrion into two internal compartments:

1. **Intermembrane Space**
   a. Narrow region between the inner and outer mitochondrial membranes.
   b. Reflects the solute composition of the cytosol, because the outer membrane is permeable to small solute molecules.

2. **Mitochondrial Matrix**
   a. Compartment enclosed by the inner mitochondrial membrane.
   b. Contains enzymes that catalyze many metabolic steps of cellular respiration.
   c. Some enzymes of respiration and ATP production are actually embedded in the inner membrane.



Three-dimensional representation and electron micrograph of a mitochondrion with its cristae penetrating the matrix space

Functions:

- Makes energy from the food we eat (break down carbohydrates and sugars into ATP)
- Cellular respiration uses oxygen and involves two main pathways (carbon pathway: where sugar is broken down into carbon dioxide and hydrogen; hydrogen pathway: where hydrogen transfers into oxygen which forms water and releases energy).

- Stores cell's energy: needed for protein manufacturing, DNA replication and consumption of new organelles; needed for muscle contraction, pumping water through membranes, and cell movement.
- Controls the concentration of water, calcium, and ions in cytoplasm. They also breakdown and recycle the energy contained in fatty and amino acids.

LYSOSOME

They are round structures surrounded by a single membrane. Lysosomes contain hydrolytic enzymes that digest all major classes of macromolecules.

- Enzymes include **lipases, carbohydrases, proteases** and **nucleases**.
- Optimal pH for lysosomal enzymes is about pH 5 (this highly acidic pH is maintained by ionic pump).
- Hydrolytic enzymes and lysosomal membrane are synthesized in the rough ER and processed further in the Golgi apparatus.
- Lysosomes pinch off from the trans-face of the Golgi apparatus.

Lysosomal membrane performs two important functions:

- Separates potentially destructive hydrolytic enzymes from the cytosol.
- Maintains the optimal acidic environment for enzyme activity by pumping H^+s inward from the cytosol to the lumen.

There are three types of lysosome:

1. **Primary:** contain only digestive enzymes.
2. **Secondary:** fused with food vacuole or organelle.
3. **Residual body (tertiary):** contain undigested wastes. It is a normal feature of cell aging (for example – in nerve cells – "age pigment" – lipofuscin).

Functions of lysosomes:

- **Intracellular digestion.**
  - Cellular process of ingestion, where the plasma membrane engulfs substances and pinches off to form a particle-containing vacuole.
  - Lysosomes may fuse with food-filled vacuoles, and their hydrolytic enzymes digest the food (*heterophagosomes*) *(see fig. 16, plate I).*
  - Human cells called macrophages phagocytize bacteria and other invaders.
- **Recycle cell's own organic material.**
  - Lysosomes may engulf other cellular organelles or part of the cytosol and digest them with hydrolytic enzymes (*autophagosomes*) *(see fig. 16, plate I).*

- Resulted monomers are released into the cytosol where they can be recycled into new macromolecules.

- **Programmed cell destruction.**
  - Destruction of cells by their own lysosomes is important during development (*autolysis*).

PEROXISOMES

Peroxisomes are membrane-bound organelles that contain speciall teams of enzymes for specific metabolic pathways; all contain peroxide-producing oxidases.

- They are bound by a single membrane.
- Often have a granular or crystalline core which is a dense collection of enzymes.
- Contain peroxide-producing **oxidases** that transfer hydrogen from various substrates to oxygen, producing hydrogen peroxide.
  - oxidase $RH_2 + O_2$ ----------> $R + H_2O_2$
- Contain **catalase**, an enzyme that converts toxic hydrogen peroxide into water.
  - catalase $2H_2O_2$ ----------> $2H_2O + O_2$
- Peroxisomal reactions have many functions, some of which are:
  - Breakdown of fats into smaller molecules (acetyl CoA). The pro-ducts are carried to the mitochondria as fuel for cellular respiration.
  - Detoxification of alcohol and other harmful compounds. In the liver, peroxisomes enzymatically transfer H from poisons to O_2.

CYTOSKELETON

It is a network of fibers throughout the cytoplasm that forms a dynamic framework for support and movement.

- Gives mechanical support to the cell and helps maintain its shape.
- Enables a cell to change shape.
- Associated with motility (cell movement, organelle movement).

The cytoskeleton is constructed from at least three types of fibers: **microtubules** (thickest), **microfilaments** (thinnest) and **intermediate filaments** (intermediate in diameter).

MICROFILAMENT

Microfilaments built from globular protein monomers, G-actin. G-actin monomers are linked into long chains of F-actin. Two F-actin chains are wound into a helix.

Schematic representation of F-actin

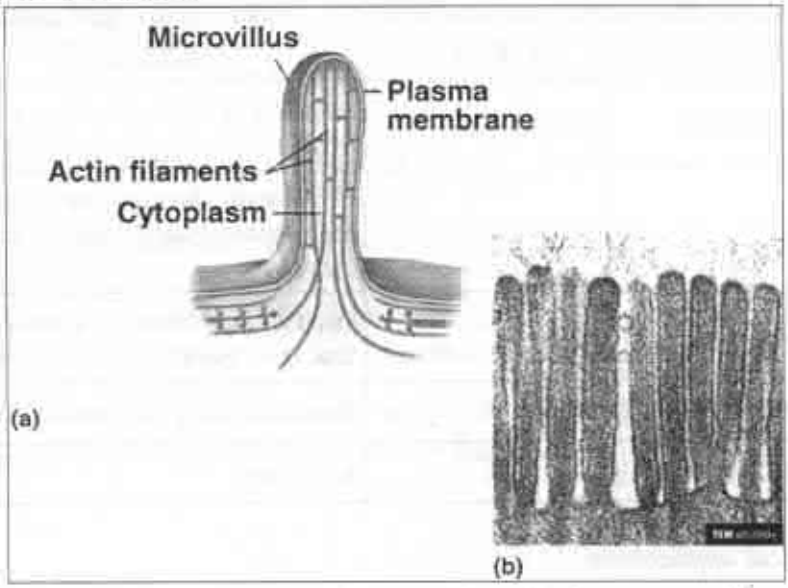
Microfilaments contain:

- ACTIN (major component)
- TROPOMYOZIN
- TROPONIN

Functions of microfilaments:

- Participate in muscle contraction.
- Provide support (e.g. bundles of microfilaments in the core of intestinal microvilli).
- Responsible for localized contraction of cells. Small actin-myosin aggregates exist in some parts of the cell and cause localized contractions. **Examples** include:
  - Contracting ring of microfilaments pinches a cell in two during cell division.
  - Elongation and contraction of pseudopodia during phagocytosis.

Presumably, most actin filament-related activities depend upon the interaction of **myosin** with actin.



Drawing of a microvillus: (a) three-dimensional representation and (b) electron micrograph of a brush border

Microvilli

- Represent extensions of plasma membrane.
- They increase the cell surface.
- Normally a lot on each cell (e.g. enterocytes).
- Form a brush border on the apical surface of the cell.
- Do not move

INTERMEDIATE FILAMENTS are tough supporting elements

- Filaments which are intermediate in diameter (8 – 12 nm) between microtubules and microfilaments.
- Diverse class of cytoskeletal elements that differ in diameter and composition depending upon cell type.
- Consist of eight subunits.
- More permanent than microfilaments and microtubules.

Functions:

- Makes the specificity of cells.
- Stabilizes the position of nucleus and organelles.
- Stabilizes position of the cell with respect to the surrounding cells.
- They resist tension.

Several proteins that form intermediate filaments have been isolated and localized by immunocytochemical means:

FILAMENT TYPE	CELL TYPE	EXAMPLES
CYTOKERATINS	Epithelial cell	Stratified keratinized and nonkeratinized epithelium.
VIMENTIN	Mesenchymal cells	Fibroblasts, chondroblasts, endothelial cells, macrophages, vascular smooth muscle.
DESMIN	Muscle	Striated and smooth muscle (except vascular smooth muscle)
NEUROFILAMENTS	Neurons	Nerve cell body and processes
GLIAL FILAMENTS	Glial cells	Astrocytes

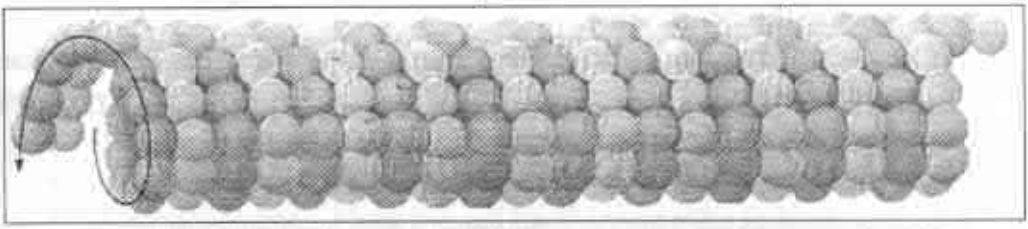
Medical application:

The presence of a specific type of intermediate filament in tumors can reveal which cell originated the tumor, information important for diagnosis and treat-

ment. Identification of intermediate filament proteins by means of immunocytochemical methods is a routine procedure.

MICROTUBULES

- Straight hollow fibers about 25 nm in diameter.
- long, hollow, stiff tubes made of 2 globular proteins [a -ß tubulin].
- subunits linked by non-covalent forces into a ring of 13 parallel protofilaments (alternating a -ß).
- each end of **microtubule** exhibits a polarity: the alpha end is "plus" and the beta end is "minus". Tubulin polymerization is under control of the concentration of Ca^{2+} and of the microtubule-associated proteins.
- half of all tubulin is free in cytoplasm and half is complexes into **microtubules.**
- The microtubule results in dynamic assembly and disassembly process.
- **Microtubules** grow from one end (mostly "plus" end). Microtubule stability is variable; for example, microtubules of cilia are stable, whereas microtubules of the mitotic spindle have a short life span.



Schematic representation of microtubule

Functions:

- Primary component of the cytoskeleton.
- Allow changes in shape.
- Allow movements of vesicles or organelles within the cell.
- Assist in cell division.
- Allow the movement of chromosomes during cell division.
- Form centrioles and cilia, tail (flagella) of spermatozoon.

Microtubules provide the basis for several complex cytoplasmic components, including centrioles, basal bodies, cilia, and flagella.

CENTROSOME

Contains:

- **centriole pair**
- **granular-looking (centrosome) matrix**

Centrioles are cylindrical structures composed primarily of short, highly organized microtubules. Each centriole consists of nine triplets of microtubules, arranged to form a hollow tube. Two centrioles direct formation of mitotic spindle. They also form the basal body of cilia and flagella *(see fig. 12, plate I)*.

In each pair, the long axes of the centrioles are at right angles to each other. Before cell division, more specifically during the S period of the interphase, each centrosome duplicates itself so that now each centrosome has two pairs of centrioles. During mitosis, the centrosomes divide in two; move to opposite poles of the cell, and become organizing centers for the microtubules of the mitotic spindle.

Functions:

- Direct the movement of chromosomes during cell division.
- Organize the cytoskeleton.
- Before cell division, centrioles divide, move to ends of cell and become spindle fibers.

CILIA AND FLAGELLA

Locomotor organelles found in eukaryotes, which are formed from a specialized arrangement of microtubules.

ULTRASTRUCTURE of cilia and flagella:

- Are extensions of plasma membrane with a **core of microtubules**.
- Microtubular core is made of **nine doublets of microtubules** arranged in a ring with two single microtubules in the center (**9+2 pattern**).
- Each doublet is a pair of attached microtubules. One of the pair shares a portion of the other's wall.
- Each doublet is connected to the center of the ring by **radial spokes** that end near the central microtubules.
- Each doublet is attached to the neighboring doublet by a pair of **side arms**. Many pairs of side arms are evenly spaced along the doublet's length.
- Side arms are made of **dynein**, a large protein motor molecule that changes its conformation in the presence of ATP as an energy source.
- A complex cycle of movements caused by dynein's conformational changes makes the cilium or flagellum bend.

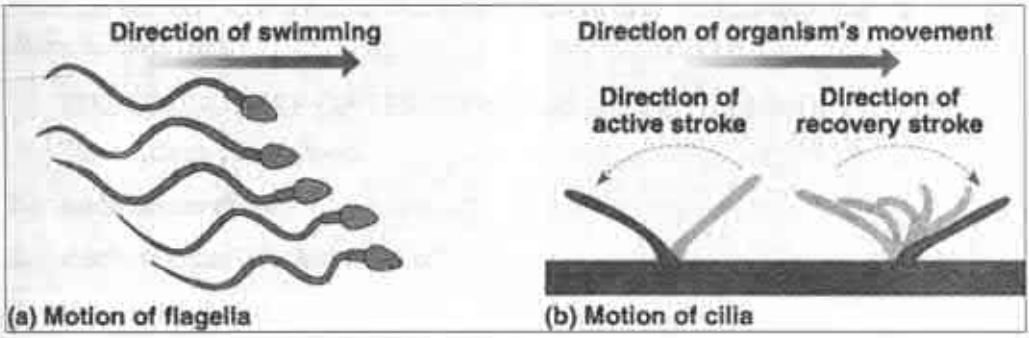
- Is anchored by a **basal body** – a cellular structure, identical to a centriole, which anchors the microtubular assembly of cilia and flagella. The basal body can convert into a centriole and vice versa; may be a template for ordering tubulin into the microtubules of newly forming cilia or flagella (as cilia and flagella continue to grow, new tubulin subunits are added to the tips, rather than to the bases) *(see fig. 13, plate I)*.

CILIA

- Occur in large numbers on cell surface.
- Move the cell or move material around a cell (the movement of cilia assures the movement of the oocyte through the oviduct, cleans the inhaled respiratory air).
- There are two types of cilia: **motile cilia**, which constantly beat in a single direction, and **non-motile cilia**, which typically serve as sensory organelles.
- Work like paddles (rowing), with a power stroke alternating with a recovery stroke. Moves the cell or fluid in a direction perpendicular to the axis of the cilium.
- Found in respiratory tract, oviduct.

FLAGELLA

- **Similar to cilia but longer.**
- Few (sometimes there is only one).
- Cell movement – **Move the cell itself in wavelike fashion.**
- Undulating motion drives the cell in the same direction as the axis of the flagellum.
- Sperm is the only flagellated animal cell.



Schematic drawing of motions of a flagella (a) and cilia (b)

INCLUSIONS

Inclusions are products of the vital activity of the cell. The inclusions are small particles of insoluble substances suspended in the cytosol. A huge range of inclusions exist in different cell types *(see fig. 14, plate I)*. They can be spent if there is necessary and be accumulated. They are divided into:

- **Nutritious** (lipid droplets, glycogen). A particularly widespread example are lipid droplets, which are spherical droplets composed of lipids and proteins that are used as a way of storing lipids such as fatty acids and sterols. Lipid droplets make up much of the volume of adipocytes, which are specialized lipid-storage cells, but they are also found in a range of other cell types.
- **Pigmental** (pigmental granules – Hb, carotene, etc). They may be synthesized by the cell (eg, in the skin melanocytes) or come from outside the body (eg, carotene). One of the most common pigments is **lipofuscin,** a yellowish-brown substance present mainly in permanent cells (eg, neurons, cardiac muscle) that increases in quantity with age. Its chemical constitution is complex. It is believed that granules of lipofuscin derive from secondary lysosomes and represent deposits of indigestible substances. A widely distributed pigment, **melanin** is abundant in the epidermis and in the pigment layer of the retina in the form of dense intracellular membrane-limited granules.
- **Secretory** (secretory granules) – under stimulation, proteins which are stored in secretory granules are periodically released into the extracellular medium.
- **Excretory.**

CHAPTER III

NUCLEUS

The nucleus can be compared to a computer, design department, construction boss, and board of directors – all rolled into one. The nucleus is a **membrane-limited compartment** that contains the genetic information. It contains the instructions needed to build nearly all the body's proteins. Nucleus dictates the kinds and amounts of proteins to be synthesized at any one time in response to signals acting on the cell. The number, size, shape and form of the nucleus are generally constant for a particular cell type.

The number of nuclei: usually each cell has a single nucleus; some cells such:

a. liver cells have 2-3 nuclei,
b. osteoclasts – cells of the bone tissue have 10-20-30 nuclei
c. skeletal striated muscular fibers have up to 100 nuclei.

The cells, which have more than 1 nucleus, are called polynucleated. There are a number of structures without nuclei – the mature red blood cells – erythrocytes, and the platelets in the human body. They are called postcellular structures.

The shape of the nuclei: the nucleus if usually spherical and is centrally located in the cell, however, in the some cells it may be spindle-shaped, oblong-shaped, tabulated.

THE STRUCTURE OF THE NUCLEUS *(see fig. 11, plate I)*

The nucleus consists of:

1. **nuclear envelope**
2. **nucleoplasm** (nuclear matrix)
3. **nuclear skeleton** (lamina)
4. **chromatin**
5. **nucleolus**

NUCLEAR ENVELOPE

The nucleus is surrounded by the nuclear envelope, which serves as a barrier between the nucleoplasm and cytoplasm and it is composed of two parallel unit membranes: **the inner** and **the outer** nuclear membranes, separated from each other by a 10 to 30 nm space called the perinuclear cisterna. It is continuous with the cisterna of the rER and is perforated by nuclear pores.

The outer nuclear membrane is continuous with the rough ER of the cytoplasm and is studded with ribosomes on the external face. Its cytoplasmic surface is surrounded by a thin, loose meshwork of intermediate filaments – vimentin.

The inner nuclear membrane doesn't contain the ribosome and is lined by a meshwork of **protein filaments** – the nuclear lamina. **NUCLEAR LAMINA** is composed of lamins A, B, and C – a specific type off intermediate filaments. Nuclear lamina lattice around periphery and more diffuse in the center. Function:

- is responsible for the disassembly and reassembly of the nuclear envelope during mitotic events.
- provides structural rigidity to nucleus and possible sites of attachment for chromatin

The outer and the inner nuclear membranes fuse at various points and form windows known as **nuclear pores** that permit communication between the cytoplasm and the nucleus. The number of nuclear pores ranges between a few dozen to several thousand, correlated directly with the metabolic activity of the cell. These nuclear pores are not open. They have a proteins and glycoproteins in their rim. The nuclear pore and its associated glycoprotein form **the NUCLEAR PORE COMPLEX.**

The nuclear pore complex is composed of three ring-like arrays of proteins:

1. **cytoplasmic ring**
2. **middle ring**
3. **nucleoplasmic ring**

Each ring consists of 8 protein subunits arranged in an octagonal array at the periphery, and one protein subunit arranged in the center.

The CYTOPLASMIC ring

Each of its 8 protein subunits has a filamentous fiber that extends into the cytoplasm. It has been suggested that these fibers may mediate import into the nucleus trough the nuclear pore complex by moving substances toward the center of the pore.

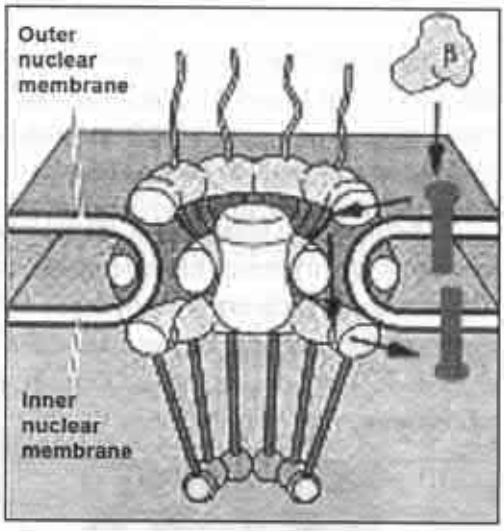
The MIDDLE ring

It is composed of a set of 8 transmembrane proteins that project into the lumen of the nuclear pore as well as into the perinuclear cistern.

The NUCLEOPLASM ring

It is analogous to the cytoplasmic ring and is located on the rim of the nucleoplasmic site of the nuclear pore and assists in the export.

The functions of the nuclear pore complex:



The nuclear pore complex

1. Regulation of the passage of substances between the nucleus and the cytoplasm. Because the nuclear envelope is impermeable to ions and molecules of all sizes, the exchange of substances between the nucleus and the cytoplasm is made only through the nuclear pores. Ions and molecules with a diameter up to 9 nm pass freely through the nuclear pore without consuming energy. But molecules and molecular complexes larger than 9 nm are transported by an active process, mediated by receptors, which uses energy from adenosine triphosphate (ATP) and takes place in two stages. First, proteins with one or several nuclear signal locations become attached to specific cytosolic proteins, originating a complex, which is temporarily attached to the nuclear pore complex without using energy. In the second stage, proteins with nuclear signal locations are transferred to the nucleus, using energy from ATP, and the cytosolic protein remains in the cytoplasm. At least part of the ATP energy may be utilized to open the nuclear pore complex to make the passage of large molecules possible.

2. Transportation of ribosomal subunits into the cytoplasm.

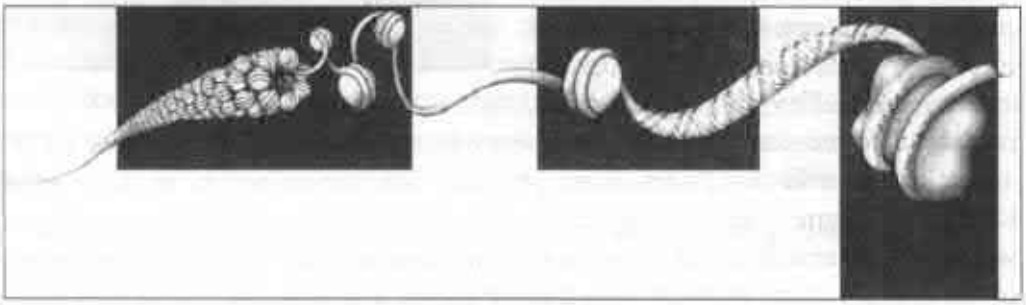
NUCLEOPLASM is the component that fills the space between the chromatin and the nucleoli in the nucleus. It is composed mainly of proteins (some of which have enzymatic activity), metabolites, and ions. When its nucleic acids and other soluble components are removed, a continuous fibrillar structure remains, forming the **nucleoskeleton.** The fibrous lamina of the nuclear envelope is part of the nuclear matrix.

CHROMATIN is a complex of DNA and globar histone proteins. DNA resides in the nucleus in the form of chromosomes, which are clearly visible during cell division. In the interval between cell divisions, there are unwound in the form of chromatin. Depending on its transcriptional activity chromatin may be condensed as **heterochromatin** and extended as **euchromatin.**

Heterochromatin is inactive form of chromatin; it is stained darker. It is situated mostly at the periphery of the nucleus. Heterochromatin predominates in the metabolically inactive cells.

Euchromatin is an active form of chromatin. Represents the active chromatin where the genetic material of the DNA molecules is being transcribed into RNA. It is present within the nucleoplasm in the clear areas between the heterochromatin. Euchromatin is prominent in metabolically active cells.

The basic structural unit of chromatin is the **nucleosome**, which consists of a core of four types of histones: two copies each of histones H2A, H2B, H3, and H4, around which are wrapped 166 DNA base pairs. An additional 48-base pair segment forms a link between adjacent nucleosomes, and another type of histone (H1 or H5) is bound to this DNA. Nonhistone proteins are also associated with chromatin.



The orders of chromatin packing

The next higher order of organization of chromatin is the 30-nm **fiber**. In this structure, nucleosomes become coiled around an axis, with six nucleosomes per turn, to form the 30-nm chromatin fiber. There are higher orders of coiling, especially in the condensation of chromatin during mitosis and meiosis.

In humans chromatin is in the form of **chromosomes,** which are structures made up of DNA and associated proteins to maintain the shape of the DNA (these proteins are involved in packaging the DNA tightly so that it will all fit into the cell.).

There are 46 chromosomes, arranged in 23 pairs – 1 set from mom and 1 set from dad.

1. Each pair is a **homologous pair** of chromosomes – same size, shape and contains the same type of genetic information, or **genes.**
2. A cell that normally has two sets is **diploid,** while a cell that normally has one set (a sperm or an unfertilized egg) is **haploid.**

NUCLEOLUS

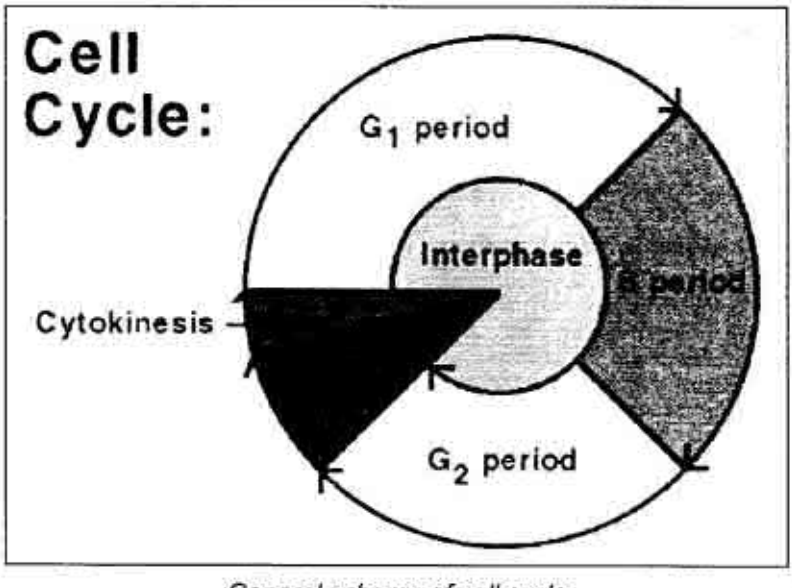
Nucleolus is the deeply staining none-membrane-bounded structure within the nucleus that is involved in rRNA synthesis and assembly of small and large ribosomal subunits. Typically, there are one or two nucleoli per cell, but they may be more. It is usually basophilic when stained with hematoxylin and eosin. They are usually very large in growing cells. It is observed only during the interphase, because it dissipates during cell division. Four distinct areas of the nucleolus have been described:

1. **fibrilar center**- consists of inactive DNA.
2. **pars fibrosa**- consists of nucleolar RNAs.
3. **pars granulose,** in which mature ribosomal subunits are assembled.
4. **nucleolar matrix**- a network of fibers active in nucleolar organization.

CHAPTER IV

THE CELL LIFE CYCLE

The cell life cycle is the life period of the cell from one division to other division or from one division to death. It is divided into two periods: interphase and cell division.



General scheme of cell cycle

INTERPHASE – a longer period of time during which the cell increases its size and content and replicates its genetic material.

1. Chromosomes are extended (not condensed) and therefore are not visible.
2. Chromosomes replicate
   a. Each chromosome is now made up of two identical sister **chromatids** joined at a centromere.
   b. There is now twice the genetic information, although the same number of chromosomes as before.

3. Centrioles replicate
4. Nuclear membrane (envelope) is still present.

Interphase is subdivided into three phases:

- G_1 (presynthesis) – during this phase cells synthesize proteins and grow. This is most variable phase in term of length. Cells with rapid division rates have G_1 phase typically lasting several minutes to hours; in those that divide slowly, it may last for days or even years.

Many times a cell will leave the cell cycle, temporarily or permanently. It exits the cycle at G_1 and enters a stage designated $\mathbf{G_0}$ (G zero). A G_0 cell is often called "quiescent", but that is probably more a reflection of the interests of the scientists studying the cell cycle than the cell itself. Many G_0 cells are anything but quiescent. They are busy carrying out their functions in the organism. e.g., secretion, attacking pathogens. Often G_0 cells are terminally differentiated: they will never reenter the cell cycle but instead will carry out their function in the organism until they die. For other cells, G_0 can be followed by reentry into the cell cycle. Most of the lymphocytes in human blood are in G_0. However, with proper stimulation, such as encountering the appropriate antigen, they can be stimulated to reenter the cell cycle (at G_1) and proceed on to new rounds of alternating S phases and mitosis.

G_0 phase represents not simply the absence of signals for mitosis but an active repression of the genes needed for mitosis. Cancer cells cannot enter G_0 and are destined to repeat the cell cycle indefinitely.

- S (DNA synthesis) – DNA replicates itself, ensuring that the two future cells will receive identical copies of the genetic material; centrioles replicate themselves. During this replication phase the chromosomes are extremely long, they are spread diffusely through the nucleus, and they cannot be recognized with the light microscope.
- G_2 (post-DNA duplication) -during this period enzymes and proteins needed for division are synthesized and moved to their proper sites.

CELL DIVISION (mitosis or meiosis), during which cell divides into two cells.

MITOSIS in multicellular organisms is responsible for:

- growth of the organism.
- repair of damaged tissues.

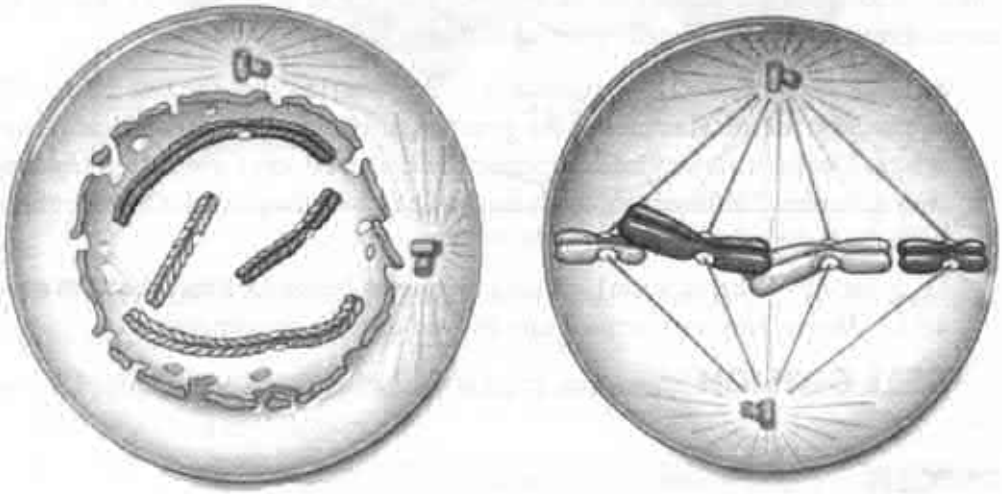
Mitosis is a form of cell division that produces two daughter cells with the same genetic component as the parent cell. Chromosomes replicated during the S phase are divided in such a way as to ensure that each daughter cell receives a copy of every chromosome.

The replicated chromosomes are attached to a 'mitotic apparatus' that aligns them and then separates the sister chromatids to produce an even partitioning of the genetic material. This separation of the genetic material in a mitotic nuclear division (or **karyokinesis**) is followed by a separation of the cell cytoplasm in a cellular division (or **cytokinesis**) to produce two daughter cells.

Stages of Mitosis:

PROPHASE

1. Throughout prophase the chromosomes continue to condense, shorten, and thicken.
2. Nucleolus (or nucleoli) disappears
3. Nuclear membrane disappear
4. **Spindle apparatus,** composed of spindle fibers. The centriole pairs separate from one another. The centrioles act as focal points for growth of a new assembly of microtubules, called the mitotic spindle.
5. Each chromosome becomes attached to a spindle fiber.



(a) (b)

Schematic drawing showing the prophase (a) and metaphase (b)

Each chromosome is actually made up of two identical chromatin treads, now called **chromatids**. The chromatids of each chromosome are held together by a small buttonlike body called a **centromere**. After the chromatids separate, each is considered a new chromosome.

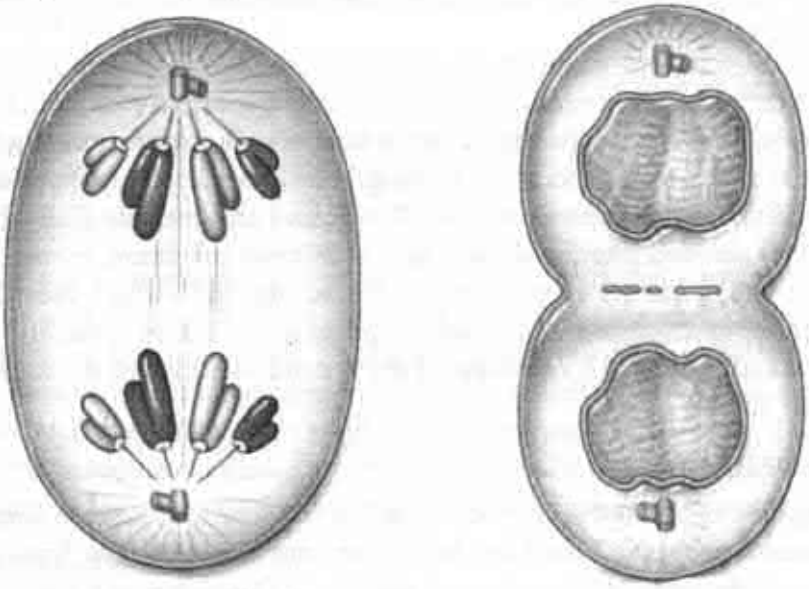
METAPHASE

During metaphase the chromosomes line up in the equatorial plane, and their doubled structure is clearly visibl. Each is attached by **microtubules** extending from the centromere to the centriole, forming the **mitotic spindle.** This arrangement of the chromosomes along a plane midway between the poles is called the **metaphase plate.**

ANAPHASE

Anaphase is easy to recognize because the moving chromosomese look V-shaped. It is the shortest stage of mitosis. It typically lasts only a few minutes. Centromere of each chromosome splits and one chromatid from each chromosome moves to centrioles at the poles of the cell.

a. The chromatids, which are now separate, are now called chromosomes.
b. There are now twice as many chromosomes in the cell as there were in the parent cell.



(a) (b)

Schematic drawing showing the anaphase (a) and telophase (b)

TELOPHASE

Telophase begins as soon as chromosomal movement stops. This final phase is like prophase in reverse.

1. Nuclear membranes reform around each group of newly divided chromosomes.
2. Nucleolus (or nucleoli) reappears.
3. Spindles disappear.
4. Chromosomes extend, becoming invisible.

The cell, for just a brief period, is binucleate (has two nuclei), and each new nucleus is identical to the original mother nucleus.

CYTOKINESIS or the division of the cytoplasm begins during late anaphase and continues through and beyond telophase. It occurs as a contractile ring of peripheral microfilaments forms at the cleavage furrow and squeezes the cells apart. Cytokinesis completes the division of the cell, into two daughter cells.

MEIOSIS

Meiosis is a process consisting of two sequential cell divisions that produces gametes containing half the number of chromosomes and half the DNA found in somatic cells.

Stages of Meiosis

Meiosis is divided into stages, much like mitosis. Unlike mitosis, however, meiosis is a two-step process, consisting of two sequential cell divisions. The two cell divisions in meiosis are called meiosis I and meiosis II. Each of these divisions is divided into the same stages as mitosis: prophase, metaphase, anaphase, and telophase. Stages are numbered according to which meiotic cell division is being discussed. For example, prophase of the first meiotic cell division is called prophase I; anaphase of the second meiotic cell division is called anaphase II.

PROPHASE I

Prophase of the first meiotic division is a very eventful time; to keep track of these events, prophase I has been broken up into five substages: leptonema, zygonema, pachynema, diplonema, and diakinesis. (Note: for each substage except diakinesis, the name of the substage ends with "-nema"; the adjective referring to each stage ends with "-tene", as in "a pachytene chromosome".)

Leptonema

During leptonema, the chromosomes begin to condense, although they are still quite diffuse. Remember that the chromosomes (DNA) have already replicated (during S phase), so each 'chromosome' consists of two sibling chromatids. Also during leptonema, each chromosome begins to search the nucleus for its homologue. The diagram to the left represents a cell with 4 chromosomes (two homologous pairs) as it would appear during leptonema.

Zygonema

In zygonema, the chromosomes continue to condense, and homologous chromosomes find each other and begin to align to each other in a process known as "rough pairing".

Pachynema

In pachynema, the aligned homologous chromosomes become much more closely associated. This process is known as synapsis. (The chromosomes are said to have synapsed.) The synapsed homologous pair of chromosomes is called a tetrad, because it consists of four chromatids. It can't be observed until the next stage, but the synapsed chromosomes may undergo crossing over in pachynema. The chromosomes continue to condense.

Diplonema

During diplonema, the homologous chromosomes in each tetrad begin to separate, but they remain connected at points of crossing over. Each point of crossing over is known as a chiasma (plural: chiasmata). Also at this stage, the nuclear envelope begins to break down.

Diakinesis

Diakinesis is the last stage of prophase I. In this stage, the homologous chromosomes separate further, and the chiasmata terminalize (proceed to the end of the chromatids, and then separate). Notice how this leaves chromatids that engaged in crossing over with exchanged genetic material (as indicated by the exchange of color). The nuclear envelope has completely disintegrated by this stage. The centromeres of the chromosomes become attached to spindle fibers. The end of diakinesis marks the end of prophase I. From here, the cell progresses into metaphase I.

METAPHASE I

During metaphase I, assembly of the spindle apparatus is completed, and the chromosome pairs line up across the center of the cell between the two centrioles. This central plane is called the metaphase plate.

ANAPHASE I

Anaphase I is marked by the separation of homologous chromosome pairs into the individual chromosomes. In other words, each half of a tetrad is pulled apart in a process called disjunction, forming two dyads. Each centromere does not divide, so sibling chromatids remain attached. This is another difference between meiosis and mitosis: in mitosis, centromeres divide during anaphase, and sibling chromatids are separated. In meiosis, this does not happen until anaphase II.

The dyads, once separated from each other, begin moving toward opposite poles of the cell.

Occasionally, the homologous chromosomes in a tetrad do not separate properly, and both homologues in the tetrad move toward the same end of the cell. This phenomenon is called nondisjunction. Nondisjunction results in half of the gametes produced having an extra copy of one chromosome, and the other half having no copies of that chromosome. If any of these gametes becomes involved in fertilization, the resulting organism will have either three copies of the chromosome in question, or only one. This can have profound consequences for the individual. For example, Down syndrome results from nondisjunction of chromosome 21 in a parent during meiosis, such that the child has three copies (trisomy) of chromosome 21.

TELOPHASE I

In telophase I, the chromosomes (each consisting of two chromatids) complete their migration to the poles. Cytokinesis occurs during telophase I, so that two cells are produced. It is important to note that each of these cells now contains half the normal number of chromosomes. For this reason, meiosis I is referred to as a **reduction division**.

Meiosis II generally resembles mitosis.

PROPHASE II

Prophase II is much less complex than prophase I, so it is not divided into substages. In prophase II, new nuclear membranes that formed in telophase I (if they formed) will now break down, and chromosomes recondense.

METAPHASE II

Centromeres connect to spindle fibers during metaphase II, and the chromosomes line up along the metaphase plate. Once the chromosomes are lined up, the centromeres begin to divide.

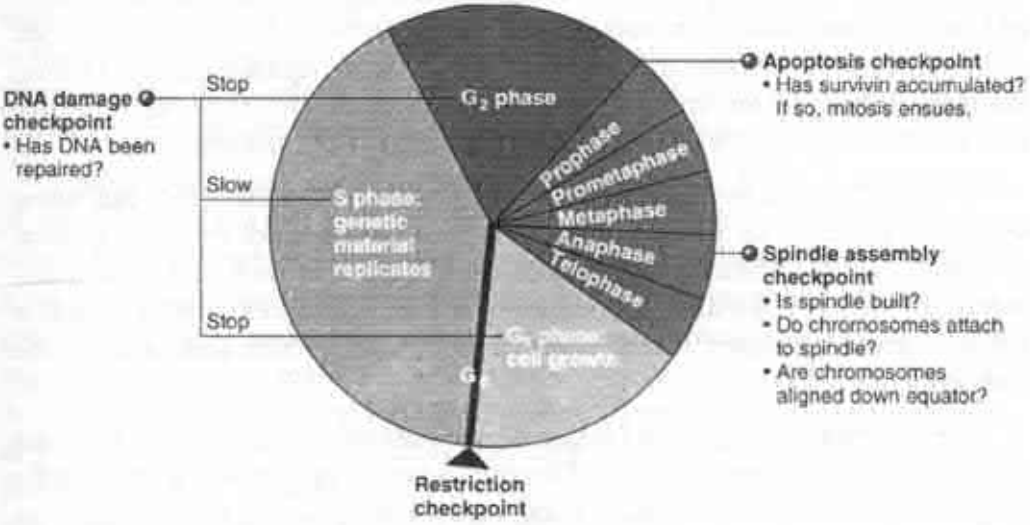
ANAPHASE II

During anaphase II, the sibling chromatids separate and begin to move toward opposite poles of the cell. Each chromatid, once separated from its sibling, can be considered a separate chromosome.

TELOPHASE II

When the chromosomes reach the poles, the cells are in telophase II. During this stage, nuclear membranes form again, and cytokinesis occurs. The overall result from one diploid cell entering meiosis is four haploid cells, which are ready to take part in fertilization.

The cell cycle is also regulated by a variety of signals that inhibit progression through the cycle. **DNA damage** arrests the cell cycle not only in G_2 but also at a checkpoint in G_1. G_1 arrest may permit the damage to be repaired before the cell enters S phase, where the damaged DNA would be replicated. In mammalian cells, arrest at the G1 checkpoint is mediated by the action of a protein known as p53. The p53 protein is also a key player in apoptosis, forcing "bad" cells to commit suicide. The gene encoding p53 is often mutated in human cancers, thus reducing the cell's ability to repair damaged DNA. Inheritance of damaged DNA by daughter cells results in an increased frequency of mutations and general instability of the genome, which may contribute to the development of cancer.



Schematic drawing of cell cycle control

Processes that occur during the G_2 phase include the accumulation of energy to be used during mitosis, the synthesis of tubulin to be assembled in mitotic mi-

crotubules, and the synthesis of chromosomal nonhistone proteins. In G_2 there is also a checkpoint at which the cell remains until all DNA synthesized with defects is corrected. In G_2 there is an accumulation of the protein complex maturation promoting factor (**MPF**) that induces the beginning of mitosis, the condensation of the chromosomes, the rupture of the nuclear envelope, and other events related to mitosis. **Spindle checkpoints**. detect any failure of spindle fibers to attach to kinetochores and arrest the cell in metaphase until all the kinetochores are attached correctly (M checkpoint – example); detect improper alignment of the spindle itself and block cytokinesis; trigger apoptosis if the damage is irreparable.

Rapidly growing tissues (eg, intestinal epithelium) frequently contain cells in mitosis, whereas slowly growing tissues do not. The increased number of mitotic figures and abnormal mitoses in tumors is an important characteristic that distinguishes malignant from benign tumors. The organism has elaborate regulatory systems that control cell reproduction by either stimulating or inhibiting mitosis. Normal cell proliferation and differentiation are controlled by a group of genes called **protooncogenes;** altering the structure or expression of these genes promotes the production of tumors. Altered protooncogenes are present in tumor-producing viruses and are probably derived from cells. Altered oncogene activity can be induced by a change in the DNA sequence (mutation), an increase in the number of genes (gene amplification), or gene rearrangement, in which genes are relocated near an active promoter site. Altered oncogenes have been associated with several tumors and hematological neoplasia. Proteins that stimulate mitotic activity in various cell types include nerve growth factor, epithelial growth factor, fibroblast growth factor, and precursors of erythrocyte growth factor (erythropoietin); there is an extensive and rapidly growing list of these proteins.

Cell proliferation is usually regulated by precise mechanisms that can, when necessary, stimulate or retard mitosis according to the needs of the organism. Several factors (eg, chemical substances, certain types of radiation, viral infections) can induce DNA damage, mutation, and abnormal cell proliferation that bypass normal regulatory mechanisms for controlled growth and result in the formation of tumors.

The term **tumor,** initially used to denote any localized swelling in the body caused by inflammation or abnormal cell proliferation, is now usually used as a synonym for **neoplasm** (Gr. *neos*, new, + *plasma*, thing formed). Neoplasm can be defined as an abnormal mass of tissue formed by uncoordinated cell proliferation. Neoplasms are either benign or malignant according to their characteristics of slow growth and no invasiveness (benign) or rapid growth and great capacity to invade other tissues and organs (malignant). **Cancer** is the common term for all malignant tumors

CHAPTER V

CELL DEATH

The cells can live 8- 10 – 130 days several years and they are getting old and than dye. The cell s can day by two ways:

1. **Apoptosis** – By programmed way
2. **Necrosis** – By accident

APOPTOSIS

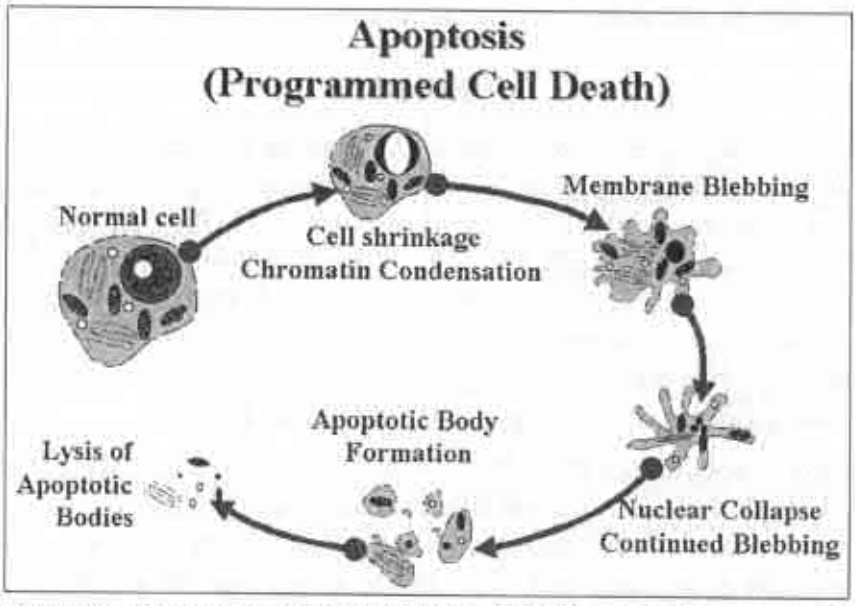
Apoptosis is a programmed cell death. The term was introduced by Kerr and the process was called shrinkage necrosis, which was the only type of cell death known at that time. This process of cell death has been termed also Programmed cell death (abbr. PCD) or active cell death (abbr. ACD) because it requires controlled gene expression, which is activated in response to a variety of external or internal stimuli or their absence. Many authors use the terms apoptosis and programmed cell death synonymously, while others consider programmed cell death a more general term that embraces different morphologies and biochemical processes.

Control of apoptosis is thought to be intimately linked with the progression of cells through the cell cycle and this process essentially guarantees a steady-state condition in which cell division is counterbalanced by cell death. Apoptosis allows selective elimination and swift clearance by phagocytosis of cells from a proliferating cell population and is an evolutionarily conserved process for killing unwanted cells in multicellular organisms. Apoptotic processes are observed, for example, during embryonal development, morphogenesis, metamorphosis, in endocrine tissue atrophy, during the normal turnover of tissues, and during tumor regression. Cellular self-destruction plays a decisive role in the elimination of self-recognizing T-lymphocytes in the thymus.

Many human diseases can be attributed directly or indirectly to a derangement of apoptosis. The disruption of normal processes leading to apoptosis results in illegitimate cell survival and can cause developmental abnormalities

and facilitate cancer development Apoptosis contributes to the adaptation of an organism to the environment and constitutes a mechanism of safe clearance of unwanted cells during resolution of inflammation through the formation of apoptotic bodies in which potentially harmful cellular contents are prevented from being released and release pro-inflammatory mediators. At the same time, macrophages appear to be capable of ingesting apoptotic cells without releasing pro-inflammatory cytokines. This process requires secreted "find-me" signals such as lysophosphatidylcholine, which attracts phagocytes, and "eat-me" signals exposed on apoptotic cells for efficient removal of such cells.

Apoptosis also provides a defense mechanism against viruses by reducing virus spread through the rapid death of virus-infected cells. Viruses often enhance their infectivity and/or evade immune responses by expressing proteins that inhibit apoptosis of their host cells.



General scheme of modifications which cell suffer during the apoptosis

Apoptosis is initiated when cells are given sufficient time to organize a number of intracellular events participating in their own destruction. The earliest indications of apoptotic cell death are morphological alterations of the cells such as chromatin condensation, disappearance of the nucleolus, and alterations of the cell surface, characterized by the occurrence of blebs. These signs are followed by a margination of the chromatin at the inner surface of the nuclear membrane. Eventually the activation of a variety of nucleases leads to the fragmentation of DNA.

DNA degradation during apoptosis generally occurs at two levels: early as high molecular weight fragments and later on as fragments of the size of nucleosomes. This requires a number of specific DNA-ses, which are activated specifically during apoptosis. DNA fragmentation can be used to identify apoptotic cells. Cells dying by apoptosis shrink and eventually break up into vesicles known as apoptotic bodies. Since intracellular contents are not released from apoptotic cells and their fragments this process is not accompanied by inflammation and the process, therefore, can be regarded as an injury-limiting mode of cell disposal.

The induction of apoptosis is an active genetically regulated process and, like other gene-directed processes such as differentiation, requires the co-ordinated expression of many genes. This process, once set in motion, is essentially irreversible.

NECROSIS

Necrosis is a passive, catabolic process that represents a cellular response to extreme accidental or toxic insults and, unlike apoptosis, is always pathological.

Morphologically, necrosis is characterized by a disruption of the cellular membrane and a swelling of the cytoplasm and mitochondria, culminating in the complete disintegration of organelles *(see fig. 15, plate I)*. The process ends with total cell lysis. Biochemical features of necrosis include loss of regulation of ion hemostasis, random digestion of DNA and DNA fragmentation after lysis. Also, the process is uncontrolled and passive and does not require energy. Severely damaged cells do not form membrane-bound vesicles (apoptotic bodies such as observed during apoptosis), and thus release their cellular contents. This normally results in inflammatory reactions with oedema and damage to surrounding cells. These effects of necrosis are exacerbated during neuronal necrosis because neurotransmitters that are released by dying cells can cause excitotoxic injury and cell death to their neighbors.

PART II

GENERAL EMBRYOLOGY

CHAPTER I

FIRST WEEK OF DEVELOPMENT

Embryology is the science that studies the development of the human embryo. Human development can be divided into two large periods: prenatal development and postnatal development.

Prenatal Development (before birth) has three stages:

1. PRE-EMBRYO – from fertilization to two weeks.
2. Period of the EMBRYO –3-8 weeks.
3. Period of the FETUS – 8-40 (birth) weeks.

Postnatal Development starts after birth.

Development begins with fertilization, the process by which the male gamete, the **sperm,** and the female gamete, the **oocyte,** unite to give rise to a **zygote.** Gametes are derived from **primordial germ cells (PGCs)** that are formed in the epiblast during the second week and that move to the wall of the yolk sac. During the fourth week these cells begin to migrate from the yolk sac toward the developing gonads, where they arrive by the end of the fifth week. Mitotic divisions increase their number during their migration and also when they arrive in the gonad. In preparation for fertilization, germ cells undergo **gametogenesis** and **cytodifferentiation**.

Stages of Development

I. **PROGENESIS** (gamete formation = gametogenesis).

II. **EMBRYOGENESIS** – is the process of embryo's development. Embryogenesis can be divided into next stages:
   - Fertilization and formation of a **zygote**.
   - Cleavage and formation of a **blastocyst**.
   - Gastrulation – formation of 3 embryonic layers (ectoderm, mesoderm, endoderm).
   - Histo- and organogenesis.
   - System genesis.

GAMETOGENESIS

Meiosis is the cell division that takes place in the **germ cells** to generate male and female gametes, sperm and egg cells, respectively. But, meiosis is not the only step in the formation of the gametes. These cells must become specialized for their role in fertilization. The overall process of gamete formation is called **gametogenesis**. In humans, there are two types of gametogenesis: spermatogenesis, which is sperm production, and oogenesis, which is production of the ovum or egg.

SPERMATOGENESIS

The sperm cell, or spermatozoon (plural: spermatozoa), is highly specialized for travel, allowing it to seek out an egg to fertilize. All spermatozoa derive from immature premeiotic stem cells called **spermatogonia** (singular: spermatogonium). They are present at birth.

Spermatogenesis begins when the male reaches puberty and continues into old age. In this process, each spermatogonium produces many **primary spermatocytes** through mitosis. Each primary spermatocyte then undergoes the first meiotic division to produce two secondary spermatocytes. Secondary spermatocytes undergo meiosis II, producing two spermatids from each secondary spermatocyte. Overall, therefore, each primary spermatocyte divides by meiosis to produce four haploid spermatids. The spermatids must undergo further differentiation and maturation to become spermatozoa.

The spermatogenesis can be divided into four stages:

- Proliferation (multiplication),
- Growth,
- Maturing,
- Spermiogenesis.

Spermatogenesis begins in the seminiferous tubules of the testis, after which the sperm move onto the epididymis to be stored and become functionally mature. The entire process takes approximately 2 months and results in gametes which are either 23,X or 23,Y.

The functionally mature spermatozoon has a **head** and acrosome cap (containing enzymes), a **neck**, and a **motile tail**.

STRUCTURE of the spermatozoon:

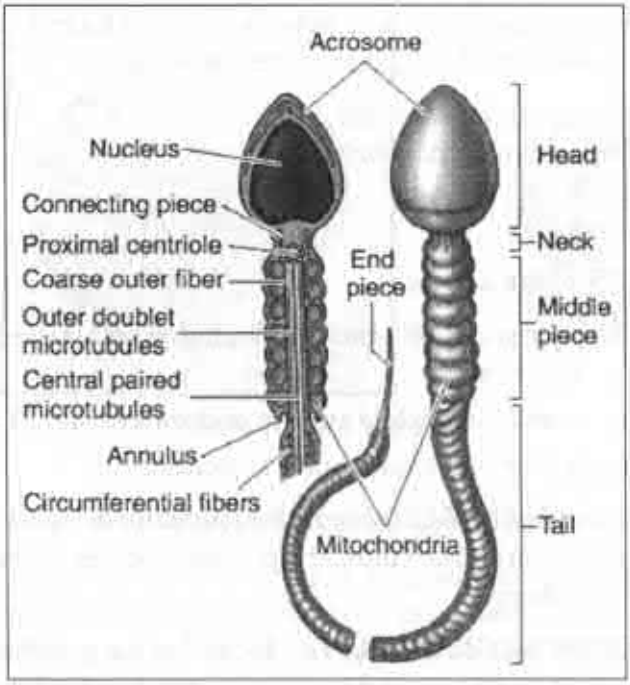
- **Head** contains:
  - Nucleus *(haploid amount of chromosomes* ***23 = 22A + 1S (X/Y)****).*

- Acrosome *(gigantic lysosome).*
- Cell membrane *(with receptors).*

• **Neck (*connecting piece*)** has:
- Centrioles (proximal & distal).

• **Tail**: flagellum has three portions:
- Middle piece
- Principle piece
- End piece

1. **MIDDLE PIECE** consists of:
   - axonema (consists of microtubules – (9×2)+2, microtubules are formed by tubulins with dinein arms) that is surrounded by a sheath of mitochondria aligned in a helix.
2. **PRINCIPLE PIECE** consists of:
   - axonema that is surrounded by dense fibrous fibers.
3. **END (terminal) PIECE** consists of:
   a. axonema that is surrounded by a small amount of cytoplasm and the cell membrane.



General structure of a spermatozoon

OOGENESIS

The ovum (plural: ova), or egg, is the largest cell type in the human body. This is because the ovum must store large quantities of materials needed for the development of the new individual after fertilization.

Unlike the male, maturation of the gamete begins before birth. In the female fetus, the primitive germ cells are called **oogonia**. The oogonia differentiate into primary oocytes before birth so that the female is born with all of the **primary oocytes** she will ever have, about 2 million of them! After birth, no more primary oocytes are formed. These primary oocytes begin the first meiotic division but are arrested at **prophase I**. They will stay in prophase until ovulation which begins at puberty. Many regress before this time, so that only 40 thousand remain at puberty. Then each month until menopause, one primary oocyte completes the first meiotic division to form ONE secondary oocyte and ONE smaller **polar body**. Cytokinesis in this case is unequal, such that one of the haploid cells receives almost all of the cytoplasm (secondary oocyte), while the other receives very little (polar body). The secondary oocyte begins the second meiotic division but again halts, this time at **metaphase**. During ovulation, it is then released from the surrounding ovarian follicle and enters the fallopian tube. If here it is penetrated by a sperm, it quickly completes the second meiotic division to become a mature ovum and a second polar body. During meiosis II, the first polar body is also thought to divide to form 2 polar bodies, so that a total of 3 polar bodies are formed.

Oogenesis can be divided into three phases:

- Proliferation (multiplication)
- Growth,
- Maturating.

STRUCTURE of the oocyte:

Oocyte is the biggest cell of human body; has round shaped, immobile and consists of:

1. Nucleus – contains a haploid amount of chromosomes ***23 = 22A + 1S (X).***
2. Cytoplasm (ooplasm) has specific structures:
    - ***YOLK GRANULES*** (spherical membrane-bound structures). They consist of proteins, lipids, and polysaccharides. Serve as nutrition for early embryo.
    - ***CORTICAL GRANULES*** (are located at the periphery. They prevent penetration of a second spermatozoon). **Cortical granules** contain:

- enzymes that cleave ZP2.
- enzymes that cleave ZP3.
- enzymes that crosslink adjacent ZP3s, making the zone impenetrable.
- reduction in the sperm-binding properties of the egg membrane.

3. **Cell membrane** with microvilli & receptors.

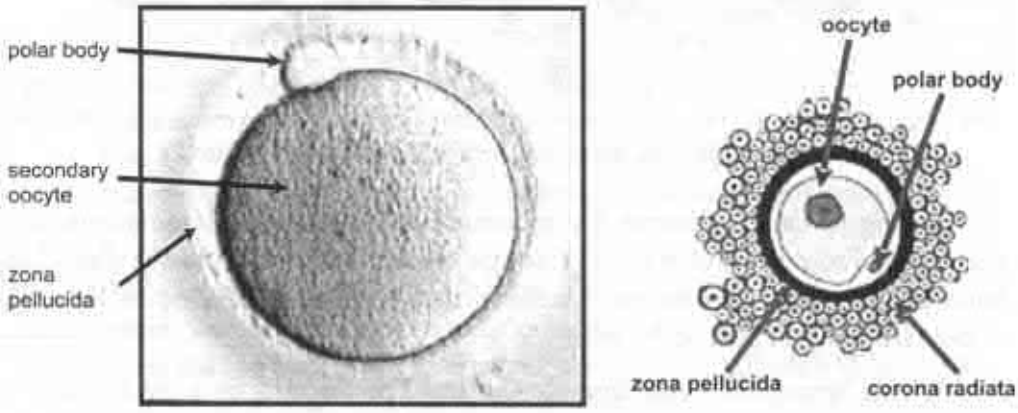
The oocyte is surrounded by two structures:

- **Zona pellucida**
- **Corona radiata**

The **ZONA PELLUCIDA** – is an amorphous layer of gel-like glycoproteine synthesized by both oocyte and follicular cells. There are 3 types of glycoproteins in the zona pellucida:

- **ZP-3** – receptors for spermatozoa.
- **ZP-2** – prevention from polyspermy.
- **ZP-1** – collects together ZP-2 & ZP-3.

The **CORONA RADIATA** is formed by several layers of follicular cells.

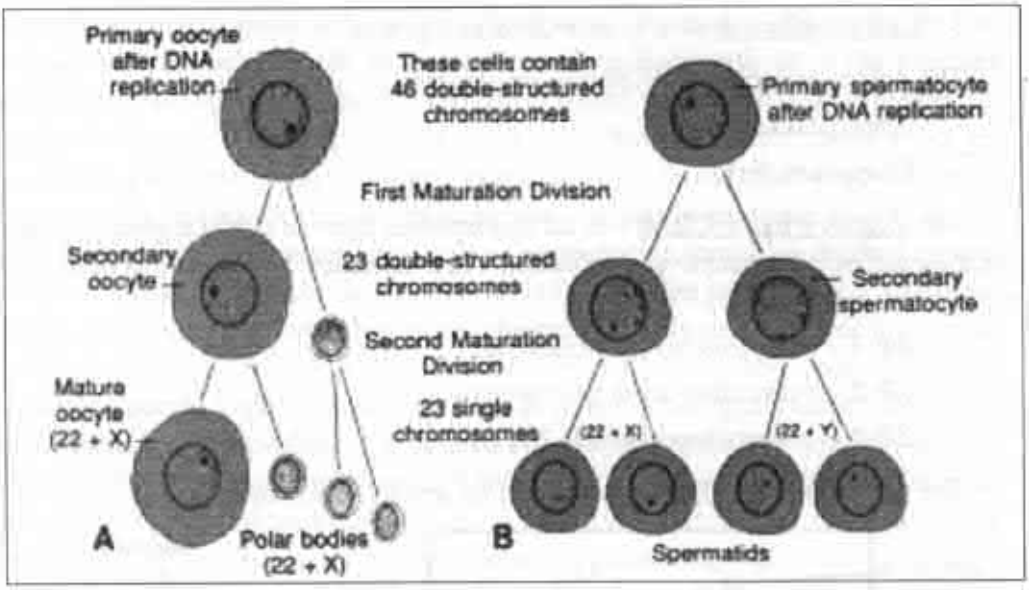


Structure of a oocyte

Important differences between Spermatogenesis and Oogenesis:

1. Time-wise: a mature sperm is created in approximately 2 months. In a female, a primary oocyte may wait up to 45 years before maturing completely. Also, complete maturation of the female gamete is dependent on fertilization by a mature sperm.

2. In the female gamete, division of the cytoplasm is unequal. The final result is ONE mature ovum and 3 smaller polar bodies versus FOUR mature sperm.
3. NO primary oocytes form after birth in the female; they are all formed in the fetus. In males, the formation of primary spermatocytes begins at puberty and continues throughout most of the male's life.



A. The primitive female germ cell (primary oocyte) produces only one mature gamete, the mature oocyte. B. The primitive male germ cell (primary spermatocyte) produces four spermatids, all of which develop into spermatozoa.

During sexual intercourse, the sperm are deposited in the vagina, outside of the cervix. From here, they travel through the cervical canal into the uterus and surround the secondary oocyte, usually in the ampulla of the fallopian tube, (the widest and longest part of the tube).

Sperm Transport in the Female Genital Tract occurs by a combination of two mechanisms:

- **Motility of spermatozoa** – they move at the speed of 2-3 mm/hour.
- **Contractions** in the female genital tract.

In their journey from vagina to oviduct, sperm must overcome a series of barriers, each of which eliminates a substantial proportion of the original population of sperm.

VAGINA

- following ejaculation, approx. 200 million sperm deposited at rear of vagina.
- pH of semen protects sperm from acidity of vagina (pH 4.2; semen neutralizes to pH 7.2).
- many sperm never reach the egg (100 to 1000 reach the oviduct; only 20-200 reach the egg).

CERVIX

- less than 1 million sperm make their way through the cervix.
- The cervix connects the vagina to the uterus.
- The cervical epithelium is richly endowed with mucus-secreting cells, and, as a consequence, the lumen is filled with mucus. The greatest barrier to sperm transport is the mucus of the cervix. Interestingly, the consistency and viscosity of cervical mucus is under endocrine control.
  - When estrogen levels are high and progesterone levels low, as occurs prior to ovulation, cervical mucus becomes watery (E-type mucus) and its mucin strands assume a parallel orientation. This state apparently greatly facilitates passage of sperm through the cervical canal.
  - Conversely, when progesterone concentrations are high, as in the luteal phase of the cycle, cervical mucus becomes exceptionally viscous and disorganized (G-type mucus), which largely precludes entry of sperm into the uterus.
- Many sperm lost in cervical folds, are reabsorbed.
- Some can remain in cervix and enter uterus at later time point.

UTERUS

- half of sperm travel into wrong oviduct.
- sperm wait at isthmus if ovulation hasn't occurred.
- when the egg is present, it "attracts" the sperm – **CHIMIOTACTISM**.
- cilia within oviduct also play a role in propelling sperm towards the egg.
- fertilization takes place in the ampulla of the oviduct.
- Less than 1% of spermatozoa reach the ampulla.

Egg Transport

Shortly before ovulation, fimbriae of the oviduct begin to sweep over the surface of the ovary, and the tube itself begins to contract rhythmically. It is thought that the oocyte surrounded by some granulosa cells is carried into the tube by these sweeping movements of the fimbriae and by motion of cilia on the epithelial lining.

Once an oocyte enters the oviduct, it is propelled by ciliary motion down into the ampulla, where fertilization takes place. The ciliary motion is regulated by the endocrine status during and after ovulation. The oviduct provides the appropriate environment not only for fertilization, but for early embryonic development, and it is important that the embryo remain there for a period of about three days.

The Fertilizable Lifespan of Gametes

Both sperm and egg have a short fertilizable lifespan, and once they are delivered into the female tract, the clock starts ticking. What this means, of course, if that mating or insemination must coincide closely with ovulation. If sperm are deposited many days before the egg reaches the oviduct, there is little chance that they will survive to fertilize. Conversely, if sperm reach the oviduct several days after ovulation, they will certainly find an egg that has long since degenerated.

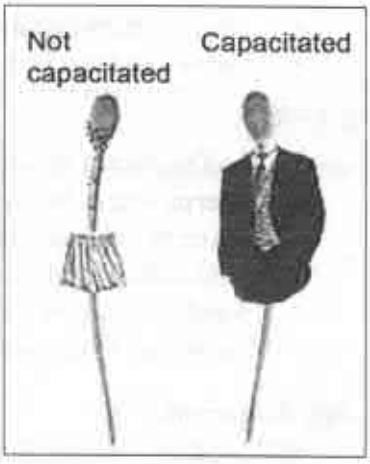
FERTILIZATION

Fertilization is the union of a human egg and sperm with formation of the zygote. Successful fertilization requires not only that a sperm and egg fuse, but that not more than one sperm fuses with the egg. Fertilization by more than one sperm – polyspermy – almost inevitably leads to early embryonic death.

- **Place**: fertilization usually occurring in the ampulla of the fallopian tube.
- The fertilization is also the initiation of prenatal development.
- **Entire process takes 24 hours.**

Freshly ejaculated sperm are unable or poorly able to fertilize. Rather, they must first undergo a series of changes known collectively as CAPACITATION (the process of activation of spermatozoa). The **capacitation** is associated with:

- removal of adherent seminal plasma proteins.
- reorganization of plasma membrane lipids and proteins.
- it also seems to involve an influx of extracellular calcium, increase in cyclic AMP, and decrease in intracellular pH.



Capacitation occurs while sperm reside in the female reproductive tract for a period of time, as they normally do during gamete transport. The length of time usually requires several hours (7 hours).

FINAL CAPACITATION = ABILITY TO FERTILIZE THE EGG.

After capacitation, sperm are able to undergo the acrosome reaction.

ACROSOME REACTION

- acrosome reaction of many sperm is necessary for one sperm to reach the egg.
- reaction takes 5-20 min.
- Is stimulated by sperm-binding glycoprotein molecules in the zona pellucida (ZP3, ZP1 and ZP2 ligands).
- Is accompanied by Ca2+ influx into sperm.
- Results in the release of acrosomal enzymes, which include hydrolytic enzymes and involves fusion of the acrosome membrane and plasma membrane.
- Is necessary for the sperm penetration through the zona pellucida.

A spermatozoon has to penetrate 3 layers before it fertilizes the oocyte:

- **corona radiata**
- **zona pellucida**
- **oocyte or vitelline membrane**

Capacitated sperm pass freely through corona cells. The constant propulsive force from the sperm's flagellating tail, in combination with acrosomal enzymes, allows the sperm to create a tract through the zona pellucida. These two factors; motility and zona-digesting enzymes; allow the sperm to traverse the zona pellucida.

Both binding and the acrosome reaction are mediated by the ligand ZP3, a zona protein. Release of acrosomal enzymes (acrosin) allows sperm to penetrate the zona, thereby coming in contact with the plasma membrane of the oocyte Permeability of the zona pellucida changes when the head of the sperm comes in contact with the oocyte surface.

The initial adhesion of sperm to the oocyte is mediated in part by the interaction of integrins on the oocyte and their ligands, disintegrins, on sperm. After adhesion, the plasma membranes of the sperm and egg fuse. Because the plasma membrane covering the acrosomal head cap disappears during the acrosome reaction, actual fusion is accomplished between the oocyte membrane and the membrane that covers the posterior region of the sperm head. In the human, both the head and tail of the spermatozoon enter the cytoplasm of the oocyte, but the plasma membrane is left behind on the oocyte surface *(see fig. 17, plate I)*.

Three changes occur in the oocyte after penetration of membrane:

1. **Cortical reaction** occurs to prevent more than one sperm from entering the egg (***polyspermy***).

The cortical reaction refers to a massive exocytosis of cortical granules seen shortly after sperm-oocyte fusion. Cortical granules contain a mixture of enzymes, including several proteases, which diffuse into the zona pellucida following exocytosis from the egg. These proteases alter the structure of the zona pellucida, inducing what is known as the **zona reaction**. Components of cortical granules may also interact with the oocyte plasma membrane. Reaction is stimulated by increased Ca^{++} from internal stores (due to presence of sperm).

The zona pellucida hardens. Runner-up sperm that have not finished traversing the zona pellucida are stopped in their tracks. Sperm receptors in the zona pellucida are destroyed. Therefore, any sperm that have not yet bound to the zona pellucida will no longer be able to bind, let alone fertilize the egg.

2. **Completion of second meiotic division** the oocyte finishes its second meiotic division immediately after the entry of the spermatozoon. NOW, the secondary oocyte becomes a mature **ovum** (*ovum activation; 2nd polar body is produced).*

3. **Formation of female and male pronuclei.**

Chromatin from both the sperm and egg are soon encapsulated in a nuclear membrane, forming pronuclei. Each pronucleus contains a haploid genome. They migrate together, their membranes break down, and the two genomes condense into chromosomes, thereby reconstituting a diploid organism.

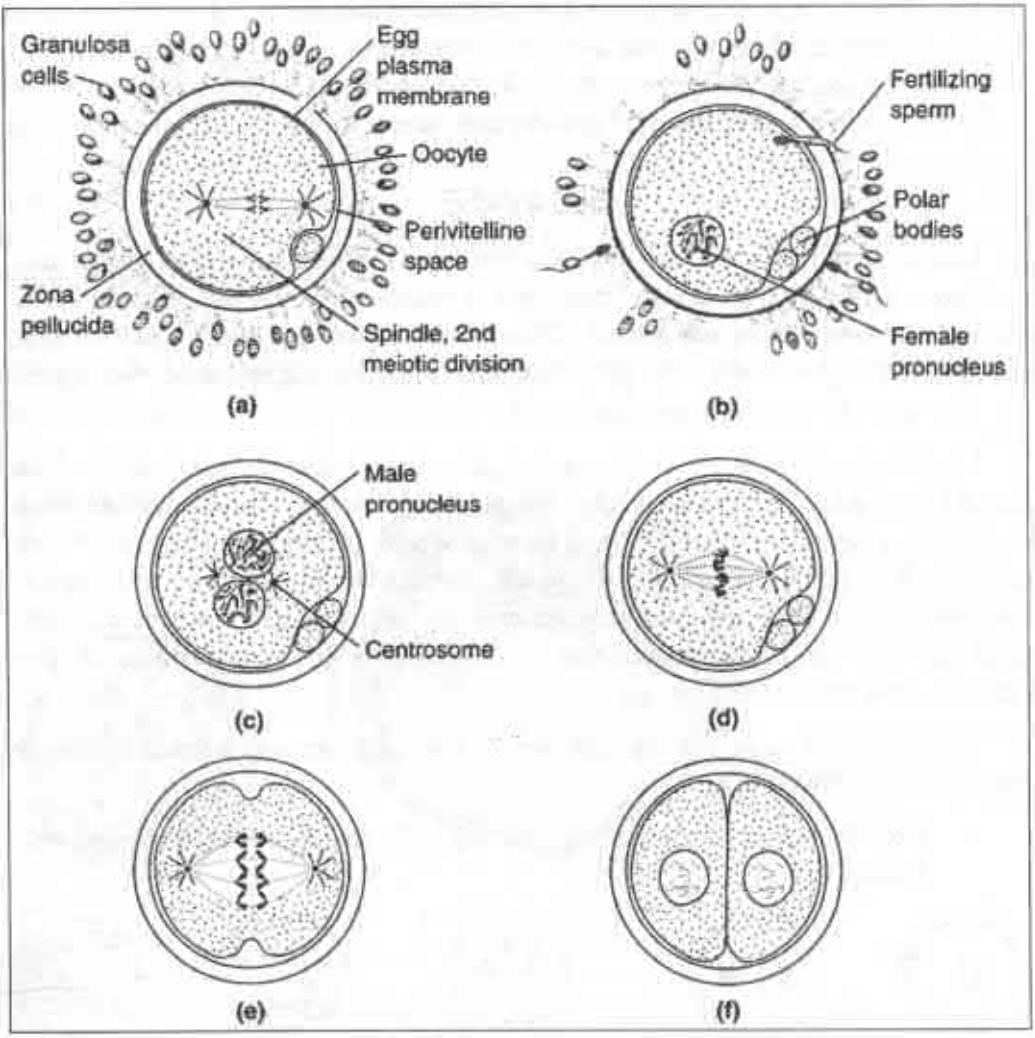
Fertilization is a process which extends over a period of 4 to 6 hours. The final product is the formation of zygote (fertilized ovum) with 46 chromosomes. The corona radiata is shed shortly afterward fertilization.

Significance of fertilization:

1) A **new life** begins (initiation of cleavage). Without fertilization, the oocyte usually degenerates 24 hours after ovulation.
2) **Restoration** of the **diploid number of chromosomes.**
3) **Determination of the sex** of the new individual: The sex of the zygote is determined by which chromosome is carried by the male gamete or sperm (either X or Y):

ova(22+X) + X-bearing spermatozoon = **girl**
ova(22+X) + Y-bearing spermatozoon = **boy**

This moment of zygote formation may be taken as the beginning or zero time point of the embryonic development.



A. Oocyte immediately after ovulation, showing the spindle of the second meiotic division. B. A spermatozoon has penetrated the oocyte. Chromosomes of the oocyte are arranged in a vesicular nucleus, the female pronucleus. C. Male and female pronuclei. D and E. Chromosomes become arranged on the spindle, split longitudinally, and move to opposite poles. F. Two-cell stage.

The cytoplasmic organelles of the zygote are <u>almost entirely</u> maternal:

- Mitochondrial DNA is almost entirely maternal.
- Mitochondrial genetic diseases are generally inherited through the mother but may affect both sons and daughters.

- The genes in mitochondrial DNA code for enzymes required for oxidative phosphorylation.
- Most mitochondrial diseases affect muscle and nerve.
- Examples of mitochondrial inheritance are:
  - mitochondrial myopathy (affects muscle).
  - Leber's optic atrophy (affects optic nerve).

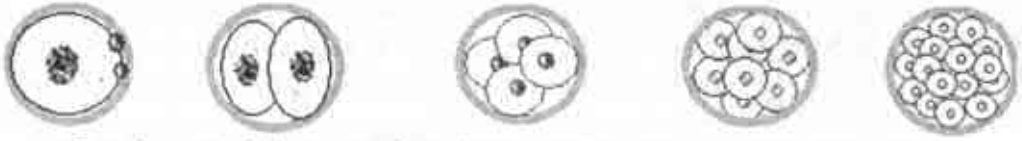
CLEAVAGE

During the first days of development the zygote travels down the oviduct and undergoes **CLEAVAGE** – *a regulated series of mitotic divisions, but these divisions are not accompanied by cell growth.* These divisions are generally equal so that each of the daughter cells is roughly half the size of the original predivision parent. The early divisions are asynchronous.

Divisions subdivide the large zygote into many smaller daughter cells called **BLASTOMERES**. Each new cell also has 46 chromosomes. The one cell embryo undergoes a series of cleavage divisions, progressing through 2-cell, 4-cell, 8-cell and 16 cell stages. The blastomeres appear identical to one another, and experimental evidence obtained from experiments with mammalian blastomeres indicates that they are still totipotent; that is, they have the ability to form any differentiated structure of the embryo.

By the 16 – 32 cell stage the embryo has the appearance of a small mulberry and is called a **MORULA**.

- Note that in all of the early stages, the embryo is encased in its zona pellucida.



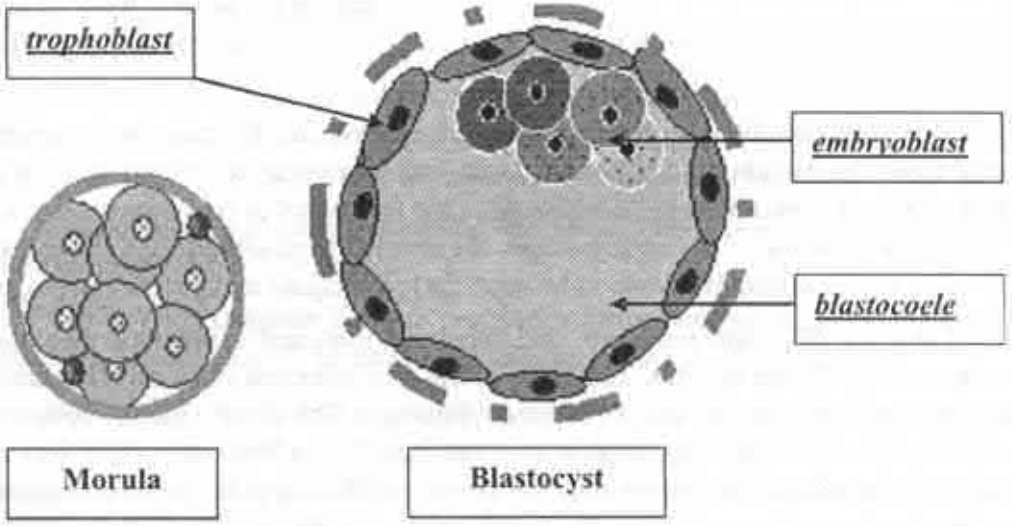
Development of the zygote from the two-cell stage to the late morula stage

BLASTOCYST

The formation of the blastocyst, beginning at the 32-64 cell stage in humans, marks the first true differentiation event. The early blastocyst forms from the morula when the cells on the outer surface near to the still intact zona pellucida form a continuous epithelial cell layer known as the trophoblast. The trophoblast layer seals off the interior of the embryo and then pumps in salt, which in turn results in the accumulation of fluid in a chamber called the blastocyst cavity or

blastocoele. This is the first of four major cavities to be formed during embryogenesis. The others are the intra and extraembryonic coeloms and the amniotic cavity. On one edge of the blastocyst cavity a group of cells remains aggregated together, the embryoblast (inner cell mass), whose appearance is approximately the same as that of the undifferentiated cells of the morula. These cells will give rise to all of the structures of the embryo proper, while the trophoblast will participate in the implantation process and contribute primarily to the formation of the placenta. The trophoblast cells fate is fixed – trophoblast cells cannot differentiate back into embryoblasts, while embryoblasts still retain the capability to produce trophoblasts.

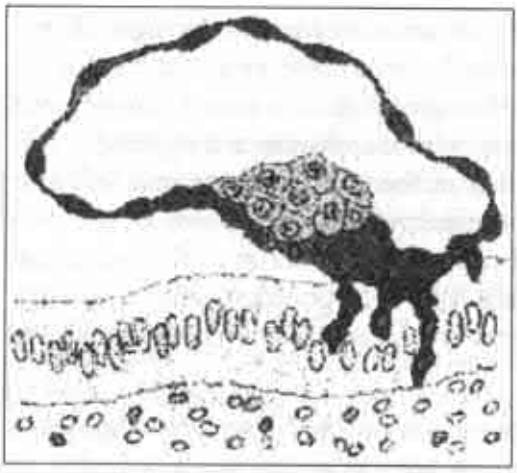
The stretched zona pellucida develops a crack and the blastocyst escapes by a process called **hatching**. This leaves an empty zona pellucida and a zona-free or hatched blastocyst lying in the lumen of the uterus.



Schematic representation of a human morula and blastocyst

IMPLANTATION (occurs 7-9 days after fertilization)

Fetal development in humans occurs completely within the endometrial layer of the uterine wall (the lining of the external surface of the uterus). The blastocyst must penetrate completely into the endometrium to allow further development to occur. After attachment to the epithelial cell layer of the endometrium at the embryonic pole, the trophoblast cells in this area undergo a differentiation process that results in their transformation into large, multinucleated, amoeba-like cells known as the syncytiotrophoblast.



Early phase of the implantation (adherence)

The syncytiotrophoblast is a highly adhesive and invasive cell that is responsible for implantation. It is able to adhere to the epithelial lining of the endometrium using several types of receptors, including the integrins, and penetrate deep into the endometrial stroma. Proteolytic enzymes that can degrade extracellular matrix (the collagen-rich substance surrounding cells) are critically involved. The remainder of the embryo is pulled in behind. The process of implantation is essentially complete by the end of the second week of development. A blood clot known as a closing plug (fibrin coagulum) then seals the site of the initial penetration.

Syncytiotrophoblast cells do not divide but are instead continuously generated from the remaining trophoblast layer, known now as the cytotrophoblast. Both types of trophoblast will contribute to the formation of the embryonic portion of the placenta. In the earliest stages, the syncytiotrophoblast (SCT) layer will develop spaces within it known as lacunae that participate in nutrient exchange.

Later the SCT will penetrate and surround maternal capillaries, then the veins and finally the arteries. This leads to the establishment of blood filled cavities within the SCT known as maternal sinusoids. The direct contact between maternal blood and the trophoblast layer provides the embryo with a rich source of nutrients, which enter the growing embryo by diffusion prior to the formation of the fetal circulatory system. The nearby maternal decidual cells also provide important nutrient substances to the embryo. Ordinarily, the invasion of blood vessels is perceived by the body as an injury and would induce a wound-healing response accompanied by clotting of the blood. However, a natural anti-clotting agent, tissue factor, is produced by the maternal decidual cells and is thought to prevent clotting from occurring *(see fig. 19, plate I)*.

Maternal blood vessels of the uterine wall and fetal vessels in the placenta are relatively deficient in smooth muscle. By contrast, most large blood vessels in the adult have extensive layers of smooth muscle cells surrounding them. Smooth muscle cells are influenced by hormones released into the circulation which in-

duce them to contract or relax, thereby regulating the size of the lumen of the vessels. Placental circulation is designed to be resistant to the actions of vasoconstrictive hormones. So, for example, when epinephrine is released into the maternal circulation due to circumstances such as sudden fright, there is little reduction in blood flow to the fetus since the placental vessels have much less smooth muscle and do not contract much. Nonetheless, the fetus is at risk when there are disturbances in maternal circulation, such as occurs in pre-eclampsia, a common condition characterized by elevated maternal blood pressure.

The process of early differentiation and implantation is an intricate one and a sizable fraction of fertilized ova are thought never to implant. Studies conducted over the last 50 years on spontaneously and electively aborted fetuses have given researchers an indication of the rates of defective embryos and fetal wastage in the general population. These studies have led to the conclusion that most spontaneous abortions occur in the first trimester. It is difficult to know precisely what is occurring during the critical first two weeks after fertilization, but it is estimated that perhaps as many as 60% of all embryos have serious defects and that a large proportion of these are weeded out very early in embryogenesis. Thus, it is likely that most of the defective embryos are eliminated before the mother is aware of anything unusual. In addition, many may never implant for unknown reasons.

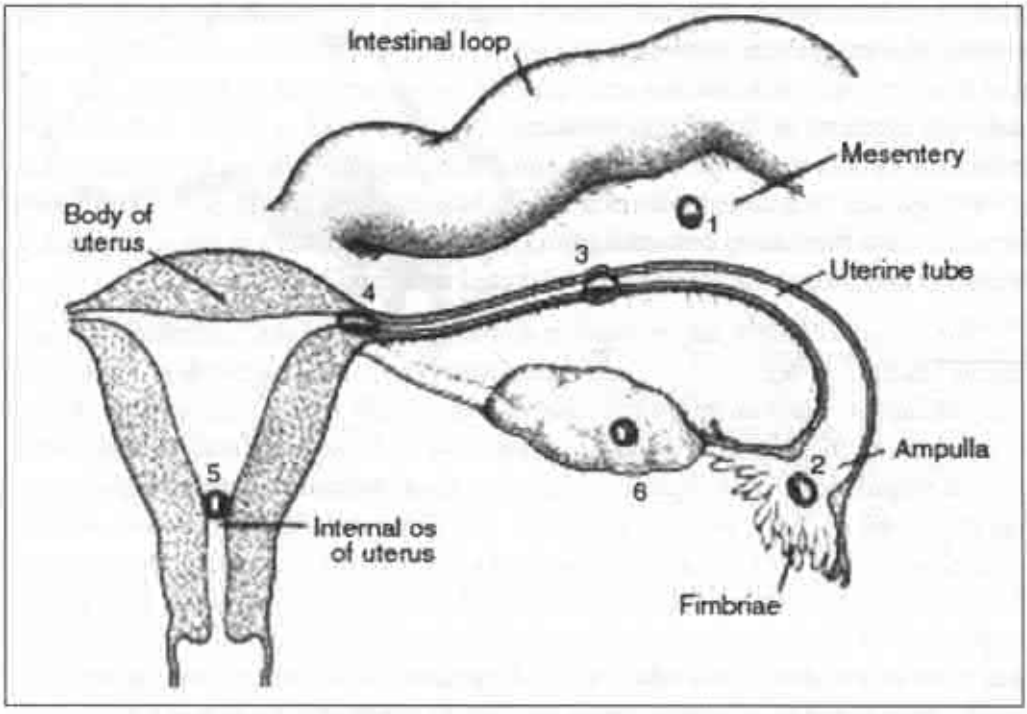
Implantation occurs in the uterine wall about 99% of the time, most often in the posterior wall.

Clinical correlations: Ectopic pregnancy, where implantation occurs at abnormal sites can be a life threatening condition to the mother and fetus.

Implantation at the cervical opening of the uterus leads to a condition where the placenta seals off the opening, known as ***placenta previa***. In this rare circumstance, the fetus can go to term but usually causes much bleeding. Delivery is often by Caesarean section.

More commonly, implantation occurs in the oviduct (***tubal pregnancy***), probably due to impeded or improper transport of the embryo. If it is in the extrauterine portion, it will generally rupture the tube by the 8th week, resulting in the death of the embryo and severe hemorrhaging and danger of infection for the mother. If implantation occurs in the intrauterine portion of the tube, appropriate placental circulation can be established and the fetus can develop somewhat further, although probably not to term.

Implantation at other sites, such as the ovary or cervix, is very rare and usually results in spontaneous abortion.



Abnormal implantation sites of the blastocyst. 1, implantation in the abdominal cavity. 2, implantation in the ampullary region of the tube. 3, tubal implantation. 4, interstitial implantation, that is, in the narrow portion of the uterine tube. 5, implantation in the region of the internal os, frequently resulting in placenta previa. 6, ovarian implantation.

Implantation in the abdominal cavity may also occur very rarely. In this case, the fetus can occasionally go to term, although this type of implantation might compromise the health of the mother by damaging internal organs.

CHAPTER II

SECOND AND THIRD WEEKS OF DEVELOPMENT

At the eighth day of development, the blastocyst is partially embedded in the endometrial stroma. Cells of the inner cell mass or embryoblast also start to differentiate – gastrulation is started. **GASTRULATION** is the process through which the inner mass cell (embryoblast) becomes a trilaminar germ disc. **During gastrulation takes place:**

- proliferation (division of cells).
- growth.
- purposeful migration of cells.
- cell differentiation.
- formation of extraembryonic organs.

Gastrulation begins at the time of implantation. It is divided into:

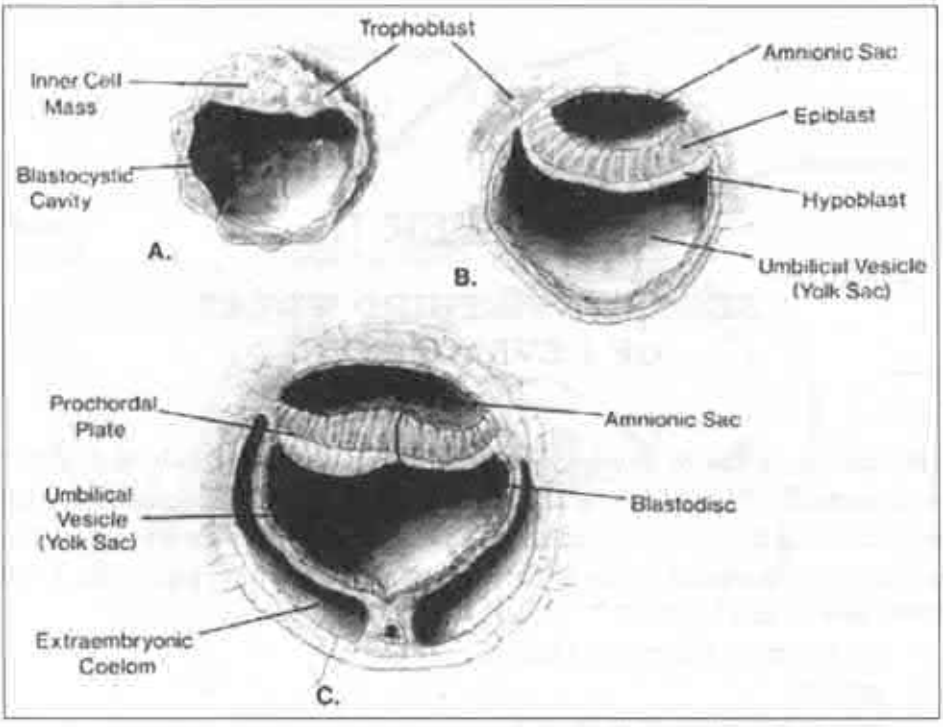
- Early gastrulation
- Late gastrulation

EARLY GASTRULATION

Early gastrulation occurs in implantation. It begins on 7 day and finishes on 14 – 15 days. During the early gastrulation the embryoblast delaminates into 2 layers:

1. a layer of small cuboidal cells adjacent to the blastocyst cavity, known as the **HYPOBLAST layer,**
2. a layer of high columnar cells adjacent to the amniotic cavity, the **EPIBLAST layer.**

Together, the layers form a flat disc – **BILAMINAR GERM DISC.**



Cross section of 11-day old blastocyst, showing the formation of two germ layers. Also shown are the amniotic cavity, the primary yolk sac, and extraembryonic coelom.

In the same time:

- A small cavity appears within the epiblast. This cavity enlarges to become the **amniotic cavity.** Epiblast cells adjacent to the cytotrophoblast are called **amnioblasts;** together with the epiblast, they line the amniotic cavity. The "floor" of the amniotic cavity is formed by the epiblast.
- A second cavity is formed below the hypoblast, called the **primitive yolk sac**. Cells of the hypoblast are continuous with the **exocoelomic membrane,** and together they line the primitive yolk sac.

The cytotrophoblast cells proliferate to form a loosely tissue, called the **extraembryonic mesoderm**. By the end of the second week, extraembryonic mesoderm fills the space between the cytotrophoblast and the amnion and exocoelomic membrane internally. When vacuoles develop in this tissue, the **extraembryonic coelom** or **chorionic cavity** is formed. This new space surrounds the amniotic cavity an the primitive yolk sac, except site where the extraembryonic mesoderm remains as a connection between the embryonic disc and the trophoblast, called the **connecting stalk** or **body stalk.**

As the result of the extraembryonic coelom grows, the large primary yolk sac pinches off, leaving behind a smaller, **secondary yolk sac** which is "permanent".

With the formation of the extraembryonic coelom (future chorionic cavity), the extraembryonic mesodermal cells become divided into two areas:

- somatopleuric mesoderm lining the ectoderm of amniotic cavity and lining the trophoblast
- splanchnopleuric mesoderm lining the endoderm of the secondary yolk sac.

The embryo surrounded by two cavities, floats in a large "bubble" (future chorionic cavity).

– The trophoblast with its lining extraembryonic mesoderm is now known as the **chorion**. The chorion growth and differentiation provide the development of the placenta.

Summary of Origin of Extraembryonic Membranes

AMNION	**bilayer**	**amniotic epithelium + extraembryonic somatic mesoderm**
CHORION	**trilayer**	**extraembryonic somatic mesoderm + cytotrophoblast + syncytiotrophoblast**
YOLK SAC WALL	**bilayer**	**yolk sac endoderm + extra-embryonic splanchnic mesoderm**

Prechordal Plate

By the end of the second week, the hypoblast cells enlarge at one end to form the **prechordal plate**. The prechordal plate marks the cranial aspect of the developing embryo and plays a large role in the development of the oral cavity.

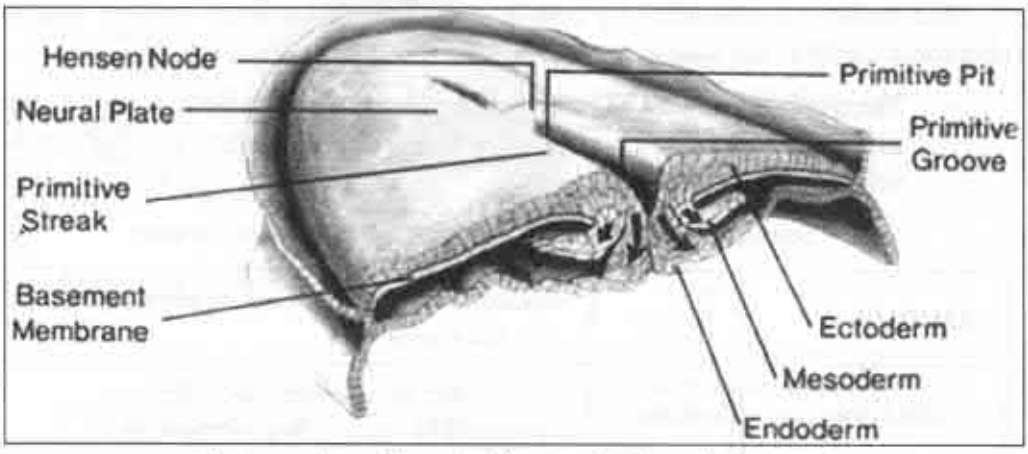
LATE GASTRULATION

The late gastrulation starts by the end of the second week (14th day) and finishes on 17th day after fertilization.

The late gastrulation begins when cells of the epiblast proliferate and migrate to the median plane of the dorsum of the embryonic disc and form a linear band called the **primitive streak** *(see fig. 21, plate I)*. The primitive streak begins at the **caudal** end of the embryonic disc and continues cranially towards the prechordial plate.

As soon as the primitive streak appears, it is possible to identify the embryo's craniocaudal axis, its cranial and caudal ends, its dorsal and ventral surfaces and its right and left sides.

The primitive streak grows by adding new cells to its caudal end. About halfway towards the prechordal plate, the cells of the primitive streak proliferate and form the **primitive node (Hensen Node)**. Running longitudinally within the primitive streak is the **primitive groove** which ends in a central indentation of the primitive node called the **primitive pit**. **Primitive groove and primitive pit** result from the invagination of epiblastic cells.



The formation of the primitive streak, the primitive node

The ectodermal cells of the primitive streak undergo further proliferation and extend laterally, cephalically, and caudally between the ectoderm and endoderm of the embryonic disc. These cells form the third germ layer, the embryonic mesoderm. In such way the 2-layered embryo becomes a 3-layered embryo that is composed of:

- ECTODERM
- MESODERM
- ENDODERM

At first the mesodermal cells have an epithelial appearance; later many become loosely arranged, irregular in shape, and display ameboid activity. Such cells form a very loose tissue referred to as mesenchyme. As the mesodermal cells extend laterally, they finally reach and fuse with the extraembryonic mesoderm of the amnion and yolk sac. At the cephalic end of the embryonic disc, the ectodermal and endodermal layers fuse over a small area which later becomes the **buccopharyngeal**

membrane. In similar manner, at the caudal end of the embryonic disc, the ectodermal and endodermal layers fuse to form the **cloacal membrane**. It should be noted that the mesoderm does not extend between the ectoderm and endoderm of the buccopharyngeal and cloacal membranes. At the caudal end of the embryonic disc, the mesoderm, having passed around the cloacal membrane, becomes continuous with the extraembryonic mesoderm of the body stalk.

While the primitive streak is giving rise to the embryonic mesoderm, a solid cord of cells grows cephalically from the primitive knot (Hensen node). The Hensen node, a specialized structure located at the cranial end of the primitive streak, serves a special role as the organizer of the embryo. As the streak elongates, prospective endodermal cells migrate through the primitive pit (in the center of the Hensen node). As the streak regresses, prospective notochordal cells begin to invaginate through the Hensen node and are laid down in the midline between the overlying neuroectoderm and underlying endoderm as the notochordal process.

Formation of the Notochord

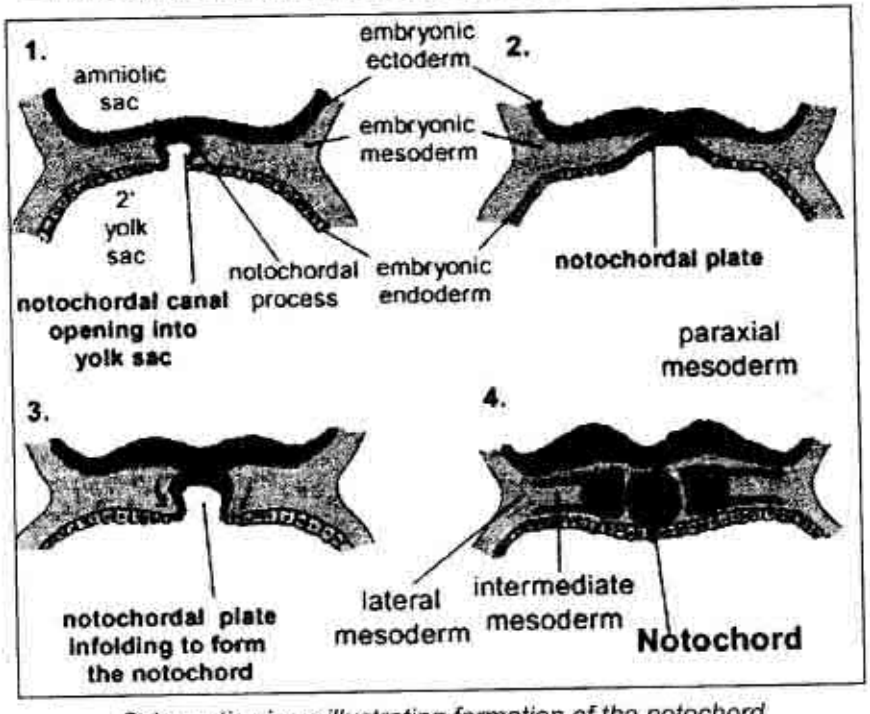
In the human, the notochordal process begins to form on day 16. The notochordal process is the precursor to the notochord. The molecular mechanism of notochord formation is unclear.

The notochordal process consists of a cord of cells radially arranged around a central lumen called the notochordal canal. The notochordal canal is continuous dorsally with the amnionic cavity through the primitive pit. The notochordal process continues to elongate between days 17 and 21. Between days 18 to 20 it fuses, or intercalates, with the underlying endoderm to form the notochordal plate. This plate is incorporated into the roof of the yolk sac, with which the notochordal canal becomes continuous. The most caudal portion of the notochordal canal is continuous both with the amnion through the primitive pit and with the yolk sac as a result of intercalation; this communication is called the neurenteric canal. By days 23 to 25, the notochordal plate folds dorsoventrally and separates (or excalates) from the endoderm and separates once again from the underlying endoderm, obliterating the neurenteric canal and ending the communication between the amnionic and yolk sacs. Thereafter, the "true" notochord exists as a solid rod of notochordal cells.

NOTOCHORD:

- defines the **primordial axis** of the embryo and gives it some rigidity.
- serves as the **basis** for development of the **axial skeleton** (bones of head and vertebral column).

- Indicates the **future site of the vertebral bodies.**
- The notochordal functions as the primary **inductor** in the early embryo – induces the ectoderm to thicken and form the neural plate.



Schematic views illustrating formation of the notochord

The notochord will mostly degenerate, persisting only as the ***NUCLEUS PULPOSUS*** of the intervertebral discs.

Clinical Note

In the formation of the **notocord**, a small canal is temporarily retained between the lumen of the notochord process and the endoderm (yolk sac). This canal is called the **neurenteric canal**, which quickly degenerates. Rarely, it persists and results in a link between the central canal of the spinal cord and the intestine. This forms a spinal enteric fistula which predisposes to recurrent meningitis, spinal cysts, and even spinal cord compression.

CHAPTER III

THIRD TO EIGHTH WEEK: THE EMBRYONIC PERIOD

ORGANOGENESIS: DIFFERENTIATION OF THE GERM LAYERS

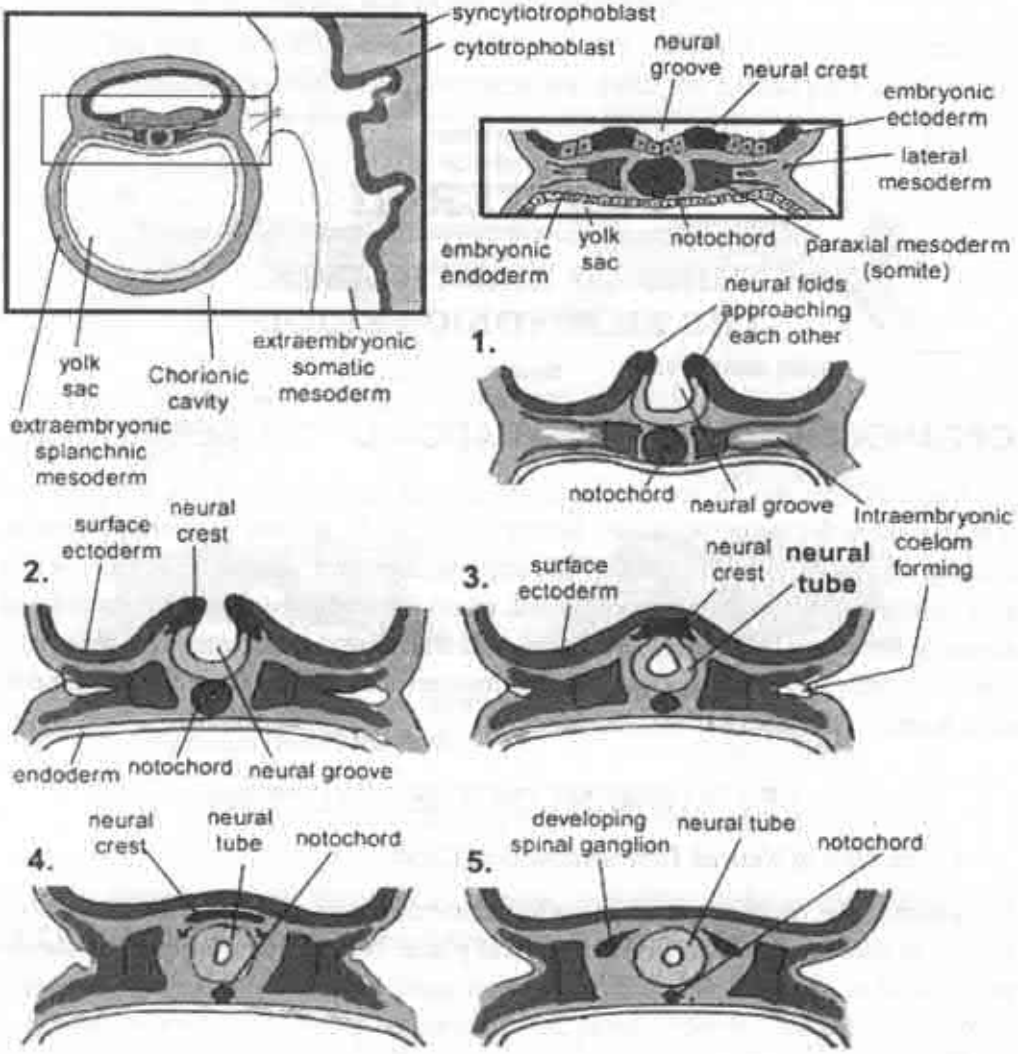
Gastrulation lays down the basic structural framework of the embryo and sets the stage for organogenesis, formation of body organs and organ system. During organogenesis, the cells of the embryo continue to rearrange themselves and, by the end of the embryonic period, when the embryo is eight weeks old and about 22 mm long from head to buttocks, all the adult organ system are recognizable. It is truly amazing how much organogenesis occurs in such a short time in such a small amount of living matter.

DEVELOPMENT OF THE ECTODERM

Formation of Neural Tube and Neural Crest

During the third week of gestation the ectoderm overlying the notochord begins to thicken and is called the **neural plate**. Neural plate appears at the cephalic end of the embryo. This plate is situated between the buccopharyngeal membrane and the primitive knot. As the embryo grows, the lateral edges along the length of the neural plate begin to elevate. The edges form the neural folds and the central region is called the neural groove. The edge of each fold is known as the neural crest.

This process continues until the edges of the neural folds begin to meet in the midline to form the neural tube. This process of fusion begins first in the region of the future embryonic neck and extends toward the cephalic and caudal ends of the embryo. The neural tube communicates for a time with the amniotic cavity through the anterior (cephalic) and posterior (caudal) neuropores. Cephalic neuropore normally closes around day 25 of gestation, caudal neuropore closes around day 29 of gestation.



Formation of neural tube (neurulation)

As the ends of the neural tube close the process of neurulation is complete and the central nervous system is now represented by this closed hollow tube which is narrow caudally and larger and more dilated cephalically. The narrow caudal end represents the future **spinal cord** while the cephalic end represents the **brain** vesicles (the forebrain, midbrain, and hindbrain).

The cells of neural crest migrate extensively and give rise to a number of diverse cell types. The particular cell type that is formed is dependent upon the area where the cells migrate as well as where along the neural tube the cells arise.

Cranial neural crest gives rise to cells forming the trigeminal, facial, glossopharyngeal, and vagal sensory ganglia. These crest cells also give rise to the ciliary, pterygopalatine, submandibular and otic parasympathetic ganglia as well as ganglia associated with the vagus. Finally, these cells give rise to cartilage cells of the head and neck, odontoblasts and mesenchymal cells that form bones of the skull.

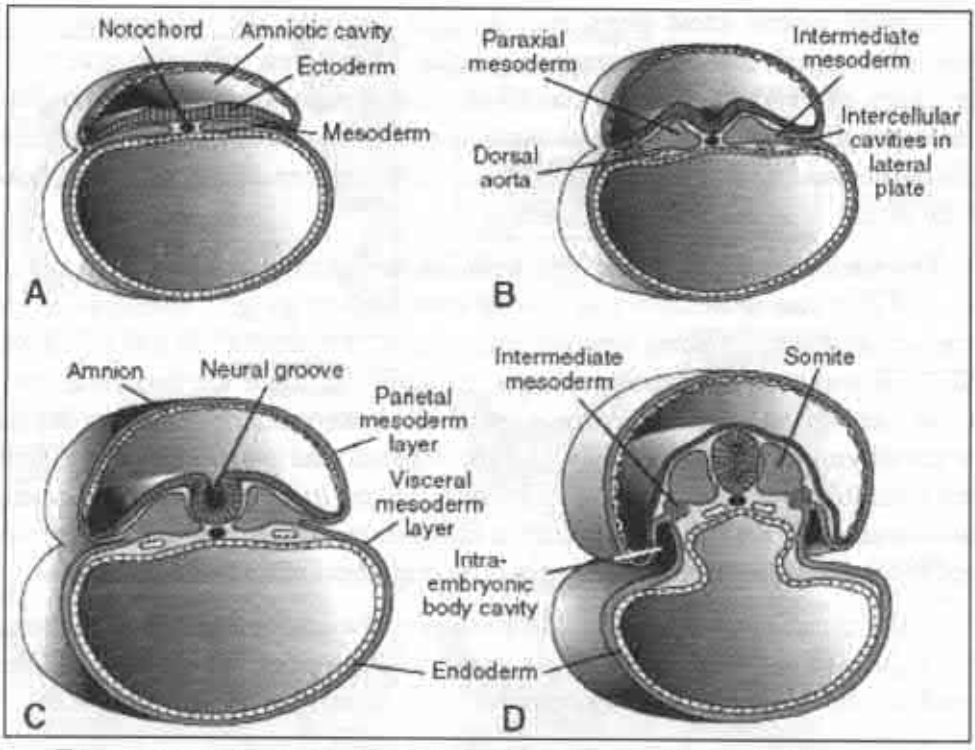
The neural crest cells associated with the neural tube that will form the spinal cord give rise to melanocytes, dorsal root sensory ganglia, the cervical, prevertebral and paravertebral sympathetic ganglia, the adrenal medulla, Schwann cells, and some parts of the meninges. In addition, there are contributions of neural crest cells to the wall of the aortic arches, the aorticopulmonary septum, the parathyroid, the carotid body and the thyroid. The pathway that the neural crest cells follow is thought to be influenced by the local environment in which the cells are located. Substances such a fibronectin, hyaluronic acid, or laminin appear to play a major role in directing the migration of neural crest.

Other central nervous system derivatives of the ectoderm include the retina, the Organ of Corti, the vestibular apparatus and the olfactory epithelium. These are all sensory neuroepithelial structures.

Outside the central nervous system and neural crest, the ectoderm gives rise to the epidermis and associated structures such as sweat glands, sebaceous glands, mammary glands, hair and nails. Structures associated with the oral cavity derived from ectoderm include the epithelia of salivary glands, enamel of teeth, covering of the tongue, anterior 2/3 of the oral cavity and part of the pituitary gland. The distal lining of the anal canal and the lining of the external auditory meatus is also derived from ectoderm. Finally, those structures associated with the eye that are derived from ectoderm include the anterior corneal epithelium, the glands associated with the eye, the lens, and pupillary muscles.

DEVELOPMENT OF THE MESODERM

In its early stages the embryonic mesoderm is a loose feltwork of cells extending laterally, anteriorly, and posteriorly between the ectoderm and the entoderm. At the edge of the embryonic disc it is continuous with the extraembryonic mesoderm that surrounds the yolk sac and amniotic cavity and lines the trophoblast. It soon becomes differentiated into three regions: paraxial mesoderm, intermediate mesoderm, and lateral mesoderm.



Transverse sections showing development of the mesodermal germ layer. A. Day 17. B. Day 19. C. Day 20. D. Day 21. The thin mesodermal sheet gives rise to paraxial mesoderm (future somites), intermediate mesoderm (future excretory units), and lateral plate, which is split into parietal and visceral mesoderm layers lining the intraembryonic cavity

Paraxial Mesoderm.

In its early stage this is a column of tissue situated on either side of the midline of the embryo. At about the fourth week it becomes divided into segmental blocks of tissue, the process being known as **segmentation** of the mesoderm. The blocks, or **somites**, can be seen through the amniotic or dorsal surface of the embryo. There are approximately 43 pairs of somites, which appear in a craniocaudal sequence and extend from the region of the developing hindbrain to the caudal end of the embryo. The first pair of somites appears at 18-19days in the future occipital region of the embryo.

Each somite becomes differentiated into next regions:

– **Sclerotome** – cells of this region take part in the formation of the bones, cartilage, and ligaments of the vertebral column (vertebral bodies, vertebral arches) and part of the base of the skull.

- **Myotome** – give rise to skeletal or voluntary muscle of its own segment.
- **Dermatome** – cells migrate laterally under the overlying ectoderm and assist in the formation of the dermis.

It is important to remember that the muscles derived from a given myotome and the dermis formed from a given dermatome always retain the nerve supply from the segment of the spinal cord which supplies that particular somite.

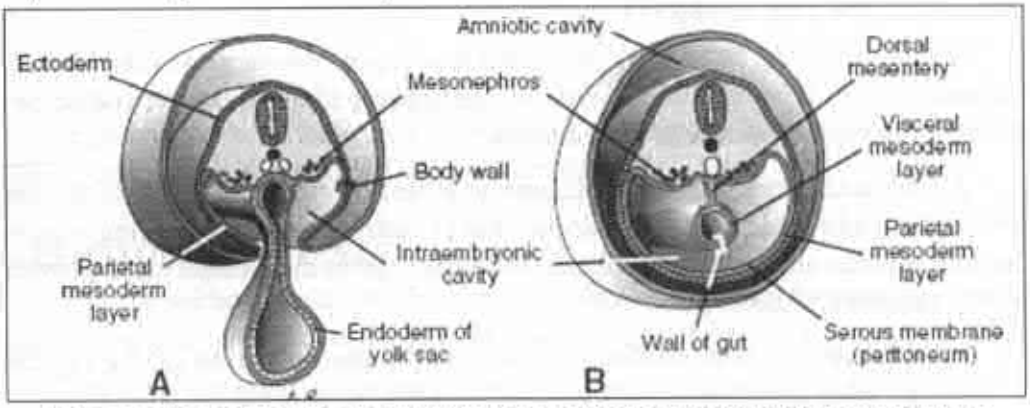
Intermediate Mesoderm

This is a second column of mesodermal tissue present on both sides of the embryo and connected to the paraxial mesoderm and the lateral mesoderm. The intermediate mesoderm gives rise to the kidneys and associated ducts, as well as the epididymis and vas deferens, accessory glands in the male, and the vagina, oviducts, uterus of the female.

Lateral mesoderm

This is directly continuous with the extraembryonic mesoderm beyond the margins of the embryonic disc. This tissue splits into a somatic (parietal) and splanchnic (visceral) layer associated with ectoderm and entoderm, respectively, and encloses a cavity, the intraembryonic coelom. The intraembryonic coelom is continous on each side of the embryo with the extra-embryonic coelom.

The intraembryonic coeloms communicate across the midline just cranial to the buccopharyngeal membrane to form a horsehoe-shaped cavity. The portion of the coelom which lies cranial to the buccopharyngeal membrane will eventually form the pericardial cavity.



A. Transverse section through a 21-day embryo in the region of the mesonephros showing parietal and visceral mesoderm layers. The intraembryonic cavities communicate with the extraembryonic cavity (chorionic cavity). B. Section at the end of the fourth week. Parietal mesoderm and overlying ectoderm form the ventral and lateral body wall. Note the peritoneal (serous) membrane

The remainder of the intraembryonic coelom will form the pleura and peritoneal cavities. The cells of the somatic and splanchnic layers of mesoderm will form the serous membranes of the pericardial, pleural, and peritoneal cavities.

The mass of mesoderm, which contains parts of the somites from cervical segments 3, 4, and 5, is known as the **septum transversum**. The latter will be used to convey blood vessels to the heart. Lateral mesoderm also gives rise to the cortex of the adrenal gland, myocardium, the spleen.

DEVELOPMENT OF THE ENDODERM

The endoderm gives origin to the following structures: the epithelium of the alimentary tract from the oral cavity down to halfway along the anal canal and the epithelium of the glands that develop from it, the thyroid, parathyroid, thymus, liver, pancreas; the epithelium of the respiratory tract; the epithelia of the pharyngotympanic tube, the middle ear (including the inner layer of the tympanic membrane), the mastoid air cells; the epithelium of the urinary bladder, parts of the female and male urethras.

The Embryonic Mesenchyme gives origin to the following structures:

- Connective tissue (proper CT, cartilage, bone and other)
- Smooth Muscle tissue
- Blood
- Lymph
- Endothelium

FOLDING OF THE EMBRYO

At the end of the third week, the germ disc begins to overgrow the yolk sac, ballooning into a convex shape, with the peripheral areas of the germ disc becoming the ventral surface of the embryo.

A significant event in the establishment of the body form is folding of the flat trilaminar embryonic disc into a somewhat cylindrical embryo. Folding occurs in both median and horizontal planes and results from differential rapid growth of the embryo's tissues.

- **Lateral** (median) **folding** is produced by the formation of the rapidly growing somites.
- **Cefalocaudal** (horizontal) **folding** is caused mainly by the rapid, longitudinal growth of the central nervous system whereas the transverse.

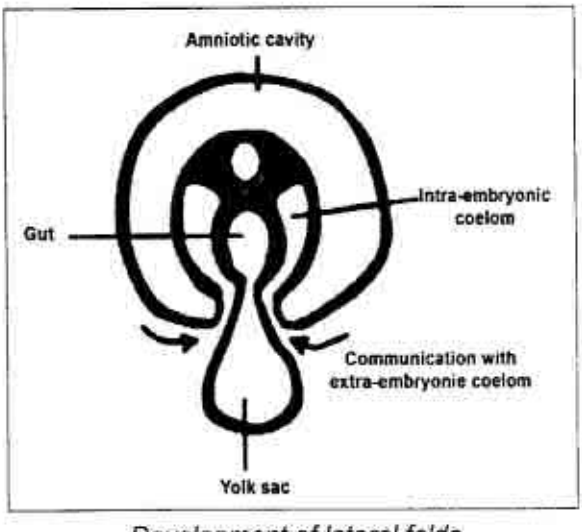
LATERAL FOLDING

The lateral edges of the germ disc bend ventrally, meet and fuse along the ventral midline from the cranial and caudal ends toward the neck of the constricting yolk sac, converting the embryo into a tubular structure with three concentric layers:

1. An outer layer of ectoderm
2. An intermediate layer of mesoderm
3. An inner layer of endoderm

Thus, ectoderm covers the whole of the embryo except for the closing neuropores and the ubilicus, from which the connecting stalk and yolk sac neck emerge.

Fusion of the lateral edges of the endoderm creates the gut tube, with blind ends in the cranial and caudal regions forming the foregut and hindgut respectively. The midgut is temporarily open to the constricting yolk sac neck, which will narrow into the slender vitelline duct by the end of the sixth week.



Development of lateral folds

The buccopharyngeal membrane, capping the foregut, ruptures at the end of the fourth week, connecting the oral cavity to the pharynx; whilst the cloacal membrane, capping the hidgut, breaks down in the seventh week, forming the anal and urogenital systems.

CEPHALOCAUDAL FOLDING

Cranial folding begins on about day 21, with cephalic folding taking place as the neural plate overgrows the yolk sac, caudal folding begins on about day 23.

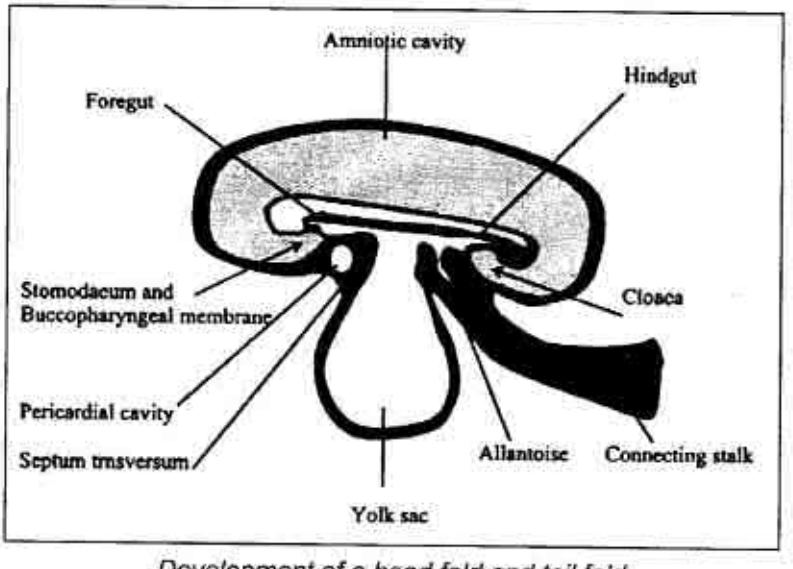
The cranial region of the embryonic disc, from caudal to cranial contains:

1. The **buccopharyngeal membrane**, just cranial to the neural plate.
2. The **cardiogenic area**, a horseshoe shaped area cranial and lateral to the buccopharyngeal membrane.

3. Thickened mesoderm called the **septum transversum** that will give rise to portions of the liver and diaphragm.

The caudal rim of the embryonic disc contains:

1. The **cloacal membrane.**
2. The **connecting stalk**, which itself contains the slender allantois.



Development of a head fold and tail fold

Caudal folding brings the cloacal membrane onto the ventral surface of the embryo, with the embryo rotating ventrally until the connective stalk lies against the neck of the yolk sac.

EXTRAEMBRYONIC ORGANS

1. Chorion
2. Yolk sac
3. Amnion
4. Allantois
5. Umbilical cord
6. Placenta

CHORION:

The chorion is formed by:

- **Cytotrophoblast,**
- **Syncytiotrophoblast**
- **Extraembryonic mesoderm**

It forms the wall of the chorionic sac, within which the embryo and its amniotic and yolk sacs are suspended by the **connecting stalk** (containing the allantois and the umbilical vessels consisting of two arteries and one vein).

YOLK SAC:

- Is the **first hemopoietic organ**.
- **Primordial germ cell**: derived from endoderm of yolk sac *(the wall is formed by entoderm and extraembryonic mesoderm).*

AMNION:

- is a membrane that is formed by amniotic epithelium (ectoderm) and extraembryonic mesoderm.
- amniotic cavity contains about 1½ litters of a clear watery amniotic fluid.

Amniotic fluid:

- Is secreted in part by amniotic **epithelial cells,** but is derived primarily from maternal blood.
- It serves as a protective watery cushion which **absorbs "jolts"** that may hurt the embryo.
- It **prevents the adhesion** of the embryo to the amnion.
- It **keeps the temperature** of the embryo nearly constant so that any rise of temperature does not cause harm to the embryo.
- It allows the embryo to **move freely**.
- It provides a space where **urine accumulates** before birth.
- It **protects** the fetus from the strong muscular contractions of the uterus in the early stages of labour.

ALLANTOIS

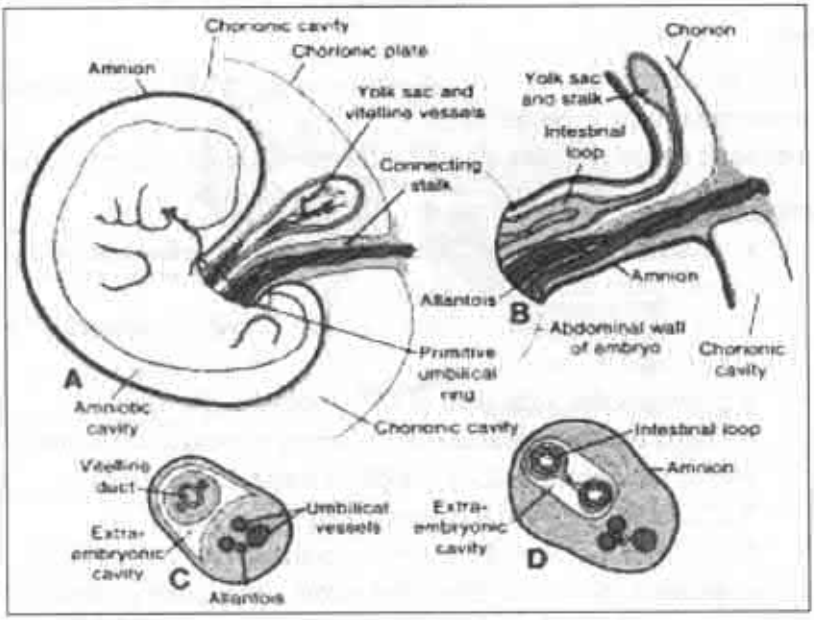
- Appears on about day 16 as a small, sausage-shaped diverticulum from the caudal wall of the yolk sac that extends into the connecting stalk. The part of the hindgut from which it arises eventually becomes the urinary bladder.
- The allantois becomes a fibrous cord, *the urachus*, which stretches from the apex of the bladder to the umbilicus.
- The allantois itself is not important in human but its significance is due to its vessels (the allantoic vessels) which then become the umbilical vessels.

UMBILICAL CORD

- Is a pathway which connects the placenta with the embryo.
- Contains: the yolk sac stalk, umbilical vessels, and the remnant of allantois.
- Cylindrical structure:
  - 40-60 cm long, 1.5-2.0 cm in Ø.

Umbilical cord contains 3 umbilical vessels: 1 vein and 2 arteries, mucous connective tissue, **Wharton's jelly** (tissue rich in mucopollysaccharides and functions as a protective layer for the blood vessels). The walls of the arteries are muscular and contain many elastic fibers, which contribute to a rapid constriction and contraction of the umbilical vessels after the cord is tied off.

* **oxygenated blood** is carried toward to fetus by the umbilical **vein.**
* **nonoxygenated blood** outward the fetus by the umbilical **arteries.**



A. A 5-week embryo showing structures passing through the primitive umbilical ring. B. The primitive umbilical cord of a 10-week embryo. C. Transverse section through the structures at the level of the umbilical ring. D. Transverse section through the primitive umbilical cord showing intestinal loops protruding in the cord.

TERATOLOGY

Birth defect, congenital malformation, and **congenital anomaly** are synonymous terms used to describe structural, behavioral, functional, and metabolic disorders present at birth. The science that studies these disorders is **teratology**.

Teratogen is any agent, viral, bacterial or environmental, that causes abnormal fetal development. A variety of agents are known to produce congenital malformations in approximately 2 to 3% of all live-born infants. These agents include viruses, such as rubella and cytomegalovirus; radiation; drugs, such as thalidomide, aminopterin, anticonvulsants, antipsychotics, and antianxiety compounds;

social drugs, such as PCP, cigarettes, and alcohol; hormones, such as diethylstilbestrol; and maternal diabetes.

Effects of teratogens depend on the **maternal and fetal genotype,** the **stage of development** when exposure occurs, and the **dose and duration of exposure** of the agent. Most major malformations are produced during the **period of embryogenesis (teratogenic period; third to eighth weeks),** but in stages before and after this time, the fetus is also susceptible, so that no period of gestation is completely free of risk *(see fig. 18, plate I).* **Prevention** of many birth defects is possible, but it depends on beginning preventative measures before conception and increasing physicians' and women's awareness of the risks. A variety of techniques are available to assess the growth and developmental status of the fetus. **Ultrasound** can accurately determine fetal age and growth parameters and detect many malformations. **Maternal serum screening** for alpha-fetoprotein can indicate the presence of a neural tube defect or other abnormalities. **Amniocentesis** is a procedure in which a needle is placed into the amniotic cavity and a fluid sample is withdrawn. This fluid can be analyzed biochemically and also provides cells for culture and genetic analysis. **Chorionic villus sampling (CVS)** involves aspirating a tissue sample directly from the placenta to obtain cells for genetic analysis.

Because many of these procedures involve a potential risk to the fetus and mother, they are generally only used for higher risk pregnancies (the exception is ultrasound). These risk factors include advanced maternal age (35 years and older); a history of neural tube defects in the family; previous gestation with a chromosome abnormality; chromosome abnormalities in both parent; and a mother who is a carrier for an X-linked disorder. Modern medicine has also made the fetus a patient who can receive treatment, such as transfusions, medications for disease, fetal surgery, and gene therapy.

PART III

GENERAL HISTOLOGY

CHAPTER I

BASIC TISSUE TYPES

All the various tissues of the human body can be divided *into **four basic tissue types (epithelial, connective, muscle, nervous).*** All organs are built from these four tissues, which have consistent characteristics and arrangements from organ to organ. Thus an appreciation of the major features of these ***four basic tissue types*** can greatly simplify your understanding of the cellular composition of the many organ systems.

Each tissue consists of:

1. **cells**
2. **extracellular matrix**

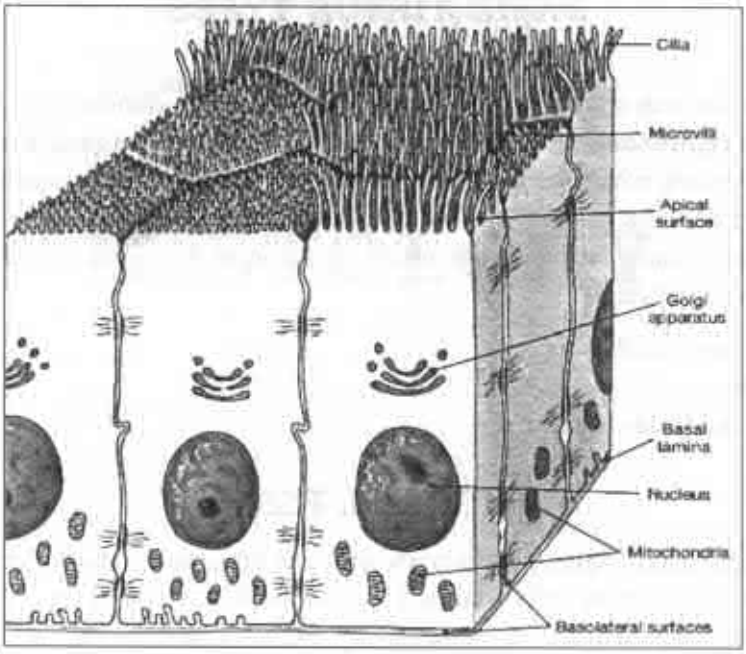
EPITHELIAL TISSUE

EPITHELIAL TISSUE covers body surfaces *(epi,* on + *thelium,* surface). Epithelial tissue consists of cells attached to one another to form an uninterrupted layer of cells that separates the underlying tissues from the outside world. The body's epithelium not only covers its obvious surfaces, but also forms glands, line passageways (such as the complex invaginations which form lungs, kidneys, sweat glands, digestive glands, liver, etc).

CHARACTERISTICS OF *EPITHELIAL TISSUE*:

1. Are formed of epithelial cells.
2. The extracellular matrix is absent practically.
3. Cells are closely apposed and adhere to one another by means of intercellular junctions.
4. Cells are located on the basement membrane.
5. Structural and functional polarity: epithelial cell has an exposed surface- **apical surface** (faces exterior surface); and an attached **basolateral surface** (attached to underlying tissue). Each surface is defined by specific

structural and functional characteristics. For exemple, the apical surface has structures important for protection of the epithelial surface (such as cilia in the respiratory tract) or for the absorption of substances (such as microvilli in the intestinal epithelium). Junctional complexes and cell adhesion molecules are present at the basolateral surface to anchor epithelial cells to each other and to the basement membrane. Organelles are distributed unevenly in these cells.



Domains of polarized epithelial cells

6. Epithelia have no blood vessels; epithelial cells receive nutrients by diffusion through apical and basal surfaces (*except stria vascularis from inner ear*).
7. Have very good nerve supply.
8. Regeneration: cells damaged or lost at the apical surface are replaced constantly.
9. Are formed from 5 embryonic layers (ectoderm, mesoderm, endoderm, mesenchyme, neural tube)

FUNCTIONS of epithelia:

1. Compartmentalization, barrier
2. Absorption and excretion

3. Protection
4. Secretion
5. Sensory
6. Transport

Most epithelial cells are separated from the connective tissue by a sheet of extracellular material called the **basal membrane.**

BASEMENT MEMBRANE

The basement membrane is visible only with the electron microscope. This structure is produced by the basal surface of epithelium and underlying connective tissue. Basal membrane are found not only in epithelial tissues but also where other cell types come into contact with connective tissue such as around muscle, Schwann cells of nervous tissue.

FUNCTIONS:

- compartmentalization – separates the connective tissue from epithelia.
- structural attachment – serves as an intermediary structure in the attachment of cells to the adjacent tissue.
- nutritive.
- barrier.
- serves as a guide during regeneration.

The BASEMENT MEMBRANE is composed of two sublayers:

1. **The basal lamina** consists of fine protein filaments embedded in an amorphous matrix, which are produced by the epithelial cells. The major components of the basal lamina are two glycoproteins - laminin and collagen (usually type IV).

   The basal lamina is organized into:
   - *lamina lucida*
   - *lamina densa*

2. **The reticular lamina** consists of reticular fibres embedded in ground substance. The fibers of the reticular lamina connect the basal lamina with the underlying connective tissue. The components of the reticular lamina are synthesized by cells of the connective tissue underlying the epithelium.

Anchoring fibrils represented by collagen fibers type VII make possible attachment of the reticular lamina to basal lamina.

CLASSIFICATION OF EPITHELIAL TISSUE

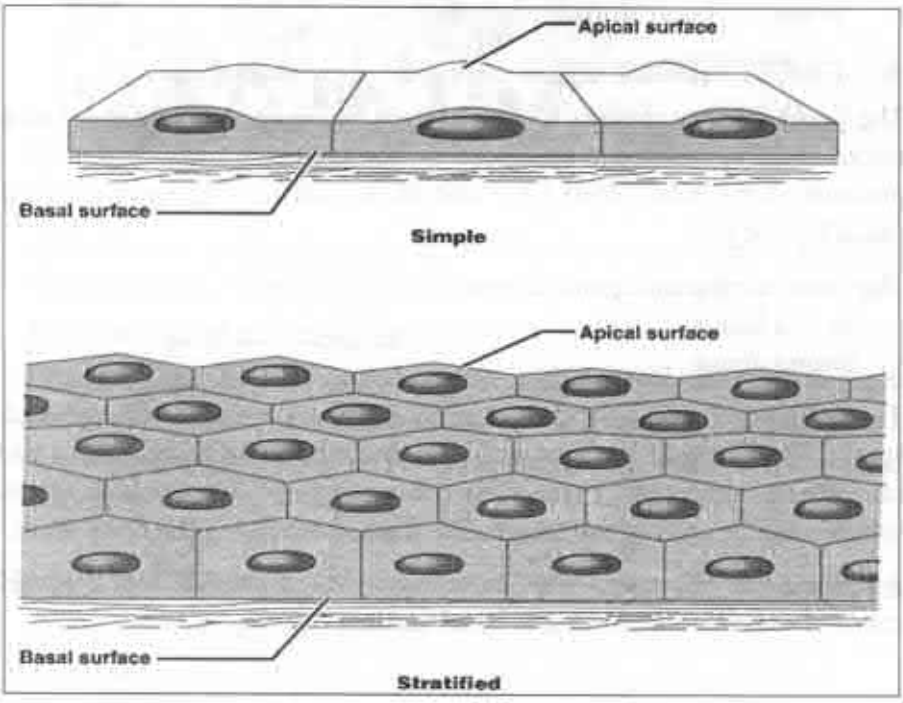
I. ***by location and function***

1. **SURFACE (lining, covering) EPITHELIA** – cover organs from outer and inner surfaces.
2. **GLANDULAR EPITHELIA** – form endocrine glands; secretory portions and line excretory ducts of exocrine glands.
3. **SENSORY EPITHELIA** – form taste buds, olfaction, organ of hearing &equilibrium.

II. ***by origin***

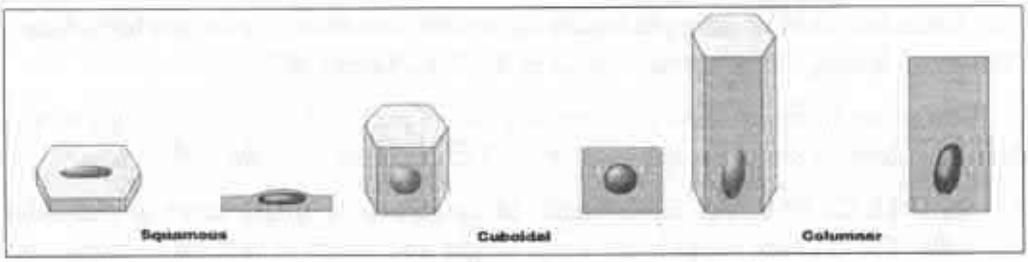
1. Ectodermal epithelia (epidermal; ependymoglial – *stratified epithelia*)
2. Endodermal epithelia (*simple columnar epithelium of the alimentary tube*)
3. Coelomic epithelia (*mesothelium*)
4. Mesodermal (nephrodermal) epithelia (*simple cuboidal epithelium of the kidney*)
5. Mesenchymal epithelia (angiodermal – *endothelium*)

III. ***by structure*** (this classification is based on the main principles: cells' relationship to the basement membrane and the shapes of the cells). In this way there are:



1. **SIMPLE epithelium** – cells are found in a single layer resting on a basement membrane.
2. **STRATIFIED epithelium** – cells are found in multiple layers resting on a basement membrane.

Cell shape is determined by the relative width and height exhibited in a vertical section. In reality, cells exist in three dimensions (they also have depth and volume). Cell shapes are squamous (flat), cuboidal, and columnar.



Additional classification and naming of specific epithelia is sometimes based on further descriptions of cell surface modifications, such as microvilli and cilia.

SIMPLE EPITHELIA can be:

- **isomorphous** (one-rowed)
- **anisomorphous** or **pseudostratified** (multi-rowed)

Simple ISOMORPHOUS epithelium comes in three basic types:

1. squamous
2. cuboidal
3. columnar

1. **SIMPLE SQUAMOUS EPITHELIUM** consists of a single, very thin layer flattened (squamous), they are **wider than they are high**. The nucleus is generally ovoid and causes a bulge at the center of the cell.

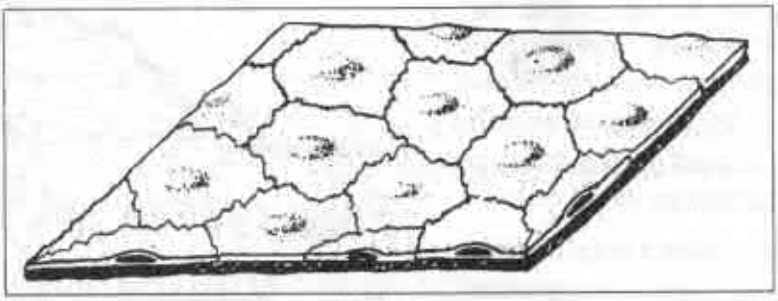


Diagram of a simple squamous epithelium

Simple squamous epithelium may be located at sites of rapid diffusion, such as the lining of lung alveoli, the lining of blood vessels (called ***endolhelium***), and at sites where very little activity is occuring, such as Bowman's capsule in the kidney and the lining of major body cavities (called ***mesothelium***).

Endothelium and **mesothelium** are special names given to the lining of certain internal surfaces.

The entire circulatory system (heart, arteries, veins, capillaries, sinusoids and lymphatics) is lined by a simple squamous epithelium that is called **endothelium.** (The inner lining of the cornea is also called "endothelium").

The major body cavities (peritoneal, pleural, pericardial) are lined by a mesodermally derived simple squamous epithelium that is called **mesothelium.**

2. **SIMPLE CUBOIDAL EPITHELIUM** consists of a **single layer of cuboidal cells.** The cells are roughly the same height and width in vertical sections, and generally have round, centrally located nuclei.

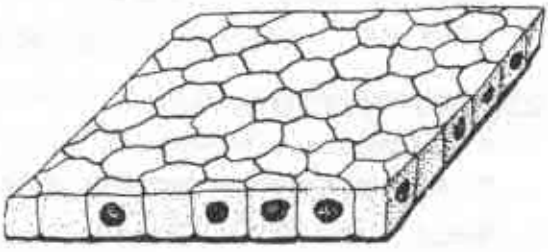


Diagram of a simple cuboidal epithelium

If stratified, **the deeper layers** are usually also cuboidal. Cuboidal epithelium is commonly encountered in glandular ducts. Cuboidal epithelial cells may be active (pumping material into or out of the lumen) or passive, depending on location and cellular specialization. **Small ducts** typically have a ***simple*** **cuboidal** epithelium. **Larger ducts** may have a ***stratified*** **cuboidal** epithelium.

3. **SIMPLE COLUMNAR (CYLINDRICAL) EPITHELIUM** contains a single layer of **columnar cells.** The cells are significantly taller than they are wide. Height can vary from low columnar to high columnar. Frequently, the cells exhibit a well defined polarity. **Nuclei** tend to be **elongated** and are often located at the basal or central regions of the cells.

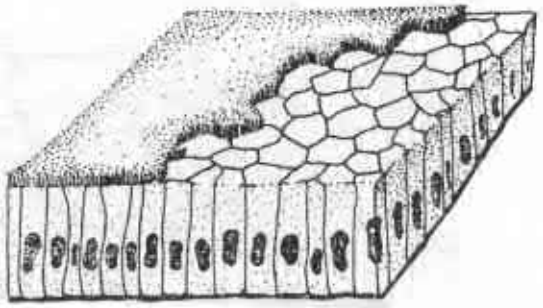


Diagram of a simple columnar epithelium

Simple columnar epithelium is usually involved in active secretion and/or absorption of material

across the single cell layer, or (if ciliated) in movement along the surface. Simple columnar epithelium lines the digestive tract and the female reproductive tract (as well as numerous other surfaces *(see fig. 25, plate I)*.

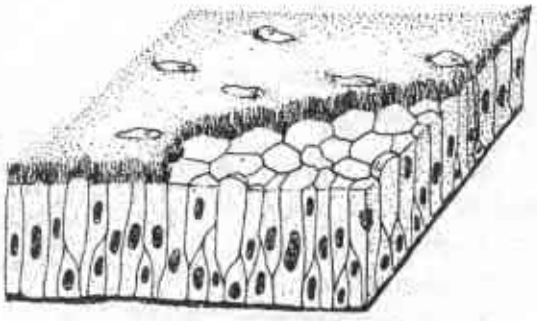
More elaborate simple columnar epithelia contain a variety of specialized cell types and surface modifications, and two prominent examples are given:

a. **Simple columnar epithelium with microvilli and goblet cells** is found in the small intestine. Microvilli are a surface modification that increases area for absorption and are found on enterocytes. NOTE, microvilli are often referred to as a brush border. Goblet cells are specialized to secrete mucus that forms a gel to coat and protect the epithelium.

b. **Simple columnar epithelium with cilia** is found in the oviducts (some microvilli may be present as well) and uterus. Cilia are a surface modification of some of the cells that have been implicated in propelling sperm and eggs.

PSEUDOSTRATIFIED COLUMNAR EPITHELIUM appears stratified, typically with nuclei located in at least two more-or-less distinct levels. But in fact every cell rests on the basement membrane, but only the columnar (tall) cells reach the luminal surface, so the epithelium is technically "simple", in spite of appearances. This epithelium consists of few major cell types:

1. **basal cells** anchored to the basal lamina, provide regeneration.
2. **intercalated cells** – similar to basal cells.
3. **columnar cells** with cilia on their apical domain, clean the air.
4. **goblet cells** – a mucus-secreting epithelial cells, produce mucous.
5. **endocrine** – produce hormones of the local action.



Diagrams of a pseudostratified epithelium

Basal and intercalated cells do not reach the lumen. A pseudostratified columnar epithelium is characteristic of the **respiratory tract** and ducts in the **male reproductive system** *(see fig. 24, plate I)*.

STRATIFIED EPITHELIA have two or more layers of cells with only the deepest cells contacting the basement membrane. Layering makes epithelia bet-

ter suited to withstand wear, resist tearing, and act as a barrier, and is thereby less suited for secretion or absorption. Make note of the fact that cell shapes in stratified epithelia will sometimes vary between surface and deeper layers, and the cell shape in the name will refer to only the surface layer. Stratified epithelium is classified according to the cell shape of its superficial layer: **squamous, cuboidal, columnar.**

1. **STRATIFIED CUBOIDAL OR COLUMNAR EPITHELIUM** consists of 2 or 3 layers of columnar or cuboidal cells. It is found in many glandular ducts. Small ducts usually have only two layers while large ones look like the drawing with only the surface cells being cuboidal or columnar.

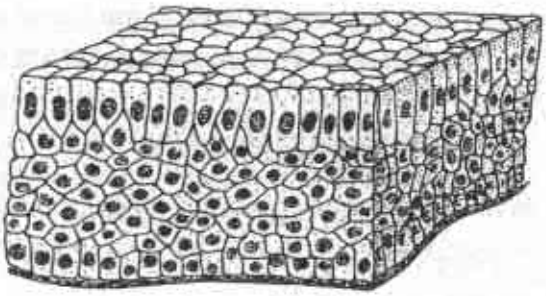


Diagram of a stratified columnar epithelium

2. **STRATIFIED SQUAMOUS NON-KERATINIZED** *(see fig. 28, plate I)* consists of three layers:
   1. ***basal***
   2. ***intermedium (spinosum)***
   3. ***superficial***

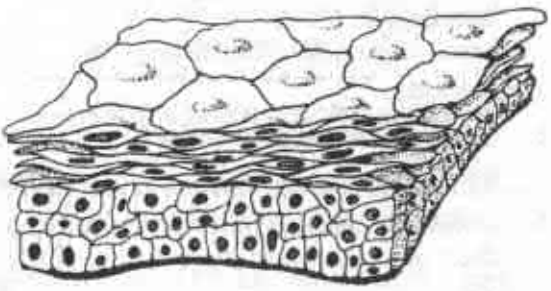


Diagram of a stratified squamous non-keratinized epithelium

Stratified squamous epithelium is found in:

- the oral cavity.
- esophagus.
- anus and rectum..
- vagina and cervix.

3. **STRATIFIED SQUAMOUS KERATINIZED** – is found only in the **SKIN** (makes up the epidermis). There are two types of skin: thick and thin.

   Epidermis of a Thick skin has five layers *(see fig. 26, plate I)*.
   1. basal
   2. spinosum
   3. granulosum
   4. lucidum
   5. corneum

Epidermis of a thin skin has four layers, no lucidum.

Superficial cells die to form tough and waterproof keratin, a surface modification which prevents mechanical damage, entry of bacteria, and keeps outside water out and inside water in. Only the surface cells are actually squamate.

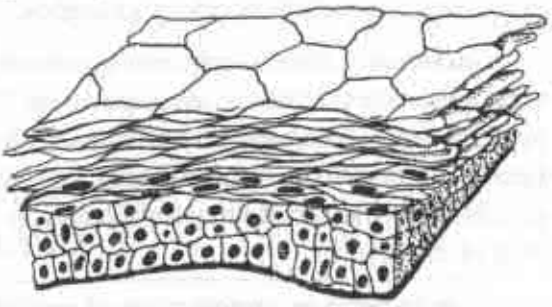
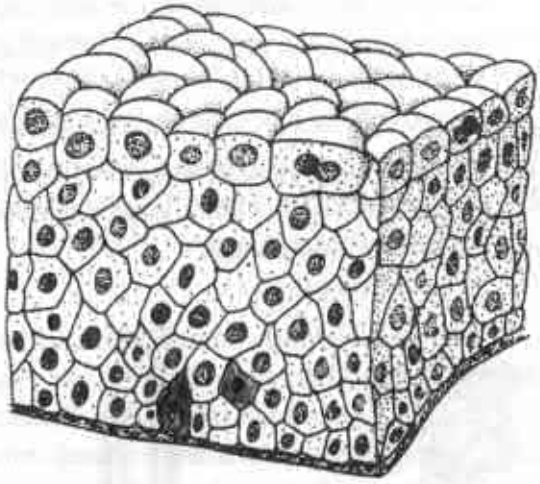


Diagram of a stratified squamous keratinized epithelium

Basal and spinosum layers combine to form stratum germinativum, for these cells are capable of division stratified squamous epithelia possess nuclear polymorphism: nuclei of the basal layer are elongated and perpendicular to basement membrane, stratum intermedium (spinosum) nuclei are around-shaped, and superficial (granular) cell nuclei are also elongated, but parallel to the basement membrane

Transitional epithelium (urothelium) has cell bodies that are at different heights, like stratified, but the cells all contact the basement membrane, like Pseudostratified. This arrangement is accomplished by long thin cytoplasmic processes, called pedicels, extending from the cell body to contact the basement membrane. The pedicels are not visible in slides and the overall appearance is that of a stratified epithelium. The superficial cells are dome-shaped with one or two nuclei. All basal cells' nuclei are round in shape.



Diagrams of a transitional epithelium

Transitional epithelium lined the distensible walls of the urinary tract *(see fig. 27, plate I)*. The name "transitional" derives from this tissue's ability to change its shape from cuboidal to squamous when stretched. In a full bladder, transitional epithelium flattens out and appears to consist of squamous cells. Although mistaken for stratified squamous epithelium, a clear identification of this is made by noting the large, ovoid surface cells.

GLANDULAR EPITHELIA

Glandular epithelia are formed by cells specialized to produce secretion. The molecules to be secreted are generally stored in the cells in small membrane-bound vesicles called **secretory granules.**

Glandular epithelial cells may synthesize, store, and secrete proteins (eg, pancreas), lipids (eg, adrenal, sebaceous glands), or complexes of carbohydrates and proteins (eg, salivary glands). The mammary glands secrete all three substances. Less common are the cells of glands that have low synthesizing activity (eg, sweat glands) and that secrete mostly substances transferred from the blood to the lumen of the gland.

Glands form as invagination of epithelial membranes during development and are lined with epithelia. Exocrine glands have ducts that reach an epithelial surface while endocrine glands don't *(see fig. 23, plate I).*

Glands are classified into:

1. **Unicellular glands** – consist of isolated glandular cells. An example of a unicellular gland is the **goblet cell. Goblet cells** are specialized to synthesize and secrete **mucin,** a glycoprotein, which upon hydration forms a lubricating solution called **mucus.** These cells are shaped like their name and are scattered among the epithelial columnar cells of the intestine and the respiratory system. Their mucus secretions cover the apical surfaces of the adjacent epithelial cells to lubricate and protect the epithelium.
2. **Multicellular glands** develop when epithelia invade the underlying connective tissue. Multicellular glands are classified as:
   - **endocrine glands**
   - **exocrine glands.**

Endocrine glands do not have ducts and their secretory products are exocytosed into bloodstream or into lymph. Endocrine glands have good blood supply, and cells form cord or follicles. Endocrine gland epithelial cells do not have a free surface. Secretions are released into surrounding loose CT and can have local effects, or they can be picked up by capillaries and distributed throughout the body. Large endocrine glands frequently have connective tissue capsules and septa. Secretory products are hormones such as insulin (pancreas) or glucocorticoids (adrenal), and other useful substances. The liver and pancreas are examples of glands that have both endocrine and exocrine functions. Endocrine secretion is regulated by complex feedback mechanisms by which secretory cells respond to circulating factors.

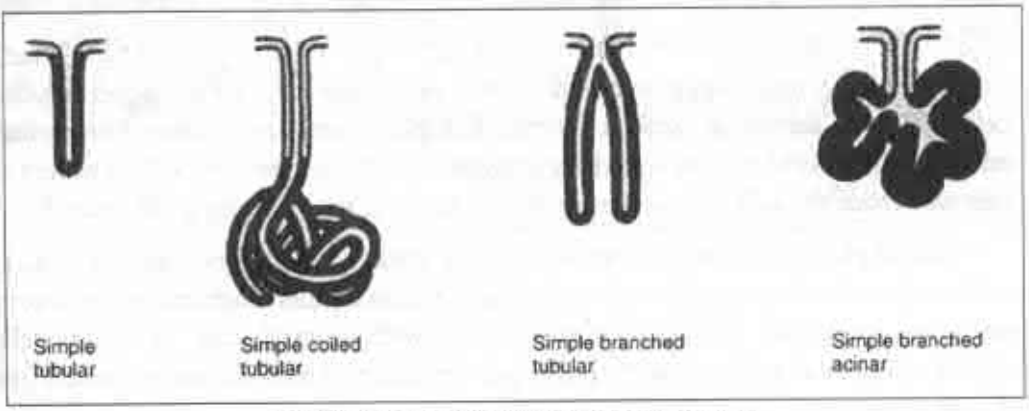
Exocrine glands have secretory units (acinus) containing the cells that produce the secretions, and ducts that carry the secretory product to an epithelial surface (internal and external). Duct cells may modify secretions as they are excreted. Secretion is involuntary and is controlled by autonomic nervous system activity or by hormones. In some instances, secretory units have contractile **myoepithelial cells** (epithelial cells similar to smooth muscle cells that contain contractile filaments) wrapped around them to squeeze out secretions. Contraction of myoepithelial cells expels the secretions into the duct system.

Exocrine glands are classified as follows:

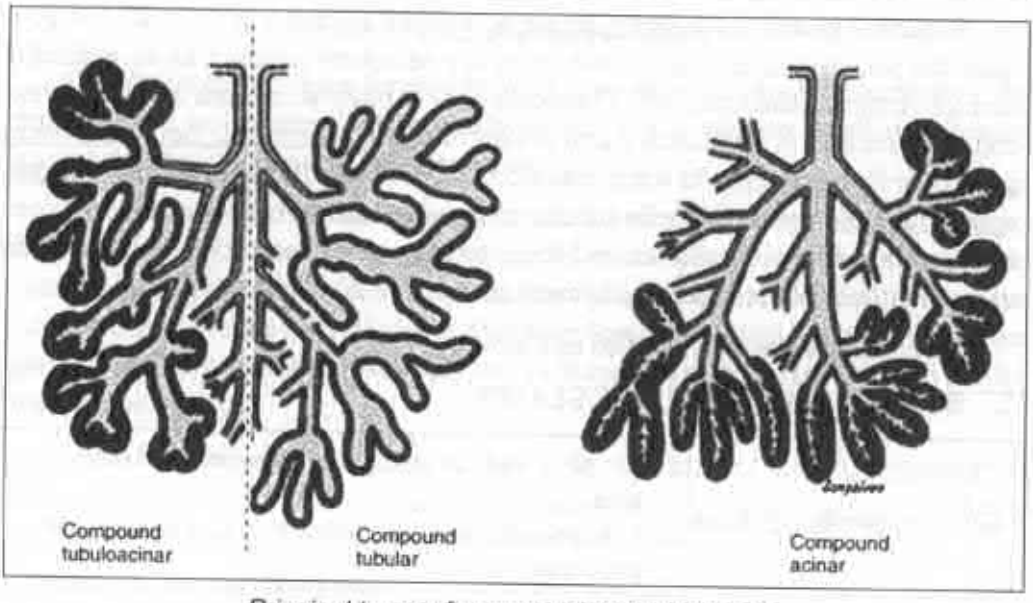
I. Based on STRUCTURE OF GLAND

Based on number of ducts	1. **Simple gland** has a single unbranched duct (e.g., sweat gland). 2. **Compound gland** has a branched duct system (e.g., pancreas).
Based on branching of secretory unit	1. **non-branched** – have non-branched terminal secretory portions; 2. **branched** – have branched terminal secretory portions.
Based on shape of secretory unit	1. **Tubular gland,** the secretory unit is tube-shaped. 2. **Alveolar (acinar) gland,** the secretory unit is rounded. 3. **Tubuloalveolar gland,** both types of secretory units are present.

Various combinations of duct and secretory portion shapes are found in the body; thus, glands may be simple straight tubular, simple coiled tubular, simple alveolar, compound tubular, compound alveolar, compound tubuloalveolar etc.



Principal types of simple exocrine glands



Principal types of compound exocrine glands

II. Based on <u>COMPOSITION OF SECRETION</u>

- **SEROUS GLAND,** which secretes **enzymes** in a watery fluid.
- **MUCOUS GLAND,** which secretes a viscous glycoprotein called **mucus**.
- **MIXED (seromucous) GLAND,** in which both types of secretion are produced in the same gland because it contains both serous and mucous cells.

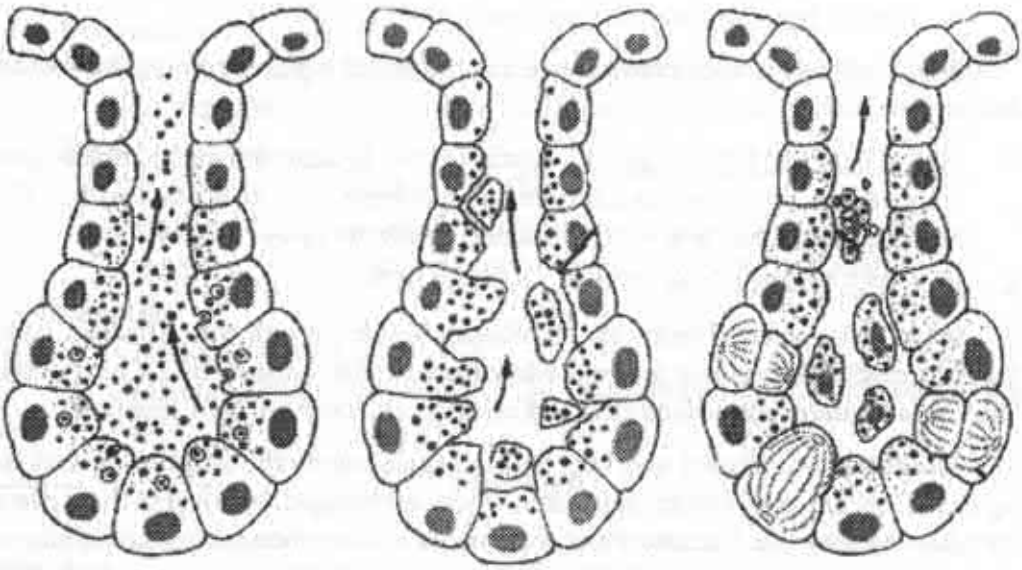
In H & E sections, **<u>serous cells</u>** exhibit the following features: The base of the cell contains a ***large, spherical centrally-located nucleus,*** and the surrounding ***cytoplasm appears intensely basophilic*** due to an abundance of RER. The apical end of the cell contains numerous *eosinophilic zymogen granules* filled with secretory *enzymes (see fig. 29, plate I).*

In contrast, **<u>mucous cells</u>** in H & E sections appear differently: the base of the cell contains a ***flattened nucleus,*** and the ***cytoplasm appears pale and vacuolated*** due to an abundance of secretory granules containing *mucin.* When mucin is released from the cell, it becomes hydrated to form *mucus (see fig. 30, plate I).*

Mixed glands consist of both serous and mucous secretory units, as well as secretory units that contain both serous and mucous cells. Such mixed secretory units are composed mostly of mucous cells, with a small "cap" of serous cells called a **serous demilune.** Both cell types are secreted into the same lumen *(see fig. 31, plate I).*

III. Based on MODE (mechanism) OF SECRETION, glands can be classified as:

- **MEROCRINE GLAND.** Secretion occurs via **exocytosis** (*salivary glands, pancreas*). This is the most common type of glandular epithelium secretion where secretory granules within the cytoplasm of the cell gather at the apical region of the cell. The secretory granules leave the cell with no loss of other cellular material.
- **HOLOCRINE GLAND.** The entire cell and its contents form the secretory product (*sebaceous gland*). This secretion consists of disintegrated cells of the gland itself. Granules fill the cell until the entire cell becomes "bloated" with secretory products. The whole cell is discharged into the lumen. Once inside the lumen, the cell degenerates and the secretory products are released.
- **APOCRINE GLAND.** Apical end of the cell is partially destroyed in the process of secretion (*mammary gland, some sweat glands*).



Secretory mechanism: merocrine (a), apocrine (b), and holocrine glands (c)

CHAPTER II

TISSUES OF THE INTERNAL ENVIRONMENT

BLOOD

- Is a fluid tissue that circulates through the cardiovascular system
- Adult has ~ 5- 5.5 L depending on size 8% of body mass
- PH of blood ~ 7,36
- Blood cells produced in bone marrow from stem cells
- Mesenchyme is the embryonic source of the blood

Blood belongs to connective tissue and like other types of connective tissues is composed of:

1. FORMED ELEMENTS (cells) – **erythrocytes, leukocytes and platelets** (are about 45%). Erythrocytes and platelets perform their functions within the vessel lumen, while leucocytes function primarily in the tissues.
2. PLASMA (extracellular matrix) – is about 55%

If blood is removed from the circulatory system, it will clot. This clot contains formed elements and a clear yellow liquid called **serum** *(liquid part of the blood that remains after blood cells and clotting proteins have been removed).*

Blood that is collected and kept from coagulating by the addition of anticoagulants (eg, heparin, citrate) separates, when centrifuged, into layers that reflect its heterogeneity. The **hematocrit** is an estimate of the volume of packed erythrocytes per unit volume of blood. The normal value is 40–50% in men and 35–45% in women.

The translucent, yellowish, somewhat viscous supernatant obtained when whole blood is centrifuged is the plasma. The formed elements of the blood separate into two easily distinguishable layers. The lower layer represents 42–47% of the entire volume of blood in the hematocrit tube. It is red and is made up of erythrocytes. The layer immediately above (1% of the blood volume), which is white or

grayish in color, is called the **buffy coat** and consists of leukocytes. These elements separate because the leukocytes are less dense than the erythrocytes. Covering the leukocytes is a fine layer of platelets not distinguishable by the naked eye.

Hematocrit tube with blood after centrifugation

BLOOD FUNCTIONS

1. **Regulation**
   - Hormonal: Carry hormones to target tissues to produce their effects.
   - Temperature: Divert blood to cool or warm the body.
2. **Transportation**
   - Respiratory: Transport 0_2 and $C0_2$.
   - Nutritive: Carry absorbed digestion products to liver and to tissues.
   - Excretory: Carry metabolic wastes to kidneys to be excreted.
3. **Protection**
   - Clotting: Prevents blood loss.
   - Immune: Leukocytes (white blood cells) protect against pathogens (disease).

PLASMA

Plasma is an aqueous solution containing substances of low or high molecular weight that make up 10% of its volume *(see fig. 33, plate I)*. Plasma consists of:

- **Water** 90-92%
- **Proteins** – albumins, globulins, clotting proteins – fibrinogens and prothrombin.
  - **Albumins** – are made in the liver. Transport hormones, metabolites, ions and generate osmotic pressure.
  - **Globulins** – **Subdivided** into α, ß, γ globulins. **α** and **ß**-globulins transport lipids and fat soluble vitamins; are secreted by the liver.

γ globulins form antibodies (a class of functional molecules of the immune system that are secreted by plasma cells).
  - **Fibrinogens** – are made in the liver and function in blood clotting.
- **Wastes** – lactic acid, urea, uric acid, creatinine, ammonium salts.
- **Nutrients** – glucose, carbohydrates, amino acids, lipids.
- **Electrolytes** – sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), chloride (Cl^-), bicarbonate (HCO_3^-), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}).
- **Respiratory gases** – oxygen and carbon dioxide.
- **Regulatory substances** – hormones, enzymes.

FORMED ELEMENTS

RED BLOOD CELLS (erythrocytes)

- Erythrocytes are the dominant (99%)
- Male – $3,9\text{-}5,5\times10^{12}$/l; female – $3,7\text{-}4,9\times10^{12}$/l
- Has no nucleus and general organelles
- Red blood cells never leave the circulatory system
- **Function:** carry gases (CO_2, O_2); transport of amino acids, antibodies, toxins, maintenance of blood buffers.

■ **Shape** – **biconcave disk.** The round, biconcave nature of the erythrocyte membrane gives it maximum surface area that is advantageous for gaseous exchange and increased deformability. Erythrocyte membrane composition is approximately 50% protein, 40% lipids, and 10% carbohydrates. Morphologically it is composed of two layers of phospholipids, arranged so that the polar surfaces face the inside and outside of the cell. The non-polar groups are directed to the center of the membrane layer. The proteins in the RBC membrane account for its shape, structure, and ability to change shape. Several peripheral proteins are associated with the inner surface of the erythrocyte membrane. The peripheral proteins seem to serve as a membrane skeleton that determines the shape of the erythrocyte. One protein associated with the inner surface of the erythrocyte membrane is the cytoskeletal **spectrin**, which links several membrane components with other cytoskeletal elements, forming a meshwork that reinforces the erythrocyte membrane. This meshwork also permits the flexibility of the membrane necessary for the large changes in shape that occur when the erythrocyte passes through capillaries. Other proteins are the channels and pumps to move ions and other molecules in, out, and across the membrane. Some of the proteins function as receptors,

many of the proteins function as the RBC antigens (ABO, and Rh), other proteins have enzymatic capability, and all in some degree or another help to stabilize the membrane. If the molecular composition of the RBC membrane changes, the membrane is affected inducing changes in its shape, or ability to transport ions and molecules. Shape also depends on water content (**osmotic effects of solutes**, especially ions). Because erythrocytes are not rigid, the viscosity of blood normally remains low *(see fig. 32, plate I)*.

If the molecular composition of the RBC membrane changes, the membrane is affected inducing changes in its shape or ability to transport ions and molecules. If the cholesterol content of the membrane increases, the membrane takes on the appearance of a target cell or spicules develop to form the acanthocyte. If abnormal proteins are incorporated into the membrane, the cell may become an elliptocyte or spherocyte. If proteins are lost, for whatever reason, the integrity of the membrane is compromised and hemolysis will result. It has been found that some of the RBC membrane antigens are essential for membrane integrity.

- **RBC contains hemoglobin (Hb)** – *Hb is a red colored, conjugated, large molecular weight protein (mw = 64,458) that makes up about 28% of the RBC mass. Most of the RBC mass is water. Each adult hemoglobin molecule consists of a quaternary protein molecule that consists of four globulin (polypeptide) sub-units. The four globulin chains constitute a tetramer. Two of the sub-units are designated as* ***α-chains*** *and the other two subunits are the* ***β-chains****. Each subunit contains one heme structure (see fig. 34, plate I).* Heme is a vitamin B_{12} derivative and contains one iron ion in the ferrous state; hemoglobin is equally efficient at binding and releasing oxygen, on the contrary its binding capacity is superior to liberation for CO_2. Erythrocytes contain an enzyme carbonic anhydrase, which catalyzes the following reaction:

  in tissues > $CO_2 + H_2O$ <=> $H^+ + HCO_3^-$ < in lungs;

  - Fetal hemoglobin is referred to as Hb F, it possesses a higher oxygen affinity; in fetuses and newborns hemoglobin and erythrocyte levels are higher than in adults. Adult hemoglobin is called Hb A;
    - **in adult: HbA (α2β2) (98%) & HbF (α2γ2) (2%)**
    - **in fetus: HbA (α2β2) (20%) & HbF (α2γ2) (80%)**

Erythrocytes lose their mitochondria, ribosomes, and many cytoplasmic enzymes during their maturation. The source of energy for erythrocytes is glucose, which is anaerobically degraded to lactate. Because erythrocytes do not have a nucleus or other organelles necessary for protein synthesis, they do not synthesize hemoglobin.

- The **younger erythrocytes**, called **RETICULOCYTES,** may have a few granules or a netlike structure in their cytoplasm (residual ribosomal RNA). Reticulocytes normally constitute about 1% of the total number of circulating erythrocytes; this is the rate at which erythrocytes are replaced daily by the bone marrow. Increased numbers of reticulocytes indicate a demand for increased O_2^- – carrying capacity, which may be caused by factors such as hemorrhage or a recent ascent to high altitude.

- **Aged red** blood cells are:
  - **echinocytes** – is a crenated erythrocyte.
  - **stomatocytes** – is characterized by a slit-like or narrow rectangular area of pallor in the cell. This cell will be concave on one side and convex on the other.
  - **spherocytes** – is an erythrocyte in which the biconcave disc profile is lost.
  - **planicytes** – with flat surfaces.

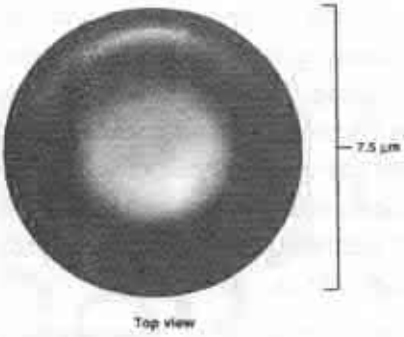
Poikilocytosis (poikilocytes) describes the variety of nonspecific shapes that may be observed in RBC's. Poikilocytosis is an irreversible alteration of the cell membrane and is an indicator of abnormal erythropoiesis due to bone marrow effects and/or abnormal RBC destruction. This is one of the most common forms of abnormal RBC morphology. There is a poikilocytosis expression that occurs as the RBC ages (senescence). The RBC will become pinched, pitted, or notched as the membrane breaks down and sloughs off.

- Human erythrocytes survive in the circulation for about 120 days. Old erythrocytes are removed from the circulation mainly by macrophages of the spleen, liver and bone marrow. The signal for removal seems to be the appearance of defective complex oligosaccharides attached to integral membrane proteins of the plasmalemma.

- **Size of RBC**

  ~7,5 – 7.8 μm diameter fresh;

  ~7,2 – 7.4 in stained smears



- **Normocytes** – 75%, Ø 7,5 µm
- **Macrocytes** – 12,5%, Ø > 9 µm
- **Microcytes** – 12,5%, Ø < 6 µm

Anisocytosis refers to variation in size of RBCs

Red Blood Cell Disorders:

1. **ANEMIA ("lack" of blood)**
   - **Anemia** is a pathological condition characterized by blood concentrations of hemoglobin below normal values. Although anemia is usually associated with a decreased number of erythrocytes, it is also possible for the number of cells to be normal but for each cell to contain a reduced amount of hemoglobin (**hypochromic anemia**).
   - During anemia decrease oxygen carrying ability of erythrocytes.
   - *Clinical symptoms*: Fatigue, chilly, pale.
   - Causes:

- Low RBC → from loss of blood (hemorrhage); insufficient production of erythrocytes by the bone marrow; or accelerated destruction of blood cells.
- Low hemoglobin → production of erythrocytes with insufficient hemoglobin, usually related to iron or B12 deficiency in the diet.

2. **POLYCYTHEMIA ("many blood cells")**
   - An increased number of erythrocytes (**erythrocytosis**, or **polycythemia**) may be a physiological adaptation. It is found, for example, in people who live at high altitudes, where O_2 tension is low.
   - Polycythemia (*polys*, many, + *kytos*, cell, + *haima*, blood), which is often associated with diseases (*cancer of the bone marrow*) of varying degrees of severity, increases blood viscosity; when severe, it can impair circula-

tion of blood through the capillaries (*increases blood pressure, less blood delivery to tissues*).

- Polycythemia might be better characterized as an increased hematocrit, ie, an increased volume occupied by erythrocytes.

LEUCOCYTES (white blood cells)

Leukocytes are involved in the cellular and humoral defense of the organism against foreign material. In suspension in the circulating blood, they are spherical, nonmotile cells, but they are capable of becoming flattened and motile on encountering a solid substrate. Leukocytes leave the venules and capillaries by passing between endothelial cells and penetrating the connective tissue by **diapedesis** (*dia,* through, + *pedesis,* to leap). Diapedesis is increased in individuals infected by microorganisms. Inflamed areas release chemicals originating mainly from cells and microorganisms, which increase diapedesis.

The attraction of specific cells by chemical mediators is called **chemotaxis,** a significant event in inflammation through which leukocytes rapidly concentrate in places where their defensive properties are needed.

- The number of leukocytes varies according to age, sex, and physiological conditions, in normal adults are **4 – 9×10⁹/l.**
- Have a nucleus and organelles.
- Can move from the capillary to the surrounding connective tissue using pseudopodia.
- Derived from stem cells in the bone marrow.
- Found in blood, lymph and loose connective tissue.
- Circulate in the blood stream for some time, but their function is performed in the connective tissue.

On the basis of presence or absence of **SPECIFIC** granules leucocytes are divided into 2 major groups:

1. **granular leucocytes (or granulocytes)** – have specific granules, nuclei of mature or nearly mature granulocytes are composed of several segments: they may have two, three or four segments.
2. **nongranular leucocytes (or agranulocytes)** – do not have specific granules and possess a spherical, oval or horseshoe-shaped nucleus.

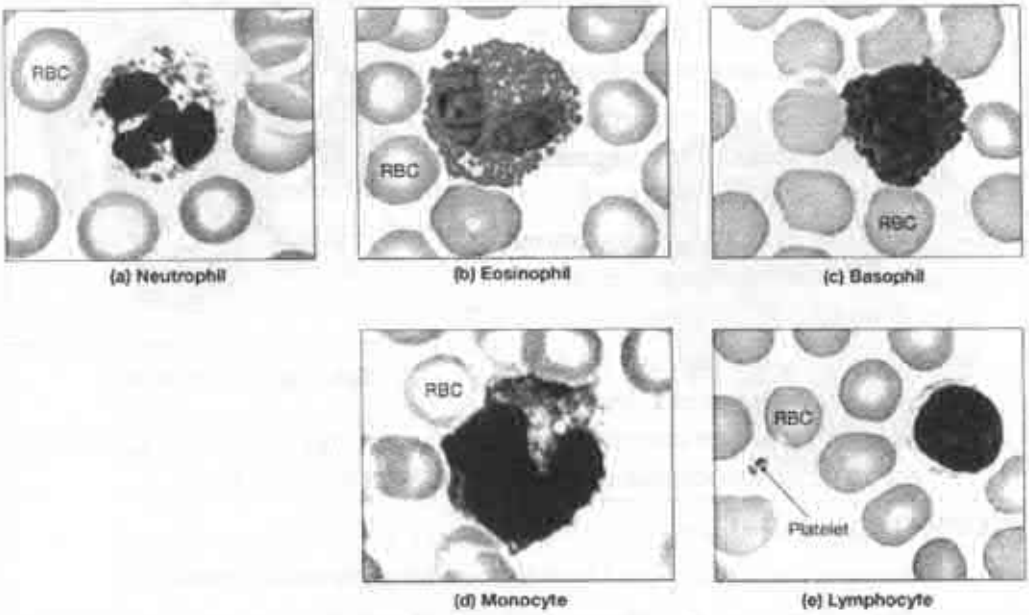
Both granulocytes and agranulocytes possess NONSPECIFIC GRANULES, which are essentially lysosomes, and their composition is similar in all leukocytes.

GRANULAR LEUCOCYTES represent a heterogeneous group that includes:

- neutrophils 65-75%
- eosinophils 1-5%
- basophils 0,5-1%

AGRANULAR LEUCOCYTES are:

- lymphocytes 20-35%
- monocytes 6-8%



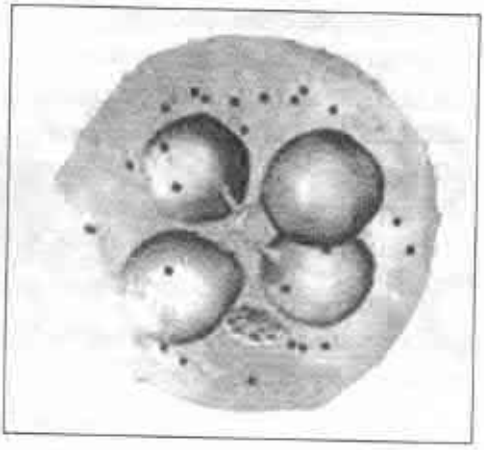
The five types of human leukocytes

NEUTROPHILS *(see fig. 36, plate I)*

Structure:

- Have 10-12 μm in diameter.
- Have multi-lobed nucleus (3-5 lobes linked by fine threads of chromatin).
- Are spherical in shape.
- The more abundant granules in the cytoplasm are **Specific granules,** which are small and contain: alkaline phosphatases, lysozyme with bacteriostatic and bacteriocidic action.
- **Azurophilic granules** are deep red/purple, which contain peroxidase and lysosomal enzymes.

- Neutrophils also contain glycogen in their cytoplasm which permits neutrophils to survive in an anaerobic environment. This ability of neutrophils is highly advantageous, since they can kill bacteria and help clean up debris in poorly oxygenated regions, eg, inflamed or necrotic tissue.
- Immature neutrophils that have recently entered the blood circulation have a nonsegmented nucleus in the shape of a horseshoe (band forms). An increased number of band neutrophils in the blood indicates a higher production of neutrophils, probably in response to a bacterial infection.
- Neutrophils with more than five lobes are called **hypersegmented** and are typically old cells.
- Half-life 6-7 hrs in circulation; lifespan 5-9 days in connective tissue (die by apoptosis).
- In females, the inactive X chromosome appears as a drumstick like appendage on one of the lobes of the nucleus (Barr Body), this characteristic is not obvious in all neutrophils in a blood smear.



Scheme of a neutrophil

PROPERTIES:

- transgression from blood into tissues, migration within tissues.
- directed migration (chemotaxis) into zones of inflammation under the influence of chemotactic factors.
- activation by mediators of the immune system and bacteria.
- intensive phagocytosis of bacteria and cellular debris (microphagocytosis).
- ability to release their granule contents into surrounding space that causes destruction of tissues and formation of **pus.**
- synthesis of a whole battery of biologically active substances.
- during phagocytosis phagocytic vacuole at first fuses with specific granules, then the phagosome-specific-granule complex fuses with nonspecific granules, i.e. lysosomes; therefore, phagocytosed material is first exposed to the actions of specific granule substances, which kills it (bacteria or cells), then lysosome (nonspecific granule) enzymes get into play, which cleave all organic biopolimers down to monomers.

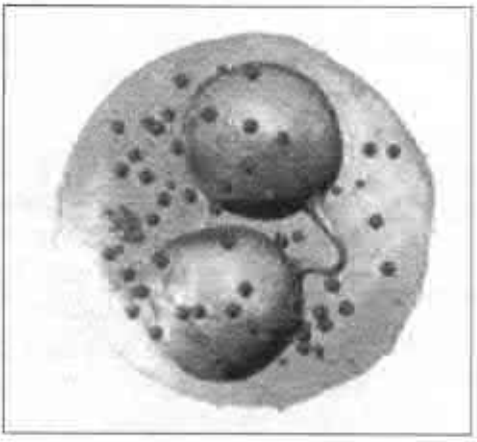
Functions are determined by substances in granules:

– Active phagocytes – **microphages;** important defense against infection.

EOSINOPHILS *(see fig. 37, plate I)*

Structure:

- Are 12-15 μm in diameter.
- Are spherical in shape.
- In the peripheral blood there are mainly the most mature forms (with segmented nuclei), having, as a rule, a **bi-lobed nucleus**.
- Their cytoplasm contains many **specific** (about 200 per cell) **and nonspecific** granules;
- **Specific granules** stain well with acidic dyes – eosin; has **red or pink** color; contain peroxidase, hydrolytic enzymes, histaminase and arginine-rich protein (major basic protein). The major basic protein also seems to function in the killing of parasitic worms.



Scheme of a eosinophil

- Specific granules have a crystalline core (**internum**) that lies parallel to the long axis of the granule. The less dense material surrounding the internum is known as the **externum,** or **matrix.**

PROPERTIES:

- ability to release granule contents into the extracellular space (degranulation).
- weak phagocytosis, in which specific granules can fuse with lysosomes and phagosomes, however, this process is not as active as in neutrophils.
- ability to attach themselves to parasites (worms), to locally release granule contents and inject into cytoplasm of a parasite.

Functions:

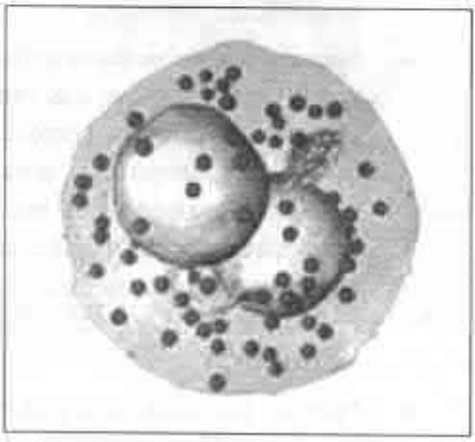
- Kill parasites, especially worms (helminths).
- Release histaminases at sites of allergic reaction neutralize the activity of mast cells (histamine).

- Phagocytose Ag-Ab complexes formed in allergy.
- They are found in large numbers in the sites of potential chronic inflammation.
- **Corticosteroids** decrease eosinophils in blood.

BASOPHILS *(see fig. 38, plate I)*

Structure:

- cells are spherical in shape,
- are about 12-15 μm in diameter.
- Rare in the peripheral blood, hard to find in smears.
- Nucleus is kidney-shaped or bisegmented, but the overlying specific granules usually obscure the nucleus.
- Their cytoplasm contains specific and nonspecific granules. Many blue (basophilic) granules – **specific granules** with heparin (acts as anticoagulant) and histamine (cause vasodilatation, increase capillary wall permeability, development of edema). Specific granules stain well with basic (alkaline) dyes – azur-2; has a **dark blue** color.



Scheme of a basophil

- May supplement mast cell function in immediate hypersensitivity reactions by migrating (under special circumstances) into connective tissues.

PROPERTIES:

- transgression from blood into tissues, migration within tissues.
- ability to release granule contents into the extracellular space (degranulation).
- weak phagocytosis.
- release of biologically active substances which are not within granules.
- uptake of histamine and serotonin from the surrounding tissues.

Functions are determined by versatile actions of granule contents as well as by elaboration and secretion of a host of biologically active substances which are

not stored within granules such as tumor necrosis factor a, prostaglandin D2, thromboxane A2, interleukin 4, leukotrien N4 and others.

LYMPHOCYTES *(see fig. 35, plate I)*

Structure:

- 6-12 μm Ø most common,
- spherical cells with round nuclei and small cytoplasmic compartment, which contains poorly developed organelles, nonspecific granules (lysosomes) could be seen; the nucleolus of the lymphocyte is not visible, but it can be demonstrated by special staining techniques and with the electron microscope.
- Lymphocytes possess receptors for antigens, immunologic mediators, hormones, and for a host of biologically active substances.
- Lymphocytes vary in life span; some live only a few days, and others survive in the circulating blood for many years. Lymphocytes are the only type of leukocytes that return from the tissues back to the blood, after diapedesis.
- **morphologically** lymphocytes are divided into: **small, medium, and large** lymphocytes. This difference between lymphocytes has functional significance in that some larger lymphocytes are believed to be cells activated by specific antigens. The small lymphocytes are predominant in the blood.
- **functionally** lymphocytes are divided into: T- and B-lymphocytes, natural killers and Null lymphocytes (undifferentiated stem cells).

- T cells function in cell-mediated immunity (make up 80% of lymphocytes) and regulation of humoral immunity. There are four types of T cells:
  - **T helper cells,**
  - **T suppressor cells,**
  - **T killer (cytotoxic) cells**
  - **T memory cells**
- Some T cells with "memory" of antigen exposure survive long periods; give immunization of body.
- B cells form **plasma cells**, function in humoral immunity via immunoglobulins and **B memory cells**.
- Initial differentiation of lymphocytes takes place **in bone marrow (B cells)** and **thymus (T cells).**

Functions:

- B-lymphocytes transform into plasma cells, which produce antibodies.
- T-lymphocytes: T-helpers – facilitate proliferation and differentiation of B- and other T-lymphocytes (killers, suppressors, memory, natural killers).
- T-killers are cytotoxic, i.e. kill foreign and cancerous cells, viruses, protozoa.
- T-suppressors inhibit proliferation and differentiation of T-killers, T-helpers and B lymphocytes.
- T-memory cells store information on antigens getting into the body.
- **Natural killers** are cytotoxic against foreign and cancerous cells, viruses, etc.

MONOCYTES *(see fig. 39, plate I)*

Structure:

- Have 17-20 μm in diameter
- Are large rounds or oval cells with horseshoe-shaped nuclei which is generally eccentrically placed. The chromatin is less condensed than that in lymphocytes (nuclei of monocytes stain lighter)
- Have quite voluminous basophilic cytoplasm, which contain multiple lysosomes (nonspecific granules), phagosomes; and a small quantity of rough endoplasmic reticulum, polyribosomes, and many small mitochondria is observed. A Golgi complex involved in the formation of the lysosomal granules is present in the cytoplasm.
- Many microvilli and pinocytotic vesicles are found at the cell surface.
- There are receptors on cytoplasmic membrane for various immunologic mediators, complement components, Fc-receptors for IgG, hormones, biogenic amines, eicosanoids, growth factors, etc.;
- Monocytes are immature cells, they are precursor cells of the mononuclear phagocyte system, upon leaving the circulation into the tissues, they differentiate into **macrophages (histiocytes, osteoclasts, alveolar macrophages, Kupffer cells and others).**
- Lifespan 12-100 hrs; **do not re-enter circulation.**

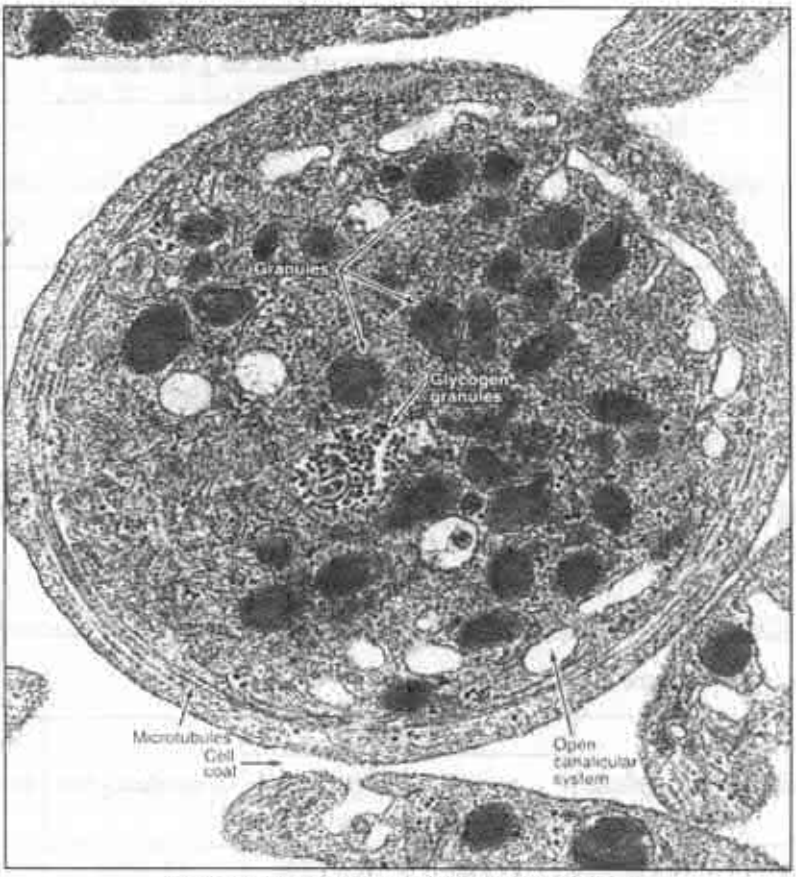
Functions:

- Participate in the phagocytosis of bacteria, other cells & tissue debris.
- Also play an important role in immune responses – presentation of antigens to lymphocytes.

PLATELETS

Structure:

- Have no nucleus;
- Result from fragmentation of **megakaryocytes**.
- Normal platelet counts range from 200 to 400×10⁹/l
- Life span 10 days in blood.
- platelets are pieces of cytoplasm with elements of Golgi complex and smooth endoplasmic reticulum, with mitochondria, ribosomes, glycogen inclusion bodies, microtubules, microfilaments, glycolytic enzymes are present as well as a number of granule types;
- There are receptors for blood clotting factors on cytoplasmic membrane.
- In stained blood smears, platelets often appear in clumps.



Electron micrograph of human platelets

All platelets have the same structure: peripheral light blue cytoplasm – **hyalomere**, central dark blue/purple granules – **granulomere**. The central granulomere possesses 3 types of membrane-bound granules:

- ***delta*** granules: contain calcium, serotonin (potent vasoconstrictor), also take up and store serotonin (5-hydroxytryptamine) from the plasma.
- ***alpha*** granules contain fibrinogen, platelet derived growth factor and other proteins.
- ***lambda*** granules – lysosomal enzymes.

Actin and myosin molecules in the hyalomere can assemble to form a contractile system that functions in platelet movement and aggregation. A cell coat rich in glycosaminoglycans and glycoproteins, lies outside the plasmalemma and is involved in platelet adhesion.

Functions: participation in blood coagulation and thrombus formation.

NORMAL VALUES	Normal quantities in 1 l of blood
erythrocytes	females – 3.7- 4.9×10^{12}/l males – 3.9- 5.5×10^{12}/l decrease in erytrocyte number – erythropenia, increase – erythrocytosis
platelets	$200\text{-}400\times10^{9}$/l
leucocytes	$4\text{-}9\times10^{9}$/l decrease in leucocyte number – leucopenia, increase – leucocytosis

HEMOGRAM is concentration of blood cells per liter plus Hemoglobin (120-140mg/l).

LEUCOCYTE DIFFERENTIAL COUNT is percentage of leucocytes

all leucocytes are 100%, of those:

basophils	eosinophils	neutrophils		lymphocytes	monocytes
		rods (juveniłe)	segmented (adult)		
0.5-1%	1-5%	1-5%	65-75%	25-35%	6-8%

One of the most important diagnostic sign is **deviation of the leucocyte differential count to the left.** This is an increase in the number of juvenile and rods neutrophils.

AGE-RELATED CHANGES IN BLOOD CELL COUNTS

- **erytrocytes:**6-7*10^{12}/l in newborns, get lower post partum and by 10-14 days of life are at adult levels, a decrease continues from 3 to 6 months, then gradualy return to adult counts at puberty.
- **leucocytes:** 10-30*10^{9}/l in neonates, in the first two weeks of life decline to 9-15*10^{9}/l, reach adult levels at puberty.
- **neutrophil/lymphocyte ratio:** at birth – comparable to adults, 4 days – concentrations equalize, and then lymphocyte counts rise through the age of 1-2 years, after that decline and turn even at 4 years, before puberty neutrophils rise and lymphocytes drop.

LYMPH

- fluid that travels through vessels called lymphatics in the lymphatic system and carries cells that help fight infection and disease.
- **pale fluid** that bathes the tissues of an organism, maintaining fluid balance, and **removes bacteria from tissues**; it enters the blood system by way of lymphatic channels and ducts.
- it is not only excess fluid from the blood that is picked up, but **also nutrients, hormones, waste products of the cells, bacteria, cancer cells, and cellular debris, a few red blood cells.**
- prominent among the constituents of lymph are **lymphocytes** and **macrophages**, the primary cells of the immune system with which the body defends itself from invasion by foreign microorganisms.

CHAPTER III

CONNECTIVE TISSUE

Connective tissue is responsible for providing structural support for the tissues and organs of the body. This mechanic function is important in maintaining the form of the body, organs and tissues. The tissue derives its name from its function in connecting or binding cells and tissues.

MESENCHYME (embryonic connective tissue) and the origin of connective tissue cells

Mesenchyme gives rise to **connective tissues.**

- Found **in the embryo**
- Mesenchymal cells are typically **star-shaped cells**, with relatively little cytoplasm.
- Cells have regular, **oval nuclei.**
- Mesenchymal cells have several **thin cytoplasmic processes**. The spaces between the cell processes are filled in gel-like ground substance with fibers.

Mesenchyme cells are only found in embryos, however some **mesenchyme-like cells** persist in adult connective tissue. These mesenchyme-like cells retain their capacity to differentiate into other connective tissue cells in response to injury. Examples include the **pericytes** (perivascular cells) of blood capillaries *(see fig. 40, plate I).*

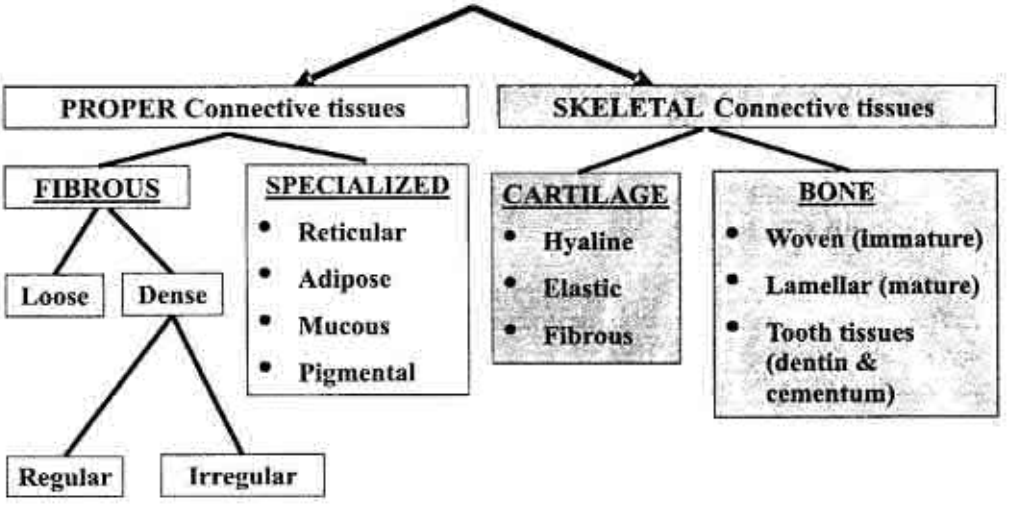
GENERAL CHARACTERISTIC OF CONNECTIVE TISSUES

- Have a **mesenchymal origin.**
- **Found throughout the body – 50% of general weight of the body.**
- Consist of cells and extracellular matrix.
- At time of histogenesis **cells appear first** then extracellular matrix is produced by the cells.

- **Never exposed to outside** environment or to the lumen of tubular organs. Epithelia separate connective tissues from them.
- Have very good **regeneration** properties.
- **Variations in blood supply:**
  - Some tissue types are well vascularized.
  - Some have poor blood supply or are avascular.
- Connective tissue is composed of:
  - cells
  - extracellular matrix.
- The extracellular material of connective tissue, which **plays a major role** in the function of the tissue, is the dominant component of the tissue. The dominance of the extracellular material is a special feature that distinguishes connective tissue from the other tissues of the body.

*Connective tissues are very heterogeneous in structure and function; however all have the three main structural components (**cells, fibers and ground substance**). The diverse composition and amount of these components in the various connective tissues can be correlated with the specific functional roles of the tissue.*

CLASSIFICATION OF CONNECTIVE TISSUES



Functions:

- **Adaptogenic** (connective tissues have adaptability to react to the external or internal factors).
- **Nutrient** (provide the nutrition for other tissues).

- **Protecting** (connective tissue is the place where immune, allergic, and inflammatory reactions take place).
- **Supporting**, surrounding and interconnecting tissues (form the skeleton, capsules of organs, septae).
- **Metabolic** serves as energy store and also provides thermal insulation. Surplus calories can be converted into lipid and stored in adipocytes.
- **Form scarring**.

PROPER CONNECTIVE TISSUE are characterized:

- Contains **varied cell** populations.
- Contains **various fiber** types.
- A syrupy **ground substance**.

LOOSE CONNECTIVE TISSUE

- is the more common type.

Localization:

- Loose connective tissue supports many structures that are normally under pressure and low friction.
- Underlies epithelial tissue.
- Surrounds small nerves, lymphatics and blood vessels.
- Fills the spaces between bundles of muscle fibers
- Forms **lamina propria mucosae**, **tunica submucosa**, **tunica adventitia**, **tunica serosa**, **septae** in glands.

Loose connective tissue comprises all the main components of proper connective tissue. There is no predominant element in this tissue. Loose connective tissue has a delicate consistency; it is flexible, well vascularized, and not very resistant to stress.

I. CONNECTIVE TISSUE CELLS

A variety of cells with different origins and functions are present in connective tissue *(see fig. 41, plate I)*. Cells are divided into 2 groups:

RESIDENT	IMMIGRANT
• **Fibroblasts** & their derivatives (**fibrocytes, fibroclasts, myofibroblasts**) • **Mast cells** • **Adipocytes** • **Melanocytes** • **Adventitial cells** • **Pericytes**	• **Leukocytes** • **Macrophages** • **Plasma cells**

FIBROBLASTS

- Are the most common cell type found in connective tissue.
- The term "**fibroblast**" is commonly used to describe the active cell type, whereas the more mature form.
- Are elongated, spindle-shaped cells with many cell processes. They have oval, pale-staining, regular nuclei with prominent nucleoli. Abundant rough endoplasmic reticulum and active Golgi bodies are found in the cytoplasm.
- Arise from mezenchymal cells.
- Capable for mitosis.
- Synthesize **collagen**, **reticular** and **elastic fibers** and the **amorphous extracellular substance** (including the glycosaminoglycans and glycoproteins).

Derivatives of fibroblast

FIBROCYTES – are definitive form of fibroblasts. Are spindle shaped, have less active synthetic activity. Last active cell does not have many organelles. **Function**: the maintenance of the extracellular matrix structure *(see fig. 42, plate I)*.

FIBROCLASTS – contain many lysosomes. **Function**: phagocytosis of the extracellular matrix (uterus after delivery).

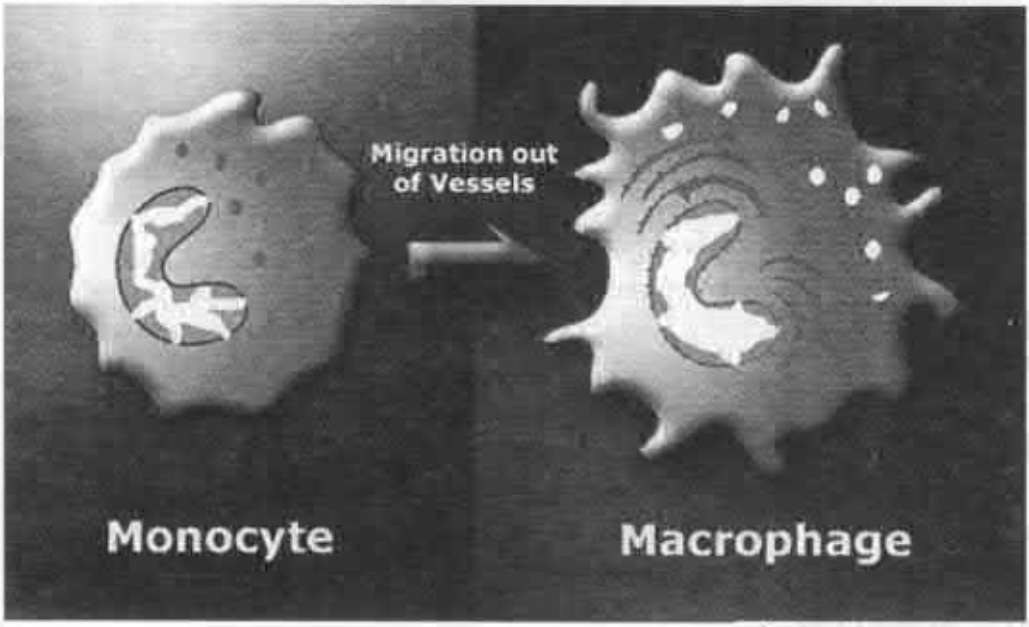
MYOFIBROBLASTS – contain myofibrils. Are similar to smooth muscle cells. Are present in granulation tissue – in wound. **Function**: promote wound repair.

MACROPHAGES

Macrophages show pronounced phagocytotic activity. Macrophages originate from **monocytes** (from precursor cells in bone marrow), which migrate to

connective tissue and differentiate into tissue macrophages. Today the various macrophages of the body are grouped in a common system called the Mononuclear Phagocyte System (MPS). Today a wide range of macrophages are included in the MPS and include: ***Kupffer cells of the liver, alveolar macrophages of the lung, osteoclasts of bone, microglia, histiocytes, osteoclasts, Langherhan's cells in epidermis, macrophages of hematopoietic organs.***

The main functions of macrophages are **ingestion** by phagocytosis of microorganisms (bacteria, viruses, and fungi), parasites, particulate matter such as dust, and they also participate in the **breakdown of aged cells** including erythrocytes, and **make Ag-presentation to lymphocytes**. The intracellular digestion occurs as a result of fusion of lysosomes with the phagosome (ingested body). With immunoglobulins derived from plasma cells attached, they can identify and phagocytose microorganisms and they can trigger B lymphocytes to become plasma cells. As dendritic cells they can become fixed in tissue and send out long branching cytoplasmic extensions (remember dendrite, like on neurons, means tree) that help them detect antigens and invading organisms. When they encounter foreign antigens they phagocytose them and complex pieces with major histocompatibility complex (MHC) molecules and express them as membrane



When a monocyte migrates out of the vessels and becomes a resident macrophage, it becomes larger but downgrades activity; peroxidase (red) present in monocyte granules is restricted to the macrophage nuclear envelope and endoplasmic reticulum.

proteins for presentation to other immune cells (such as plasma or mast cells), and another name for them is antigen presenting cells.

Macrophages also have an important role in removing cell debris and damaged extracellular components formed during the physiological involution process. For example, during pregnancy the uterus increases in size. Immediately after parturition, the uterus suffers an involution during which some of its tissues are destroyed by the action of macrophages. Macrophages are also secretory cells that produce an impressive array of substances, including enzymes (eg, collagenase) and cytokines that participate in defensive and reparative functions, and they exhibit increased tumor cell-killing capacity.

Macrophages are normally long-living and survive in the tissues for several months. In some cases where a foreign body (such as a small splinter) has penetrated the inner tissues of the body, several macrophages may fuse together to form multinuclear foreign body giant cells. These large cells accumulate at sites of invasion of the foreign body and sites of inflammation.

MAST CELLS (histiocytes, tissue basophile)

Mast cells arise from **myeloid stem cells**.

Mast cells are oval or round cells (20-30mm in diameter) in connective tissue characterized by cytoplasm packed with large round basophilic granules (up to 2mm in diameter). The rather small, spherical nucleus is centrally situated; it is frequently obscured by the cytoplasmic granules. The granules are stained metachromatically (purple after toluidine blue staining). Two of the main components of mast cell granules are **histamine** ***(vasodilator, increases capillary permeability and produce bronco-constrictor)*** and **heparin** ***(anticoagulant and lipolytic agent)***. Mast cells also release leukotrienes (C_4, D_4, E_4) or slow-reacting substance of anaphylaxis (SRS-A), but these substances are not stored in the cell. Rather, they are synthesized from membrane phospholipids and immediately released to the extracellular microenvironment upon appropriate stimulation, such as interaction with fibroblasts. The molecules produced by mast cells act locally in paracrine secretion.

The principal function of mast cells is the storage of chemical mediators of the inflammatory response. **Perivascular location** of mast cells form the first line of defense, interact with allergens, bacteria, microorganisms *(see fig. 43, plate I)*.

There are at least two populations of mast cells in connective tissue. One type, called the connective tissue mast cells, is found in the skin and peritoneal cavity; these cells measure 10-12 μm in diameter and their granules contain the

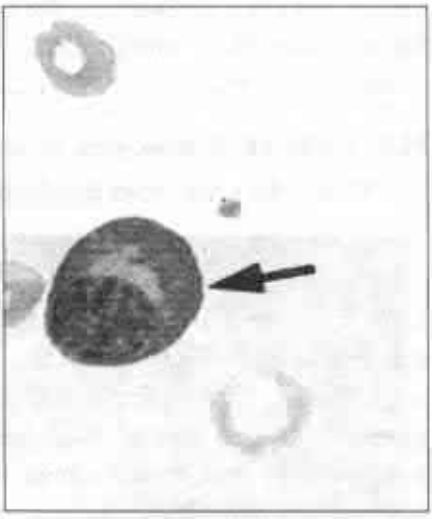
anticoagulant heparin. The second type, the so-called **mucosal mast cell,** is present in the connective tissue of the intestinal mucosa and in the lungs. These cells are smaller than the connective tissue mast cells and their granules contain chondroitin sulfate instead of heparin.

The surface of mast cells contains specific receptors for immunoglobulin E (IgE), a type of immunoglobulin produced by plasma cells. Most IgE molecules are bound to the surface of mast cells and blood basophils; very few remain in the plasma.

PLASMA CELLS

Plasma cells are derivatives of **B-lymphocytes.**

Plasma cells are responsible for **antibody production**. They are involved in the humoral immune response. Antibodies are immunoglobulins produced in response to penetration by antigens. Each antibody is specific for the one antigen. They are the only cell type that secretes antibodies.



A light micrograph of a plasma cell

These large cells have eccentric nuclei, clock-face pattern of heterochromatin, basophilic cytoplasm (much rough endoplasmic reticulum associated with protein synthesis) and well-developed Golgi complex located near nucleus. The juxtanuclear Golgi complex and the centrioles occupy a region that appears pale in regular histological preparations.

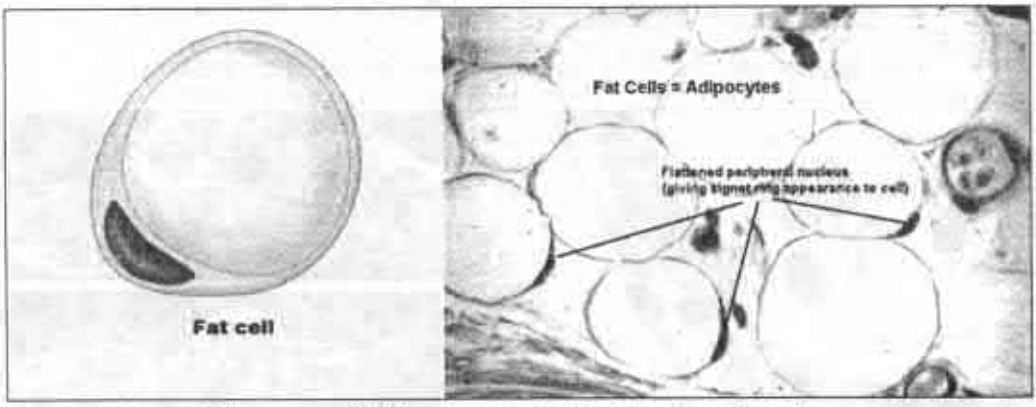
Plasma cells are relatively short-living (10-20 days) and are found in sites of chronic inflammation or sites of high risk in bacteria invasion or foreign proteins (such as the lamina propria of the intestinal and respiratory tracts).

ADIPOCYTES *(Adipose or Fat Cells)*

- Are large **spherical** cells, distended by stored lipids.
- They **store, synthesize, and secrete lipids** under hormonal and neuronal regulation.
- These cells are **long-living** (boo!), but do **not proliferate** (yeah!).

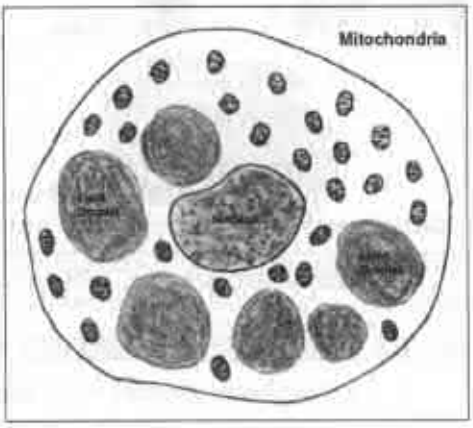
There are 2 types of adipocytes:

- **Unilocular adipocytes** have **a single, central lipid droplet**. They are common in well vascularized loose connective tissue. Their eccentric **nucleus is flattened** giving a "signet ring" appearance in section.



Scheme and light micrograph of unilocular adipocytes

- **Multilocular adipocytes** each include **many lipid droplets**, a **central nucleus**, and **numerous mitochondria** (which give this type of adipose tissue the name "brown fat"). These cells function in heat generation; humans have few, primarily in infants.



Schematic drawing of the multilocular adipocyte

MELANOCYTES

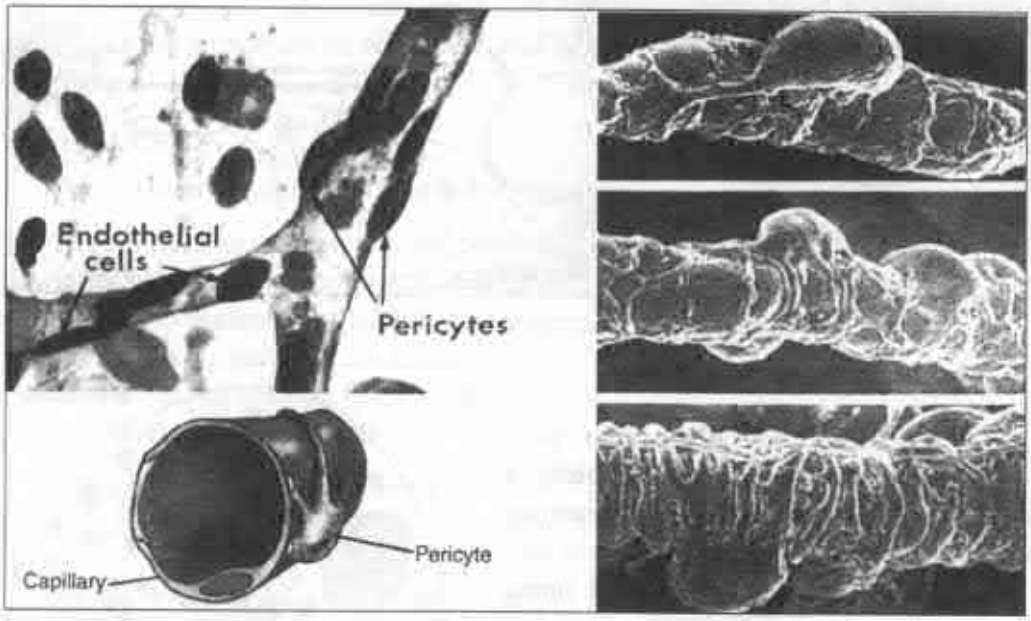
- are star-shaped
- synthesize and store melanin (pigment).
- have neural origin (from neural crests).
- **Function**: provide color of skin, eyes.

ADVENTITIAL CELLS

- are small differentiated cells.
- are located along of small blood vessels.
- Fibroblasts and adipocytes are differentiated from these cells.

PERICYTES

- are mesenchymal stem cells that can generate fibroblasts and other CT cells that are not of the blood immune cell line.
- are found around capillaries and venules.
- **Function**: synthesis of the basement membrane of epithelium.



Schematic drawing, light and scanning micrographs of the pericytes

II. EXTRACELLULAR MATRIX is composed of:

- amorphous ground substance
- protein fibers (collagen fibers, reticular fibers, elastic fibers)

a. GROUND SUBSTANCE

- Is highly hydrated.
- Is colorless, transparent, jelly-like material in which the cells and fibers are embedded.
- Consists of:
  - **Interstitial (tissue) fluid** – fairly high water content, ions.
  - **Adhesion proteins** – fibronectin and laminin. **Fibronectin** (*fibra*, fiber, + *nexus*, interconnection) is a glycoprotein synthesized by fibroblasts and some epithelial cells. This molecule has binding sites for

cells, collagen, and glycosaminoglycans. Interactions at these sites help to mediate normal cell adhesion and migration. Fibronectin is distributed as a network in the intercellular spaces of many tissues. **Laminin** is a large glycoprotein that participates in the adhesion of epithelial cells to the basal lamina, a structure rich in laminin.

- **Proteoglycans** *(large compounds of protein)* and **Glycosaminoglycans** and **Glycoproteins** (GAGs; *=large poplysaccharides*).

Glycosaminoglycans (originally called **acid mucopolysaccharides**) are linear polysaccharides formed by repeating disaccharide units usually composed of an uronic acid and a hexosamine. The **proteoglycans** are composed of a core protein associated with the four main glycosaminoglycans: **dermatan sulfate, chondroitin sulfate, keratan sulfate,** and **heparan sulfate.**

Functions as:

- A matrix to hold cells and fibers together.
- A molecular sieve through which nutrients diffuse between blood capillaries and cells

All substances passing to and from cells must pass through the ground substance.

The amount of tissue fluid is fairly constant and there is equilibrium between the water entering and leaving the intercellular substance of the connective tissue. In pathological conditions (traumatic injury, inflammation) fluid may accumulate in the connective tissue, a condition known as ***edema****.*

b. CONNECTIVE TISSUE FIBERS

Connective tissue fibers are composed of structural proteins that polymerize into elongated structures. The three main types of fibers are:

- collagen fibers
- elastic fibers
- reticular fibers

These fibers are distributed unequally among the types of connective tissue.

COLLAGEN FIBERS

The collagens constitute a family of proteins selected during evolution for the execution of several (mainly structural) functions. Collagen fibers are formed from the protein **collagen**. Collagen is the most abundant protein in the body (up

to 30% dry weight). There are more than 12 different types of collagen, though the most common types are **Types I to V.**

Collagen type	Main sites	Special features
Type I	Bones, tendons, organ capsules, dentin	Most abundant, Typical collagen fibers (64nm banding)
Type II	Hyaline cartilage, Elastic cartilage	Very thin fibrils
Type III	Reticular fibers	Often associated with Type I
Type IV	Basal lamina associated with epithelial and endothelial cells	Amorphous (non fibrous)
Type V	Basal lamina associated with muscle	Amorphous (non fibrous)

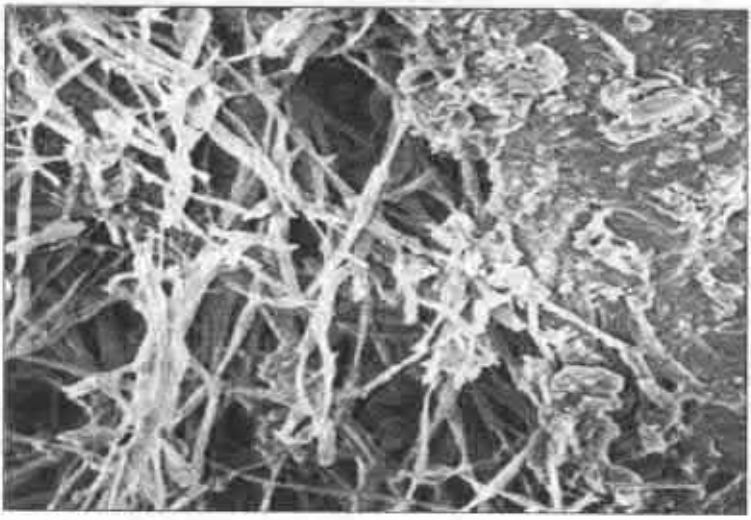
- Collagen is synthesized by a wide number of cell types (including: fibroblasts, osteoblasts, chondroblasts, odontoblasts, reticular cells, epithelial cells, endothelial cells, smooth muscle cells, Schwann cells).
- The main amino acids of collagen are:
  - glycine (33.5%)
  - proline (12%)
  - hydroxyproline (10%)

The protein unit that polymerizes to form collagen fibrils is the elongated molecule called **tropocollagen,** which measures 280 nm in length and 1.5 nm in width. Tropocollagen consists of three subunit polypeptide chains intertwined in a triple helix. Differences in the chemical structure of these polypeptide chains are responsible for the various types of collagen.

- Collagen fibers **are thick, not branched**, appear **white**. In histological preparations after regular staining they are acidophilic (pink staining with eosin).
- They have transverse striations with a characteristic periodicity of 64 nm. The transverse striations of the collagen fibrils are determined by the overlapping arrangement of the tropocollagen molecules.
- Collagen fibers are flexible, but very **inelastic** with extremely high tensile **strength**.

Collagen synthesis involves several steps:

1. Polypeptide chains are assembled on polyribosomes bound to rough endoplasmic reticulum membranes and injected into the cisternae as **preprocollagen** molecules. The signal peptide is clipped off, forming **procollagen.**
2. Hydroxylation of proline and lysine occurs after these amino acids are incorporated into polypeptide chains. Hydroxylation begins after the peptide chain has reached a certain minimum length and is still bound to the ribosomes. The two enzymes involved are **peptidyl proline hydroxylase** and **peptidyl lysine hydroxylase.**
3. Glycosylation of hydroxylysine occurs after its hydroxylation. Different collagen types have different amounts of carbohydrate in the form of galactose or glycosylgalactose linked to hydroxylysine.
4. Each chain is synthesized with an extra length of peptides called **registration peptides** on both the amino-terminal and carboxyl-terminal end. Registration peptides probably ensure that the appropriate chains (α1, α2) assemble in the correct position as a triple helix. In addition, the extra peptides make the resulting **procollagen molecule** soluble and prevent its premature intracellular assembly and precipitation as collagen fibrils. Procollagen is transported as such out of the cell to the extracellular environment.
5. Outside the cell, specific proteases called **procollagen peptidases** remove the registration peptides. The altered protein, known as **tropocollagen,** is able to assemble into polymeric collagen fibrils. The hydroxyproline residues contribute to the stability of the tropocollagen triple helix, forming hydrogen bonds between its polypeptide chains.
6. Collagen fibrils aggregate spontaneously to form fibers. Proteoglycans and structural glycoproteins play an important role in the aggregation of tropocollagen to form fibrils and in the formation of fibers from fibrils.
7. The fibrillar structure is reinforced by the formation of covalent cross-links between tropocollagen molecules. This process is catalyzed by the action of the enzyme **lysyl oxidase,** which also acts in the extracellular space.



Scanning micrograph of collagen fibers

RETICULAR FIBERS

- Are very **thin**.
- Are special branched forms **of collagen (Type III).**
- Form fine-meshed **flexible network** around cells and cell groups in diverse organs.
- They are abundant in lymphatic organs (lymph nodes, spleen, red bone marrow), smooth muscle (in the sheath surrounding each myocyte), in endoneurium (connective tissue surrounding peripheral nerve fibers), and supporting epithelial cells of several glands (liver, endocrine glands).

Reticular fibers are not visible in normal histological preparations after regular staining (H & E), however they can be visualized and stained black after impregnation with silver salts. This affinity for silver is called ***argyrophilia****.*

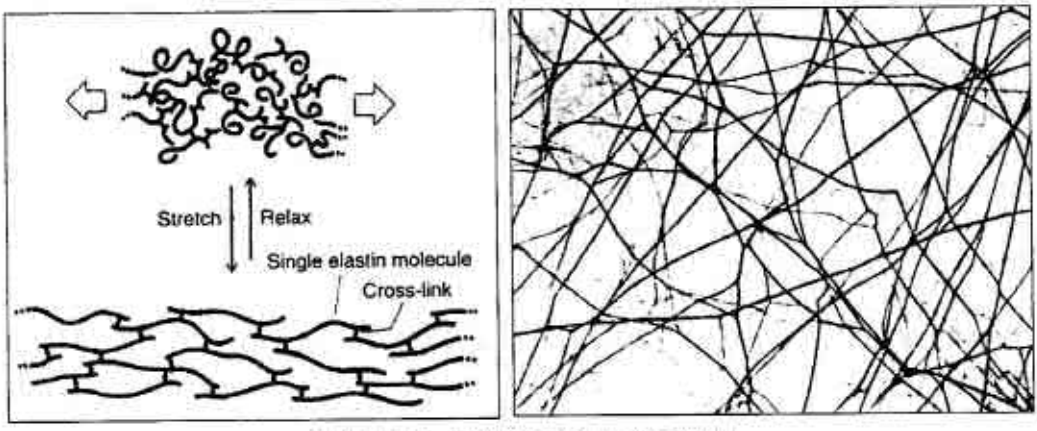
ELASTIC FIBERS

Elastic fibers, as the name suggests, are highly **elastic** and **stretch** in response to tension.

- Are formed from the protein **elastin**. Elastin is rich in glycine and proline, but in addition has two unusual amino acids, desmosine and isodesmosine. A precursor of elastin is **proelastin,** a globular molecule produced by fibroblasts in connective tissue and by smooth muscle cells in blood vessels. Proelastin polymerizes, producing elastin, the amorphous

rubberlike glycoprotein that predominates in mature fibers. Elastic fibers also have a high content of valine.

- The elastin imparts a **yellow color** to the tissue.
- Are very prominent in elastic tissues such as the elastic ligaments.



Schematic drawings of elastic fibers

Elastin can be stained in histological preparations using orcein. Elastin also occurs in a nonfibrillar form as ***fenestrated membranes*** *(elastic laminae) present in the walls of some blood vessels.*

DENSE CONNECTIVE TISSUE

- Is adapted to offer resistance and protection.
- Contains relatively **few cells** with **much** greater **number of collagen fibers**.
- Dense connective tissue is less flexible and far more resistant to stress than is loose connective tissue.
- Is divided into two sub-categories:
  - dense ***irregular*** connective tissue
  - dense ***regular*** connective tissue

1. Dense REGULAR Connective Tissue

- Has closely-packed densely-arranged fiber bundles with **clear orientation** (such as in tendons) and relatively few cells *(see fig. 44, plate I).*
- Main matrix elements are parallel collagen fibers with a few elastic fibers.
- Rows of fibroblast cells between collagen fibers.

- Found in **tendons** (attach muscle to bone), **ligaments** (attach bone to bone), and **aponeuroses.**
- Offer great resistance to traction forces.

Tendons

Tendons are the most common type of dense regular connective tissue. Tendons connect skeletal muscles to bone. Owing to the dominance of the collagen fibers, the tendons have a **white color** (stains acidophilic in regular staining). The collagen bundles in tendons are arranged in bundles **(primary bundles).** Several primary bundles, each surrounded by loose connective tissue **(endotendineum),** are grouped into larger bundles **(secondary bundles).** The loose connective tissue surrounding the primary and secondary bundles contains blood vessels and nerves. The whole tendon is surrounded by a denser connective tissue **(peritendineum).**

Each primary bundle has orderly-arranged rows of fibrocytes, when seen in longitudinal section. These fibrocytes have relatively little cytoplasm.

Ligaments

Ligaments are a special type of dense regular connective tissue that connects bones to bones. They have a similar structural arrangement to tendons, but differ in their **yellow color**, which is due to the abundance of elastic fibers in the tissue. The elastic fibers are stained in dark brown-red with orcein. Elastic fibers provide the ligament with remarkable elasticity (in contrast to tendons).

2. **Dense IRREGULAR Connective Tissue**
   - Irregularly arranged collagen fibers with some elastic fibers *(see fig. 45, plate I).*
   - Tensions in various directions
   - Occurs in sheets
   - Found in **dermis of skin, periosteum, perichondrium,** form **capsules** of organs

ADIPOSE CONNECTIVE TISSUE

- is specialized connective tissue that functions as the major storage site for fat in the form of triglycerides.
- is found in two different forms: **white adipose tissue and brown adipose tissue.** Most adipose tissue is white.

1. WHITE ADIPOSE TISSUE *(see fig. 46, plate I)*
   - serves three functions:
     - **heat insulation**
     - **mechanical cushion**
     - and most importantly, **a source of energy**.
   - Subcutaneous adipose tissue, found directly below the skin, is an important heat insulator in the body, because it conducts heat only one third as readily as other tissues. The degree of insulation is dependent upon the thickness of this fat layer. For example, a person with a 2-mm layer of subcutaneous fat will feel as comfortable at 15°C as a person with a 1-mm layer at 16°C.
   - white adipose tissue also surrounds internal organs and provides some protection for these organs from jarring.
   - white adipose tissue composes as much as 20% of the body weight in men and 25% of the body weight in women.
2. BROWN ADIPOSE TISSUE *(see fig. 47, plate I)*
   - has **rich vascularization** and **densely** packed **mitochondria**
   - is found in various locations, primarily in the **interscapular region** and **the axillae**, minor amounts are found near the thymus and in the dorsal midline region of the thorax and abdomen.
   - During maturation brown adipose tissue is metabolically less active, most of the mitochondria (which are responsible for the brown color) in brown adipose tissue disappear, and the tissue becomes similar in function and appearance to white fat – as a mere fat deposit.
   - Brown adipose tissue is important for regulating body temperature via non-shivering thermogenesis.
   - The mechanism of heat generation is related to the metabolism of the mitochondria. Mitochondria from brown adipose tissue have a specific carrier called uncoupling protein that transfers protons from outside to inside without subsequent production of ATP.

In neonates (new born babies), brown fat, which then makes up about 5% of the body mass and is located on the back, along the upper half of the spine and towards the shoulders, is of great importance to avoid lethal cold (hypothermia is a major death risk for premature neonates). Numerous factors make infants more susceptible to cold than adults:

- The **higher ratio of body surface** (proportional to heat loss) **to body volume** (proportional to heat production).
- The higher proportional surface area of the head.
- **The low amount of musculature** and the inability or reluctance to shiver.
- A **lack of thermal insulation**, e.g. subcutaneous fat and fine body hair (especially in prematurely born children).
- The **inability to move** away from cold areas, air currents or heat-draining materials.
- The **inability to use additional ways** of keeping warm (e.g. turning up a heater, drying their skin, changing clothes or performing physical exercise).
- The **nervous system is not fully developed** and does not respond quickly and/or properly to cold (e.g. by contracting blood vessels in the skin).

PIGMENTAL CONNECTIVE TISSUE

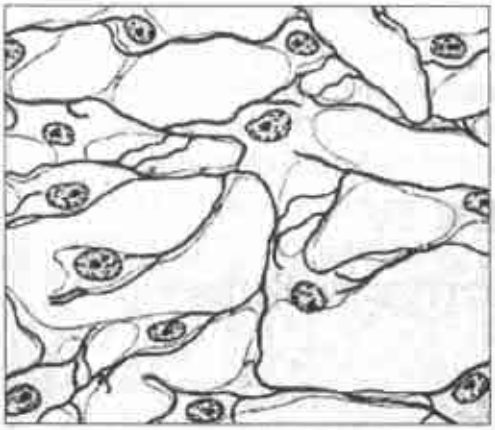
- Is a loose connective tissue in which pigmental cells predominate.
- Found in **areola of mammary** gland, **perianal** zone, **genitals** region, pigmented **nevus** (birthmarks).

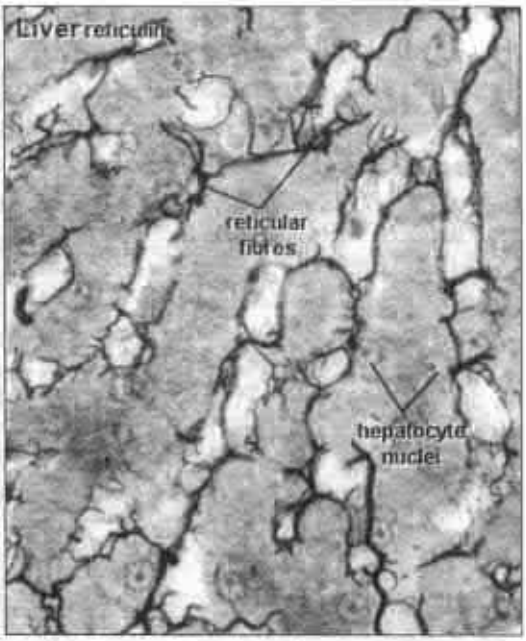
MUCOUS CONNECTIVE TISSUE

- It is a loose connective tissue composed of fibroblasts with several long cytoplasmic processes.
- The intercellular space is filled with a jelly-like amorphous ground substance, rich in hyaluronic acid and fibers.
- This is found in the umbilical cord (Wharton's jelly); the ocular vitreous also fit tolerably well in this class.

RETICULAR TISSUE

- Composed of **reticular cells** and **reticular fibers**.
- Create a complex, 3-D **network (stroma)** that supports the cells of an organ.
- Forms the **stroma** (skeleton of red bone marrow, spleen, liver).





Reticular connective tissue showing the attached cells and the fibers

CHAPTER IV

SKELETAL CONNECTIVE TISSUES

CARTILAGE

Cartilage is important for:

- **skeletal support** in the embryo prior to the development of the bony skeleton.
- **elongation** of developing long bones (endochondral ossification).
- **articulating joints** (articular cartilage).
- **flexible support** in the ear and eartubes, and in the larger tubes of the respiratory tract (trachea, bronchi).

Cartilage is the skeletal connective tissue.

Cartilage is **an avascular** tissue which has no blood vessels of its own. Cartilage receives its nutrients from blood vessels from a surrounding dense connective tissue, the perichondrium. Nutrients and metabolites pass to and from the cells via matrix by diffusion.

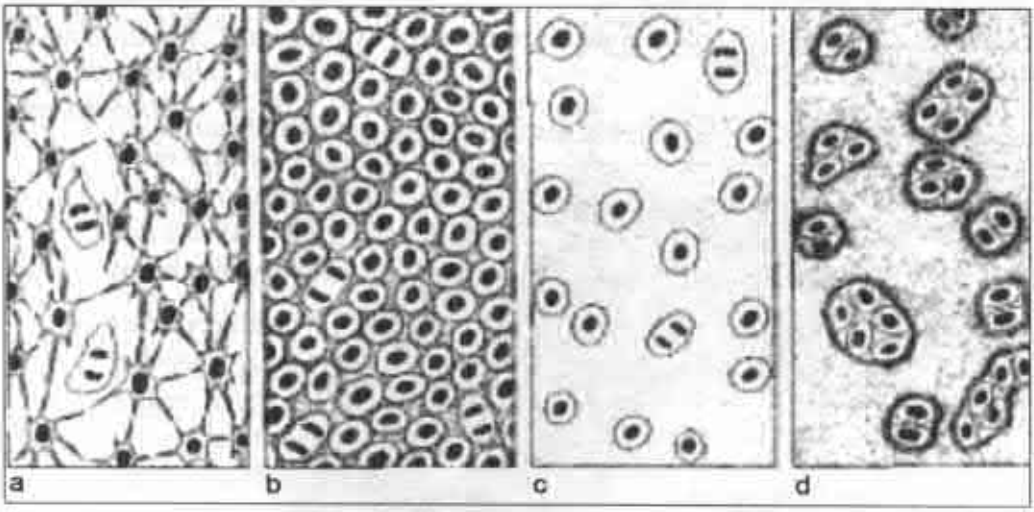
Cartilage is a **tissue of very low metabolic activity** and cell turnover (except the embryo).

Nerves are not present in cartilage, but nerves and nerve endings are present in the perichondrium.

CHONDROGENESIS (cartilage formation)

- Like all connective tissue, cartilage is derived in the embryo from mesenchyme.
- **Mesenchyme cells** grow and retract their extensions, multiply rapidly, and form mesenchymal condensations of **young cartilage cells** or **chondroblasts**, which are very active in secreting the surrounding matrix. Synthesis and deposition of the matrix then begin to separate the chondroblasts from one another. During development, the differentiation of

cartilage takes place from the center outward. The chondroblasts grow and develop in lacunae. These chondroblasts further differentiate into mature cartilage cells or **chondrocytes**. The more central cells of future cartilage have the characteristics of chondrocytes, whereas the peripheral cells are typical chondroblasts. The superficial mesenchyme develops into the perichondrium.



Histogenesis of hyaline cartilage. **A:** *The mesenchyme is the precursor tissue of all types of cartilage.* **B:** *Mitotic proliferation of mesenchymal cells gives rise to a highly cellular tissue.* **C:** *Chondroblasts are separated from one another by the formation of a great amount of matrix.* **D:** *Multiplication of cartilage cells gives rise to isogenous groups, each surrounded by a condensation of territorial (capsular) matrix.*

The cartilage consists of **cells and extracellular matrix**:

- the extracellular **matrix is strong and pliable.**
- cells are called **chondroblasts and chondrocytes** found in small cavities called lacunae.

CELLS

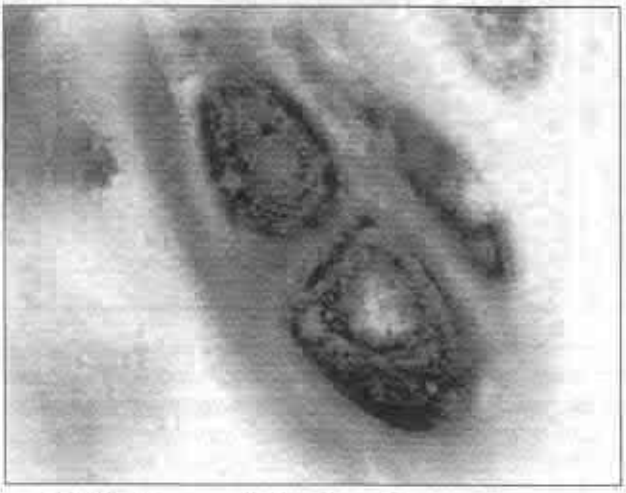
CHONDROGENIC CELLS are derived from mesenchymal cells. They can differentiate into chondroblasts. **Chondrogenic cells** are spindle-shaped, located in the perichondrium.

CHONDROBLASTS are young flattened cells which have the ability to undergo mitosis, are active in secreting the intercellular matrix. The chondroblasts typically have basophilic cytoplasm. These cells have well-developed RER, an extensive Golgi complex, mitochondria. The chondroblasts further differentiate into chondrocytes.

- FUNCTION:
  - They produce extracellular matrix
  - They provide the appositional growth of cartilage

CHONDROCYTES are mature cartilage cells which are also capable of cell division. Those located superficially are ovoid-shaped with their longitudinal axis parallel to the cartilage surface. Those located deeper are spherical in shape and may occur in groups called **isogenous groups**. The cytoplasm of chondrocytes is less basophilic.

- FUNCTION: they **provide the interstitial growth** of cartilage.



A light micrograph showing a isogenous group

EXTRACELLULAR MATRIX

- The most important component of cartilage which provides the biomechanical characteristics of the tissue is the **extracellular matrix.**
- The matrix is composed of **amorphous substance,** in which fibers are embedded.

Amorphous substance is composed of:

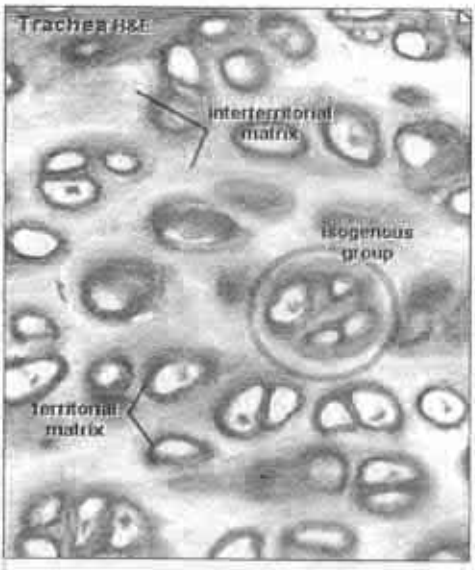
- **Water**
- **Proteoglycans**
- **Other glycoproteins (e.g. glycosaminoglycans, chondronectin)**
- **Hyaluronic acid** (gelatinous mucopolysaccharide)
- **Minerals**

Type II collagen fibers (constitute about 40% of the dry weight of cartilage) are embedded in the matrix and provide structural support.

In normal histological preparations the collagen fibers are not seen as they have submicroscopic dimensions and their refractive index is similar to that of the amorphous matrix.

Extracellular matrix is subdivided into 2 regions:

- **Territorial matrix** – is adjacent to chondrocytes. Is poor in collagen and rich in chondroitin sulfate – **intense basophilic staining.**
- **Interterritorial matrix** – is between isogenous groups. Is **richer in type II collagen** and poorer in proteglycans than the territorial matrix.



Light micrograph of a hyaline cartilage showing subdivisions of the extracellular matrix

CLASSIFICATION OF CARTILAGE

- **Hyaline cartilage**
- **Elastic cartilage**
- **Fibrous cartilage (fibrocartilage)**

HYALINE CARTILAGE

- is the most common form of cartilage *(see fig. 48, plate 1)*.
- **Location:**
  - respiratory tract (nasal septum, larynx, trachea, bronchi)
  - the ventral part of ribs (connection with the stern)
  - articulating surfaces of long bones and joints (articular cartilage)
  - Embryo's skeleton (important role in long bone development)
- is a semi-transparent (translucent), milky-white tissue, that is both flexible and resilient to mechanical forces.
- Chondrocytes may occur in isogenous groups of 4-8 cells.

Because cartilage is devoid of blood capillaries, chondrocytes respire under low oxygen tension. Hyaline cartilage cells metabolize glucose mainly by anaero-

bic glycolysis to produce lactic acid as the end product. Nutrients from the blood cross the perichondrium to reach more deeply placed cartilage cells. Mechanisms include diffusion and transport of water and solute promoted by the pumping action of intermittent cartilage compression and decompression. Because of this, the maximum width of the cartilage is limited.

Chondrocyte function depends on a proper hormonal balance. The synthesis of sulfated glycosaminoglycans is accelerated by growth hormone, thyroxin, and testosterone and is slowed by cortisone, hydrocortisone, and estradiol. Cartilage growth depends mainly on the hypophyseal growth hormone **somatotropin.** This hormone does not act directly on cartilage cells but promotes the synthesis of **somatomedin C** in the liver. Somatomedin C acts directly on cartilage cells, promoting their growth.

Damage repair is limited. When cartilage is fractured, protochondral cells migrate in from the chondrogenic layer of the perichondrium and produce cartilage, but the repair is usually not a good one. More often the break is filled with scar tissue by fibroblasts. This problem is particularly severe for articular cartilage which does not have a perichondrium.

Articular Cartilage is a special type of hyaline cartilage *(see fig. 49, plate I).*

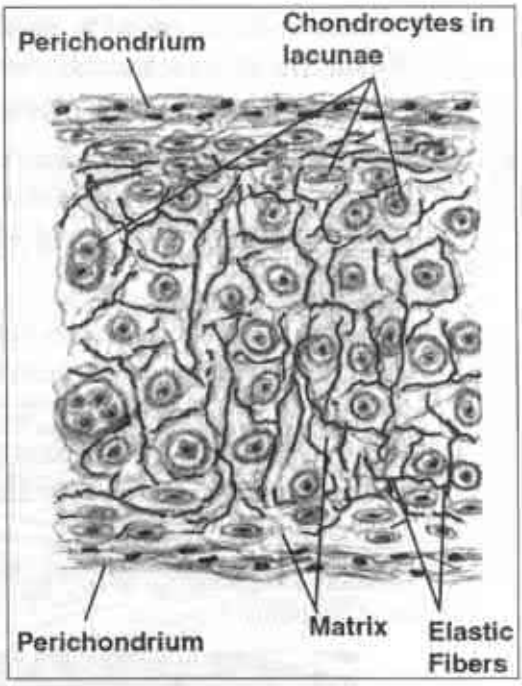
It is smooth and slippery to provide the gliding surfaces of joints. The weight bearing surface has no perichondrium and it is bathed in synovial fluid, which acts as a lubricant and provides the cartilage with nutrients by diffusion. Collagen is oriented perpendicular to the surface for strength, but it bends parallel near the surface to reduce wear. When joints are subjected to excessive wear the parallel surface fibers can be worn away so that the perpendicular ends stick out and grind against the opposing surface and wear is accelerated. Where the articular cartilage attaches to the end of a bone, the matrix is calcified.

- **Damage**
  - Matrix can breakdown.
  - Exposes collagen fibers.
  - Increased friction

ELASTIC CARTILAGE

- The inter-group matrix material is considerably lessened in volume, and on the whole the chondrocytes of elastic cartilage are bigger than those of hyaline.

- The net result is that the isogenous groups are closer to each other and the distinct separation seen in hyaline cartilage isn't as obvious. There's still a pattern of isogenous groups but it's much harder to make them out.
- Isogenous groups are not so well defined as in hyaline cartilage, but they still exist.
- The nature of the fibrillar component is also different; it consists principally of elastic fibers. There's little collagen, but the elastic fibers are predominant. They give this type of cartilage the ability to be deformed and return to shape *(see fig. 50, plate I)*.



Scheme of elastic cartilage

Location:

- Auricle of external ear, auditory canal
- Tip of nose
- the walls of the Eustachian tube
- Epiglottis, cuneiform cartilages of larynx.

Function: Provides support with more flexibility.

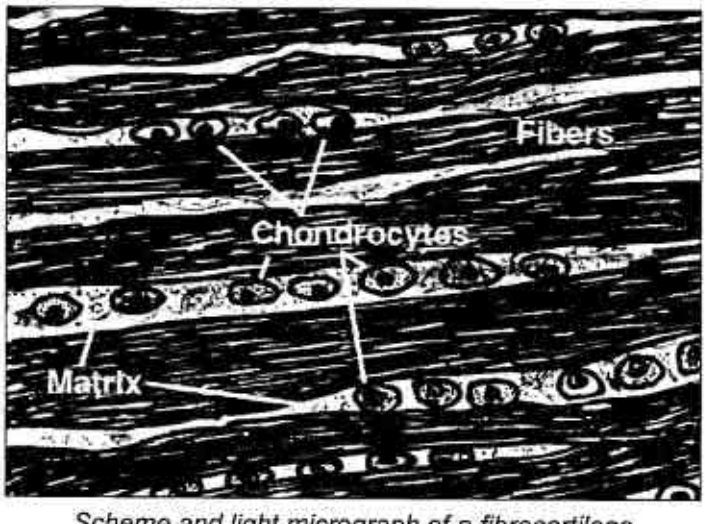
FIBROCARTILAGE *(see fig. 51, plate I)*

- is found in areas of the body subject to high mechanical stress or weight bearing.
- it lacks the flexibility of the other cartilage types.
- Is present in:
  - **intervertebral disks**
  - **pubic symphysis**
  - **temporo-mandibular joints**
  - at **sites of connection of many ligaments to bones** (e.g. Ligamentum teres femoris)
  - **tendon insertions**

- is a sort of halfway state between "true" cartilage and the fibrous CT's.
- Matrix similar to hyaline cartilage but less strong with thick collagen fibers.
- Chondrocytes arranged in parallel rows between bundles of collagen. They are in lacunae, though often a lacuna may be incomplete.
- Fibrocartilage is **not** surrounded by **perichondrium**.
- is characterized by **large numbers and concentrations of collagen fibers** (no elastic fiber) in the matrix. These collagen fibers are the dominant feature of the matrix and with relatively **little amorphous matrix**.

Intervertebral disks

- consist of fibrocartilage plates between the vertebrae and act as mechanical shock absorbers. In sections they are seen to be formed of two components:
- **annulus fibrosus**, which is the outer region consisting of orderly concentric arrangements of cells and matrix dominated by type I collagen (as in tendons).
- **nucleus pulposus** (large vacuolated cells, that are vestiges of the embryonic notochord).



Scheme and light micrograph of a fibrocartilage

CARTILAGE AS AN ORGAN

1. **Perichondrium** is the connective tissue sheath covering that lies over the most cartilage. It **is vascular**, and its vessels supply nutrients to the cells of cartilage.

- **It has 2 layers:**
  - **Outer – fibrous,** formed by dense irregular connective tissue. Provide: *mechanical support, protection, attachment.*
  - **Inner – cellular layer – contains chondrogenic cell, chondroblasts.** Provide: *cartilage growth, maintenance.*

Articular cartilage and fibrocartilage have no perichondrium. Cartilage cells take the nutrients from the synovial fluid.

2. **Zone of young cartilage** – chondrocytes are located alone.
3. **Zone of mature cartilage** – chondrocytes form the isogenous groups.

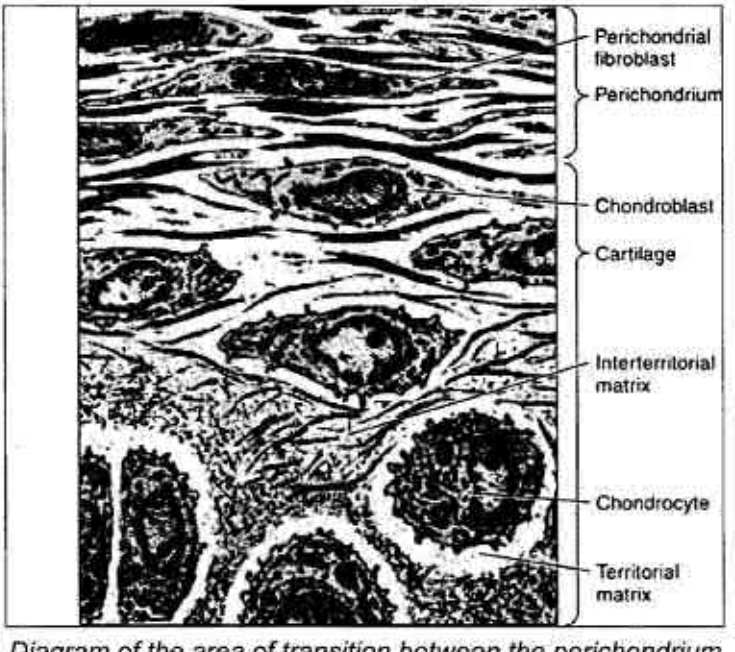


Diagram of the area of transition between the perichondrium and the hyaline cartilage

GROWTH OF CARTILAGE

There are two different types of growth:

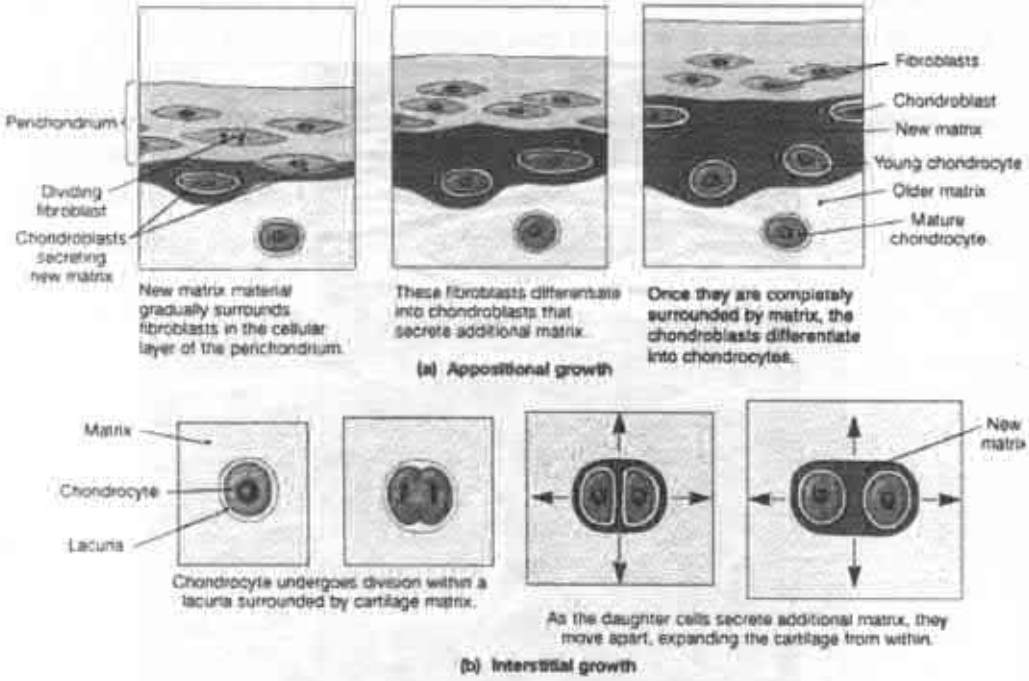
- appositional growth
- interstitial growth

I. Interstitial growth

- Chondroblasts within the existing cartilage divide and form small groups of cells, isogenous groups, which produce matrix to become separated from each other by a thin partition of matrix.
- Occurs **mainly in immature cartilage.**

II. Appositional growth

- Cells of the inner layer of perichondrium divide continually; the innermost cells diferentiate into chondroblasts or chondrocytes.
- Occurs also **in mature cartilage.**



Schematic drawing of types of growth of the cartilage

BONE

Functions of the bone:

- **Support** – Bone provides a framework for the body by supporting soft tissues and providing points of attachment for most of the skeletal muscles.
- **Protection** – Bones protect many internal organs from injury very well, such as the brain and spinal cord. In addition, the heart, lungs, and reproductive organs are given some degree of protection.
- **Movement** – Most skeletal muscles are attached to bones. When the muscles contract, they pull on bones to activate lever systems and movement is produced.
- **Mineral homeostasis** – Bone tissue stores a number of minerals, particularly calcium and phosphorus. Under control of the endocrine system, bone releases the minerals into the blood or stores the minerals in bone matrix to maintain critical mineral balances.
- **Blood cell production** – In all bones of the infant and certain bones of the adult, a connective tissue known as red marrow produces blood cells by the process of hematopoiesis.
- **Storage of energy** – In some bones, yellow bone marrow stores lipids, creating an important energy reserve for the body.

MACROSCOPIC STRUCTURE OF BONE

There are two main categories of bone:

- **Spongy bone** (trabecular bone, cancellous bone)
- **Compact bone** (cortical bone)

- **Spongy bone** consists of lamellae (layers) of bone matrix arranged in an irregular latticework of thin plates of bone called trabeculae. The spaces between the trabeculae are a part of the medullary cavity of the bone and contain red bone marrow.
- **Compact bone** contains very few spaces. The layers of bone matrix are packed together tightly, forming osteons (Haversian systems). It forms the external layer of all bones, providing protection and support and helps the long bone resist the stress of weight applied to them.

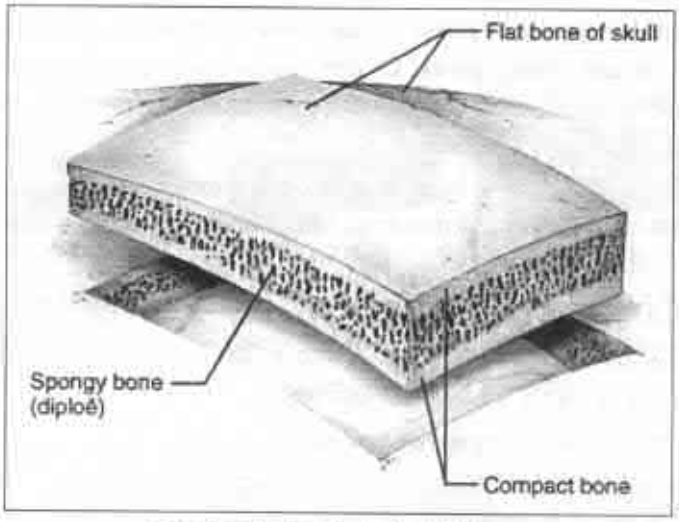
ANATOMICAL classification of bones

- Bones are characterized anatomically as:
  - **long bones** (e.g. humerus, femur)
  - **flat bones** (membrane bones)
  - **irregular bones** (such as the vertebrae)

- All these bone types, regardless of their anatomical form, are composed of both spongy and compact bone.

Structure of Short, Irregular, and Flat Bones

The flat bones or "membrane" bones of the skull are composed in a sandwich-like fashion of an **outer layer of compact bone** (outer table), **a middle layer of spongy bone (diploe),** and **an inner layer of compact bone** (inner table). Periosteum covers the flat bone on the outer side (near the scalp) and on the inner side the periosteum is thicker and continuous with the dura mater (outer meningeal layer of the brain).



Schematic drawing of a flat bone

Structure of Long Bones

The shaft (or diaphysis) is composed of compact (cortical or diaphyseal) bone. The epiphyses are mainly composed of trabeculae of spongy bone.

HISTOLOGY of bone

- **Cells: osteoblasts, osteocytes, osteoclasts** – constitute only a very small percentage of the bone tissue.
- Whereas the bulk of the tissue is occupied by the intercellular, **calcified, bone matrix**.
- **The bone matrix has two main components :**
  - **Organic matrix**– is composed of type I collagen, chondroitin sulfate and keratan sulfate. **Type I collagen fibers** are 90% of the volume

of bone. They are 60 nm fibrils that are laid down in parallel arrays within each lamella. They run in a spiral around haversian lamellae along the long axis of a Haversian system and spiral at different angles and in different directions in each lamella. If a whole bone is decalcified it becomes rope-like.

- **Inorganic salts** – is composed of calcium, phosphate, magnesium, potassium, sodium. It consists primarily of hydroxyapatite crystals **($Ca_{10}(PO_4)_6(OH)_2$).** Hydroxyapatite crystals are long, thin, and lie parallel to the collagen fibrils. If collagen is dissolved out of a bone, it becomes as brittle as china.

- The morphofunctional unit of bone tissue is a **BONE LAMELLAE**.

BONE CELLS

OSTEOBLASTS

- are involved in the formation of bone and are found in the boundaries of developing and growing bone.
- are oval-shaped cells, with a large eccentric nucleus, and the cytoplasm is basophilic. These cells are very active in synthesizing and secreting the components of the bone matrix and have well-developed rough endoplasmic reticulum (RER), Golgi bodies and granules.
- are rich in the enzyme alkaline phosphatase, which plays a major role in the formation of the mineral deposits in the matrix.
- The matrix closest to the osteoblasts is not yet calcified and is known as **osteoid or prebone**. This osteoid is rich in collagen fibers.
- They maintain contact with one another with slender cytoplasmic processes and produce bone matrix on bone surfaces. Osteoblasts become embedded in bone when they other neighbouring osteoblasts secrete osteoid (new bone matrix) around them to form lacunae.
- Once made, osteoid immediately begins to calcify. During this process, the osteoblast filapodial processes maintain contact and are also embedded to form canaliculi. The buried osteoblast becomes relatively dormant and is therefore called an osteocyte.

OSTEOCYTES

- are mature bone cells that develop from osteoblasts and are located in lacunae within the bony matrix.
- Osteocytes have cytoplasmic processes located in canaliculi, which penetrate the bony matrix.

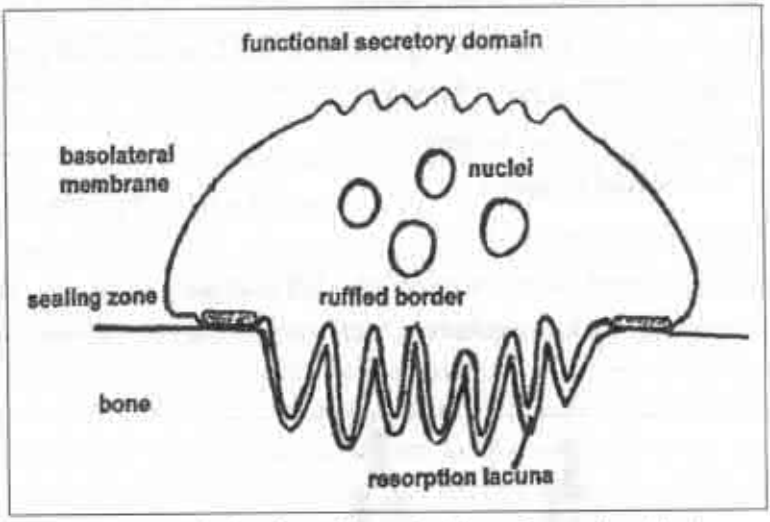
- Cytoplasmic processes from one osteocyte make contact with the processes from neighboring osteocytes and can communicate via gap junctions. Because the bony matrix is calcified there is no possibility of diffusion except via the network of canaliculi.

Section of bone tissue showing an osteocyte with its cytoplasmic processes surrounded by matrix

OSTEOCLASTS *(see fig. 52, plate I)*

- Originate from monocytes and are included in the mononuclear phagocyte system.
- Are the largest of the bone cells (20-100 mm diameter) and are multinucleated (with up to 50 nuclei) with a pale acidophilic cytoplasm.
- Osteoclasts are involved in bone resorption and can be found on the eroding surfaces of bone, often in cavities known as Howship's lacunae.
- The osteocytic cell membrane closest to the bone undergoing resorption has multiple invaginations and is known as the "ruffled border".
- They have a smooth clear zone around the ruffles that seals them to the bone to maintain a high concentration of the enzymes and acid.
- The cells are metabolically very active, possess large numbers of mitochondria (resulting in the acidophilia of regular staining) and have well-developed Golgi bodies.
- The cytoplasm of osteoclasts has vacuoles and lysosomes, since the mechanism of bone resorption is partly an enzymatic digestion, by cathepsins and collagenase, and also from acid made by an osteoclastic proton pump.

- In dense bone, many osteoclasts act together to erode resorption tunnels, which are later partially filled in with lamellar bone to become osteons.
- They dissolve bone at the same rate as osteoblasts make it by exocytosing the contents of special lysosomes.



Schematic drawing of the structure of an osteoclast

MICROSCOPIC STRUCTURE of bone

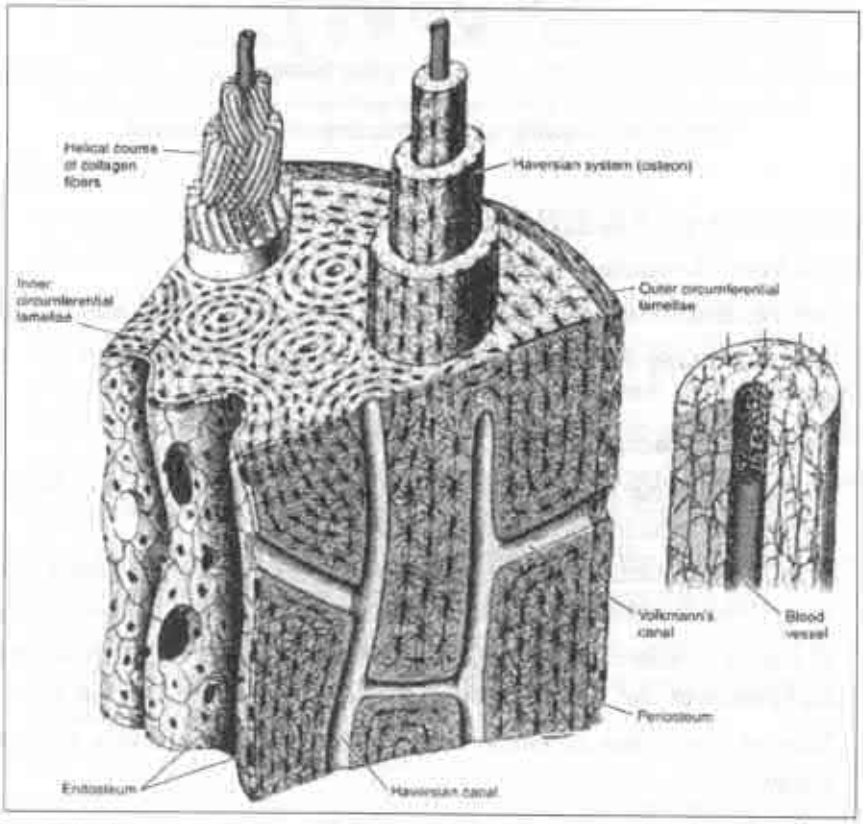
I. **Woven bone (Immature bone, Primary bone)**
   - can be identified by the **lack** of order of the lacunae (of **osteocytes**).
   - contains **many osteoblasts** and large **irregularly** arranged type I **collagen** bundles.
   - has a **low mineral content.**
   - is the first bone produced during the fetal development & bone repair and is replaced by the secondary bone.
   - is found temporarily **in the developing embryo** *(before undergoing rearrangement (remodeling) resulting in the development of lamellar bone).*
   - is found in some specific locations including the vicinity of **sutures of flat bones of the skull, in tooth sockets, and some tendon insertions.**
   - Woven bone also develops temporarily **in cases of bone fracture and repair.**

II. **Lamellar bone (Mature bone, Secondary bone)**
   - is the bone of adults in which the tissue is well organized and regular.

- The lacunae (of osteocytes) are regularly arranged as are the collagen fibers of the matrix. The term lamella ("leaf") refers to the layer of matrix between two rows of lacunae.
- The lamellar arrangements are best illustrated in the cortical (compact) bone of the diaphysis of long bones.
- Mature compact bone is composed of three lamellar arrangements :
  - **Osteons (Haversian Systems)**
  - **Circumferential Systems**
  - **Interstitial Systems**

OSTEONS (Haversian Systems)

- is the **basic unit** of structure in an adult compact bone.
- are long cylindrical structures, that run parallel to the long axis of the diaphysis.



Schematic drawing of the wall of a long-bone diaphysis showing three types of lamellar bone: haversian system and outer and inner circumferential lamellae

- in transverse section are seen to be formed of **4-20 regular** CONCENTRIC LAMELLA surrounding a central vascular channel (**Haversian canal**). The collagen fibers in each lamella are regularly arranged. The direction of the collagen fibers alternates from lamella to lamella.
- Each Haversian canal contains nerves, **lymphatic** and **blood vessels** involved in the common nutrition of the osteon *(see fig. 53, plate I)*.
- The blood vessels of the Haversian canals are supplied with blood out of vessels from the periosteum. These blood vessels penetrate the osteons in a transverse direction and are known as **Volkmann's canals**.

CIRCUMFERENTIAL SYSTEMS

- Immediately below the periosteum, at the periphery of compact bone of the diaphysis, the lamellae surround the bone in a continuous manner. These are known as the **outer circumferential lamellae**.
- A similar system of continuous lamellae adjacent to the endosteum is also found and is known as the **inner circumferential lamellae**.
- Bundles of collagen fibers, known as Sharpey's fibers or perforating fibers, anchor the periosteum to the outer circumferential lamellae, especially in sites of tendon insertions.

INTERSTITIAL SYSTEMS

- Remodeling of bone is a continuous process involving resorption of osteons and the rebuilding of new osteons. Interstitial systems of compact bone represent fragments of older compact bone (remnants of osteons) found between newer osteons, after remodeling.
- They are present between regular osteons and can be identified as irregular lamellar structures that lack a central Haversian canal.

Remodeling

Bone quality is maintained and renewed because osteoclasts are constantly tearing it down and osteoblasts are constantly building it up. Their action is in a dynamic balance so that the net amount of bone remains constant. This process is termed remodeling.

Remodeling is accomplished by osteoblasts and osteoclasts in both a random process that renews the surface of bone, and a well organized one that creates new haversian systems inside compact bone:

- The **resorption of osteons involves osteoclasts** from the Haversian canals eroding parts of lamella leading to the formation of resorption cavities.
- These may connect with resorption cavities from adjacent osteons.

- When sufficient resorption has occurred, **osteoblasts appear in the resorption cavity and start building a new generation of osteons.**
- When the new osteon is completed, the remnants of the previous osteon result in an interstitial system. This process of remodeling continues throughout life.

PERIOSTEUM

- is the double-layered connective tissue surrounding the bone except where the articular cartilage is present.
- It is divided into an outer fibrous layer and an inner osteogenic layer.)
  - **outer fibrous** layer is composed of dense irregular CT containing blood vessels, lymphatics, and nerves that pass into the bone.
  - **inner osteogenic** layer contains elastic fibers and various bone cell types, particularly osteoprogenitor cells, that give rise to new osteoblasts when stimulated.

The periosteum is anchored to bone by fine Sharpey's fibers which run perpendicular to the surface and are embedded in bone. Sharpey's fibers are also found in tendon insertions in bone.

- Functions of periosteum:
  - **nutritive** – contains blood vessels and nerves
  - **growth** of the bone in thickness and regeneration
  - **supporting and integrity** – provides the link between the bone and tendons, ligaments and muscles.

ENDOSTEUM is a single layer of cells which adhere to all inner surfaces of bone.

The endosteum does not contain a fibrous component, but the cells are almost always in direct contact with each other. Endosteum is found on bony trabeculae, in haversian and Volkmann's canals, and on surfaces lining the medullary cavity. Endosteum cell types are osteoprogenitor cells, osteoblasts, and osteoclasts, and the endosteum is therefore the equivalent of the cellular periosteum, but without its overlying fibrous periosteum and it has a greater abundance of osteoblasts and osteoclasts.

HISTOGENESIS OF BONE

Occurs in 2 processes:

- **INTRAMEMBRANOUS BONE FORMATION (development direct from mesenchyme)** – is the process by which most of the flat bones are formed.

- **ENDOCHONDRAL BONE FORMATION (through the hyaline cartilage model)** – is the process by which long bones are formed.

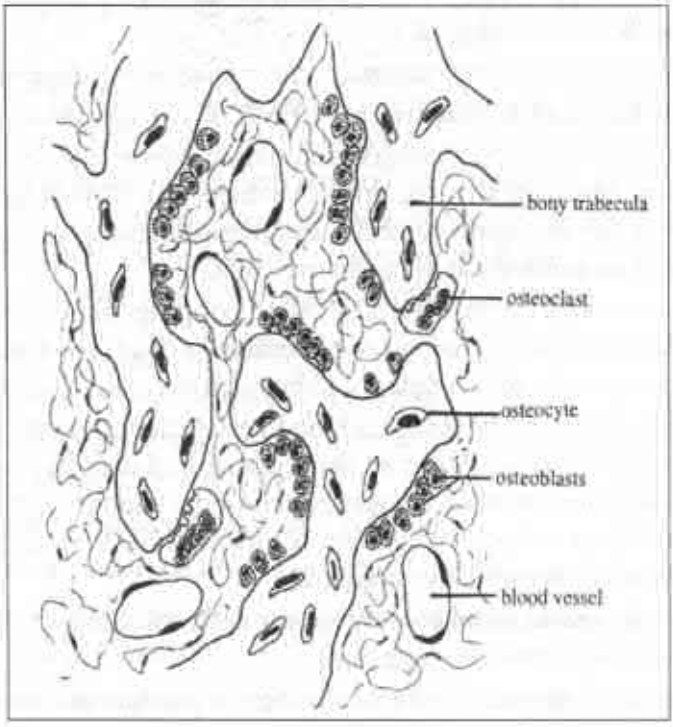
In both cases the first bone tissue to be formed is primary (woven or immature) bone, which is temporary only, prior to its replacement by secondary (lamellar or mature) bone.

INTRAMEMBRANOUS OSSIFICATION

- This mode of ossification **is typical of flat bones**, such as some of those in the skull (e.g., the frontal, parietal, occipital, and temporal) and a few others *(see fig. 55, plate I)*.

Steps:

1. Mesenchymal cells differentiate into **OSTEOBLASTS** which begin to secrete bone matrix **osteoid** (prebone). Aggregations of osteoblasts form **BONE ISLET (primary ossification center).**
2. Many ossification centers develop and eventually fuse, forming a network of anastomosing **TRABECULAE,** the called spongy bone or primary bone. Osteoblasts are present on the surface of the developing trabeculae. Some



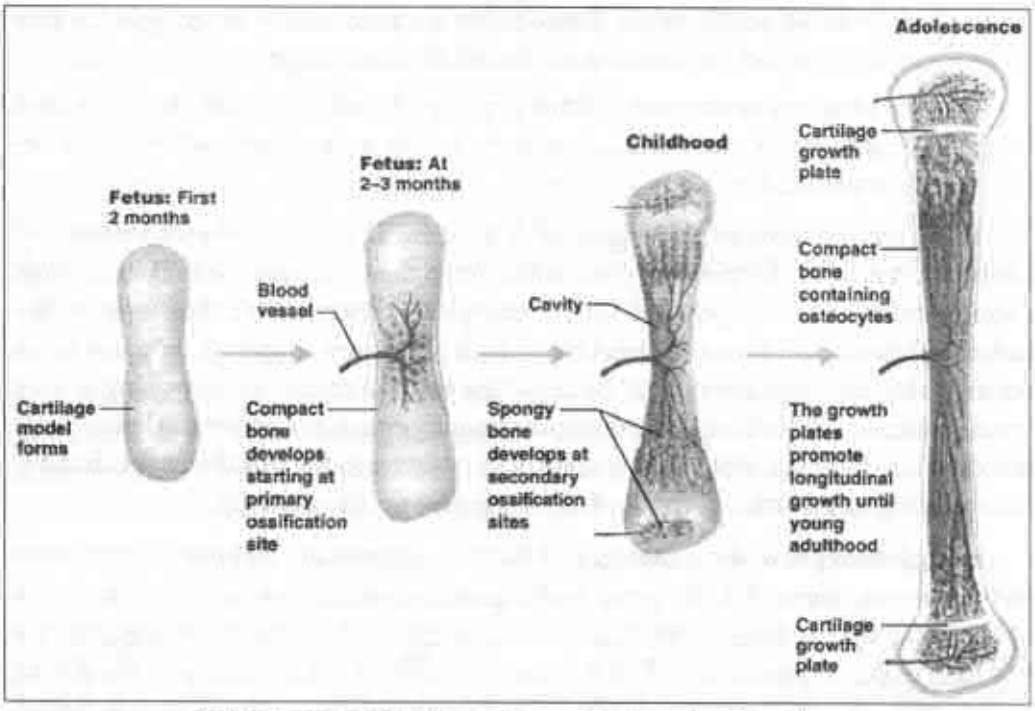
Intramembranous ossification

osteoblasts differentiate into **OSTEOCYTES**. Osteocytes become trapped within the calcified **osteoid**. Osteocytes remain connected to each other by cytoplasmic processes. The hollow space where osteocytes are placed is called **LACUNAE**. At this early stage **OSTEOCLASTS** are present on the surface of the trabeculae and are active in bone resorption. Primitive blood vessels are seen in the connective tissue located between the trabeculae.

3. **MINERALIZATION** – in this process is used Ca which is transported by blood vessels.
4. Transformation of primary bone into lamellar bone. At a later stage the connective tissue surrounding the developing flat bone forms the **periosteum**.

ENDOCHONDRAL OSSIFICATION – doesn't end at birth.

- The first stages involve the development of a hyaline cartilage model with surrounding perichondrium.
- A layer of woven bone (the **PERIOSTEAL COLLAR**) develops around the central shaft of the cartilage as a result of intramembranous ossification.
- The chondrocytes in the developing central shaft (primary center of ossification) **hypertrophy** (enlarge with swollen cytoplasm) and their lacunae also become enlarged.
- There is no diffusion via matrix and the chondrocytes **degenerate** and **die**.
- Appear **PRIMARY (diaphyseal) CENTER** of ossification.
- At the same time, blood vessels and mesenchyme-like cells from the periosteum penetrate this region of the diaphysis. **Osteoblasts differentiate from the mesenchyme cells and begin forming primary bone tissue on the calcified cartilage framework.**
- A bone marrow cavity forms in the developing diaphysis as a result of osteoclastic activity eroding the primary spongy bone trabeculae. The bone cavity enlarges accompanied by further vascularization.
- At a later stage of development blood vessels penetrate the epiphyses accompanied by hypertrophy of the more central cartilage cells and calcification of the matrix and degeneration of the chondrocytes. Osteoblasts start building trabecular bone on the skeleton of the calcified cartilage. The trabecula are radially arranged.
- **Secondary ossification centers** appear at the swellings in the extremities of the cartilage model (epiphyses).
- During their expansion and remodeling, the primary and secondary ossification centers produce cavities that are gradually filled with bone marrow.



Formation of a long bone on a model made of cartilage

In the secondary ossification centers, cartilage remains in two regions: the **articular cartilage,** which persists throughout adult life and does not contribute to bone growth in length, and the **epiphyseal cartilage,** also called the **epiphyseal plate,** which connects the two epiphyses to the diaphysis. The epiphyseal cartilage is responsible for the growth in length of the bone, and it disappears in adults, which is why bone growth ceases in adulthood.

CARTILAGE GROWTH PLATE (epiphyseal plate)

- Located between diaphysis and epiphysis
- Several zones can be identified according to the arrangement and appearance of the chondrocytes:
  - **resting zone** (small flattened lacunae)
  - **zone of proliferation** (site of mitoses, and larger elliptical lacunae)
  - **zone of hypertrophy** (greatly enlarged and rounded chrondrocytes in enlarged lacunae)
  - **zone of calcification** of the matrix and degeneration of the chondrocytes

- **zone of ossification**. Osteoblasts are involved in forming bone trabeculae on the remains of the calcified cartilage.
- **primary spongiosa** (primary spongy bone) where the newly-formed trabeculae are continuously eroded by osteoclastic activity and remodelled.

In summary, **growth in length of a long bone** occurs by proliferation of chondrocytes in the epiphyseal plate adjacent to the epiphysis. At the same time, chondrocytes of the diaphyseal side of the plate hypertrophy; their matrix becomes calcified, and the cells die. Osteoblasts lay down a layer of primary bone on the calcified cartilage matrix. Because the rates of these two opposing events (proliferation and destruction) are approximately equal, the epiphyseal plate does not change thickness. Instead, it is displaced away from the middle of the diaphysis, resulting in growth in length of the bone *(see fig. 56, plate II)*.

Long bones grow in diameter, and flat bones grow in thickness, by addition to the outer surfaces with the periosteal intramembranous method. On the inside of the bone, the medullary cavity also increases in size because the osteoclasts are winning over the osteoblasts. The balance is such that the thickness of the cortex increases a little as the bone diameter increases. As the cortex grows outward, capillaries that enter the bone from the periosteum are surrounded to form Volkmann's canals. As the cortex of all bones thickens it is converted from immature woven bone into compact bone by remodelling into haversian systems.

PHYSIOLOGY OF BONE

- Most of the calcium stored in the body is in bone tissue and can be released to the blood according to physiological demands or alternatively can be used to produce new bone.
- Calcium levels in the extracellular fluid of the body are very closely regulated. Three hormones, in particular, are involved in calcium homeostasis:
  - **Parathyroid hormone** – involved in increasing blood calcium levels by stimulating osteoclastic activity and bone resorption.
  - **Calcitonin** – involved in reducing blood calcium levels by stimulating osteoblasts.
  - **Vitamin D3**

Stages in the Healing of a Bone Fracture

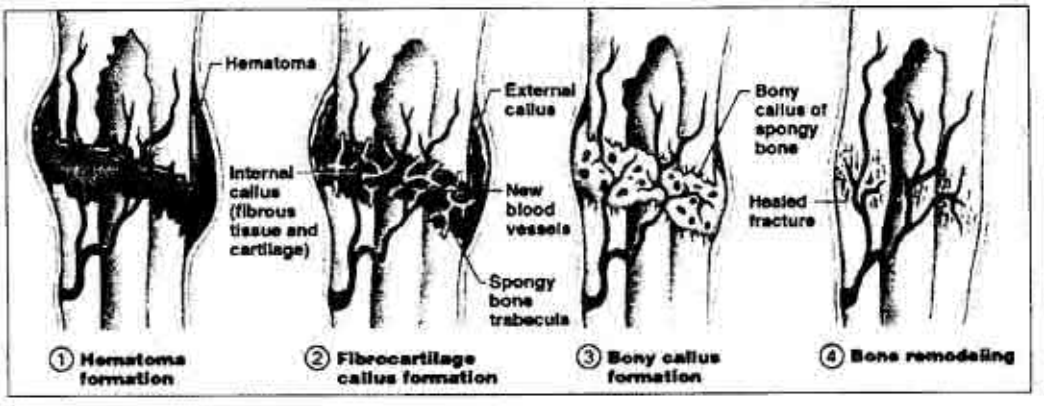
Fracture repair can be understood as an extension of the development and growth processes.When a bone is fractured, bone matrix is destroyed and bone

cells adjoining the fracture die. The damaged blood vessels produce a localized hemorrhage and form a blood clot. During repair, the blood clot, cells, and damaged bone matrix are removed by macrophages.

Cells of the periosteum and endosteum respond to the injury. There is a rapid proliferation of fibroblasts, which are involved in the formation of cartilage and fibrocartilage (fibrocartilaginous callus) that fills the injured gap. On the basis of the fibrocartilaginous callus, osteoclasts begin forming bone matrix, resulting in a bony callus of primary (woven, immature bone).

Stresses imposed on the bone during repair and during the patient's gradual return to activity serve to remodel the bone callus. If these stresses are identical to those that occurred during the growth of the bone and therefore influence its structure the primary bone tissue of the callus is gradually resorbed and replaced by secondary tissue, remodeling the bone and restoring its original structure.

Unlike other connective tissues, bone tissue heals without forming a scar.



Stages in the Healing of a Bone Fracture

CHAPTER V

MUSCULAR TISSUE

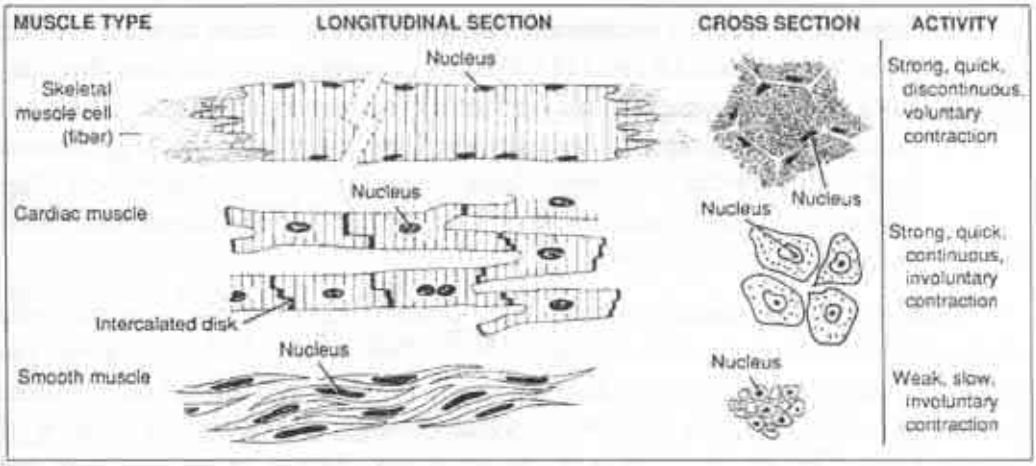
- Muscle (contractile) tissue is composed of muscle fibers or muscle cells.
  - That contains actin filaments and myosin filaments.
- Muscle tissue is characterized by its well-developed properties of contraction.
- Muscle is responsible for the movements of the body and the various parts of the body.
- Specific nomenclature associated with muscle commonly involves the prefix **sarco** – or **myo**.
  - **SARCOPLASM** – the cytoplasm of muscle fibers or cells.
  - **SARCOPLASMIC RETICULUM** – the endoplasmic reticulum of fibers or cells.
  - **SARCOLEMMA** – the Plasmalemma of fibers or cells and Basement membrane.
  - **MYOFIBRILS** – are long cylindrical bundles of **MYOFILAMENTS**; these are the contractile elements of each muscle fiber.

Functions:

- Skeletal muscles are responsible for the entire locomotion.
- Cardiac muscle is responsible for coursing the blood through the body.
- Smooth muscle helps maintain blood pressure, and squeezes or propels substances (i.e., food, feces) through organs.
- Muscles also maintain posture, stabilize joints, and generate heat.

The two major categories of muscle are **STRIATED** and **SMOOTH**.

STRIATED MUSCLE has a distinctive pattern of cross-banding (striations) which is visible microscopically. There are two types of striated muscle, **SKELETAL** and **CARDIAC**.



Structure of the three muscle types

STRIATED SKELETAL MUSCLE

The striated skeletal muscle develops from myotome (paraxial mesoderm). Embryonic muscle cells are myoblasts which have a single nucleus and no myofibrils. Before birth myoblasts repeatedly divide, and daughter cells fuse to form large multinucleated cells – **MYOTUBES** that produce myofilaments. Development tapers off through the first year of life.

The striated skeletal muscle is packed in skeletal muscles that attach to and cover the bony skeleton. It is striated because it displays cross striations (have visible banding). The skeletal muscle is a **voluntary** (subject to conscious control) muscle responsible for locomotion. However, it is also controlled involuntarily in reflex behaviors. Contraction in this type is faster than the other types.

The morphofunctional unit of the skeletal muscle is **MUSCLE FIBER (myofiber).**

Microscopic Structure of Myofiber

- A skeletal muscle fiber is a single long multinucleated cell, although an unusual one. These very large cell/fibers are up to 0.1 mm in diameter and several cm in length.
- The multiple nuclei are flattened and found around the periphery just under the cell membrane (sarcolemma). This characteristic nuclear location is helpful in distinguishing skeletal muscle from cardiac and smooth muscle, both of which have centrally located nuclei.
- Fibers are covered by the basement membrane.

- Between the plasma membrane and the basement membrane are located **Undifferentiated SATELLITE CELLS** (a stem cell population which allows more regeneration or a further increase in muscle mass). Their cytoplasm lacks myofibrils, but they have the potential to undergo mitosis and to differentiate into **myoblasts** following injury to the muscle. The fusion of myoblasts into myotubes occurs within the external lamina of the original damaged fiber.

With increase in exercise, satellite cells divide and one of the daughter cells from each division fuses with existing muscle fibers, causing a hypertrophic increase in muscle mass (exercise hypertrophy). Growth in diameter of a muscle is due to an increase in the number of myofibrils within the constant number of muscle fibers. Growth in length is the result of the addition of more sarcomeres in myofibrils within muscle fibers.

The remaining daughter cells become a **stem cell population** which allows more regeneration or a further increase in muscle mass.

The cytoplasm of a skeletal muscle fiber (sarcoplasm) contains a lot of organelles, such as:

- many elongated **mitochondria** – are found located between the myofibrils or in accumulations just under the sarcolemma. The numbers and activities of the mitochondria are greater in muscle fibers with high metabolic activity.
- **sarcoplasmic reticulum** (specialized smooth endoplasmic reticulum which stores calcium ions).
- **lysosomes**.
- small amounts of **rough endoplasmic reticulum** and **ribosomes** (low level of protein synthesis in this tissue).

Other Components of the Sarcoplasm:

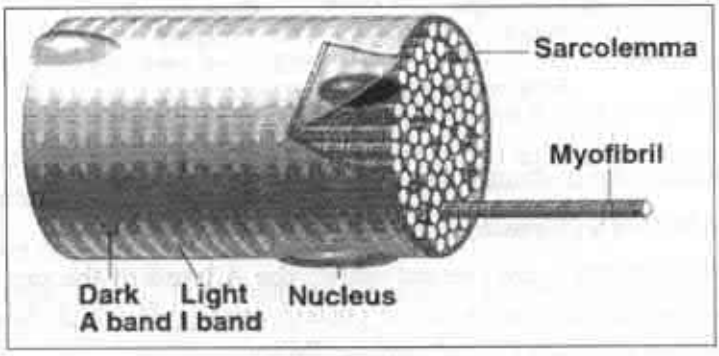
- **Glycogen** is found in abundance in the sarcoplasm in the form of coarse granules. It serves as a depot of energy that is mobilized during muscle contraction.
- **Myoglobin** – this oxygen-binding protein, which is similar to hemoglobin, is principally responsible for the dark red color of some muscles. Myoglobin acts as an oxygen-storing pigment, which is necessary for the high oxidative phosphorylation level in this type of fiber. Muscles that must maintain activity for prolonged periods usually are red and have high myoglobin content.

- In aged muscle fibers **lipofuscin** deposits (brown pigment) are common. These are now known to be large secondary lysosomes.
- The large part of the skeletal muscle fiber is filled with longitudinal parallel-arranged **MYOFIBRILS (special type of organelles).**

Microscopic Structure of Myofibril

Myofibril consists of two types of **protein filaments** called "thick filaments", and "thin filaments".

- **Bundles of myofilaments form:**
  - **I band** = light band – isotropic
  - **A band** = dark band – anisotropic.



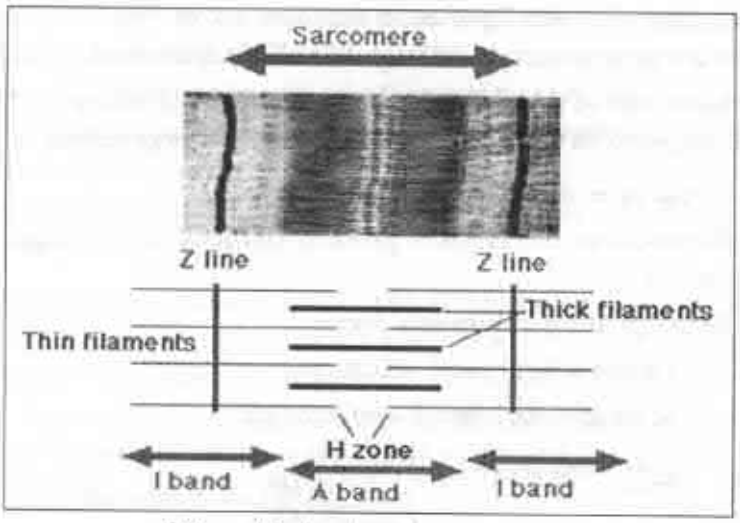
Schematic representation of a segment of a muscle fiber (cell)

Myofibrils are made up of a continuous chain of sarcomeres.

SARCOMERE – is the smallest structural and functional unit of myofibril, which extends from one Z line to the next Z line and which is composed of: **½ I band, A band and ½ I band.**

A less-stained region in the middle of the A-bands, is called the H-band (Hensen's band), which contains only myosin fibers.

The stained bands are called **A-bands**, and in between these are non-stained **I-bands**. If the same myofiber is examined by polarizing microscopy the A-bands are seen to be birefringent or anisotropic (bright against a dark background with crossed polars), whereas the I-bands are non-birefringent or isotropic. (The origin of the nomenclature comes from these polarizing properties: **A = Anisotropic, I = Isotropic**).

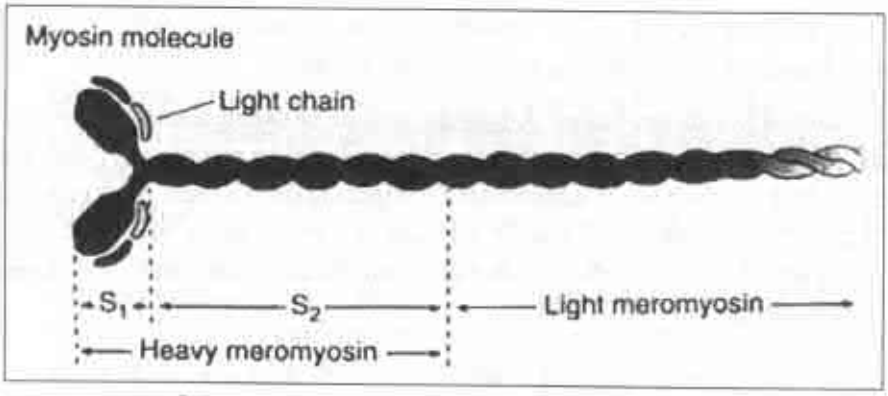


Schematic drawing of a sarcomere

Ultrastructure of Myofilaments:

THICK FILAMENTS (Myosin)

- Thick filaments – are present within the **A band** of the sarcomere; and are composed primarily of the protein **myosin II** which has important properties of elasticity and contractibility.
- Each myosin II molecule has a rod-like tail, hinge region and two globular heads:
  - Tails – two interwoven, heavy polypeptide chains.
  - Hinge region.
  - Heads – two smaller, light polypeptide chains called cross bridges.



Schematic representation of the thick filament

The individual myosin II molecules have heavy chains (each with a head and tail region) and light chains. There are two types of light chains; one of each is associated with the head region of each heavy chain. A thick filament consists of about 300 myosin molecules, with the heads oriented away from the center of the filament. The myosin heads have binding sites for **actin** and for **ATP;** they have ATPase activity and have a hinge region where they are connected to the tail that allows the head to move (bend).

In striated muscle, 6 thin filaments surround each thick filament in the region of the A band where thin and thick filaments overlap.

THIN FILAMENTS

- These are the only filaments present in the **I band** and are composed of the protein **ACTIN** and its associated regulatory proteins **TROPOMYOSIN** and **TROPONIN.**
- The main component of the thin filaments is a protein called actin.
- Actin molecules join together forming chains twisted into a helix configuration.

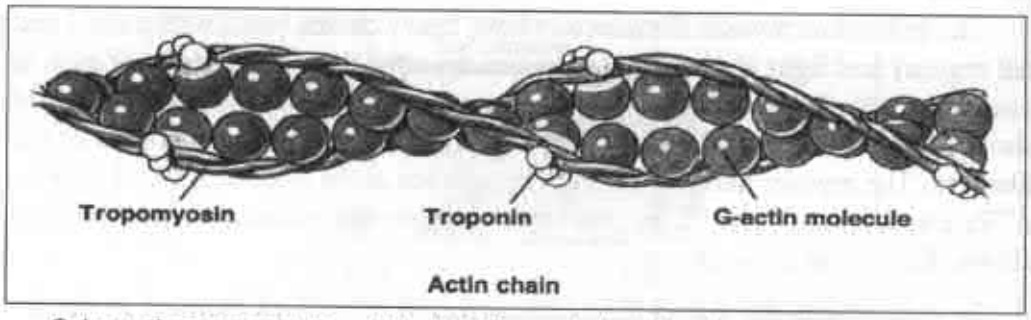
Two types of actin are found:

- **G-actin** (globular) consists of spherical monomers of about 5.6nm in diameter. The monomers are polarized, with one hemisphere having specific binding sites for myosin.
- **F-actin** (fibrous) consists of chains or strings of G-actin molecules.

Each actin molecule has a single "**myosin-binding site**" (myosin heads attach during contraction).

The other two protein molecules that form the **thin filaments** are called troponin and tropomyosin. The molecules of **tropomyosin** cover the **myosin-binding sites** on the **actin** molecules when the muscle fibres are relaxed. **Tropomyosin** is a long polypeptide molecule which lies in a spiral groove on the actin (like a string of pearls). **Troponin** is composed of 3 globular subunits that are bound at specific sites along the tropomyosin molecule:

1) **T subunit** (TnT) binds to the tropomyosin,
2) **C subunit** (TnC) has the binding site for calcium,
3) **I subunit** (TnI) that, when bound to actin, inhibits the actin-myosin binding site.



Schematic representation of the thin filament, showing the spatial configuration of three major protein components: actin, tropomyosin, and troponin

ACCESSORY PROTEINS – other proteins within the sarcomere act to maintain the relative position of the myofilaments and regulate the length of the polymerized filaments:

For **Thin filaments:** the **Z line** anchors the ends of thin filaments of the adjacent sarcomeres.

- **α-ACTININ** bundles the thin filaments together and anchors them to the Z lines.
- **NEBULIN** is a long, inextensible filament that is attached to the Z line and runs parallel to the thin filaments. It maintains the regular geometric arrangement of the thin filaments, attaches them to the Z line and regulates the number of G-actin monomers that polymerize to form thin filaments during development.
- **TROPOMODULIN** caps the free end of each thin filament (facing the M line) to regulate the length of the thin filaments within the sarcomere.

For **Thick filaments:** firmly attached to the M line; also attached to the Z line.

- **TITIN** is a very large fibrous protein that is an integral part of the thick filaments; it spans the distance from M line to Z line and maintains the central position of the A band by attaching the thick filaments to the Z line. The portion of the molecule within the I band is quite elastic (allows for stretching and contraction) and serves to keep the filaments in the appropriate orientation so that the sarcomere doesn't structurally deteriorate during stretching and contraction.
- **MYOMESIN and C PROTEIN** attach the thick filaments together at the M line and keeps them in order (in register).

Other accessory proteins that keep the arrangement of the myofibrils within each muscle fiber:

- **DESMIN** forms a lattice at the level of the Z line that **links adjacent myofibrils to each other.**
- **DYSTROPHIN** links laminin (present in the external lamina of each muscle fiber) to actin filaments. In ***Duchenne's muscular dystrophy***, dystrophin is absent and muscles become progressively weaker.

Excitation contraction coupling is the relationship between the depolarization of an action potential and the release of Ca^{++} onto myofilaments. Two microanatomical structures are involved in this process. The action potential is conducted into the interior of the muscle fiber by T tubules to where Ca^{++} is stored and released by the sarcoplasmic reticulum.

TRANSVERSE TUBULES (T tubules)

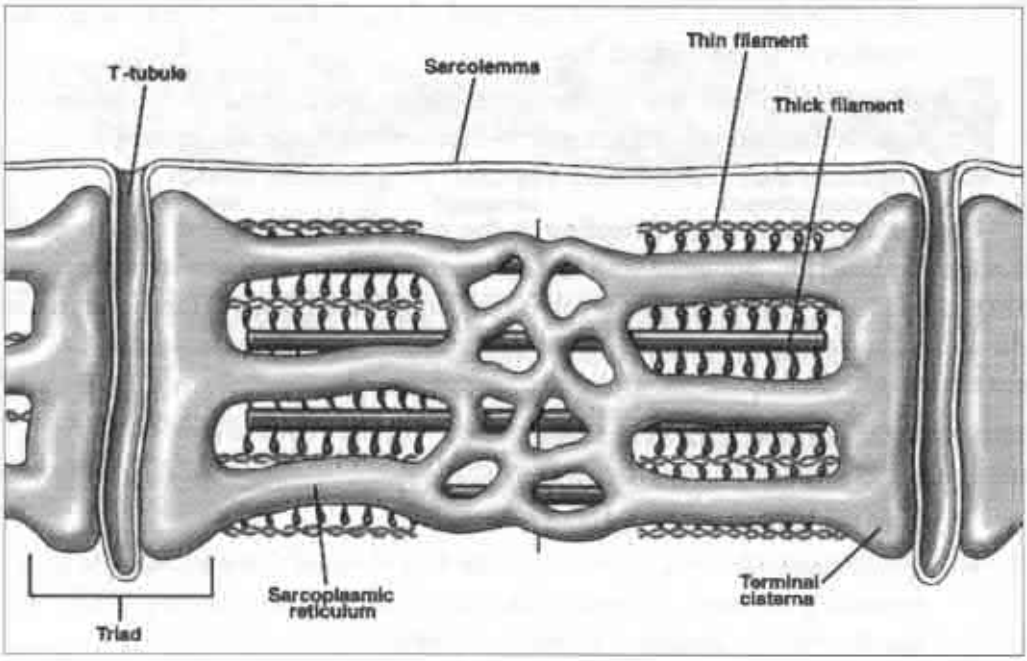
- Sarcolemma invaginate into the sarcoplasm to form a transverse distributed tubular system – T tubules.
- These fingerlike invaginations of the sarcolemma form a complex anastomosing network of tubules that encircles the boundaries of the A-I bands of each sarcomere in every myofibril
- Since the T tubule membrane is continuous with the sarcolemma, an action potential that spreads over the sarcolemma also spreads into the interior of a muscle fiber on T tubules; in this way they conduct impulses to the deepest regions of the muscle cytoplasm.
- These impulses signal for the release of Ca2+ from adjacent terminal cisternae.

SARCOPLASMIC RETICULUM (SR)

- is smooth endoplasmic reticulum that mostly runs longitudinally and surrounds each myofibril.
- Paired terminal cisternae form perpendicular cross channels.
- These terminal cisternae are sites of accumulation of calcium ions during muscle relaxation and play an important role in the contraction process.

TRIADS

- Two terminal cisternae are associated with one T-tubule to form structures (visible by transmission electron microscopy) known as **TRIADS**.



Excitation contraction coupling

THE SLIDING FILAMENT THEORY OF STRIATED MUSCLE CONTRACTION

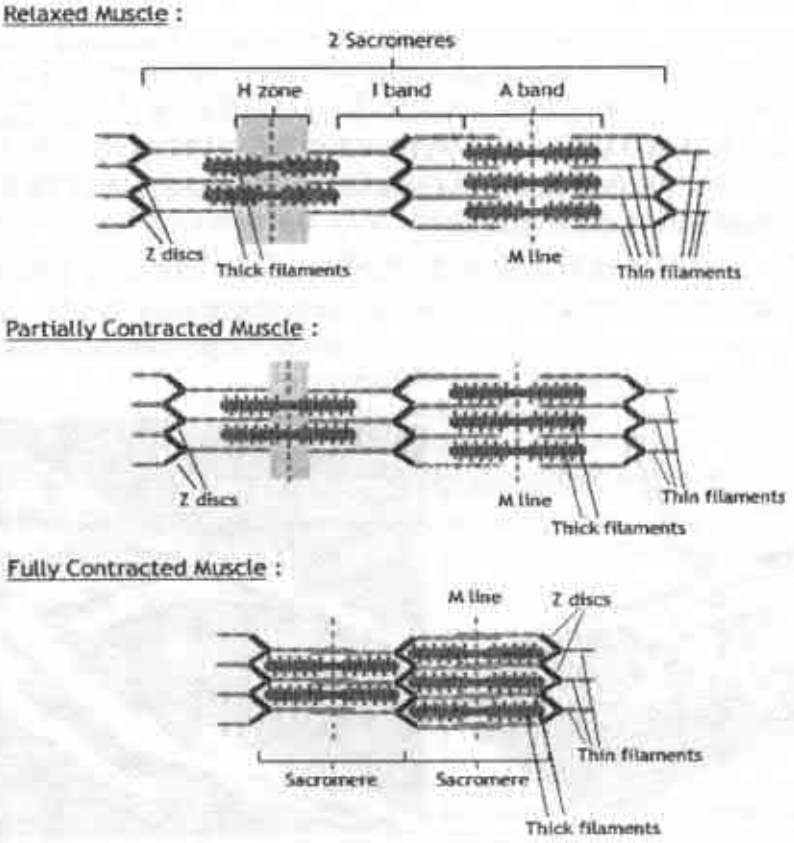
How does contraction take place? ATP and **calcium ions** (released from sarcoplasmic reticulum) are necessary for contraction to occur.

Myosin cannot interact with actin when a muscle is at rest because **tropomyosin blocks the binding site on actin**. When calcium is present, it binds to troponin and causes a **configurational change**, dislodging tropomyosin and exposing the actin-myosin binding site.

1. Myosin heads then bind tightly to the exposed actin binding site. This is the **RIGOR CONFIGURATION.**
2. The myosin head region also has a binding site for ATP. ATP binds and induces conformational changes that result in **detachment** of the myosin heads from the actin binding sites.
3. **ATPase enzyme** activity is resident within the myosin heads. ATP is hydrolyzed to ADP and P_i and the myosin head bends toward the Z line (~ 5 nm).
4. The myosin heads again bind to the thin filament. When P_i is released, the binding of actin to myosin becomes even tighter, resulting in **crossbridges**.

The myosin heads return to the original unbent position, causing them to swing and pull the thin filaments toward the center of the sarcomere (this is the **POWER STROKE**). The thin filaments are pulled past the stationary thick filaments, bringing the Z lines closer together, thus shortening the I band and consequently shortening the sarcomere. At this time, the ADP molecule drops off.

5. The myosin heads are again tightly bound to the actin binding sites. If calcium is still bound to troponin, the cycle will start over and contraction will continue; new crossbridges will form and the muscle will continue to shorten.
   - Note that **the myofilaments themselves do not shorten – the sarcomere does!**
   - When a muscle contracts, it is the result of **hundreds of cycles** of crossbridges formed and broken in a coordinated manner, causing the entire length of the muscle to shorten.



Scheme showing the contraction of a sarcomere

How Muscle Fibers Produce Movement?

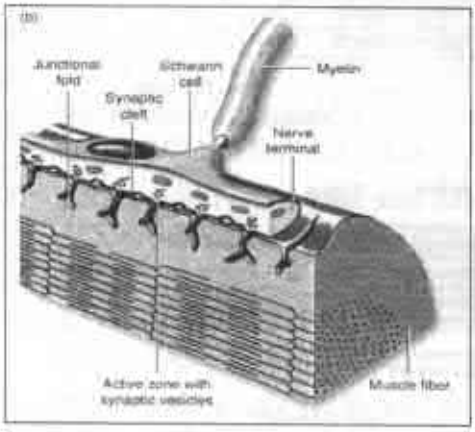
- Skeletal muscle is controlled by central nervous system (voluntary control).
- Skeletal muscle must have neural innervation to function.
- In order to produce movement, skeletal muscles must be stimulated by a motor neuron.
- **Motor unit** contains:
  - One neuron.
  - Muscle cells stimulated by that neuron.

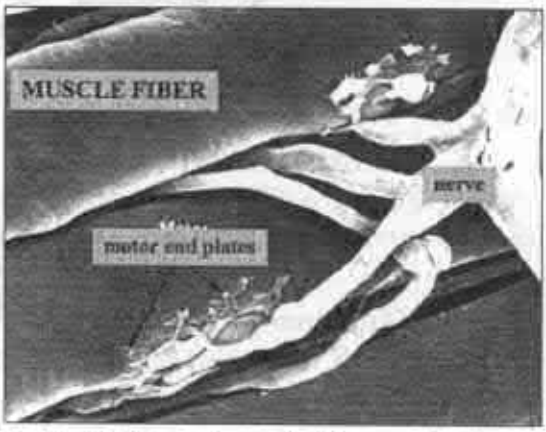
One axon can innervate 1 or more muscle fibers (a **motor unit**). The more motor units activated, the stronger the contraction.

Neuromuscular junctions – is a plaque-like synapse between the end of the axon and the plasma membrane of a muscle cell. As with any synapse, transmission of the signal is chemical.

MOTOR END PLATE

- Each motor endplate (myoneural junction; axon terminal) is located in a hollow in the sarcolemma called the **PRIMARY SYNAPTIC CLEFT**. Within this cleft, the sarcolemma is further folded into smaller ridges, the **SECONDARY SYNAPTIC CLEFTS (JUNCTIONAL FOLDS)**. Small vesicles containing the neurotransmitter **ACETYLCHOLINE** are present within the endplates.
- The neurotransmitter acetylcholine is released into the gap between the terminus of the neuron and the surface of the muscle; this brings about a depolarization of the plasma membrane of the myofiber, internal chemical changes in the cell, and contraction.





Drawing and scanning micrograph of a motor end plate

- **In humans**, each **skeletal muscle fiber — no matter how long it may be — has only one such neuromuscular junction.**

1. When an action potential travels down the nerve and depolarizes it, the vesicles fuse with the axon terminal membrane and are released into the synaptic cleft. The acetylcholine binds to specific receptors located on the sarcolemma, resulting in increased permeability of the sarcolemma to Na^+. Acetylcholinesterase, an enzyme located in the sarcolemma, breaks down acetlylcholine when the action potential is finished.

   - ***Myesthenia gravis*** is an autoimmune disease in which the acetylcholine receptors are destroyed, resulting in progressive muscle weakness.

2. The influx of Na^+ generates an action potential, causing a wave of depolarization to travel along the sarcolemma and into deep invaginations of the sarcolemma called **TRANSVERSE (T) TUBULES**, present at every **A – I junction.**

3. Depolarization is then conducted to a network of smooth endoplasmic reticulum cisternae.

4. The T tubules and terminal cisternae are separated by a 15 nm gap; they communicate via **JUNCTIONAL CHANNEL COMPLEXES** which sense the voltage change and stimulate the release of Ca^{++} from the terminal cisternae. The rest of the sarcoplasmic reticulum is distributed around the myofibril and acts as a calcium sink (via a Ca^{++} binding protein, **calsequestrin**) until it is depolarized and calcium is released, allowing contraction to occur.

5. Active transport mechanisms cause re-uptake of calcium into the sarcoplasmic reticulum when the muscle is at rest.

When a motor axon is activated, all the muscle fibers of its unit contract completely (or try to). When only a few motor units in a muscle are activated the whole muscle contracts, but with little strength. The strength of a muscle contraction is determined by how many of its motor units are activated. In muscles of the same size, one with more motor units can be contracted with smaller increments of force and, therefore, the more motor units within a muscle the more accurately it can be controlled. Back and leg muscles have fewer motor units than tongue or finger muscles, and oculomotor muscles have the most motor units. Tonus, or prolonged partial contraction, in skeletal muscle is the result of motor units taking turns.

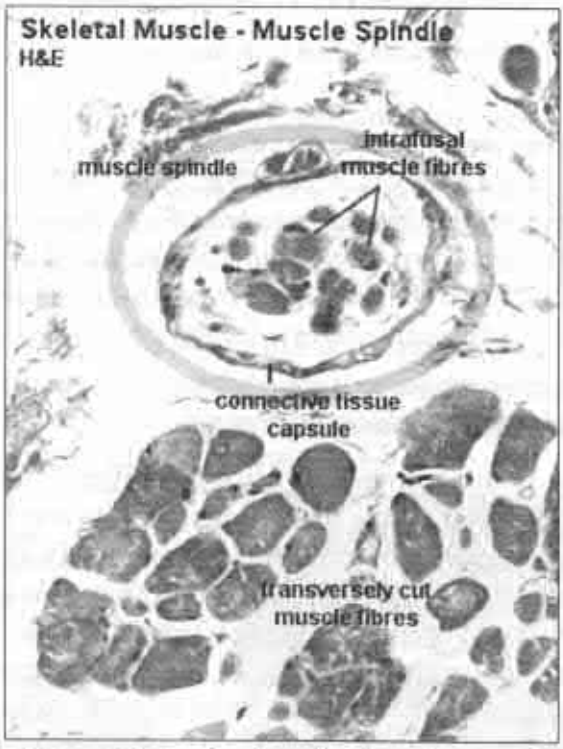
Muscle Spindles

All human striated muscles contain encapsulated proprioceptors known as **MUSCLE SPINDLES**. These structures consist of a connective tissue capsule

surrounding a fluid-filled space that contains a few long, thick muscle fibers and some short, thinner fibers (collectively called **intrafusal fibers**). Several sensory nerve fibers penetrate the muscle spindles, where they detect changes in the length (distention) of extrafusal muscle fibers and relay this information to the spinal cord. Here, reflexes of varying complexity are activated to maintain posture and to regulate the activity of opposing muscle groups involved in motor activities such as walking *(see fig. 60, plate II)*.

Golgi Tendon Organs

In tendons, near the insertion sites of muscle fibers, a connective tissue sheath encapsulates several large bundles of collagen fibers that are continuous with the collagen fibers that make up the myotendinous junction. Sensory nerves penetrate the connective tissue capsule. These structures, known as **GOLGI TENDON ORGANS**, contribute to proprioception by detecting tensional differences in tendons.



A light micrograph showing the structure of a muscle spindle

Classification of muscle fibers

Muscle fibers are classified into three main categories, based on their color and function:

1. **RED FIBERS** or slow-twitch high-oxidative fibers ("dark meat").
   - Have relatively small diameters, much myoglobin, many well-developed mitochondria, a rich blood supply and much ATP-ase.
   - Found in muscles with very high metabolic activity involved in slow sustained contractions (predominate in the limbs, back musculature). The energy source is from oxidative phosphorylation.
2. **WHITE FIBERS** or fast-twitch glycolytic-anaerobic fibers ("white meat")
   - Have larger diameters, less myoglobin and fewer mitochondria, relatively poorer blood supplies and less ATP-ase, rich in glycogen and glycolytic enzymes.
   - They are capable of rapid contraction that generates a large force, but they fatigue quickly. Their energy is derived mainly from anaerobic glycolysis.
   - They have more neuromuscular junctions than red fibers and are therefore capable of very precise movements.
   - Extraocular muscles and muscles of the digits (fingers) contain mostly white fibers.
3. **INTERMEDIATE FIBERS**
   - Have structural and functional properties intermediate between red and white fibers.

The classification of muscle fibers has clinical significance for the diagnosis of muscle diseases, or myopathies (*myo;* muscle + Gr. *pathos,* suffering). In humans, skeletal muscles are frequently composed of mixtures of these various types of fibers.

Repair and regeneration after injury

If muscles are used intensively, trained or exercised, they increase in mass as a result of increase in protein synthesis and sarcomere production. This results in hypertrophy of use ("Use it or lose it"). On the contrary, limb immobilization (e.g. in plaster casts, or as a result of inactivity due to hospitalization, or lack of gravity) causes loss of muscle mass (disuse myopathy or atrophy).

Myofibers are syncytial and post-mitotic, with very limited regenerative abilities after trauma. After trauma such as muscle crush, pathological changes occur in muscle and may lead to breakdown of myofibers and release of myoglobin, which can affect renal function and be life-threatening. In the limited repair processes, satellite cells are activated, divide and can form new myotubes and myo-

cytes. In some cases the satellite cells can fuse with existing fibers and contribute to the repair processes.

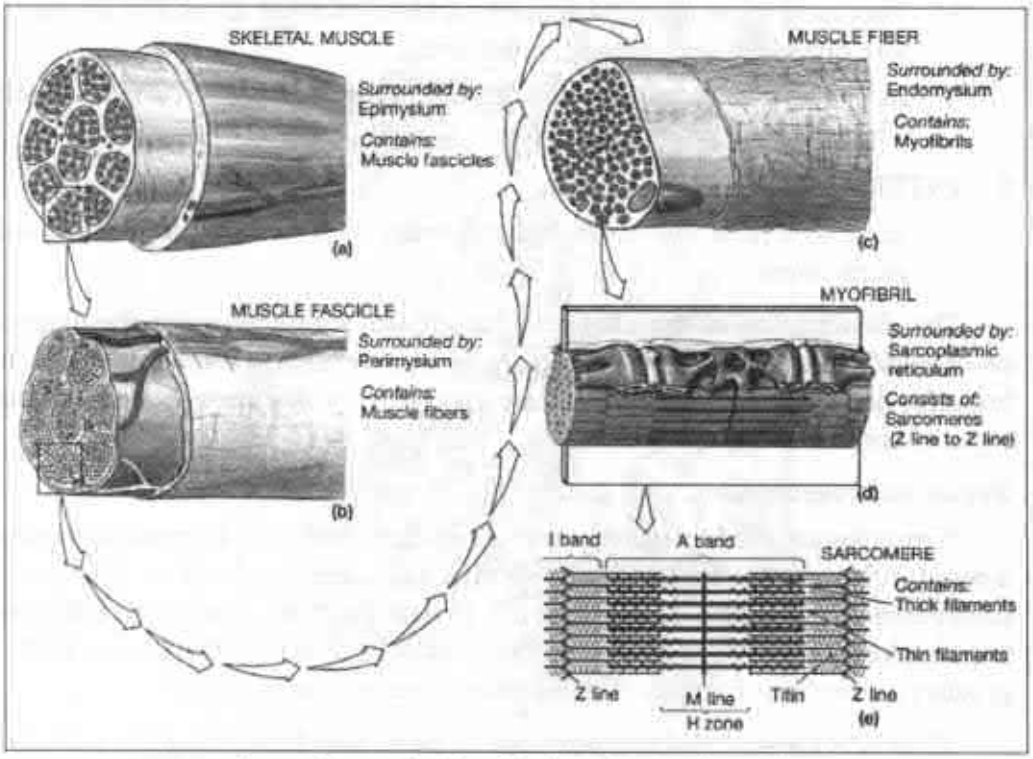
Organization of Skeletal Muscle

Each skeletal muscle has several **connective tissue sheaths** containing blood and lymphatic vessels and nerves; the sheaths also help to transmit the force of contraction between the fibers.

a) **Epimysium** – outermost sheath of dense connective tissue, continuous with tendon.

b) **Perimysium** – thin collagenous septa which extend inward from epimysium and divide muscle fibers into groups called **fascicles.**

c) **Endomysium** – is composed of loose CT; it is represented by a delicate network of collagen fibers and basal lamina which invests each muscle fiber within the fascicles.



General organization of striated skeletal muscle

ATYPICAL STRIATED MUSCLE

- Some striated muscles of the body with typical histological appearance of striated muscle are involuntary muscles. An example of such involuntary striated muscle is the cremaster muscle (near the spermatic cord).
- In some cases striated muscles are not really "skeletal" as they are not attached to the skeleton (e.g. esophageal striated muscle, external urethral sphincter, external anal sphincter).

CARDIAC MUSCLE TISSUE
(found only in the heart)

The cardiac muscle tissue **is an involuntary** muscle forms the bulk of the heart wall. The main function is to pump blood.

The cardiac muscle tissue develops from myoepicardial plate. During embryonic development, the splanchnic mesoderm cells of the primitive heart tube align into chainlike arrays. Rather than fusing into syncytial (Gr. *syn*, together, + *kytos*, cell) cells, as in the development of skeletal muscle, cardiac cells form complex junctions between their extended processes. Cells within a chain often bifurcate, or branch, and bind to cells in adjacent chains. Consequently, the heart consists of tightly knit bundles of cells, interwoven in a fashion that provides for a characteristic wave of contraction that leads to a wringing out of the heart ventricles.

The morphofunctional unit of the cardiac muscle tissue is **the cardiac muscle cell.**

Each cardiac muscle cell possesses one or two nuclei per cell centrally placed. Cardiac muscle cells contain numerous mitochondria, which occupy 40% or more of the cytoplasmic volume, reflecting the need for continuous aerobic metabolism in heart muscle. By comparison, only about 2% of skeletal muscle fiber is occupied by mitochondria. Fatty acids, transported to cardiac muscle cells by lipoproteins, are the major fuel of the heart. Fatty acids are stored as triglycerides in the numerous lipid droplets seen in cardiac muscle cells. A small amount of glycogen is present and can be broken down to glucose and used for energy production during periods of stress. Lipofuscin pigment granules (aging pigment), often seen in long-lived cells, are found near the nuclear poles of cardiac muscle cells.

The contractile filaments are not organized in fibrils, but are arranged in loose bundles and this gives cells the appearance of longitudinal as well as cross striations.

Heart muscle contracts spontaneously in a rhythmic manner and connections of the autonomic nervous system only modulate the repetition rate of con-

tractions; axon terminals do not make direct contact with muscle fibers or initiate action potentials in them.

Surrounding the muscle cells is a delicate sheath of endomysial connective tissue containing a rich capillary network.

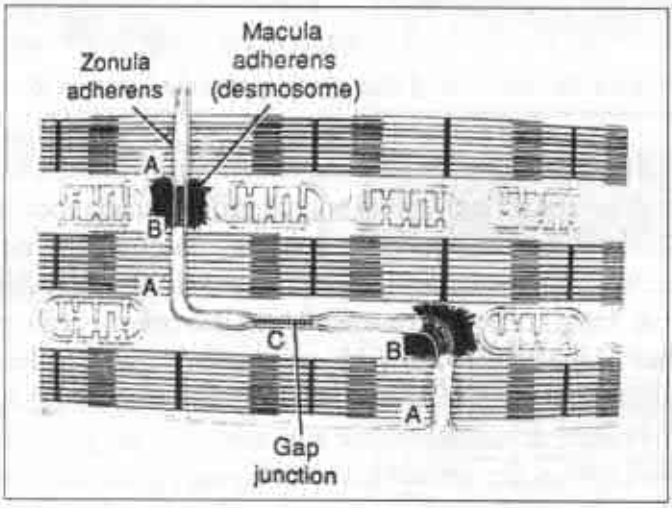
Cells attached to other cardiac muscle cells **at intercalated disks.**

INTERCALATED DISKS

These are step-like areas of interdigitation between adjacent cardiac muscle cells. At the ultrastructural level the intercalated disks are seen to have two main components:

- **transverse** regions, rich in desmosomes and tight junctions. These are important in providing good cell adhesion between adjacent myocytes.
- **longitudinal** regions, parallel to the direction of the myofilaments. These regions have many gap junctions, which are areas of low electrical resistance and permit the spread of excitation from myocyte to myocyte, so that muscle fiber contractions are synchronous.

Intercalated discs are junctional specializations that connect the cell end to end; facilitate the formation of a **FUNCTIONAL SYNCYTIUM.**



Junctional specializations making up the intercalated disk

Types of cardiac muscle cells:

- Cardiac **working** cells (contractile)
- Cardiac **conducting** cells (conductive)

- Cardiac **secretory** cells – are located in the atria. They produce 2 hormones: atrial natriuretic factor (ANF) and brain natriuretic factor (BNF) – they are diuretics and inhibit renin secretion in the kidney and aldosterone secretion in the adrenal gland, stimulate relaxation of vascular smooth muscle, in such way promote lowered blood pressure and decreased blood volume.

Contractile mechanisms.

The contractile mechanisms of cardiac muscle are slightly different than in skeletal muscle. The organization of actin and myosin, and the role for tropomyosin and troponin are very similar to skeletal muscle. However, T tubules enter the cell at the Z lines (instead of at the A-I border in skeletal muscle) so there are one half as many, and T tubules are larger than in skeletal muscle. The sarcoplasmic reticulum sarcoplasmic reticulum is not well-developed, form less terminal cisternae, so there are no triads, but exist **DIAD**: one T-tubule and one terminal cisternae.

Cardiac muscle does not regenerate at all. It has been thought that this is because there are no cardiac muscle stem cells that remain in the heart after it develops (there is some intriguing new evidence that they are there, and it might be possible to activate them). When regions of the heart are damaged, areas are filled with scar tissue. Existing fibers can hypertrophy with increased demands and can partially compensate for loss of muscle tissue.

SMOOTH MUSCLE TISSUE

Smooth muscle does not have striations or sarcomeres, and the contractile mechanism is different. Contraction is slow and can be sustained in individual fibers for long periods (tonus) with little expended energy. One of its most important functions is to line and regulate the lumen of hollow organs. In blood vessels, smooth muscle maintains vascular tone and is thereby one of the factors in regulating blood pressure and flow. In the intestines, smooth muscle is usually arranged such that both autonomic nervous control, and spontaneous rhythmic contractions, gives rise to appropriate motions such as peristaltic waves.

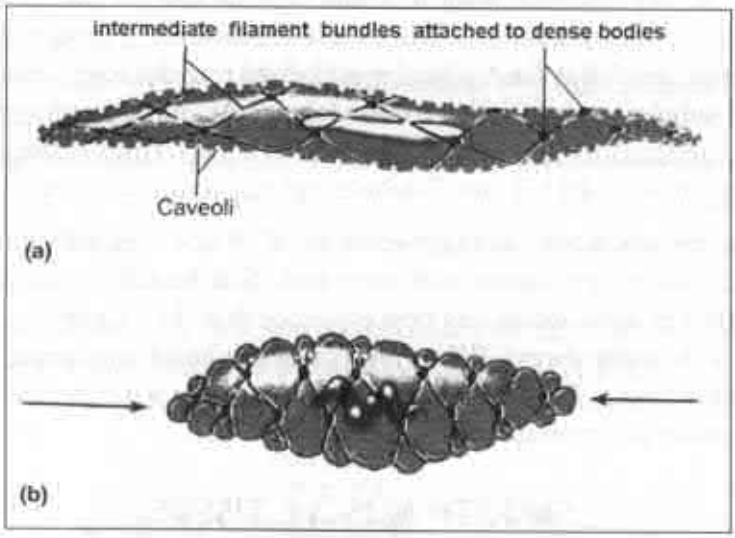
- The smooth muscle is involuntary muscle.
- It is capable for regeneration.
- Morpho-functional unit is the **SMOOTH MUSCLE CELL.**

Cells are spindle-shaped (**fusiform**); they are largest at their midpoints and taper toward their ends. One nucleus per cell centrally placed. The organelles are located close to the nucleus in two distinct poles (are mitochondria, polyribo-

somes, cisternae of rough endoplasmic reticulum, and the Golgi complex). Pinocytotic vesicles are frequent near the cell surface. The sarcoplasmic reticulum is poorly developed and consists of narrow tubules with no terminal cisternae.

The rest of the sarcoplasm is filled with myofilaments, though these are not arranged in ordered sarcomeres as in striated muscle, but generally lie in the long axis of the cell.

Each smooth muscle cell is enclosed by a basal lamina and a network of reticular fibers.



Smooth muscle cells relaxed (a) and contracted (b)

Three types of myofilaments may be seen:

- thin myofilaments (5-7nm thick), which are the most common type
- thick myofilaments (about 16nm thick), which are less common
- intermediate filaments (about 10nm thick). These may be grouped as "dense bodies" and are also found in contact with the sarcolemma (attachment plaques). It is thought that these intermediate filaments provide some sort of structural support for the cells.

The contraction mechanism of smooth muscle cells is still not very clear. **Contraction** may be initiated by autonomic nerve impulses (blood vessels), hormonal stimulation (uterus) or stretch (G.I. tract).

The actin and myosin do not appear to be regularly arranged. Myosin is present in relatively low amounts. A calcium ion target protein, calmodulin, is present. The myocytes lack a T-system, though the sarcolemma has numerous small

fixed saccules, known as caveolae. These caveolae may possibly have a role analogous to that of the T-system of striated muscle.

1. **Neuromuscular junctions** with smooth muscle are not as elaborate as in skeletal muscle. In some instances, the axon terminal does not even contact muscle fibers, but lies in the intercellular CT, and contraction is affected by diffusion of neurotransmitter. This results in a wider dispersion of the neurotransmitter to more cells, resulting in slower and less precise control.
2. Smooth muscle often occurs in sheets, as in the walls of hollow viscera **(visceral smooth muscle)** or bundles **(uterus)**; usually, smooth muscle fibers have a relatively poor nerve supply, but function as a syncytium due to the numerous gap junctions between the fibers.

Unitary smooth muscle is a style of innervation typified by the viscera. Only a few of the muscle fibers are supplied with neural control. The muscles regulate themselves by responding to environmental factors, hormones, and spread of contraction within the muscle so contraction is only modulated by neural control. The muscles respond to neural control in a unitary manner like one big motor unit.

Multiunit smooth muscles are richly innervated (1 axon/smooth muscle fiber) and are capable of precise contractions, as in the iris and ciliary muscles of the eye. Axons pass near the smooth muscle cells (**en passant**) but do not have motor end plates as in skeletal muscle. Innervation is more precise because the muscle is organized into motor units.

- Thin filaments containing **F actin** and **tropomyosin** are present in the sarcoplasm, but they appear to be distributed in a net-like configuration with attachment points at the sarcolemma (via **α-actinin**) and possibly at **DENSE BODIES** within the sarcoplasm. The dense bodies are the functional equivalents of the Z lines of striated muscle.
- The type of **myosin II** that is present in smooth muscle is folded until the **regulatory myosin light chain** is phosphorylated. Once phosphorylation occurs, thick filaments are formed by myosin II molecules, which then bind with F actin of the thin filaments. Contraction occurs using the same sliding filament mechanism as in striated muscle. Although **troponin is lacking** in smooth muscle fibers, calcium availability is absolutely necessary for contraction to occur.
- Calcium ions bind to **calmodulin**; the calcium/calmodulin complex activates **myosin light chain kinase**, which then phosphorylates the regulatory myosin light chain. The myosin can then assemble into thick filaments

and subsequently interact with actin. The calcium may be stored in the vesicles (**caveolae**) lining the sarcolemma or extracellular Ca^{++} may simply diffuse into the fibers. **Hormones** activate ligand-gated Ca^{++} channels:

- **estrogens** and **oxytocin** cause myosin phosphorylation to produce contraction.
- **progesterone** decreases phophorylation and causes relaxation.

- Phosphorylation occurs slowly and ATP is hydrolyzed at only 10% of the rate of skeletal muscle, resulting in slow, sustained contractions that use relatively little energy. Maximal contraction may take up to a second to occur, and may take place throughout the entire fiber or may pass through it like a wave. Gap junctions between smooth muscle fibers in gut allow the spread of excitation throughout the muscle mass, resulting in **contraction bands**.

Location of smooth muscle

- Smooth muscle is found in the walls of the hollow internal organs (hollow viscera), where it plays a role in maintaining the patency of the lumen. Smooth muscle forms the contractile layers of the intestinal tract, where it is important in peristaltic contractions involved in the movement of food.
- Smooth muscle is found in the walls of the respiratory tracts.
- Smooth muscle is present in the walls of blood vessels (vascular smooth muscle, especially in arterial vessels).
- Smooth muscle is found in the dermis of the skin (arrector pili).
- Smooth muscle is found in the eye (iris diaphragm, controlling the amount of light reaching the retina).
- Smooth muscle is a major component in the wall of the uterus.

Smooth muscle is also found in many other sites in the body.

Origin of smooth muscle

Smooth muscle is derived from mesenchyme.

Some glands of ectodermal origin, such as salivary glands, sweat glands or mammary glands, possess smooth muscle cells surrounding their secretory units (myoepithelial cells). These myoepithelial cells are ectodermal in origin.

Some of smooth muscles derive from neural tube (dilator pupillae and sphincter pupillae muscles of the iris).

CHAPTER VI

THE NERVOUS TISSUE

Nerve tissue is distributed throughout the body as an integrated communications network.

Functions:

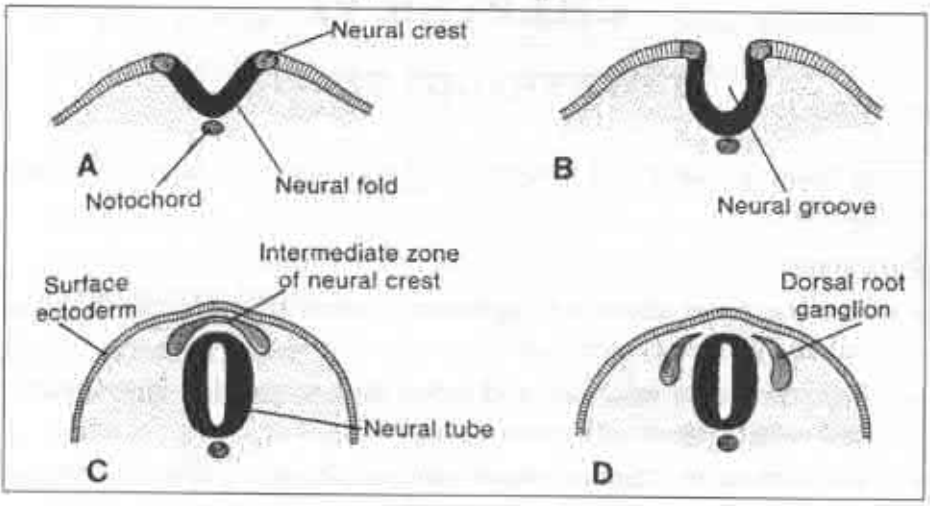
- Nervous tissue **allows an organism to sense stimuli in both the internal and external environment.**
- The stimuli are **analysed and integrated to provide appropriate, coordinated responses in various organs.**
- The afferent or sensory neurons **conduct nerve impulses from the sense organs and receptors to the central nervous system.**
- Internuncial or connector neurons **supply the connection** between the afferent and efferent neurons as well as different parts of the central nervous system.
- Efferent or somatic motor neurons **transmit the impulse from the central nervous system to a muscle (the effector organ) which then reacts to the initial stimulus.**
- Autonomic motor or efferent neurons **transmit impulses to the involuntary muscles and glands.**

Morphogenesis of the neural tissue includes:

- Proliferation;
- Determination & differentiation;
- Address migration of cells;
- Address growth of processes of neurons;
- Formation of intercellular junctions – synapses;
- Apoptosis.

DEVELOPMENT OF NERVE TISSUE

Nerve tissues develop from embryonic ectoderm that is induced to differentiate by the underlying notochord. First, a neural plate forms; then the edges of the plate thicken, forming the neural groove. The edges of the groove grow toward each other and ultimately fuse, forming the neural tube.



Neurulation (formation of the neural tube)

Neural tube gives rise to the entire central nervous system, including neurons, glial cells, ependymal cells, and the epithelial cells of the choroid plexus.

Cells lateral to the neural groove form the **neural crest.** These cells undergo extensive migrations and contribute to the formation of the peripheral nervous system, as well as a number of other structures.

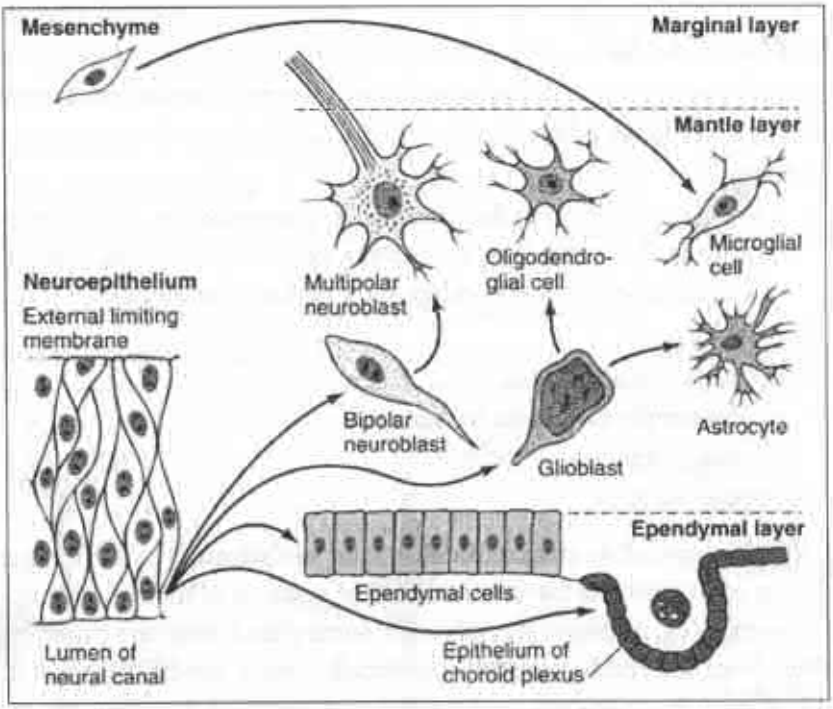
Development of Neuron

A. There is a fantastic variety in the neuronal family. Nevertheless, the differentiation of a motor neuron in the spinal cord will serve to illustrate the major principles of neuronal differentiation.

1. The motor neurons in the spinal cord develop from neuroblasts that have very few processes The neuroblasts, once formed, migrate away from the lumen of the spinal cord, and as they do so they begin to form a small number of processes.
2. Some of these processes develop into a dendrites, group of moderately long processes that receive inputs form other cells via synapses.

3. One axonal process becomes extremely elongated and may grow extensively in the marginal layer if it is destined to carry impulses parallel to the long axis of the spinal cord, or it may grow a process that begins to project through the marginal layer out of the central nervous system into the peripheral nervous system.
4. Motor neurons eventually will contract developing muscle fibers and form motor end plates with them.

B. Some neuroblasts form motor neurons, while others form small interneurons, or large pyramidal neurons of the cerebral cortex, or Purkinje cells of the cerebellum, or other types of neurons. These diverse cell types differ in the size and shape of their cell bodies, extent of dendritic arborization, and length of axons and in functional criteria as well.



Schematic drawing showing the derivates of the neuroepithelium of the neural tube

In summary:

Neural tube contributes to development of:

- CNS (brain &spinal cord)
- Retina of the eye
- Olfactory organ

Neural crest:

- Neural ganglia (spinal &cranial; autonomic)
- Neurolemmocytes
- Adrenal medulla
- Diffuse endocrine cells
- Pigmental cells
- Cells of arachnoid & pia mater

Placodes:

- Sensoepithelial cells of organ of Corti & equilibrium
- Receptor cells of taste organ
- Epithelium of lens

NEURAL TISSUE CELLS

Structurally, nerve tissue consists of two cell types: **nerve cells,** or **neurons** and several types of **glial cells.**

- **Neurons:**
  - Are responsible for the reception, transmission, and processing of stimuli; the triggering of certain cell activities; and the release of neurotransmitters and other informational molecules.
- **Neuroglia:**
  - Support neural tissue
  - Help supply nutrients to neurons.
  - Protect neurons.
  - Form barriers.

NEURONS respond to environmental changes (**stimuli**) by altering electrical potentials that exist between the inner and outer surfaces of their membranes. Cells with this property (eg, neurons, muscle cells, some gland cells) are called **excitable,** or **irritable.** Neurons react promptly to stimuli with a modification of electrical potential that may be restricted to the place that received the stimulus or may be spread (propagated) throughout the neuron by the plasma membrane. This propagation, called the **action potential,** or **nerve impulse,** is capable of traveling long distances; it transmits information to other neurons, muscles, and glands.

MORPHOLOGICAL classification of neurons (based on a number of processes found in the cell body)

- **Unipolar**
- **Pseudounipolar**

- **Bipolar**
- **Multipolar**

UNIPOLAR NEURONS

- Have only one process (axon).
- True unipolar is very rare in adult.

PSEUDOUNIPOLAR NEURONS

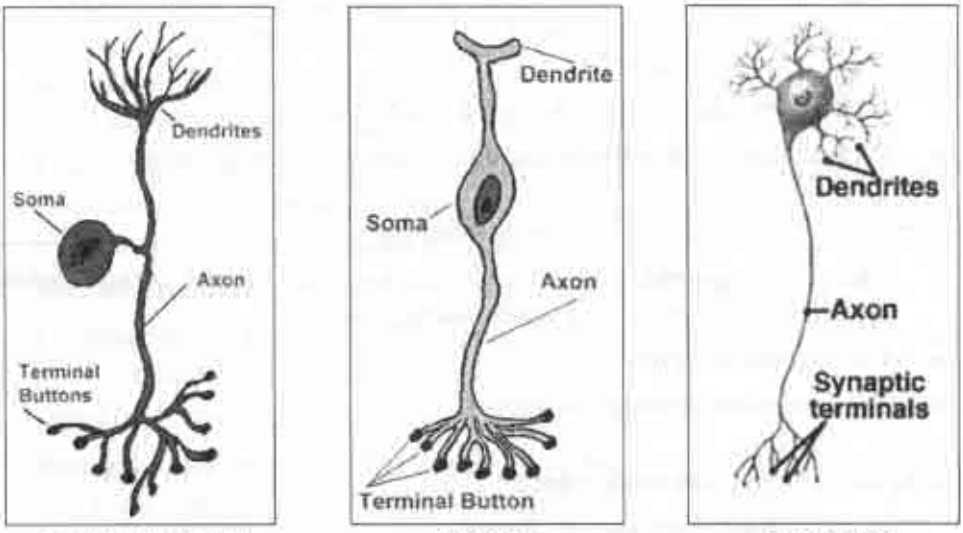
- Have a single process that extends from the cell body and subsequently branches into an axon and dendrite.
- Found in sensory ganglia of dorsal roots of spinal nerves & cranial ganglia.

BIPOLAR NEURONS

- **neurons** are spindle-shaped, with a **dendrite at one end and an axon at the other.**
- Are present in some sense organs: retina, spiral ganglion.

MULTIPOLAR NEURONS

- Have more than 2 processes. Have two or **more dendrites** and only **one axon.**
- Most common type of neuron > 99% of neurons.
- **Location**: gray matter of brain and spinal cord, peripheral ganglia.



Simplified view of the three main types of neurons, according to their morphological characteristics

FUNCTIONAL classification of neurons

- ***MOTOR* neurons** – efferent (conduct impulses from CNS to other neurons, muscles or glands).
- ***SENSORY* neurons** – afferent (receive stimuli from the internal and external environment). Conduct nerve impulses to the CNS.
- ***INTERNEURONS*** act as connectors of neurons in chain. They most commonly connect sensory and motor neurons. During mammalian evolution a great increase in the number and complexity of interneurons has occurred.
- ***NEUROSECRETORY*** neurons synthesize and secrete hormones:
  - neurons of hypothalamus: supraoptic and paraventricular nuclei – neurons produce hormones: vasopressin and oxytocin).

NEURON

- Is the **morphofunctional unit** of the nervous system.

Special neuronal characteristics.

- **Convey APs (excitable)**
- **Longevity**
- **Do not divide**
- **High metabolic rate**

Neurons have 2 special properties:

- **Irritability** (the ability to respond to a stimulus)
- **Propagation of impulses** (the ability to conduct impulses).

Neurons have 3 physiological parts or segments:

- **Receptive segment** (dendrites and perikaryon). The perikaryon also has an additional trophic and synthesizing role.
- **Conductive segment** (axon).
- **Transmissive segment** (synapse).

Most Neurons have **three main parts:**

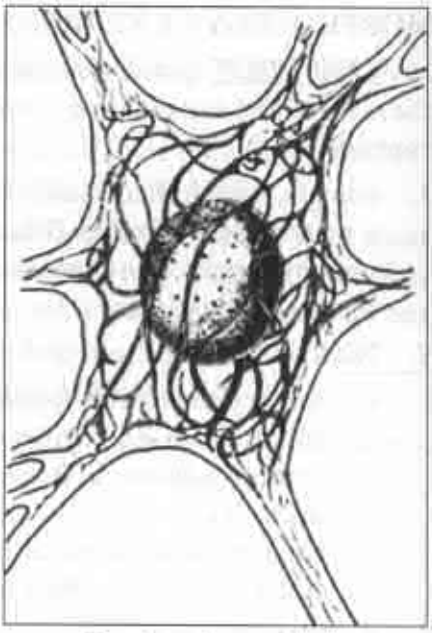
- **Cell body** (perikaryon, soma)
- One or more **dendrites**
- Only one **axon**

MORPHOLOGY OF NEURONS

CELL BODY (perikaryon or soma) – is the part of the neuron that contains the nucleus and surrounding cytoplasm, exclusive of the cell processes. Cell body contains:

1. a single, spherical, unusually large, euchromatic (pale-staining) **nucleus** with a prominent nucleolus. Binuclear nerve cells are seen in sympathetic and sensory ganglia. The chromatin is finely dispersed, reflecting the intense synthetic activity of these cells.
2. **Nissl bodies** (chromatophilic substance):
   - Are represented by highly developed rough endoplasmic reticulum organized into aggregates of parallel cisternae and numerous free ribosomes (polysomes) between the cisternae. When appropriate stains are used, rough endoplasmic reticulum and free ribosomes appear under the light microscope as basophilic granular areas. The number of Nissl bodies varies according to neuronal type and functional state.
   - *Location*: in the cytoplasm of perikarion and dendrites, absent in axon and axon's origin (**axon hillock** – ***clear conical area at the origin of axon from the soma***).
   - Nissl bodies react to injury by breaking up and diffusing throughout the cytoplasm called ***chromatolysis.***
3. **Golgi complex** – is located only in the cell body and consists of multiple parallel arrays of smooth cisternae arranged around the periphery of the nucleus
4. **Mitochondria** – are especially abundant in the axon terminals. They are scattered throughout the cytoplasm of the cell body.
5. **Lysosomes:** Primary lysosomes are common, located near a golgi apparatus, associated with hydrolysis of end products of cellular metabolism. Secondary lysosomes increase in number with age, some becomes lipofuscin granules.
6. **Microfilaments** (actin) associated with the cell membrane.
7. **Lipofuscin** is a yellow pigment clumps which represent a residue of undigested material by lysosomes (harmless aging).
8. **Lipid inclusions** commonly found in perikaryon.
9. **Iron granules** present in nerve cells of various regions like in globus pallidus, tend to increase in number with age.
10. Melanin is present in:
    - substantia nigra of midbrain,
    - locus ceruleus in 4th ventricle floor.

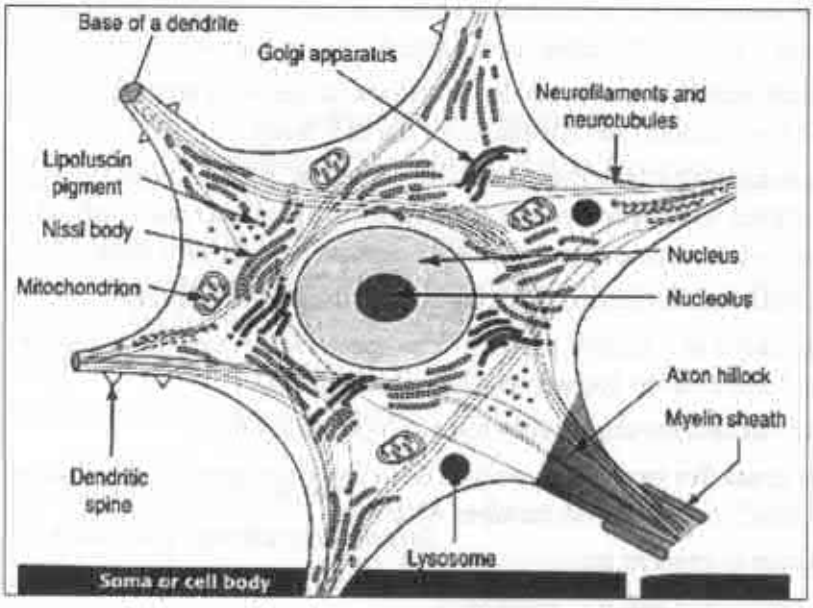
11. **Microtubules** (neurotubules) are identical to those found in many other cells; move material inside cell.
12. **Neurofilaments** (specific type of intermediate filaments) give the cell shape and support. Neurofilaments are abundant in perikaryons and cell processes.



Drawing of neurofibrils

Neurofilaments and neurotubules form **neurofibrils** – is artefact (are visible with the light microscope). Neurofibrils appear at time of slide preparing and can be distinguished inside of neurons.

Cell body is the trophic center of the neuron involved in protein synthesis (neurotransmitters & repair proteins). **Cell body** also has receptive capabilities. The perikaryon of most neurons receives a great number of nerve endings that convey excitatory or inhibitory stimuli generated in other nerve cells.



Scheme of the ultrastructure of a neuron's soma

DENDRITES

- Receptive to stimuli and bring impulses from the environment (sensory epithelial cell or other neurons) to the cell body.
- Are short, highly branched.
- Surfaces specialized for contact with other neurons (**dendrite spines** – mushroom-shaped structures) – increase the area useful for synapse formation. Dendritic spines participate in the plastic changes that underlie adaptation, learning, and memory. They are dynamic structures with a morphological plasticity based on the cytoskeletal protein actin, which is related to the development of the synapses and their functional adaptation in adults.
- Have **arborized terminals** – permit a neuron to receive stimuli at the same time from many other neurons.
- The cytoplasmic composition of the dendrite base, close to the neuron body, is similar to that of the perikaryon but is devoid of Golgi complexes.
- Also contains neurofibrils and Nissl bodies.

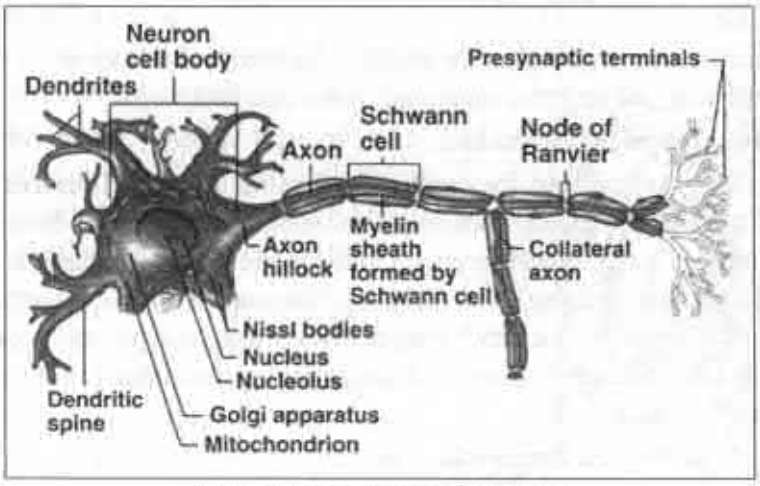
AXON

- Is a long process emerging from the cell body, which conducts impulses away from cell body
- Represents long, thin cylindrical process of cell.
- Cytoplasm (axoplasm) possesses mitochondria, many microtubules and neurofilaments, and some cisternae of smooth endoplasmic reticulum. The absence of polyribosomes and rough endoplasmic reticulum emphasizes the dependence of the axon on the perikaryon for its maintenance. If an axon is severed, its peripheral parts degenerate and die.
- May has **collaterals** (branching at right angles from the main trunk)
- The distal portion of the axon is usually branched – **the telodendron.**
- Each terminal branch of the telodendron has an enlarged ending – the **synaptic button** (contains vesicles filled with neurotransmitters)
- All axons originate from a short pyramid-shaped region, the **axon hillock that** usually arises from the perikaryon.

The plasma membrane of the axon is termed the **AXOLEMMA**, and the cytoplasm of the axon is termed the **AXOPLASM**.

The **diameter** of the axon is fairly **constant**.

The **length** of the axon is fairly **variable**, and some reach up to 100 cm



The general structure of a neuron

TRANSPORT

There are two types of neuronal transport:

- **Dendritic** – the movement of substances and organelles through the dendrites.
- **Axonal** – the movement of substances and organelles through the axon.

AXONAL Transport – there is a bidirectional transport of small and large molecules along the axon.

- **Anterograde transport** – carries material away from the soma to the terminal synapse.

Anterograde transport occurs at two distinct speeds.

- **slow axonal flow**
  - movement in one direction only – away from the cell body.
  - movement at 1-5 mm per day.
  - conveys components needed for growth and regeneration of the axon.
- **fast axonal flow**
  - transports in either direction.
  - at 100-500 mm per day.
  - moves organelles & materials along the surface of microtubules.
  - involves transport of enzymes needed for synthesis of neurotransmitters. within the terminal synapse.

- **Retrograde transport** – carries material toward the soma for reutilization, recycling, or degradation. This process is used to study the pathways of neurons; peroxidase or another marker is injected in regions with axon terminals, and its distribution is followed after a certain period of time.

MEMBRANE POTENTIALS

The nerve cells have molecules in their membranes that act as pumps and channels, transporting ions into and out of the cytoplasm. The axolemma or limiting membrane of the axon pumps Na^+ out of the axoplasm, maintaining a concentration of Na^+ that is only a tenth of that in the extracellular fluid. In contrast, the concentration of K^+ is maintained at a level many times greater than that prevailing in the extracellular environment. Therefore, there is a potential difference across the axolemma of –65 mV with the inside negative to the outside. This is the **resting membrane potential.** When a neuron is stimulated, ion channels open and there is a sudden influx of extracellular Na^+ (an ion whose concentration is much higher in the extracellular fluid than in the cytoplasm) that changes the resting potential from –65 mV to +30 mV. The cell interior becomes positive in relation to the extracellular environment, which determines the beginning of the **action potential** or **nerve impulse.** However, the +30 mV potential closes the sodium channels, and the axonal membrane again becomes impermeable to this ion. In axons, in a few milliseconds, the opening of potassium channels modifies this ionic situation. As a result of the elevated intracellular concentration of potassium, this ion leaves the axon by diffusion, and the membrane potential returns to –65 mV, ending the action potential. The duration of these events is very short (about 5 ms) and takes place in a very small membrane area. However, the action potential propagates along the membrane, that is, the electrical disturbance opens neighboring sodium channels and, in sequence, potassium channels. In this way the action potential propagates at high speed along the axon. When the action potential arrives at the nerve ending, it promotes discharge of stored neurotransmitter that stimulates or inhibits another neuron or a nonneural cell, such as a muscle or gland cell.

NEUROGLIA

(means nerve glue in Greek)

- Glial cells are specialized cells of the nervous system which have got two objectives:
  - glue neurons together.
  - and associated neurons.
- Make up 90% of the cells of CNS.
- Occupy about 50 % of the total volume of nervous tissue.

NEUROGLIA differ from **NEURONS**

- Neuroglia **have no action potentials** and cannot transmit nerve impulses
- Neuroglia **are able o divide** (and are the source of tumors of the nervous system)
- Neuroglia **do not form synapses**
- Neuroglia **form the myelin sheathes** of axons.

Neuroglia can divide into two main groups: macroglia and microglia *(see fig. 61, plate II)*.

1. **MACROGLIA** contain:
   - **Ependymal cells**
   - **Astrocytes**
   - **Oligodendrocytes**
2. **MICROGLIA** is represented by macrophages of the nervous tissue.

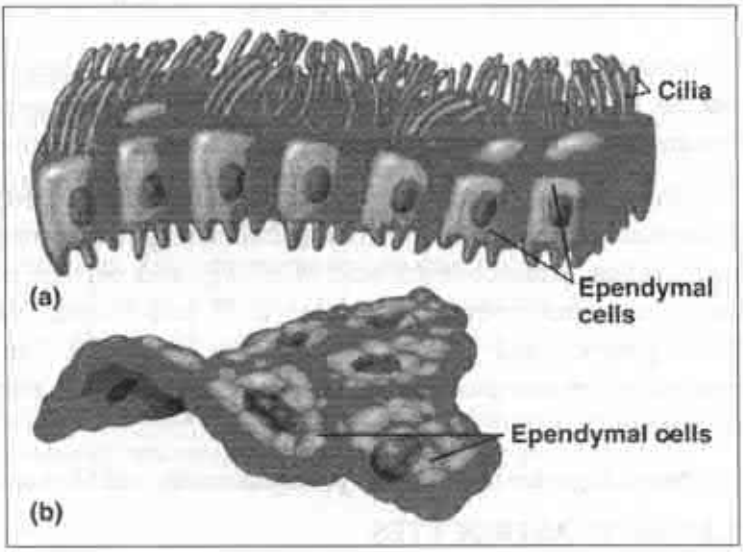
Functions of the neuroglia:

- **PHYSICAL SUPPORT**
  - Because of their number and their long processes, the astrocytes appear to be the most **important supporting elements in the CNS.**
- **REPAIR**
  - Glial cells, especially astrocytes, participate in the repair process following injury to the CNS. Repair consists of proliferation of glial cells that fill the detect left by the degeneration of neurons and their processes.
- **METABOLIC INSULATION**
  - Astrocytes may also participate in the formation of the blood-brain barrier. Oligodendroglial cells are frequently found adjacent to neurons, and there appears to be a metabolic dependency between them.

EPENDYMAL CELLS

- Line ventricles of the brain, central canal of the spinal cord, choroid plexus.
- These epithelium-like cells are cuboidal or columnar-shaped.
- Are ciliated in the embryo stage. During adult cells are with few cilia.
- Have numerous microvilli.
- Cytoplasm contains fibrils which may extend into the basal cytoplasmic processes.
- Desmosmes complex are present between cells.
- Secrete and move liquor.
- Form **blood-liquor barrier** which has next structural components:
  1. **Endothelium** of capillary
  2. **Basement membrane of endothelium**
  3. **Loose connective tissue**
  4. **Ependymal cells**
  5. **Basement membrane of ependymal cells**

Cells covering the white matter are more flattened and have fewer cilia than those covering grey matter.



Drawings of ependymal cells

TANYCYTES

Tanycytes are modified ependimal cells distinguished by their long, radially orientated and unbranching basal processes, which usually reach subependymal capillaries.

ASTROCYTES (astroglia)

- Are present only in the central nervous system (CNS).
- Are the largest of the neuroglia, possessing numerous long processes.
- Are star-shaped cells with multiple radiating processes.
- Processes end in expanded pedicles that attach to the wall of blood capillaries. These pedicles are called the **"vascular feet".**

Functions of astrocytes:

1. **Scavenge ion and debris (wastes) from neuron** metabolism and supply energy for metabolism.
2. **Provide structural support** for nervous tissue.
3. **Form a protective barrier** between pia mater and the nervous tissue of the brain and spinal cord.
4. **Eat parts of dead neurons.**
5. **Form scar tissue** after injury to the CNS.
6. Can influence neuronal survival and activity through their ability to regulate **the content of the extracellular environment,** absorb local excess of neurotransmitters, and release metabolic and neuroactive molecules.
7. Also play a role in regulating the numerous functions of the central nervous system. Astrocytes *in vitro* exhibit adrenergic receptors, amino acid receptors (eg, -aminobutyric acid [GABA]), and peptide receptors (including natriuretic peptide, angiotensin II, endothelins, vasoactive intestinal peptide, and thyrotropin-releasing hormone). The presence of these and other receptors on astrocytes enables them to respond to several stimuli.

There are **two categories** of astrocyte: **protoplasmatic** and **fibrous astrocytes.**

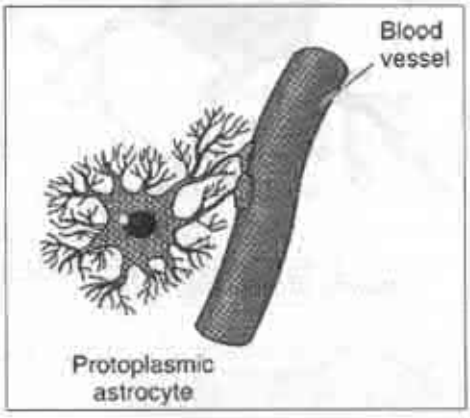
PROTOPLASMATIC ASTROCYTES

- Found in the gray matter of the brain and spinal cord.
- Have abundant granular cytoplasm.

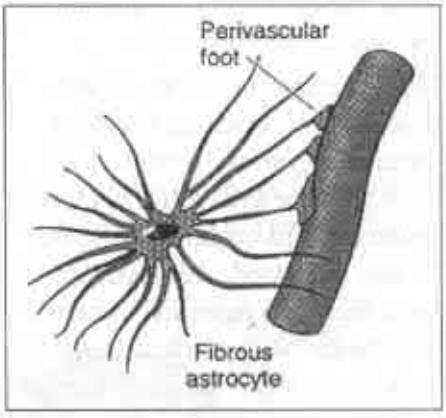
- Their processes have many branches, are shorter than those of fibrous astrocytes, and are relatively thick.

FIBROUS ASTROCYTES

- Found chiefly in the white matter.
- Have long, slender, smooth processes that branch infrequently.



Drawing of a protoplasmatic astrocyte



Drawing of a fibrous astrocyte

Astrocytes promote tight junctions to form blood-brain barrier:

1. **endothelium of the capillary** (between endothelial cells there are tight junctions).
2. **basement membrane** of endothelium.
3. **perivascular membrane**– is formed by foot processes of astrocytes.

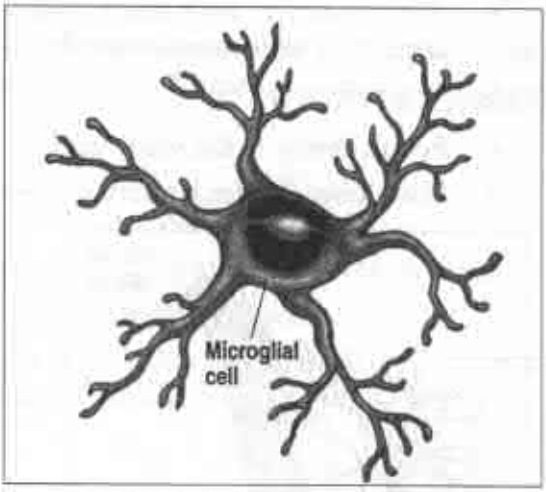
OLIGODENDROCYTES

- Much smaller than astrocytes
- Processes are less numerous and shorter
- Found both in gray matter and in white matter
- Produce the **myelin sheath** which provides the electrical insulation for certain neurons in the CNS and PNS
- Function: electrical insulation, maintaining metabolic exchange and microenvironment around the neuronal body and processes.

MICROGLIA

- The cell bodies are small, dense, and elongated.
- Have short processes covered by numerous small expansions, giving them a thorny appearance.

- Are not numerous, but they are found in both white and gray matter.
- Are specialized macrophages.
- Act as Ag-presentation cells.
- Has mesenchymal origin and derived from monocyte.
- They are involved with inflammation and repair in the adult central nervous system, and they produce and release neutral proteases and oxidative radicals. When activated, microglia retract their processes and assume the morphological characteristics of macrophages, becoming phagocytic and acting as antigen-presenting cells).



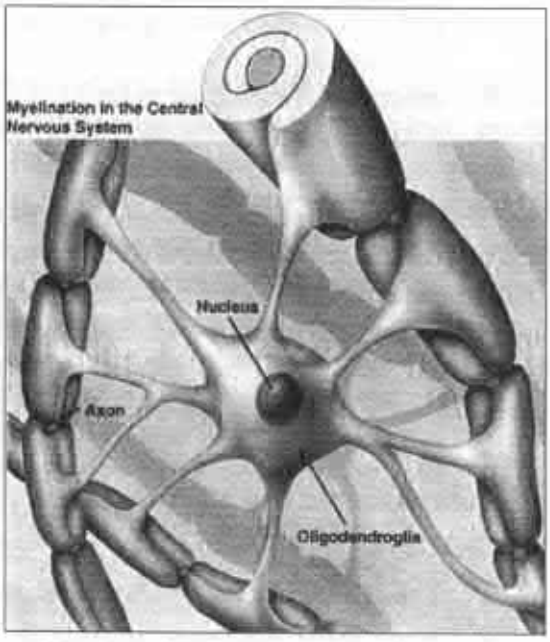
Drawing of a microglial cell

- Microglia secrete a number of immunoregulatory cytokines and dispose of unwanted cellular debris caused by central nervous system lesions.

NEUROGLIA
and **nervous system**

- **Neuroglia of CNS** is represented by: astrocytes, oligodendrocytes, ependymal cells, microglia.
- **Neuroglia of PNS** is represented by: neurolemmocytes (Schwann cell), satellite cells.

1. **Schwann cells or neurolemmocytes** have the same function as oligodendrocytes. Their wrap around portion of only one axon to form myelin sheath, in contrast to the ability of oligodendrocytes to branch and serve more than one neuron and its processes.



Drawing of a oligodendrocyte of central nervous system

2. **Satellite cells** are flattened cells that surround neuron cell bodies in ganglia, provide support and nutrients.

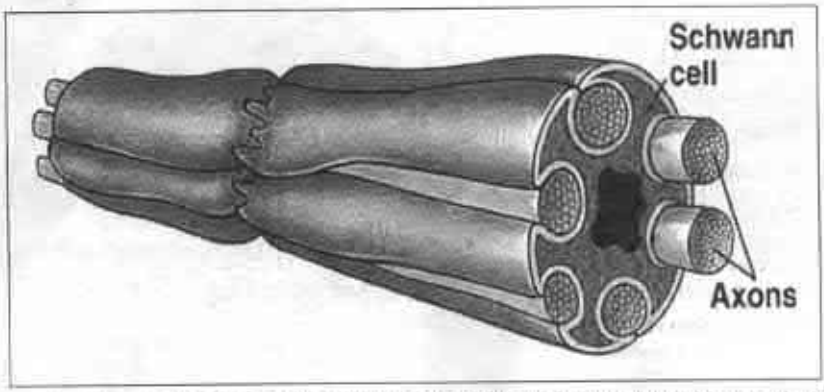
NERVE FIBERS

NERVE FIBERS consist of axons enveloped by special sheaths formed by oligodendrocytes. There are two types of nerve fibers:

- **Myelinated fibers**
- **Unmyelinated fibers**

UNMYELINATED NERVE FIBERS

The axons are enclosed in simple clefts of oligodendrocytes or Schwann cells. Each cell may enclose several non-myelinated axons. Axons of small diameter are usually **unmyelinated nerve fibers.**



The most frequent type of unmyelinated nerve fiber, in which isolated axons are surrounded by a Schwann cell and each axon has its own mesaxon

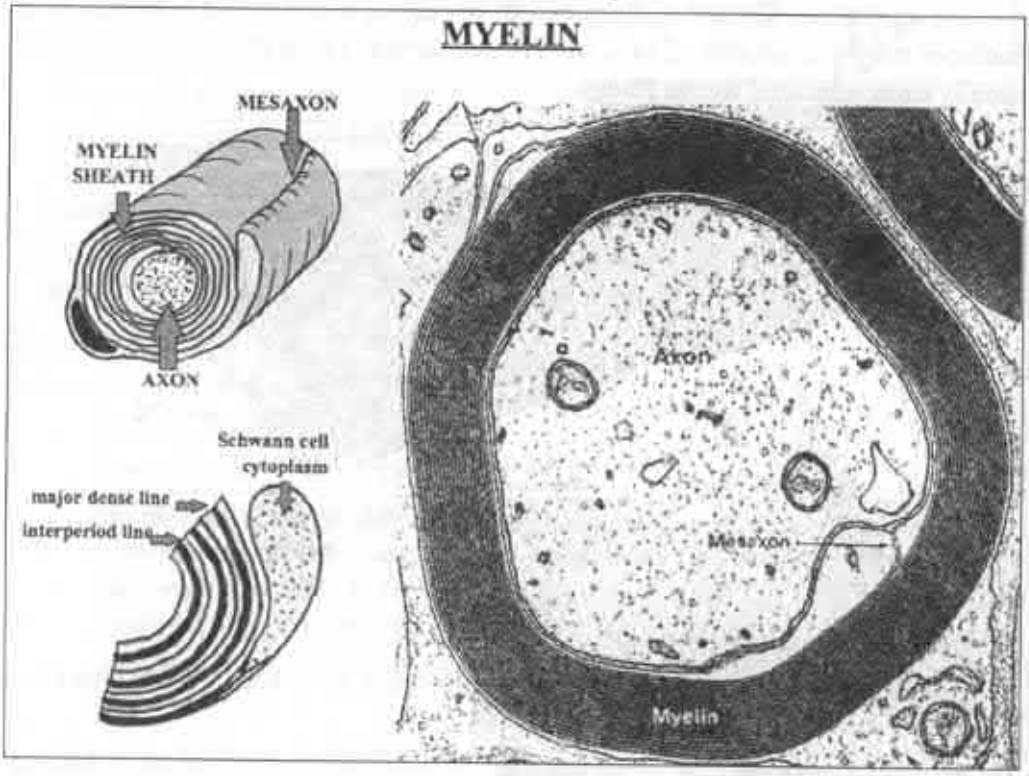
MYELINATED nerve fiber

- The myelin sheath is segmented because it is formed by numerous Schwann cells.
- The junctions where two adjacent Schwann cells meet are devoid of myelin – junction is called **nodes of Ranvier** (they are the sites where collaterals can arise).
- Areas of incomplete fusion of the Schwann cell membrane occur – **Schmidt-Lanterman clefts** (defects in the myelin formation). The cleft is formed by Schwann cell cytoplasm that is not displaced to the periphery during myelin formation.

- The distance between 2 nodes is called an **internode** and consists of one Schwann cell.

MYELIN SHEATH

- Consists of **many layers of modified cell membrane** having a higher proportion of lipids than other cell membrane.
- Myelin is responsible for the color of the white matter of the brain and spinal cord.
- CNS myelin contains 2 major proteins called **myelin basic protein** and **proteolipid protein**. *Several human demyelinating diseases are due to the deficiency or lack of one or both of these proteins.*



In the structure of the myelin sheath can be distinguish 2 types of lines:

- *Major dense lines* = regular mature myeline lamination of concentric, dense nature, 3 nm thick separated by light iintervals of 10 nm.
- *Interperiod lines* = finer dense lines of 2 nm thickness bisecting the spaces between major dense lines.

Functions of the myelin sheath are:

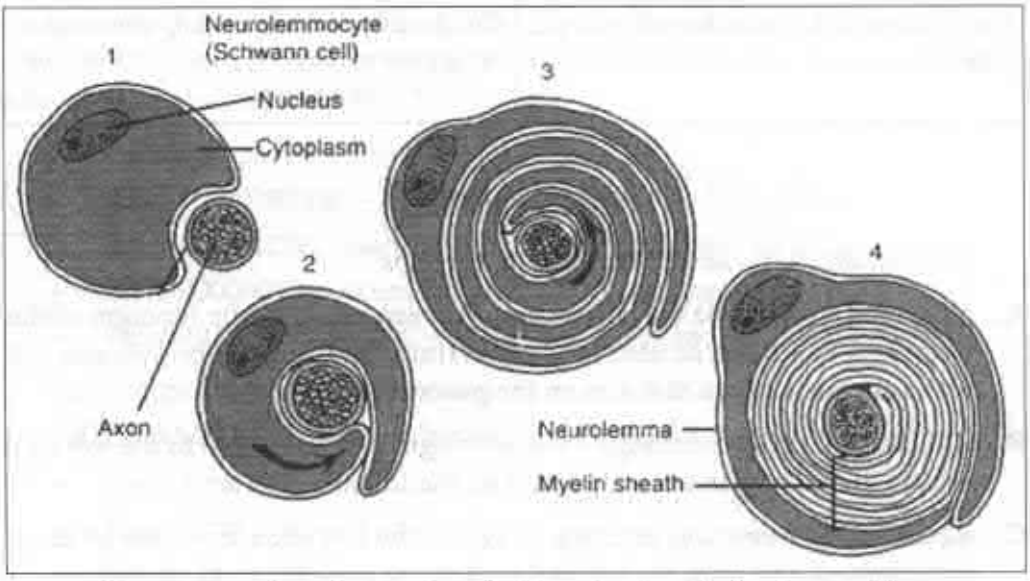
- **Protection** of the axon
- **Electrically insulating** fibers from one another
- **Increasing the speed** of nerve impulse transmission

Myelin sheath of the central nervous system is formed by oligodendrocytes (the process wrap around the axon). The same oligodendrocyte forms myelin sheaths for several (3–50) nerve fibers. In the central nervous system, processes of other cells sometimes cover the nodes of Ranvier, or there is considerable extracellular space (ES) at that point.

Myelin sheaths in the peripheral nervous system **are formed by Schwann cell.** Schwann cells have the same function as oligodendrocytes.

Schwann cells *envelop all nerve fibers of the PNS from attachment to the spinal cord and brain stem.* They have a heterochromatic nucleus, flattened centrally located in the cell, numerous mitochondria, microtubules and microfilaments, lysosomes, some granular endoplasmic reticulum and small golgi apparatus. One Schwann cell forms myelin around a segment of one axon, in contrast to the ability of oligodendrocytes to branch and serve more than one neuron and its processes.

- The first step in myelin formation is axon penetration of existing groove of the Schwann cell cytoplasm. The edges of the groove come together



Four consecutive phases of myelin formation in peripheral nerve fibers

to form a mesaxon, so that the plasm membranes of the 2 edges fuse together on their outer surface.

- Next, the mesaxon wraps itself around the axon several times, the number of turns, determining the thickness of the myelin layer.

UNMYELINATED	MYELINATED
Localization	
Mostly in the **autonomic NS**	In the **CNS and PNS**
Speed of the conduction of the nerve impulse	
Low (0,5-2 m/s)	**High (5-120 m/s)**
Nerve fiber of the **cable type** (cytoplasm of the Schwann cell can contains 10-20 axons of different neurons).	Nerve fiber **contains only 1 axon.** But in the CNS 1 oligodendrocyte can take part in the process of myelinization until 40-50 nerve fibers.
Structural components	
1. axon (many axons) 2. cytoplasm of the Schwann cell + short mesaxon (mesaxons) 3. basement membrane.	1. axon 2. myelin sheath with Schmidt-Lanterman clefts and node of Ranvier. 3. cytoplasm and nucleus of the Schwann cell. 4. basement membrane.
Conduction of the nerve impulse is **continuous.**	Conduction of the nerve impulse is **salutatory** (from the node to node of Ranvier – nerve impulse jumps).

NERVE ENDINGS

Functionally they can be divided into 3 groups:

A. **SYNAPSES** – provide the connection between neurons; the function of the synapse is to convert an electrical signal (impulse) from the presynaptic cell into a chemical signal that acts on the **postsynaptic** cell.

B. **EFFERENT** (motor) endings – transmit signals from the NS to the working organs (muscles, glands); are present on the axons.

C. **RECEPTOR** (sensitive) endings – receive the irritation from the external environment and from the internal organs; are present on the dendrites.

A. SYNAPSES are divided into:

1. ELECTRICAL

In mammals are rarely present. They are as nexus – provide the passive transport of the electric current through the cleft from the cell to other in the both directions and without delay.

2. CHEMICAL (mostly distributed)

The conduction of the nerve impulse is only in one direction and with delay.

The conduction of the nerve impulse is determined by the special substance – **neurotransmitters**. Neurotransmitters are generally synthesized in the cell body; they are then stored in vesicles in the presynaptic region of a synapse. During transmission of a nerve impulse, they are released into the synaptic cleft by **exocytosis.** The extra membrane that collects at the presynaptic region as a result of exocytosis of the synaptic vesicles is recycled by **endocytosis.** Retrieved membrane fuses with the smooth endoplasmic reticulum of the presynaptic compartment to be reused in the formation of new synaptic vesicles. Some neurotransmitters are synthesized in the presynaptic compartment, using enzymes and precursors brought by axonal transport. Most neurotransmitters are amines, amino acids, or small peptides (neuropeptides). Several peptides that act as neurotransmitters are used elsewhere in the body, as hormones in the digestive tract.

Neuromodulators are chemical messengers that do not act directly on synapses but modify neuron sensitivity to synaptic stimulation or inhibition. Some neuromodulators are neuropeptides or steroids produced in the nerve tissue; others are circulating steroids.

Chemical synapses are divided into:

- ***AXO-DENDRITIC*** – occurs between axons and dendrites.
- ***AXO-SOMATIC*** – occurs between axons and the cell body.
- ***AXO-AXONIC*** – occurs between axons and axons.
- ***DENDRO-DENDRITIC*** – occurs between dendrites and dendrites.

The synapse is formed by:

o **PRESYNAPTIC element** – that delivers the signal. It contains:

- Axon terminal
- Synaptic vesicles
- Neurotransmitters
- Mitochondria

- **SYNAPTIC CLEFT**
- **POSTSYNAPTIC elements**
  - Neurotransmitter receptors
  - May generate Action Potential

Sequence of Events during Chemical Synapse Transmission

Nerve impulses that sweep rapidly (in milliseconds) along the cell membrane promote an explosive electrical activity (depolarization) that is propagated along the cell membrane. This impulse briefly opens calcium channels in the presynaptic region, promoting a calcium influx that triggers the exocytosis of synaptic vesicles. The neurotransmitters released at the sites of exocytosis react with receptors present at the postsynaptic region, promoting a transient electrical activity (depolarization) at the postsynaptic membrane. These synapses are called **excitatory,** because their activity promotes impulses in the postsynaptic cell membrane. In some synapses the neurotransmitter–receptor interaction has an opposite effect, promoting **hyperpolarization** with no transmission of the nerve impulse. These are called **inhibitory** synapses. Thus, synapses can excite or inhibit impulse transmission and thereby regulate nerve activity.

Once used, neurotransmitters are quickly removed by enzymatic breakdown, diffusion, or endocytosis mediated by specific receptors on the presynaptic membrane. This removal of neurotransmitters is functionally important because it prevents an undesirable sustained stimulation of the postsynaptic neuron.

B. EFFERENT (motor) nerve endings

1. **MOTOR** nerve endings are present in the striated and smooth muscles. In structure they are like synapses, but there are some features:
   - nearly to the muscle fiber the axon loses the myelin sheath and gives some small branches.
   - they are covered by the Schwann cells and basement membrane.

The transmission of the excitation is provided by the neurotransmitter – **acetylcholine**.

2. **SECRETORY** nerve endings are present in the glands.

They can make next influences:

- **hydrokinetic** (mobilization of the water);
- **proteokinetic** (secretion of the proteins);
- **synthetic** (to increase the synthesis);
- **trophic** (to maintain the normal structure and function).

C. RECEPTOR (sensitive) nerve endings

- **EXTERORECEPTORS** (receive the signals from the external environment). They are: visual, auditory, olfactory, taste, tactile receptors.
- **INTERORECEPTORS.** They are divided into:

o ***viscероceptors*** – receive signals from the inner organs;

o ***proprioreceptors*** – receptors of the locomotor system.

<u>**Physiological classification**</u> of the receptor nerve endings:

- **mechanoreceptors** (pressure, vibration).
- **chemoreceptors** (taste, smell).
- **thermoreceptors** (cold, warm).
- **pain receptors.**

<u>**Morphological classification**</u>

1. Free (simple)

They consist of terminal branches of the dendrites of the sensory neuron. They provide the perception of pain, cold, warm, tactile signals. The free nerve endings are present inside the epithelium and in the loose connective tissue, which is located beneath.

They consist of only the dendrite.

2. Restricted (compound). There are two subtypes:

I. Encapsulated – they are surrounded by the connective tissue capsule.

<u>Structure:</u>

- branches of the dendrite
- surrounding Schwann cells
- connective tissue capsule

<u>Examples:</u>

- Vater-Pacini corpuscles
- Meissner's tactile corpuscles
- Ruffini's corpuscles
- Bulb of Krause
- Neuromuscular spindles
- Tendon organ of Golgi

II. **Un-encapsulated.** They consist of the branches of the dendrites that are surrounded by the Schwann cells. They are present in the dermis of the skin and in the lamina propria of the tunica mucosa.

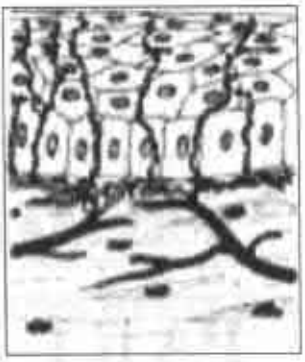
Receptor nerve endings

- ***Vater-Pacini corpuscles*** – are present on the connective tissue of the skin and inner organs. They are responsible for the sensation of the pressure and vibration.
- ***Meissner's tactile corpuscles*** – are located in the papillary layer of the dermis in skin, mostly: tips of fingers, lips, nipple and area which is around.
- ***Ruffini's curpuscles*** – are located in the connective tissue of the skin and in the capsules of the articulations. Implicate in reception of pressure.
- ***Bulb of Krause*** – is present in the papillary layer of the dermis, lamina propria of the tunica mucosa in the oral cavity. It is mechanoreceptor.
- ***Neuromuscular spindle*** – receptors of the sprain of the muscle fibers. It has motor and sensory innervations.
- ***Tendon organ of Golgi*** – receptor of the sprain. It is located in the places where the skeletal muscle fibers join to the tendon.

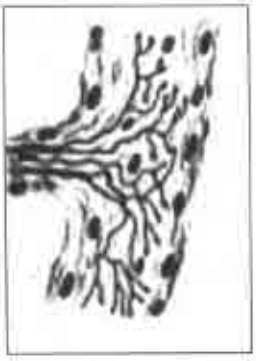
Vater-Pacini corpuscle
(vibration, deep pressure)

Meissner's tactile corpuscle
(touch)

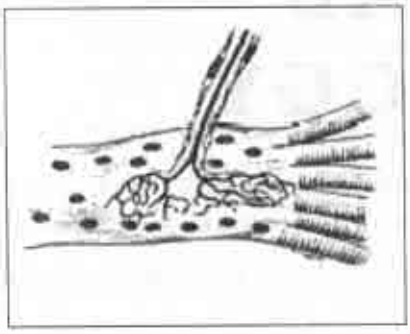
Bulb of Krause *(touch)*



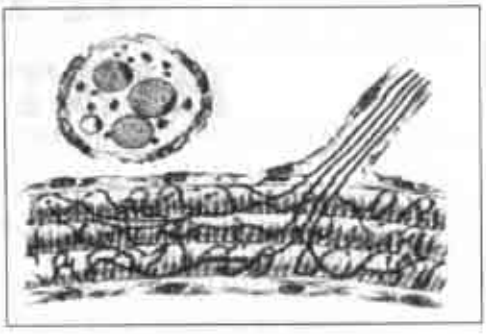
Free nerve ending
(pain, temperature, light touch)



Ruffini's corpuscle
(stretch)



Neural spindle proprioreceptor
(sprain)



Neuromuscular spindle proprioreceptr *(sprain)*

Several types of sensory nerve endings

PART IV

SPECIAL HISTOLOGY

CHAPTER I

NERVOUS SYSTEM

Function:

- **To detect, analyze, utilize and transmit all the information** generated by the sensory stimuli (such as heat & light) and by the mechanical and chemical changes that take place in the internal and external environment
- **Controls and integrates all body activities** within limits that maintain life
- **To organize and coordinate most functions** of the body, especially the motor, visceral, endocrine and mental activities

Development of the Nervous System

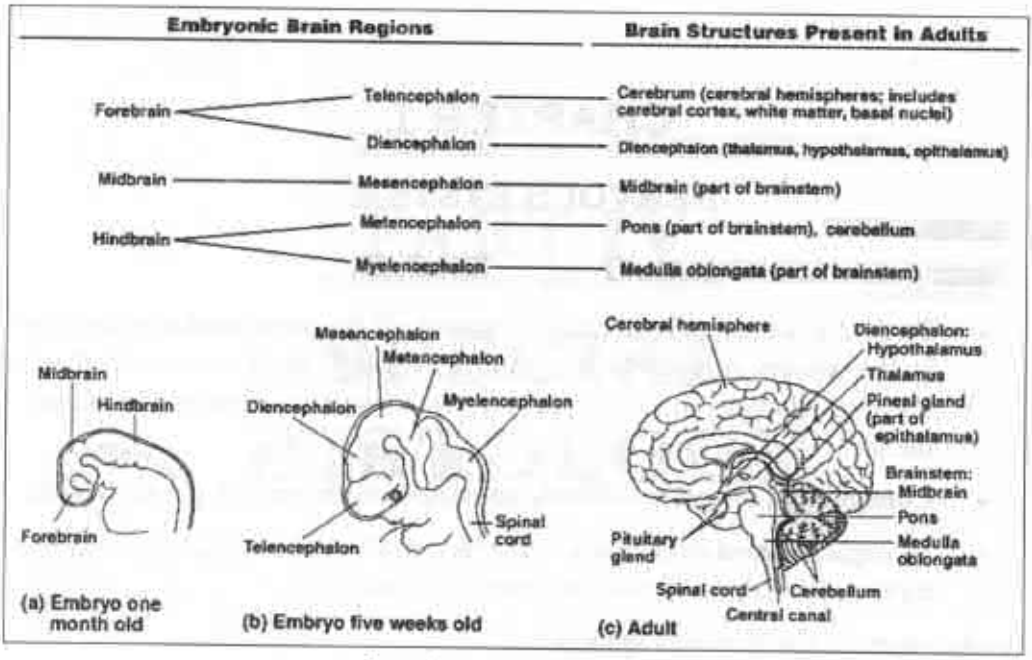
The neural crest and neural tube together give rise to nervous system.

The cranial end becomes expanded to form brain. The expanded brain forms vesicles from which different parts of brain develop.

I. Stage of 3 brain "bubbles" (at the end of the 4-th week of embryonic development).
   - **Prosencephalon**
   - **Mesencephalon**
   - **Rhombencephalon**

II. Stage of 5 brain "bubbles" (6-th week).
   - **Prosencephalon**
     - Telencephalon --- cerebral hemispheres
     - Diencephalon + nervous components of the eye
   - **Mesencephalon** – arise reflex centers of the vision, hearing, t & tactile sensitivity.
   - **Rhombencephalon**

- Metencephalon – cerebellum, brain stem
- Myelencephalon

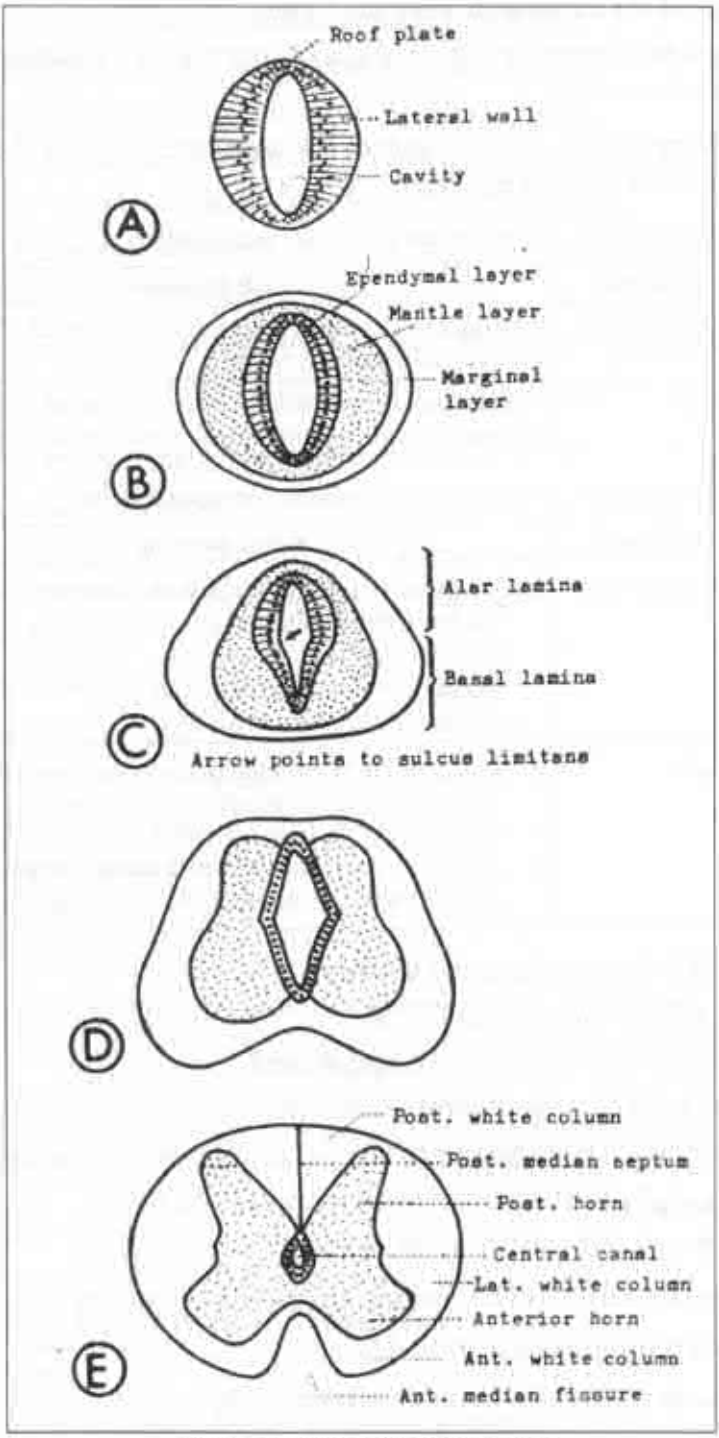


Development of the brain

The caudal end of the neural tube becomes elongated to form spinal cord. Different parts & components of it develop as follows: the wall of the tube is subdivided into 3 layers – ependymal layer, mantle layer and marginal layer. These are again subdivided into dorsal alar lamina and ventral basal lamina. Basal lamina gives rise to structures that are motor in function and alar lamina into those that are sensory in function.

Grey Column: from mantle layer of lateral wall of neural tube.

While matter: comes from marginal layer of lateral wall of neural tube.



Development of the spinal cord

Development of the autonomic nervous system

The sympathetic nervous system originates from the basal plate of the neural tube and neural crest cells.

The parasympathetic nervous system also originates from the basal plate of the neural tube and neural crest cells.

Table 1: Origination of the Sympathetic Nervous System

Embryonic Structure	*Adult Derivative*
Basal plate of neural tube	Preganglionic sympathetic neurons within the intermediolateral cell column
Neural crest cells	Postganglionic sympathetic neurons within the sympathetic chain ganglia and prevertebral ganglia

Table 2: Origination of the Parasympathetic Nervous System

Embryonic Structure	Adult Derivative
Basal plate of neural tube	1. Preganglionic parasympathetic neurons within the nuclei of the midbrain (III), pons (VIII), and medulla (IX, X) 2. Preganglionic parasympathetic neurons within the spinal cord nucleus at S2-S4
Neural crest cells	1. Postganglionic parasympathetic neurons within the ciliary (111), pterygopalatine (VII), submandibular (VII), otic (IX), and enteric (X) ganglia 2. Postganglionic parasympathetic neurons within the ganglia of the abdominal and pelvic cavities

ANATOMICAL Nervous System Divisions

- **Central nervous system** (CNS)
  - consists of the ***brain*** and ***spinal cord.***
- **Peripheral nervous system** (PNS) consists of:
  - ***cranial*** and ***spinal nerves*** that contain both sensory and motor fibers.
  - ***ganglia*** (collections of neurons outside the CNS).
  - motor and sensory ***nerve endings.***

Function: connects CNS to muscles, glands and all sensory receptors.

FUNCTIONAL Subdivisions of the PNS

- **Somatic** (voluntary) **nervous system** (SNS)
  - neurons from cutaneous and special sensory receptors to the CNS.

  - motor neurons to skeletal muscle tissue.
- **Autonomic** (involuntary) **nervous systems** (ANS)
  - sensory neurons from visceral organs to CNS.
  - motor neurons to smooth & cardiac muscle, glands & blood vessels.
    - ***sympathetic division*** (speeds up heart rate).
    - ***parasympathetic division*** (slow down heart rate).
- **Enteric** nervous system (ENS)
  - involuntary sensory and motor neurons control GI tract.
  - neurons function independently of ANS and CNS.

HISTOLOGY OF NERVOUS SYSTEM

White matter consists of:

- aggregations of **myelinated nerve fibers**,
- **few unmyelinated nerve fibers**
- **neuroglia**
- blood vessels

Gray matter contains:

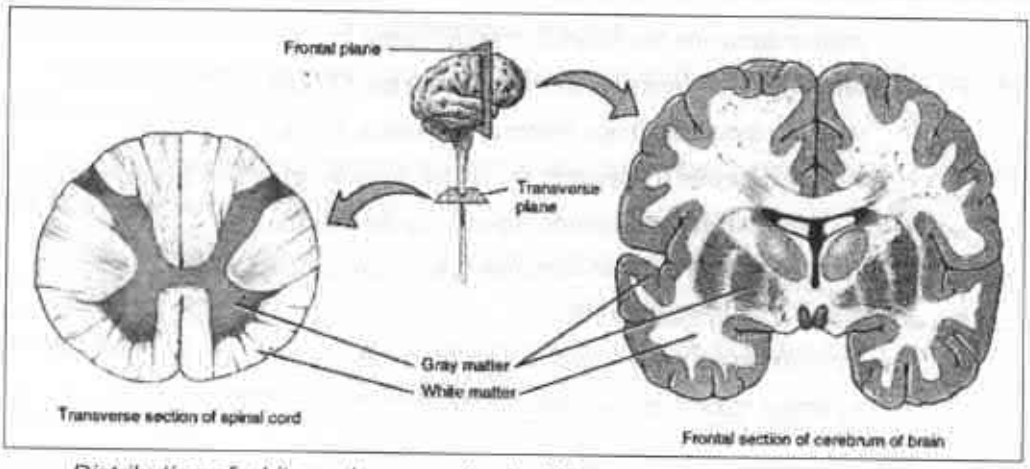
- **neuron** cell bodies (perikarya),
- **mostly unmyelinated nerve fibers**
- **few myelinated nerve fibers**
- **neuroglia**
- blood vessels
  - In the spinal cord, gray matter forms an H-shaped inner core (*central position*) that is surrounded by white matter (*peripheral position*).
  - In the brain, a thin superficial shell of gray matter covers the cerebrum (form cortex), the white matter is located in the center (medulla).

In the CNS

- Groups of nerve cell bodies are called **nuclei.**
- Bundles of nerve fibers are called **tracts.**

In the PNS

- Groups of nerve cell bodies are called **ganglia.**
- Bundles of nerve fibers form **nerves.**



Distribution of white and gray matter in different portions of nervous system

CENTRAL NERVOUS SYSTEM

SPINAL CORD

- Spinal cord is a cylindrical structure that is directly continuous with the brain and is situated in the vertebral canal but not reaching up to its end.
- In cross section, it exhibits a butterfly-shaped grayish-tan inner substance, the **gray matter** and a whitish peripheral substance, the **white matter.**
- The **white matter** is composed of myelinated and unmyelinated nerve fibers that represent ***ascending, descending and transverse pathways.***
- The **gray matter** consists of mirror-image lateral gray masses connected by a cross-bar of gray matter called the ***gray commissure*** that encloses the central canal.

The **central canal** is the small channel in the gray matter which connects the gray matter of left and right sides. It is lined by ependymal cells.

The spinal cord has two grooves that mark its surface: **Anterior median fissure and Posterior medial sulcus.**

GRAY MATTER of the spinal cord is divided into the:

- **Dorsal** (posterior) horns – are fine-bored and long
- **Ventral** (anterior) horns – are wide and short
- **Lateral** horns

According to topography of axon's neurons **of spinal cord** are divided into:

- **Radicular** – axons of neurons form anterior roots.

- **Inner** – axons of neurons are located inside of the gray matter.
- **Fascicular** – axons of neurons form bundles of nerve fibers inside of the white matter – tracts.

DORSAL (posterior) **HORNS**

Inside of them are distinguished:

- **Spongy layer** – contains many interneurons.
- **Substantia gelatinosa** – mostly is formed by neuroglia.
- **Proper nucleus** – is formed by interneurons that axons come to the opposite part of spinal cord and inside of tracts go to the cerebellum and thalamus.
- **Thoracic nucleus** (Clara's nucleus) – is formed by interneurons that axons come to the cerebellum.

VENTRAL (anterior) **HORNS**

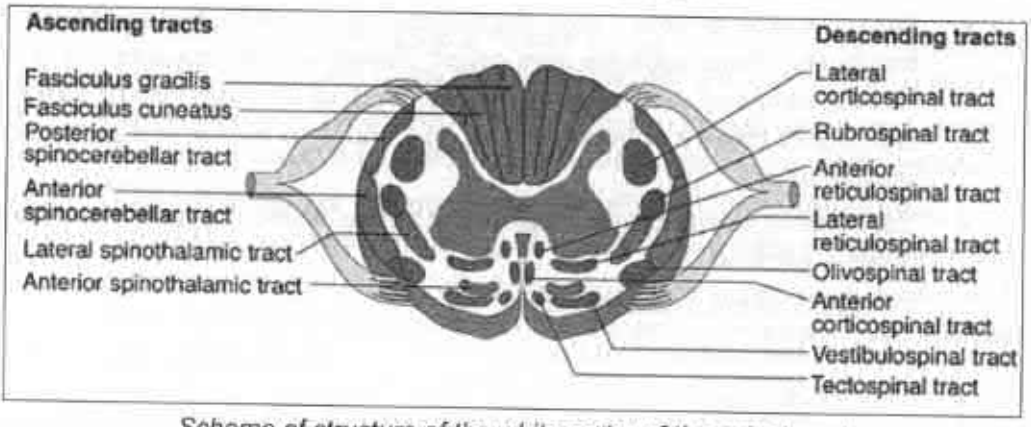
- Contain multipolar motor neurons.
- **Spinal motor neurons** have the largest nerve cell bodies in the ventral horn. These cells are also called "***lower motor neurons***", or just "***motor neurons***". Axons of these cells extend through ventral (anterior) roots into peripheral nerves, and hence to motor end plates on muscle fibers.
- The anterior horns are the largest in the areas where the innervation for limbs is present:
  - Cervical enlargement (arms).
  - Lumbar enlargement (legs).
- Most evident are:
  - **Medial group of motor neurons** that innervate muscles of the trunk (body).
  - **Lateral group of motor neurons** that innervate muscle of upper and lower extremities.

LATERAL HORNS

- Are well developed in thoracic (C8-L2) & sacral (S2-S4) regions.
- The lateral horn neurons are ***autonomic (sympathetic) motor neurons*** that serve the visceral organs.
- Their axons also leave the cord via the ventral root.

WHITE MATTER

- The white matter of the spinal cord is composed of **myelinated** and **unmyelinated nerve fibers** that allow communication between different parts of the spinal cord and between the cord and the brain.
- Nerve fibers run in three directions:
  - ***Ascending*** / up to higher centres (sensory inputs).
  - ***Descending*** / down to the cord from the brain or from within the cord to lower levels (motor outputs).
  - ***Transversely*** / across from one side of the cord to the other (commissural fibers).
- The white matter on each side of the column is divided into three ***white columns*** or ***funiculi*** and labeled according to their position (posterior, lateral, anterior).
- Each funiculi contains several fiber tracts, and each tract is made up of axons with similar destinations and functions.



Scheme of structure of the white mater of the spinal cord

CEREBELLUM

- Has three portions – 2 lateral hemispheres and the middle portion (vermis).

Functions: coordination of voluntary muscles, maintenance of balance, maintenance of muscle tone.

Cerebellum consists of gray (cortex) and white matter (medulla).

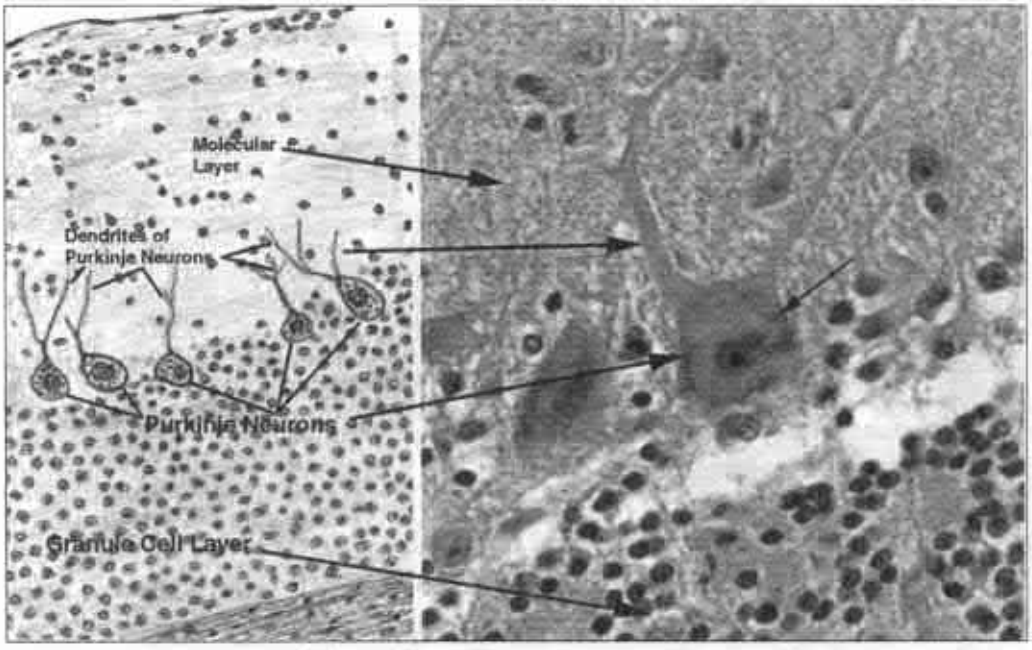
GRAY MATTER – CEREBELLAR CORTEX

The cortex of the cerebellum consists of **three well-defined layers:**

I. **Molecular layer** is relatively acellular and contains:
- Stellate neurons
- Basket neurons
- Dendrites of Purkinje neurons
- Parallel fibers (axons of granule cells)
- Neuroglia

II. **Purkinje cell layer** contains:
- Cell bodies of Purkinje neurons that are surrounded by the basket (axons of basket and stellate neurons)

III. **Granule cell layer** is rich in cells and contains:
- Granule neurons (cells)
- Golgi cells (type I and type II)
- cerebellar Glomeruli
- Neuroglia



Section of the cerebellum with distinct Purkinje cells

NEURONS of Cerebellar Cortex

I. **PURKINJE CELLS**

- Are the largest neurons in the brain.
- Have extensive dendritic trees that are broad perpendicular and compressed parallel to the length of the folium.
- The dendrites fill the molecular layer.
- The axons run through the white matter and synapse in cerebellar nuclei (or in vestibular nuclei).
- Purkinje axons engage in inhibitory synapses; they release GABA as a neurotransmitter.

II. **BASKET CELLS** are located in the depth of the molecular layer.

- Their dendrites receive excitatory synaptic input from parallel fibers.
- Their axons run transversely in the folium and inhibit bands of Purkinje cells that border the excited zone. Axonal branches form basket synaptic contacts around initial segments of Purkinje axons and release GABA.

III. **STELLATE CELLS** are intrinsec neurons named for their star-like shape, which results from dendrites arising in many directions.

- serve as inhibitory interneurons.

IV. **GOLGI NEURONS** in the granule cell layer like as stellate cells in the molecular layer serve as inhibitory interneurons. They are large in size.

V. **GRANULE CELLS**

- are the smallest neurons in the brain.
- they are the only excitatory neurons in the cerebellar cortex; they release glutamate as a neurotransmitter.
- have a few short dendrites within the granule cell layer.
- axons of granule cells enter the molecular layer, bifurcate, and run along the length of the folium (parallel fibers).
- each granule cell makes synaptic contact with numerous Purkinje neurons along a longitudinal band.

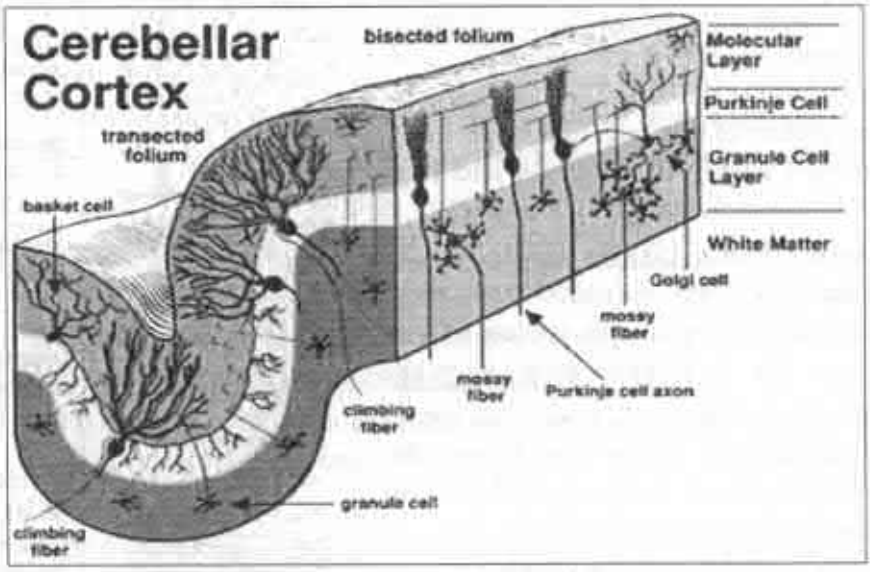
Cortical AFFERENT fibers

Two types of afferent fibers enter the cerebellar cortex. Both afferent types **are excitatory** to the cortex and they send excitatory collateral branches to cerebellar nuclei before entering the cortex. The afferent fibers are:

- **Mossy fibers** – have terminations that resemble moss. The moss endings synapse in glomeruli, with dendrites of granule cells. Mossy afferents come from all sources (spinal cord, pontine nuclei, vestibular nuclei & nerve) except the reticular formation.
- **Climbing fibers** – terminate by climbing the dendritic tree of a Purkinje neuron, exciting it greatly via numerous synaptic contacts. All climbing fibers come from the olivary nucleus. Like mossy fibers, climbing fibers send excitatory collaterals to cerebellar nuclei before terminating in the cortex.

Cortical Circuitry

- Afferent fibers, both **climbing and mossy**, are excitatory. The fibers send collaterals to cerebellar nuclei before terminating in cerebellar cortex.
- ***Climbing fibers*** *excite* Purkinje cells directly. ***Mossy fibers*** *excite* granule cells. Synapse between mossy fiber and granule cell is called cerebellar glomerulus. ***Granule cells*** *excite* bands of Purkinje cells and basket cells along the length of a folium. ***Basket cells*** *inhibit* Purkinje cells along the bilateral margins of the excited band.
- Purkinje cell axons terminate in cerebellar nuclei (or vestibular nuclei) where they selectively inhibit output neurons. Efferent axons from cerebellar nuclei constitute cerebellar output.



Schematic drawing of cerebellar cortex

Note: Granule cells are the only excitatory cells in the cerebellar cortex, all other cells are inhibitory. Purkinje cell axons are the only axons that leave the cerebellar cortex.

THE BRAIN

Functions: interprets sensations, determines perception, stores memory, reasoning, makes decisions, coordinates muscular movements, regulates visceral activities, and determines personality.

- The brain has **Gray** matter (cerebral cortex), and **White** matter.

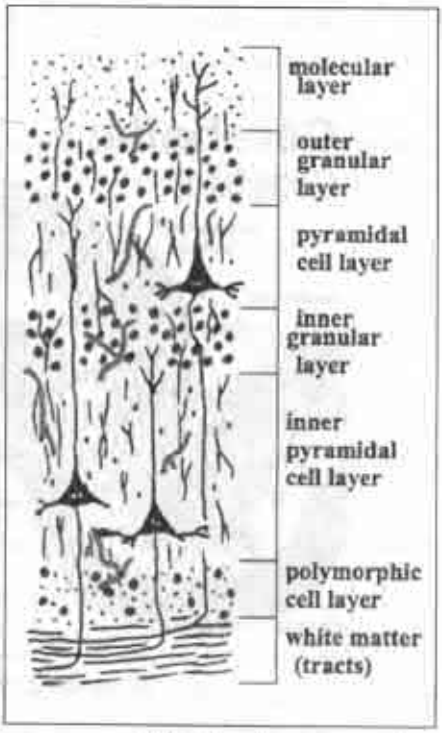
CEREBRAL CORTEX

The cerebral cortex forms the surface of gyri and sulci over each entire cerebral hemisphere. There are 6 layers:

- **Molecular** layer
- **Outer granular** layer
- **Outer pyramidal** cell layer
- **Inner granular** layer
- **Ganglionic** (inner pyramidal) layer (large pyramidal cells – Betz cells) – is well developed in the motor area.
- **Fusiform** (***polymorphic***) cell layer

Layers:

- **Layer I ("MOLECULAR layer")** is the outermost layer. This layer contains relatively few nerve cell bodies. The odd name "molecular layer" derives from the fine texture of this layer, due to its composition largely of dendrites and fine axon terminals (and glia, of course).
- **Layer II ("OUTER GRANULAR layer")**, typically contains many very small cells (granule cells, stellate cells).
- **Layer III ("OUTER PYRAMIDAL layer")** contains cell bodies of *small pyramidal cells*. Axons from these cells typically project to the upper layers of neighbouring cortical regions.
- **Layer IV ("INNER GRANULAR layer")** contains axonal ramifications of afferent fibers, such as sensory axons from the thalamus.



The structure of the cerebral cortex

- **Layer V ("INNER PYRAMIDAL layer")** contains cell bodies of large pyramidal cells. Axons from these cells typically project to more distant cortical regions, to other parts of the brain, or to lower centres (such as spinal motor neurons). The larger size of these pyramidal cells is associated with the greater length of their axons.
- **Layer VI ("layer of PLEIOMORPHIC cells)** typically contains cells of assorted size and shape (hence, "pleiomorphic").

Neurons of Cerebral Cortex

- The cortex of the cerebrum consists of: ***stellate cells, horizontal cells, granule cells*** – intrinsic neurons, and ***pyramidal cells*** – the efferent (long-axon) cells of the cerebral cortex.

PYRAMIDAL CELLS may be recognized by their relatively large soma and by their prominent apical dendrites (the upward "apex" on the "pyramid"). The diagrammatic pyramidal cells alongside the image suggest the extent of these dendrites (although most lateral branches are truncated here as well).

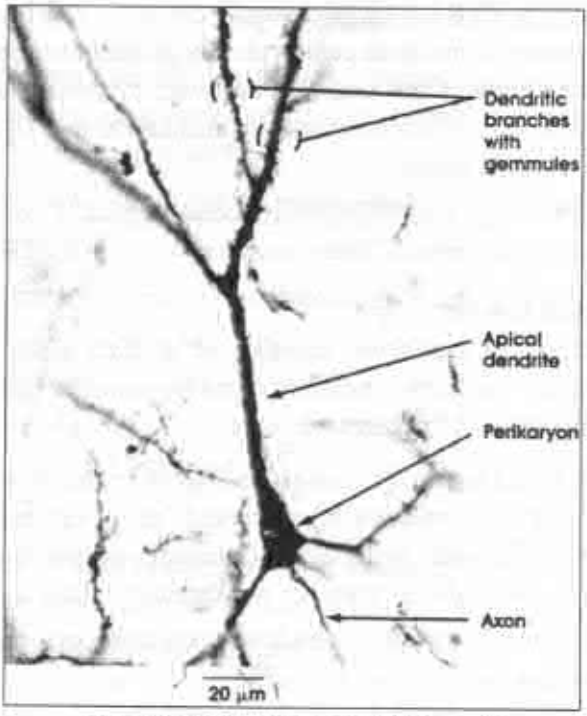
- Although typically an apical dendrite is conspicuous only near the soma of its pyramidal cell, these are actually very long processes which may extend (with many branches) into the molecular layer (layer I).
- Basal dendrites (beginning the lower "corners" of each "pyramid") may extend to either side far beyond the width of this image.
- Axons from pyramidal cells project distances which, at this magnification, could equal many tens of meters. Some go to adjacent areas of cortex ("association fibers"). Others cross through the corpus callosum.

THE GIANT BETZ CELLS

- are extremely large pyramidal cells of the **motor (precentral) cortex.** These pyramidal cells comprise some of the upper motor neurons.
- Axons from these cells **descend in the corticospinal tract, or pyramidal tract**, to synapse with lower motor neurons.
- Their exceptionally large size is presumably associated with their need to sustain extremely long axons.

Types of cerebral cortex

- **UNGRANULAR** – is specific for its motor centers. III, V, VI layers are well developed.
- **GRANULAR** – is specific for its sensitive centers. II, IV layers are well developed.



The structure of the giant Betz cell

WHITE MATTER

The white matter of the cerebrum is composed of **myelinated** and **unmyelinated nerve fibers** that allow communication between different parts of the brain and between the brain and the spinal cord.

- There are three types of nerve fibers:
  - **Associative** nerve fibres (connect gyri of one hemisphere).
  - **Commissural** nerve fibres (connect gyri of opposite hemispheres).
  - **Projective** nerve fibres (connect a cortex to underlying departments of nervous system).

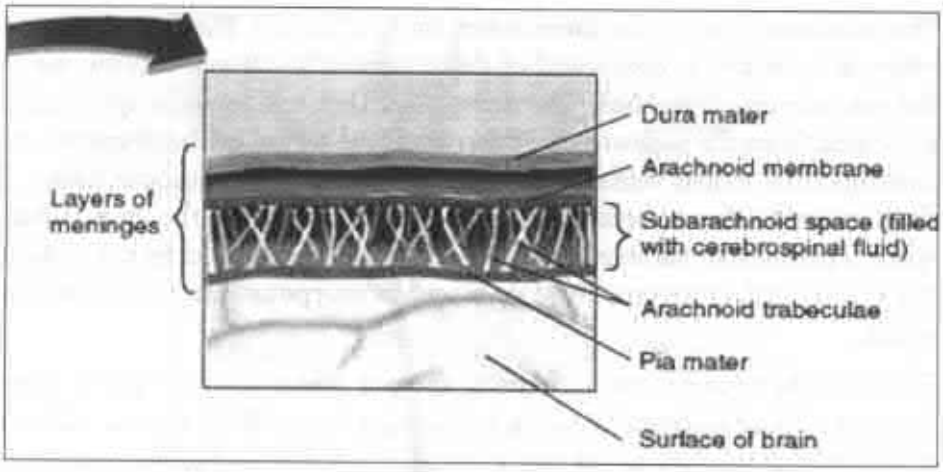
MENINGES

Development of Meninges

- The dura mater arises from mesenchyme that surrounds the neural tube.
- The pia mater and arachnoid membrane arises form neural crest cells.

The central nervous system is enveloped by specialized layers of connective tissue.

- The outermost layer is the **dura mater** (or just "dura"). The dura mater is the external layer and is composed of dense connective tissue continuous with the periosteum of the skull. The dura mater that envelops the spinal cord is separated from the periosteum of the vertebrae by the epidural space, which contains thin-walled veins, loose connective tissue, and adipose tissue. The dura mater is always separated from the arachnoid by the thin subdural space. The internal surface of all dura mater, as well as its external surface in the spinal cord, is covered by simple squamous epithelium of mesenchymal origin.
- Immediately adjacent to the brain is the **pia mater** (or just "pia"). The pia mater is a loose connective tissue containing many blood vessels. Although it is located quite close to the nerve tissue, it is not in contact with nerve cells or fibers. Between the pia mater and the neural elements is a thin layer of neuroglial processes, adhering firmly to the pia mater and forming a physical barrier at the periphery of the central nervous system. This barrier separates the central nervous system from the cerebrospinal fluid. The pia mater follows all the irregularities of the surface of the central nervous system and penetrates it to some extent along with the blood vessels. Squamous cells of mesenchymal origin cover pia mater. Blood vessels penetrate the central nervous system through tunnels covered by pia mater – the **perivascular spaces.** The pia mater disappears before the blood vessels are transformed into capillaries. In the central nervous system, the blood capillaries are completely covered by expansions of the neuroglial cell processes.
- Between dura and pia is the **arachnoid.** The arachnoid has two components: a layer in contact with the dura mater and a system of trabeculae connecting the layer with the pia mater. The cavities between the trabeculae form the **subarachnoid space,** which is filled with cerebrospinal fluid and is completely separated from the **subdural space.** This space forms a hydraulic cushion that protects the central nervous system from trauma. The subarachnoid space communicates with the ventricles of the brain. The arachnoid is composed of connective tissue devoid of blood vessels. The same type of simple squamous epithelium that covers the dura mater covers its surfaces. Because the arachnoid has fewer trabeculae in the spinal cord, it can be more clearly distinguished from the pia mater in that area. In some areas, the arachnoid perforates the dura mater, forming protrusions that terminate in venous sinuses in the dura mater. These protrusions, which are covered by endothelial cells of the veins, are called **arachnoid villi.** Their function is to reabsorb cerebrospinal fluid into the blood of the venous sinuses.



Drawing of the meninges

CHOROID PLEXUS and CEREBROSPINAL FLUID

The choroid plexus consists of invaginated folds of pia mater, rich in dilated fenestrated capillaries that penetrate the interior of the brain ventricles. It is found in the roofs of the third and fourth ventricles and in part in the walls of the lateral ventricles.

The choroid plexus is composed of loose connective tissue of the pia mater, covered by a simple cuboidal or low columnar epithelium. The main function of the choroid plexus is to elaborate cerebrospinal fluid, which contains only a small amount of solids and completely fills the ventricles, central canal of the spinal cord, subarachnoid space, and perivascular space. Cerebrospinal fluid is important for the metabolism of the central nervous system and acts as a protective device against mechanical shocks.

Cerebrospinal fluid is clear, has a low density and is very low in protein content. A few desquamated cells and two to five lymphocytes per milliliter are also present. Cerebrospinal fluid is continuously produced and circulates through the ventricles, from which it passes into the subarachnoid space. There, arachnoid villi provide the main pathway for absorption of cerebrospinal fluid into the venous circulation. (There are no lymphatic vessels in brain nerve tissue.)

PERIPHERAL NERVOUS SYSTEM

GANGLIA are aggregations of neuron cell bodies outside of the CNS.

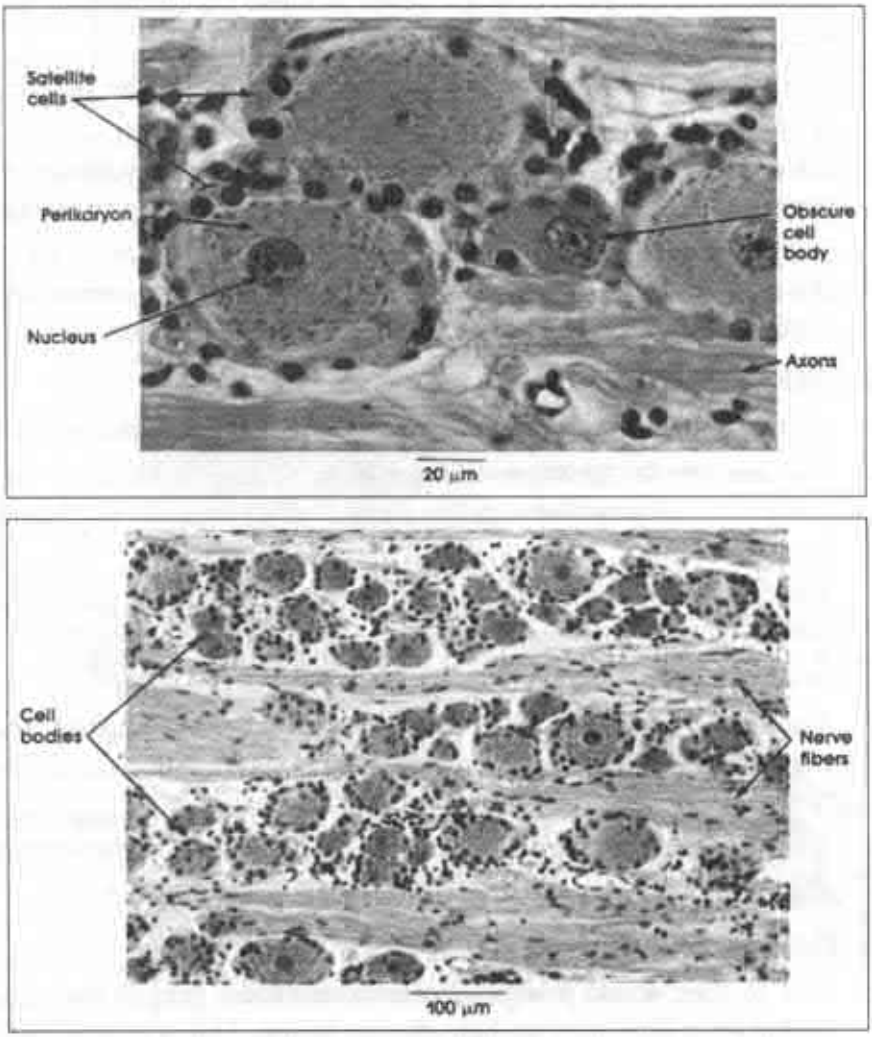
There are two principal types, distinguished mainly by their function and sometimes by location. **One type is sensory, the other motor.**

The first type of ganglia: are cranial ganglia and spinal ganglia. Sensory ganglia – contain pseudounipolar or bipolar afferent neurons.

- They are located by course of posterior roots of the spinal cord (spinal ganglia) and cranial nerves (V, VII, VIII, IX, X).

The second type of ganglion is the autonomic ganglion. They're functionally motor ganglia. Autonomic ganglia can be:

- **Paravertebral**
- **Prevertebral**
- **Intramural**



Photomicrograph of a spinal ganglion (high and low magnifications)

SPINAL GANGLION

- is encapsulated by a dense CT tissue, which is continuous with the epineurium and perineurium of the sensory neuron.
- The ganglion cells are **grouped together on the periphery.**
- The ganglion cells are **pseudo-unipolar** neurons, **have centrally-located nuclei**.
- They are **surrounded by flattened satellite cells** with round (or ovoid) nuclei. The satellite cells serve similar functions as the Schwann cells: structural and metabolic support (don't produce myelin).
- Between the ganglion cells **are septa of loose CT** (fibroblasts and connective tissue fibers)
- Fascicles of myelinated nerve fibers course through the middle of the spinal ganglion. There are two types of fibers:
- the **afferent fiber** – comes into the soma from a sensory structure in the periphery. This can be any kind of sensory structure: a touch receptor, cold receptor, pain fiber, pressure sensor, etc.
- the **efferent fiber** – leaves the soma in the ganglion and carries the information into the central nervous system for further action.

SYMPATHETIC GANGLION

- Contains the soma of the second neuron in the autonomic motor chain.
- The neurons in an autonomic ganglion receive input from the CNS, process it, and send the signal to some effector organ other than skeletal muscle.
- Typical effector organs for autonomic ganglia are sweat glands, salivary glands, smooth muscle, etc.
- In sympathetic ganglia are multipolar neurons.
- The neuron soma is smaller than those of a sensory ganglion, and their nuclei are more eccentric, some ganglion cells are binucleated. There are fewer satellite cells, only 2 or so per soma.
- The nerve fibers, scattered between the cell bodies, have little to no myelin.
- The capsule surrounding the ganglion cells is less distinct.

PARASYMPATHETIC GANGLIA

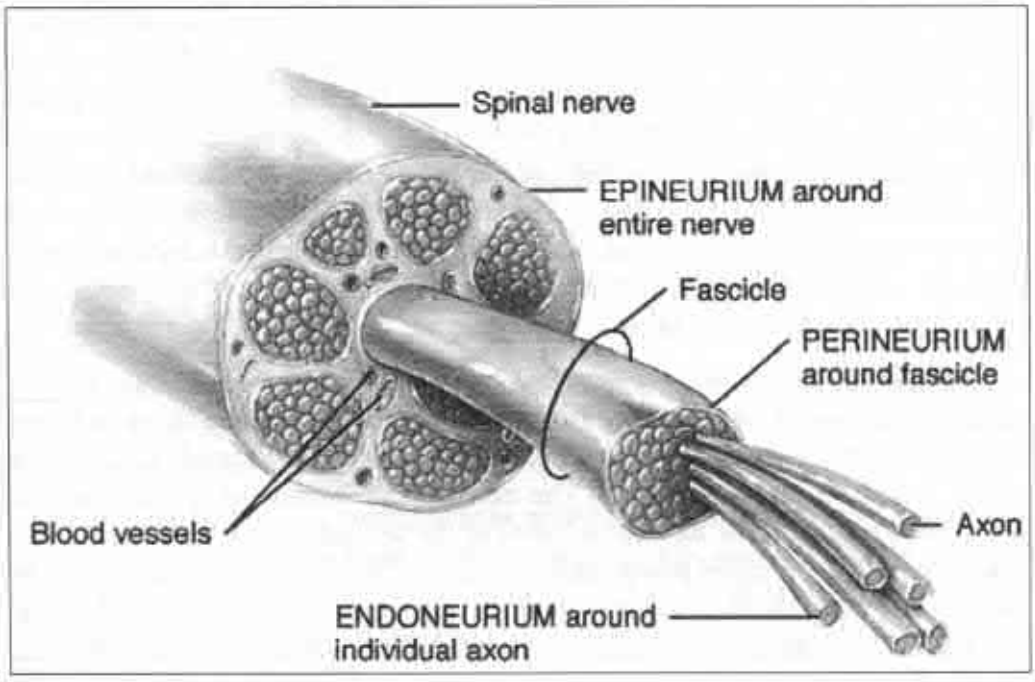
- Due to their short, postganglionic axons, these ganglia are often seen within or in proximity to their effector region. This is evident within the

walls of the gastrointestinal tract (as parasympathetic ganglia are located within the submucosa or between the circular and longitudinal layers of smooth muscle).

NERVES

A "*nerve*" is a grossly visible anatomic structure, and is a bundle of axonal processes from many different neurons, wrapped in a connective tissue sheath.

Nerves have an external fibrous coat of dense connective tissue called **epineurium,** which also fills the space between the bundles of nerve fibers. Each bundle is surrounded by the **perineurium,** a sleeve formed by loose connective tissue. The cells of each layer of the perineurial sleeve are joined at their edges by tight junctions, an arrangement that makes the perineurium a barrier to the passage of most macromolecules and has the important function of protecting the nerve fibers from aggression. Within the perineurial sheath run the Schwann cell-sheathed axons and their enveloping connective tissue, the **endoneurium**. The endoneurium consists of a thin layer of reticular fibers, produced by Schwann cells.



Schematic representation of a nerve

The nerves establish communication between brain and spinal cord centers and the sense organs and effectors (muscles, glands, etc). They possess afferent and efferent fibers to and from the central nervous system. **Afferent** fibers carry the information obtained from the interior of the body and the environment to the central nervous system. **Efferent** fibers carry impulses from the central nervous system to the effector organs commanded by these centers.

Nerve's classifications:

A. I. **Cranial nerves** – originate from brain, go to other body parts
   II. **Spinal nerves** – originate from spinal cord , go to other body parts

B. 1. **Sensory nerves** – impulses conducted into CNS.
   2. **Motor nerves** – impulses conducted away fom CNS to effector organs (muscles, glands).
   3. **Mixed nerves** – contain both sensory & motor nerves.

AUTONOMIC NERVOUS SYSTEM

The term "autonomic" is not correct–although it is widely used–inasmuch as most of the functions of the autonomic nervous system are not autonomous; they are organized and regulated in the central nervous system. The concept of the autonomic nervous system is mainly functional. Anatomically, it is composed of collections of nerve cells located in the central nervous system, fibers that leave the central nervous system through cranial or spinal nerves, and nerve ganglia situated in the paths of these fibers. The term "autonomic" covers all the neural elements concerned with visceral function. In fact, the so-called autonomic functions are as dependent on the central nervous system as are the motor neurons that trigger muscle contractions.

The autonomic nervous system is a two-neuron network. The first neuron of the autonomic chain is located in the central nervous system. Its axon forms a synapse with the second multipolar neuron in the chain, located in a ganglion of the peripheral nervous system. The nerve fibers (axons) of the first neuron are called **preganglionic fibers;** the axons of the second neuron to the effectors–muscle or gland – is called **postganglionic fibers.** The chemical mediator present in the synaptic vesicles of all preganglionic endings and at anatomically parasympathetic postganglionic endings is **acetylcholine,** which is released from the terminals by nerve impulses.

CHAPTER II

SENSE ORGANS

Analyzers are complex systems that provide the communication between nervous system and external or internal environment.

Each analyzer is composed of:

1. **Peripheral part** – sense organs.
2. **Intermediate** – the chain of interneurons.
3. **Central** – brain (cortex).

Classification of sense organs

- **I group** – eye and olfactory organ – are developed from the neural plate. Their receptors are specialized neurosensitive cells that transform mechanical impulse into the neural impulse.
- **II group** – organ of hearing and equilibrium, taste organ. Their receptors are specialized epithelial cells.
- **III group** – receptor nerve endings.

EYE

The eye is a complex and highly developed photosensitive organ that permits an accurate analysis of the shape, light intensity, and colors reflected from objects. The eyes are located in protective bony structures of the skull, the **orbits.**

Each eye includes a tough, fibrous globe to maintain its shape, a lens system to focus the image, a layer of photosensitive cells, and a system of cells and nerves whose function is to collect, process, and transmit visual information to the brain. Near the eye are located accessory structures: muscles, eyelids, lacrimal apparatus, and conjunctiva.

Each eye is composed of three concentric layers (tunics):

- **Outer** – fibrous consists of the sclera and the cornea.
- **Middle** – vascular coat consisting of the choroid, ciliary body and iris.
- **Inner** – retina

The eye contains three compartments: the **anterior chamber,** which occupies the space between the cornea and the iris and lens; the **posterior chamber,** between the iris, ciliary process, zonular attachments, and lens; and the **vitreous space,** which lies behind the lens and is surrounded by the retina. Both the anterior and posterior chambers contain a protein-poor fluid called **aqueous humor.** The vitreous space is filled with a gelatinous substance called the **vitreous body.**

FUNCTIONAL APPARATUS OF THE EYE

I. DIOPTRIC **(refractive)** – formed by following components of the eye:
- Cornea
- Fluid of the anterior and posterior chambers
- Lens
- Vitreous humor

II. ACCOMMODATION **of eye**:
- Iris
- Ciliary body
- Lens

III. PHOTORECEPTOR:
- Retina

I. FIBROUS TUNIC

The fibrous tunic is divided in three compartments:
- **Sclera** – the anterior five-sixths of the fibrous tunic.
- **Cornea** – the anterior one-sixths of the fibrous tunic.
- **Limbus** is an area of transition from the transparent collagen bundles of the cornea to the white opaque fibers of the sclera.

SCLERA (white of eye)
- It is opaque white structure.
- The sclera consists of dense regular connective tissue made up mainly of flat collagen bundles intersecting in various directions while remaining parallel to the surface of the organ, a moderate amount of ground substance, and a few fibroblasts.

- The sclera is **relatively** avascular.
- Functions:
  - Support.
  - Protection.

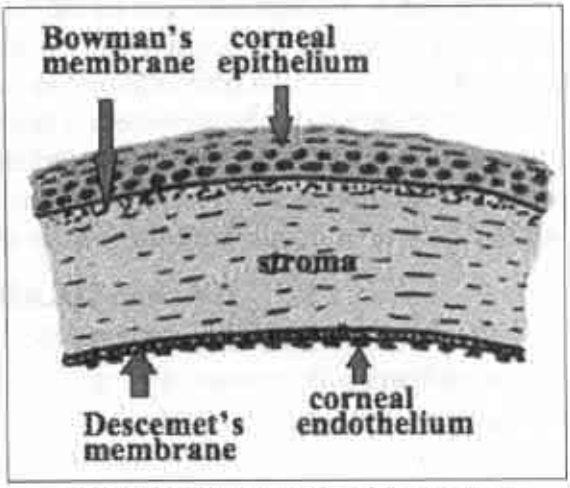
CORNEA

In contrast to the sclera, the **cornea** is colorless and transparent. It is the **principal light refracting structure** of the eye. The cornea is **avascular** (*the lack of blood vessels means the cornea is isolated from the immune system, and corneal transplants hence are not subjected to the normal process of graft rejection seen with other organs*).The cornea has **much pain receptor nerve endings.**

- **It consists of 5 layers:**

1. ANTERIOR CORNEAL EPITHELIUM is a stratified squamous nonkeratinized epithelium and consists of five or six layers of cells. In the basal part of the epithelium are numerous mitotic figures that are responsible for the cornea's regenerative capacity. The turnover time for these cells is approximately 7 days. The surface corneal cells show microvilli protruding into the space filled by the precorneal tear film.

Note: the typical epithelial property of rapid proliferation permits damage of cornea to be rapidly repaired. (It also makes the cornea sensitive to irradiation, and one symptom of overexposure to radioactivity–or other insults that affect proliferative cells – is corneal opacification.)



Scheme of the structure of the cornea

2. BOWMAN'S MEMBRANE – provides strength to the cornea and as a barrier to the spread of infections. It consists of collagen fibers crossing at random, a condensation of the intercellular substance, and no cells.

3. STROMA (substantia propria) represents about 90% of the cornea's thickness. It is composed of parallel bundles of collagen fibrils, fibrocytes, fibrob-

lasts, and ground substance. Although the stroma is avascular, migrating lymphoid cells are normally present in the cornea.

4. DECEMET'S MEMBRANE is a thick homogeneous structure composed of fine collagenous filaments organized in a three-dimensional network.
5. POSTERIOR CORNEAL EPITHELIUM (endothelium) is a simple squamous epithelium. These cells possess organelles for secretion that are characteristic of cells engaged in active transport and protein synthesis and that may be related to the synthesis and maintenance of Descemet's membrane.

Corneal posterior endothelium and anterior epithelium are responsible for maintaining the transparency of the cornea. Both layers are capable of transporting sodium ions toward their apical surfaces. Chloride ions and water follow passively, maintaining the corneal stroma in a relatively dehydrated state. This state, along with the regular orientation of the very thin collagen fibrils of the stroma, accounts for the transparency of the cornea *(see fig. 70, plate II)*.

CORNEOSCLERAL JUNCTION, or LIMBUS

It is highly vascularized, and its blood vessels assume an important role in corneal inflammatory processes. The cornea, an avascular structure, receives its metabolites by diffusion from adjacent vessels and from the fluid of the anterior chamber of the eye. In the region of the limbus in the stromal layer, irregular endothelium lined channels, the trabecular meshwork, merge to form **Schlemm's canal**, which drains fluid from the anterior chamber of the eye. Schlemm's canal communicates externally with the venous system.

II. VASCULAR TUNIC

The vascular tunic of the eye consists of three parts:

- Choroid
- Ciliary body
- Iris

CHOROID

The choroid is located between sclera and retina. It is formed by loose connective tissue, which is rich in fibroblasts, macrophages, lymphocytes, mast cells, plasma cells, collagen fibers, and elastic fibers. It is:

1. **Highly vascular tunic** (supplies retina).
2. **Pigmented tunic** (absorbs light). The choroid contains many melanocytes which give it its characteristic black color.

The choroid has 4 layers:

- **Suprachoroidal lamina** bounds the choroids to the sclera; forms by a loose layer of connective tissue rich in melanocytes, fibroblasts, and elastic fibers.
- **Choriovascular**
- **Choriocapillary layer** (is richer than the others layer in small vessels). It has an important function in nutrition of the retina, and damage to this tissue causes serious damage to the retina.
- **Bruch's membrane** (basal complex) – a thin hyaline membrane separates the choriocapillary layer from the retina. Bruch's membrane extends from the optic papilla to the ora serrata.

CILIARY BODY

- **Ciliary processes**

The ciliary processes are ridge-like extensions of the ciliary body. They have a loose connective tissue core and numerous fenestrated capillaries. They are covered by the two simple epithelial layers (ciliary epithelium). These cells secrete aqueous humor. This fluid has an inorganic ion composition similar to that of plasma but contains less than 0.1% protein (plasma has about 7% protein).

- **Suspensory ligament** of the lens – begin from the ciliary processes to the capsule of the lens and anchors it in place. Importance:
  - Controls lens shape.
  - Focusing on close objects.

IRIS

The iris is a colored part of eye. It is an extension of the choroid that partially covers the lens, leaving a round opening in the center called the **pupil** (*controls amount of light*). The anterior surface of the iris is irregular and rough, with grooves and ridges. It is formed from a discontinuous layer of pigment cells and fibroblasts. Beneath this layer is a poorly vascularized connective tissue with few fibers and many fibroblasts and melanocytes. The next layer is rich in blood vessels embedded in loose connective tissue. The smooth posterior surface of the iris is covered by two layers of epithelium, which also cover the ciliary body and its processes. The inner epithelium, in contact with the posterior chamber, is heavily pigmented with melanin granules. There are also a few strands of smooth muscle in the loose connective tissue that form the core of the iris.

The melanocytes of the stroma of the iris are responsible for the color of the eyes. If the layer of pigment in the interior region of the iris consists of only a few

cells, the light reflected from the black pigment epithelium in the posterior surface of the iris will be blue. As the amount of pigment increases, the iris assumes various shades of greenish-blue, gray, and finally brown. Albinos have almost no pigment, and the pink color of their irises is due to the reflection of incident light from the blood vessels of the iris.

The function of the iris is to **control light** input to the retina, exactly the way the aperture diaphragm of a camera does. It's capable of closing or opening the hole in it–the pupil–by contraction of smooth muscles that form two groups: ***radial dilators and peripheral sphincters.***

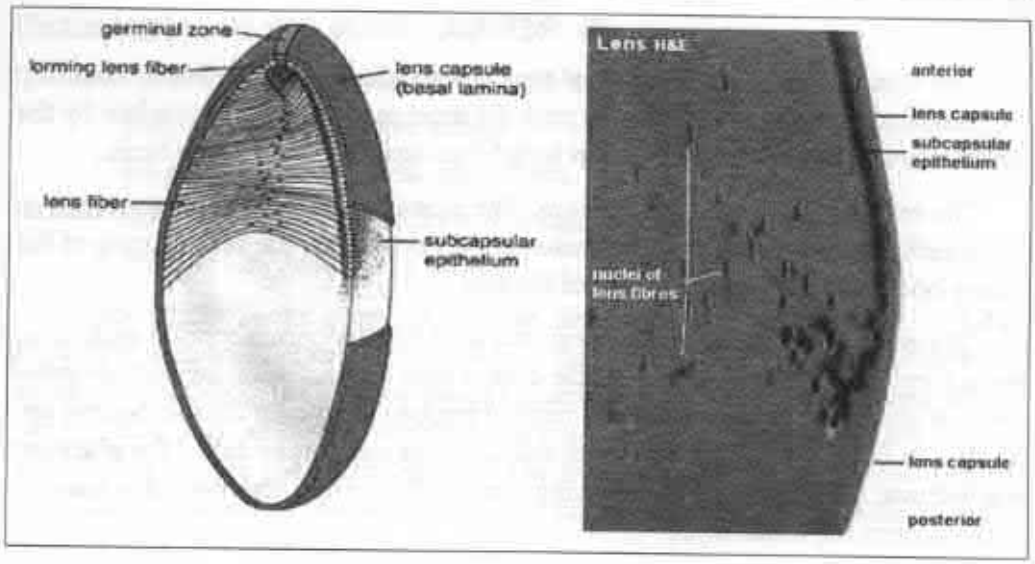
- Contraction of a set of dilator muscles (Dilator MM) disposed fibers running radially away from the pupil opens it when light levels are low. An apposed sphincter muscle, forming a ring around the opening (Sphincter MM) does exactly the opposite: it contracts to close the pupil in bright light.
- The contraction of these muscles in response to light is mediated through the autonomic nervous system.
- Though complete adjustment of vision to low levels of light is physiologic and takes a few minutes, the **pupillary reflex** is almost instantaneous.

Note: If you've been through an ophthalmologic examination (or if you've done one) you will know that when the doctor examines the retina, he uses a bright light to illuminate it. Naturally this would trigger closure of the pupil; so he prevents it. Flooding the eye with an agent that blocks neurotransmitter receptor sites on the muscle prevents contraction of the sphincter.

LENS

The lens is a transparent, avascular, biconvex structure characterized by great elasticity, a feature that is lost with age as the lens hardens. The lens has three principal components.

1. **Lens capsule.** The lens is enveloped by a thick, homogeneous, refractile, carbohydrate-rich capsule coating the outer surface of the epithelial cells. It is a very thick basement membrane and consists mainly of collagen type IV and glycoprotein.
2. **Subcapsular epithelium** that is present only on the anterior surface. Subcapsular epithelium consists of a single layer of cuboidal epithelial cells. The subcapsular epithelium secretes the lens capsule. The lens increases in size and grows throughout life as new lens fibers develop from cells located at the equator of the lens. The cells of this epithelium exhibit many interdigitations with the lens fibers.



The structure of lens

3. **Lens fibers** that are filled with crystallins. Lens fibers are elongated and appear as thin, flattened structures. They are highly differentiated cells derived from cells of the subcapsular epithelium. Lens fibers eventually lose their nuclei and other organelles and become greatly elongated.

- *Loss of transparency of the lens* – **CATARACT**.

VITREOUS HUMOR

The vitreous body occupies the region of the eye behind the lens. It is jelly-like substance that consists of water (about 99%), a small amount of collagen, and heavily hydrated hyaluronic acid molecules.

Function:

- Helps maintain globe shape.
- Helps maintain intraocular pressure (IOP).

CHAMBERS OF THE EYE

There are 2 chambers: **anterior and posterior.**

Anterior chamber is located between cornea and iris, and is bounded by the cornea on one side and the iris on the other; behind it sits the lens.

The lens separates the **posterior chamber** (between iris and lens) from the much larger vitreous chamber. Fluid produced in the posterior chamber flows through the pupil to the anterior chamber and is drained away.

III. RETINA

The retina is a complex array of neural and glial elements. It's structurally an extension of the brain, at least in part. Its innermost surface is overlain by the vitreous body; its outermost portion is right up against the vascular tunic.

The retina consists of two portions. The posterior portion is photosensitive; the anterior part, which is not photosensitive, constitutes the inner lining of the ciliary body and the posterior part of the iris.

The retina derives from an evagination of the anterior cephalic vesicle, or prosencephalon. As this **optic vesicle** comes into contact with the surface ectoderm, it gradually invaginates in its central region, forming a double-walled **optic cup.** In adults, the outer wall gives rise to a thin membrane called the **pigment epithelium;** the optical or functioning part of the retina "**the neural retina**» is derived from the inner layer.

The retina has an inverted structure, for the light will first cross the ganglion layer and then the bipolar layer to reach the rods and cones.

Cell types of retina

- Photoreceptor cells **with rods & cones.**
- Conducting neurons (**bipolar & ganglion neurons**).
- **Associative neurons** (horizontal & amacrine).
- **Supporting cells** (Muller's cells & neuroglial cells).

Photoreceptor cells

The rods and cones, named for the forms they assume, are polarized neurons. They have three regions:

1. a single photosensitive **dendrite** which can be divided into outer and inner segments. The outer segments are modified cilia and contain stacks of membrane-limited saccules with a flattened, disk-like shape. The photosensitive pigment of the retina is in the membranes of these saccules. The inner segment contains organelles for synthesis of pigment of the outer segment. **Conclusion**: the outer segment is the site of photosensitivity; the inner segment contains the metabolic machinery necessary for the biosynthetic and energy-producing processes of these cells.
2. **body** of cell (nuclear region).
3. **axon** makes synapses with dendrite of a bipolar neuron.

Photoreceptor cells with **RODS**

Rods cells are the most numerous (120 million) cells.

- They are more sensitive to light.
- Responsible to darkness adaptation.
- Have low resolution and form images without clear details.
- Provide Gray and fuzzy vision.

Discs of the outer segments of the dendrites contain pigment – **RHODOPSIN,** which is located in the outer surface of the lipid bilayer of the flattened membranous disks. The **outer segment** is separated from the **inner segment** by a constriction. Just below this constriction is a basal segment from which a cilium arises and passes to the outer segment. The inner segment is rich in glycogen and has a large amount of ribosomes, a remarkable accumulation of mitochondria, most of which lie near the constriction.

Photoreceptor cells with **CONES**

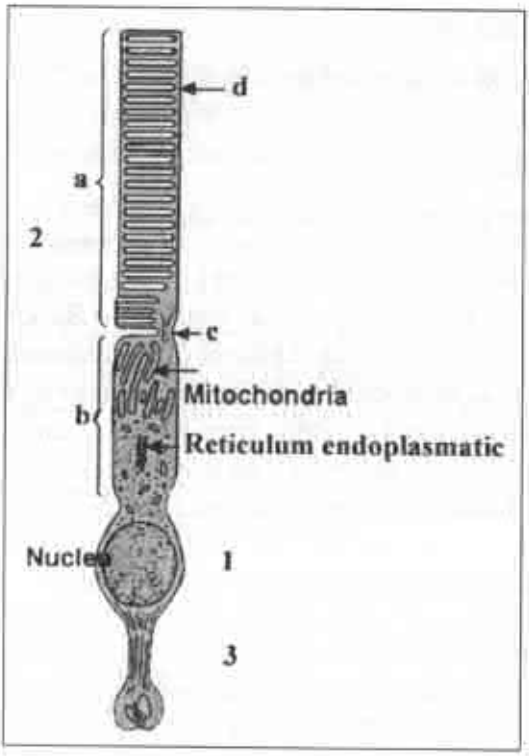
Cone cells are also elongated neurons. Each human retina has about 6 million cone cells. The structure is similar to that of rods, with outer and inner segments, a basal body with cilium, and an accumulation of mitochondria and polyribosomes.

- Have higher resolution (form images with clear details).
- Responsible for color sensitiveness.
- Provides acute vision (daily vision).
- High concentration of cones is in the **fovea** (site with high level of vision).

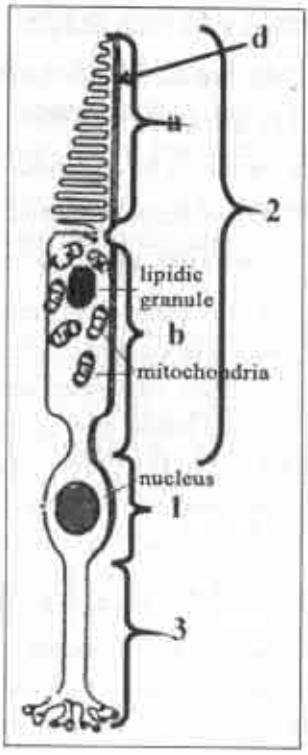
Hemidiscs of the outer segment of dendrites contain pigment – **IODOPSIN.**

Cones differ from rods in their form (conical) and the structure of their outer segments. Newly synthesized protein is not concentrated in recently assembled hemidisks, as it is in rods, but is distributed uniformly throughout the outer segment. The inner segment of dendrite contains **elipsoid** – a complex formed by mitochondria and droplets of lipid.

There are at least three functional types of cones that cannot be distinguished by their morphological characteristics. Each type contains a variety of the cone iodopsin, and its maximum sensitivity is in the red, green, or blue region of the visible spectrum.



Structure of the photoreceptor cell with rod
1 – cell body
2 – dendrite
a – outer segment
b – inner segment
c – connecting stalk
3 – axon
d – cell membrane



Structure of the photoreceptor cell with cone
1 – cell body
2 – dendrite
a – outer segment
b – inner segment
c – connecting stalk
3 – axon
d – cell membrane

Conducting neurons and **Associative neurons**

– **Bipolar neurons** establish synaptic connections with the axons of photoreceptor cells, and the dendrites of ganglion cells. The layer of bipolar cells consists of two types of cells: **diffuse bipolar cells,** which have synapses with two or more photoreceptors; and **monosynaptic bipolar cells,** which establish contact with the axon of only one cone photoreceptor and only one ganglion cell. Certain numbers of cones therefore transmit their impulses directly to the brain.

- **Horizontal cells** establish contact between different photoreceptors. Their exact function is not known, but they may act to integrate stimuli. They also establish synapses with bipolar neurons.
- **Amacrine cells** are various types of neurons that establish contact between the ganglion cells and bipolar cells. Their function is also obscure.
- **Ganglion cells** are multipolar neurons that establish contacts with the bipolar neurons. They contain a large euchromatic nucleus and basophilic Nissl bodies. The ganglion cells project their axons to a specific region of the retina, where they come together to form the **optic nerve**. This region, which is devoid of receptors, is known as the **blind spot** of the retina, the **papilla of the optic nerve,** or the **optic nerve head.**

Supporting cells

Supporting cells of the retina are neuroglia. Supporting cells are represented by: astrocyte, microglial cell types, and by som large, extensively ramified cells, called **Muller's cells.** The processes of these cells bind the neural cells of the retina and extend from the internal to the external limiting membranes of the retina. The external limiting membrane is a zone of adhesion (tight junctions) between photoreceptors and Muller's cells. Muller's cells provide support, nourish, and insulate the retinal neurons and fibers.

The specific arrangement and associations of the nuclei and processes of all these cells result in the retina being organized in 10 layers *(see fig. 77, plate II)*:

1. Outer pigmented layer.

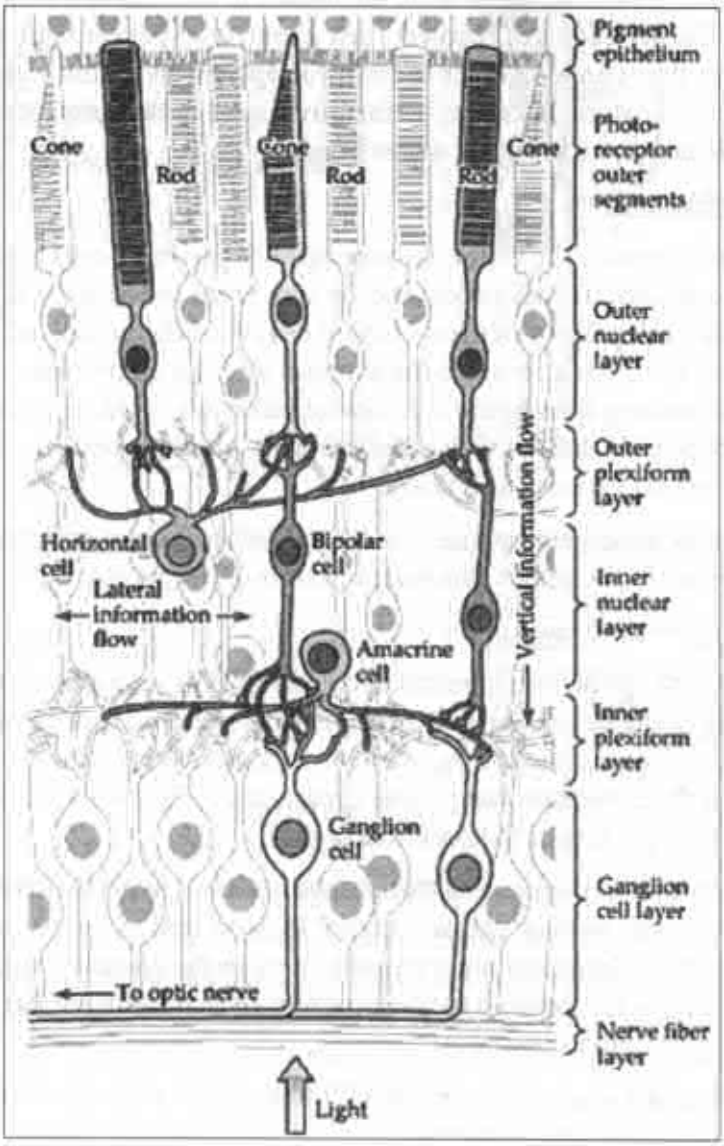
The pigment epithelium consists of columnar cells with a basal nucleus. The basal regions of the cells adhere firmly to Bruch's membrane, and the cell membranes have numerous basal invaginations. Mitochondria are more abundant in the region of the cytoplasm near these invaginations. These characteristics suggest an ion-transporting activity for this region.

The cytoplasm of pigment epithelial cells has abundant smooth endoplasmic reticulum, believed to be a site of vitamin A esterification and transport to the photoreceptors. Melanin granules are numerous in the apical cytoplasm and microvilli. Melanin is synthesized in these cells by a mechanism similar to that from the melanocytes in the skin.

The cell apex has abundant microvilli that envelop the tips of the photoreceptors.

Functions:

- Absorbs light
- Prevents light scatter
- Supports neural retina
- Phagocytosis of the used-up rod and cone material (which is replaced) and participations in the cycle by which visual pigments are formed.



Schematic drawing of the retina

2. **Layer of rods and cones** contains the dendrites of the rod and cone cells.
3. **The outer limiting membrane** – isn't a membrane; it's a region of occluding junctions, between Müller cells and the "waists" of the rod and cone cells.
4. **The outer nuclear layer** (ONL) – reside the soma of the rods and cones.
5. **The outer plexiform layer** (OPL) contains the dendrites of the bipolar cells, and their synapses with the axons of rod and cone cells.
6. **The inner nuclear layer** (INL) comprises cell bodies of the bipolar neurons. Assosiative neurons (the horizontal and amacrine cells) also have their bodies in the inner nuclear layer.
7. **The inner plexiform layer** (IPL) contains the dendrites of the ganglion cells and their synapses with the axons of bipolar cells.
8. **The ganglion cell layer** (GCL) is formed by the cell bodies of ganglion cells. The ganglion cells are the multipolar neurons and are the final neuron in the chain that sends information to the visual nucleus.
9. **The nerve fiber layer** (NFL) is formed by the axons of ganglion cells All unmyelinated nerves fiber together form the optic nerve.
10. **The inner limiting membrane** (ILM) is innermost limit of the retina. This isn't a membrane. It's the fused feet of the Müller cells, the retina's glial element.

Accessory Structures of the Eye

CONJUNCTIVA

The conjunctiva is a thin, transparent mucous membrane that covers the anterior portion of the eye up to the cornea and the internal surface of the eyelids. It has a stratified columnar epithelium with numerous goblet cells, and its lamina propria is composed of loose connective tissue.

EYELID

The inner surface is lined with conjunctival epithelium, specifically the tarsal conjunctiva. The outer surface is thin integument, with typical integumental adnexa, such as hairs and sebaceous glands. The conjunctival surface is moist. The secretions of its goblet cells and tears prevent the surface of the cornea from drying out. They also control bacterial proliferation.

There are three types of glands in the lid:

- The **Meibomian glands** are long sebaceous glands in the tarsal plate. They do not communicate with the hair follicles. The Meibomian glands produce a sebaceous substance that creates an oily layer on the surface of

the tear film, helping to prevent rapid evaporation of the normal tear layer. *If you've ever awakened the morning after a wild party with your eyes sort of glued shut by a crusty material, that's the secretion of this gland.*

- The **glands of Zeis** are smaller, modified sebaceous glands connected to the follicles of the eyelashes.
- The sweat **glands of Moll** are unbranched sinuous tubules that begin in a simple spiral and not in a glomerulus like ordinary sweat glands. They empty their secretion into the follicles of the eyelashes.

LACHRYMAL GLANDS

The lacrimal glands are classified structurally as **compound tubuloacinar**, and in terms of their secretion, they **are serous**. Their secretion–***tears***–lubricates the surface of the eyeball and the conjunctival pocket.

- Tears are produced continuously, to keep the surface of the eye from drying out, and also to trap dust particles and other airborne material on a film of liquid. The "windshield wiper" action of the eyelids during a blink then can sweep the surface clean every few seconds.
- Tears also have an antibacterial action, one of their components being lysozyme.

THE EAR

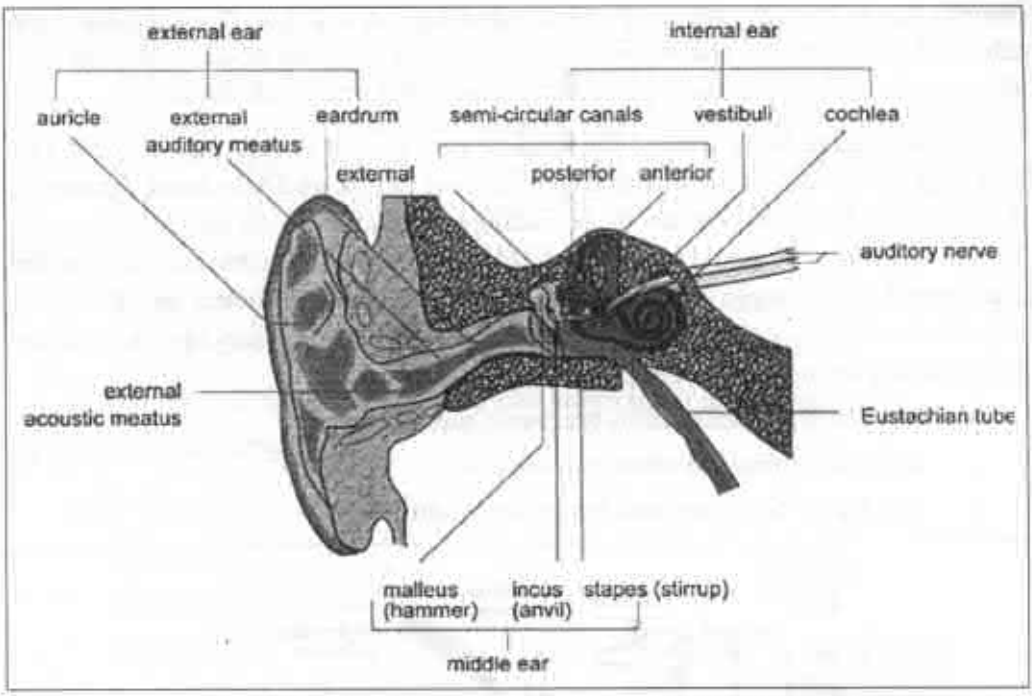
- is an extraordinarily complex organ with two functions:
  - **sound reception** and
  - **maintenance of positional equilibrium**.

The ear has three distinct parts:

- **the OUTER EAR** which receives sound waves;
- **the MIDDLE EAR** in which sound waves are transmitted from air to bone and by bone to the internal ear;
- **the INNER EAR** in which these vibrations are transduced to specific nerve impulses that pass via the acoustic nerve to the central nervous system. The internal ear also contains the vestibular organ, which maintains equilibrium.

OUTER EAR includes:

- **AURICLE or PINNA** (the visible ear) – collects sound waves that are conducted across the external acoustic meatus to the tympanic mem-



The structural components of the external, middle and internal ear

brane.The auricle consists of a core of elastic cartilage surrounded by perichondrium. From outside the cartilage is covered by thin skin layer with hair follicles and sebaceous glands.

- **EXTERNAL AUDITORY CANAL** – the outer 1/3 is cartilage, the inner 2/3 is part of the temporal bone. It is lined by skin (stratified squamous keratinized epithelium) that has hair and ceruminous **glands** (a type of modified apocrine sweat glands) secreting a brown product called cerumen. Hairs and cerumen probably have a protective function.
- **TYMPANIC MEMBRANE** (Eardrum) – is a thin membrane separating the outer ear from the middle ear. It is sandwich of tissues, with keratinized stratified squamous epithelium facing the outer ear, simple squamous epithelium facing the middle ear, and a very thin layer of connective tissue in between.

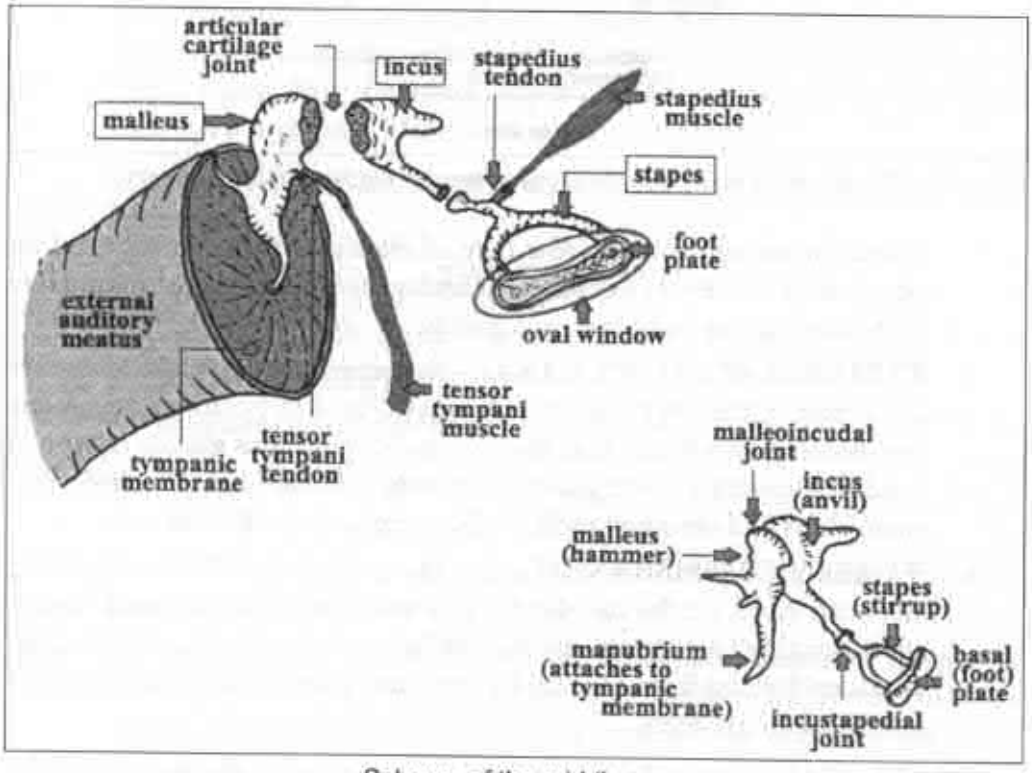
MIDDLE EAR is basically a space, communicating via the eustacian tube with oropharynx.

It is located within petrous temporal bone. The middle ear is lined by simple squamous epithelium to simple cuboidal epithelium resting on a thin lamina

propria that is strongly adherent to the subjacent periosteum. Near the auditory tube and in its interior, the simple epithelium that lines the middle ear is gradually transformed into ciliated pseudostratified columnar epithelium.

In the medial bony wall of the middle ear are two membrane-covered oblong regions devoid of bone; these are the **oval** and **round windows.** Spanning the space of the middle ear are three middle ear ossicles (malleus, incus, stapes). These bones are articulated by synovial joints and, like all structures of this cavity, are covered with simple squamous epithelium. In the middle ear are two small muscles that insert themselves into the malleus and stapes. They have a function in regulating sound conduction.

- **MALLEUS** – attached to tympanic membrane.
- **INCUS** – joined to other two ossicles.
- **STAPES** – footplate attached to oval window.



Scheme of the middle ear

Function:

- Transmit sound vibrations from tympanic membrane to inner ear
- Amplify vibrations

PHARYNGOTYMPANIC TUBE (auditory/Eustachian tube)

- Extend from middle ear to pharynx.
- The walls of the Eustachian tube are usually collapsed, but they are separated during the process of swallowing, thus balancing the pressure of the air in the middle ear with the atmospheric pressure.

INNER EAR is composed of 2 labyrinths.

- **Bony labyrinth** consists of series of cavities in petrous temporal bone.
- **Membranous labyrinth** located within bony labyrinth

It's necessary to remember that the one labyrinth is inside the other!

BONY LABYRINTH

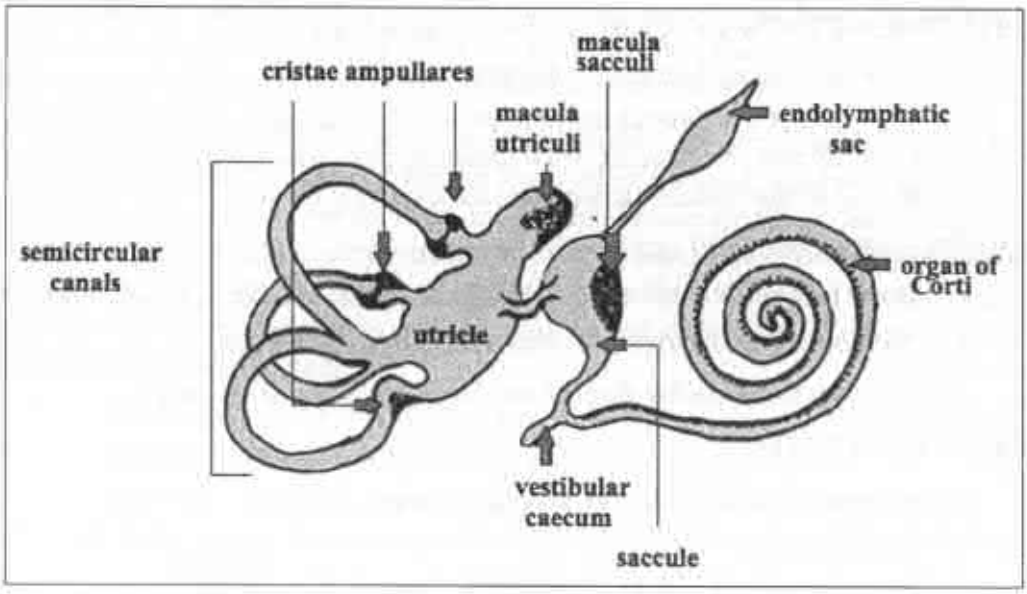
Bony labyrinth is divisible into several regions:

- the vestibule,
- the semicircular canals,
- the cochlea.
  - The first two contain those parts of the membranous labyrinth that are involved in the **balance sense**.
  - The last part contains the portion of the membranous labyrinth that is involved in **hearing perception.**

The vestibule, cochlea, and semicircular ducts all are connected!

MEMBRANOUS LABYRINTH is a continuous epithelium-lined series of cavities of ectodermal origin. It derives from the auditory vesicle that is developed from the ectoderm of the lateral part of the embryo's head. During embryonic development, this vesicle invaginates into the subjacent connective tissue, loses contact with the cephalic ectoderm, and moves deeply into the rudiments of the future temporal bone. During this process, it undergoes a complex series of changes in form, giving rise to two specialized regions of the membranous labyrinth: **utricle and saccule. Semicircular ducts** originate from the utricle, whereas the elaborate **cochlear duct** is formed from the saccule. In each of these areas, the epithelial lining becomes specialized to form sensory structures such as the **maculae** of the utricle and saccule, the **cristae** of the semicircular ducts, and the **organ of Corti** of the cochlear duct.

The bony labyrinth is filled with **perilymph,** which is similar in ionic composition to extracellular fluids elsewhere but has very low protein content. The main cation is sodium.



Membranous labyrinth of the ear

The membranous labyrinth contains **endolymph,** which is characterized by its low sodium and high potassium content. The protein concentration in endolymph is low. Cochelear endolymph is also unique. It has extremely low calcium content. It is held at a positive voltage with respect to perilymph, of approximately 85 mV. This is called the **endocochlear potential**. Both the low Ca level and endocochlear potential are extremely important for the cochlea to function normally. Even small changes from the normal state result in a decrease of hearing sensitivity.

COCHLEA

- Is a spiral canal that winds more than 2.5 times around a central bony axis, the **MODIOLUS**.
- The spiralling tunnel that forms the cochlea of the bony labyrinth is divided into three spiraling channels by a portions of the membranous labyrinth attached to bony ridges:
  - The **COCHLEAR DUCT** (scala media) represents the central chamber and contains endolymph.
  - The **SCALA VESTIBULI**, starting at the oval window.
  - The **SCALA TYMPANI**, ending at the round window.

The scalea vestibuli and tympani are filled with PERILYMPH and communicate at the apex of the cochlea, the **HELICOTREMA** *(see fig. 71, plate II)*.

COCHLEAR DUCT contains **endolymph** and has the following histological structures:

I. **Vestibular membrane** (Reissner's membrane). The **vestibular membrane** consists of two layers of squamous epithelium, one derived from the scala media and the other from the lining of the scala vestibuli. Cells of both layers are joined by means of extensive tight junctions that help preserve the very high ionic gradients across this membrane.

II. **Basilar membrane** with basement lamina extends across the cochlear canal from spiral lamina to the spiral ligament. It separates the scala media from the scala tympani and supports the Organ of Corti.

Basilar membrane has 2 zones:

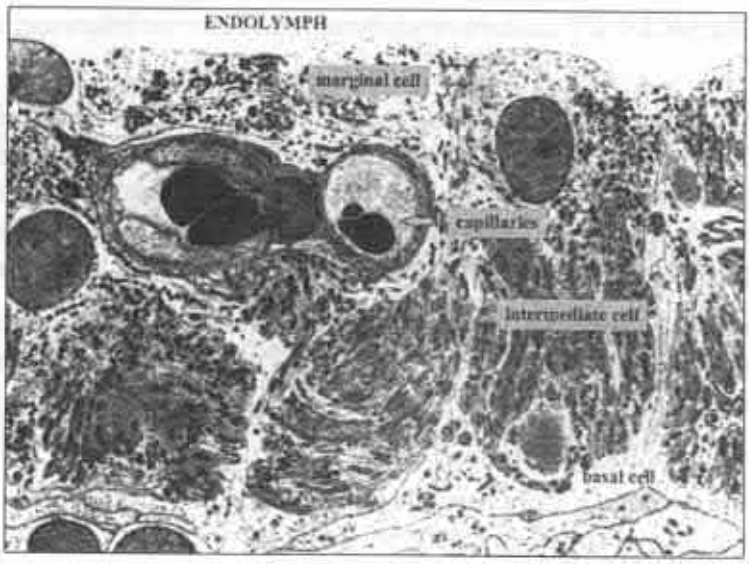
- **Zona arcuata** = thin, extends from medial attachment to base of outer most cells of organ of Corti. Supports the organ of Corti, with radially oriented 10 nm collagen like fibrils.
- **Zona pectinata** = thicker, from organ of Corti to spiral ligament; trilaminar in structure. Upper layer is meshwork of transverse fibers. Lower layer is of longitudinal fibers. In between is structureless intermediate layer of few fibroblasts like cells.

The width of fibers vary from 0.20 mm to 0.36 mm at the apex, the diameter of component fibers gradually decreases → vibrate at higher frequency near base and lower frequency near the Helicotrema, thereby discriminate frequency or pitch of sounds.

III. **Stria vascularis** that is attached to the spiral ligament (dense regular connective tissue). The **stria vascularis** is an unusual vascularized epithelium located in the lateral wall of the cochlear duct. It consists of cells that have many deep infoldings of their basal plasma membranes, where numerous mitochondria are located. These characteristics indicate that they are ion- and water-transporting cells, and it is generally believed that they are responsible for the characteristic ionic composition of endolymph.

Stria vascularis contains three types of cells:

1. **Basal cells** (light staining) contain few mitochondria; their ascending processes form a cuplike structure that partially surround and isolate marginal cells.
2. **Marginal cells** (dark stained) – their convex free surface is covered by microvilli; have dense cytoplasm, many small vesicles and mitochondria.

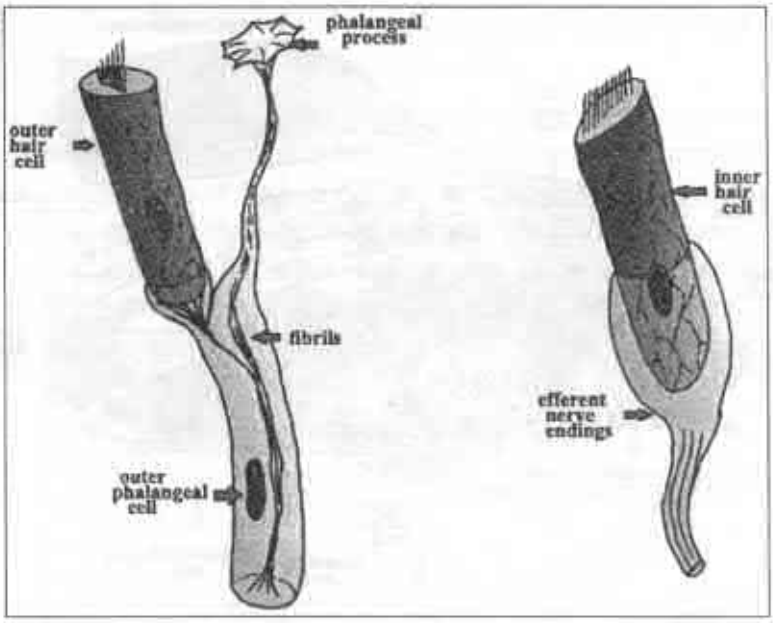


Electron micrograph of a stria vascularis

3. **Intermediate cells** have few mitochondria, their radiating processes interdigitate with marginal cells.

IV. **ORGAN OF CORTI** is the sensory epithelium of the cochlea. It is formed by:

- **HAIR cells** (inner & outer) with steriocilia. Hair cells are columnar epithelial cells. At the apical end of each hair cell are cytoplasmic projections – **stereocilia**, embedded in a tectorial membrane. The most characteristic feature of these cells is the V-shaped (outer hair cells) or linear (inner hair cells) array of stereocilia *(see fig. 73, plate II)*. Three to five rows of **outer hair cells** can be seen, depending on the distance from the base of the organ, and there is a single row of **inner hair cells.** At the basal end of each hair cell are synapses with dendrites of bipolar neurons from spiral ganglion.
- **SUPPORTING cells**: inner & outer (phalangeal cells). They support and surround the basal portion of the sensory cells. **Outer phalangeal cells** of Deiters are the supporting cells for the 3 – 4 rows of outer hair cells. Base is columnar with cup shaped upper end. The apex does not reach the free surface of the organ of Corti. **Inner phalangeal cells** are arranged in a row on the inner side of the inner pillar cells. Contiguous with slender Border cells marking the inner boundary of the organ of corti. Lining epithelium is low cuboidal or squamous cells.



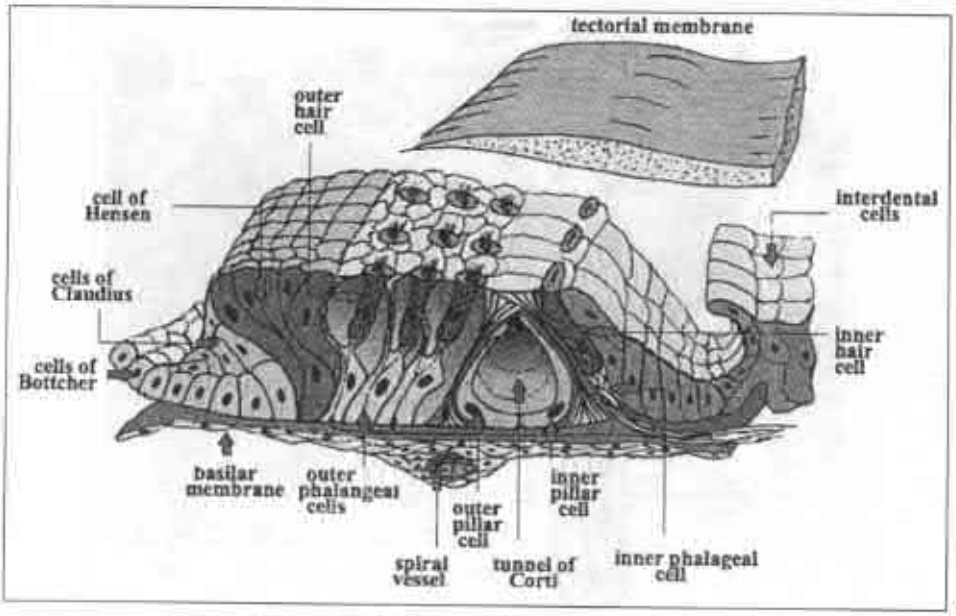
Hair cells and phalangeal cell

Cells of Hensen delimit the outer border of the organ of Corti arrange in rows decreasing in height continuous with the cells of Claudius and cells of Boettcher. **Cells of Claudius** are cuboidal cells at external spiral sulcus. **Cells of Boettcher** form a small group of polyhedral cells found only in basal coil of cochlea.

- **PILLAR** cells: outer & inner. Pillar cells contain a large number of microtubules that seems to impart stiffness to these cells. They outline a triangular space between the outer and inner hair cells – the **inner tunnel**. This structure is important in sound transduction.
- **Tectorial membrane** – an amorphous acidiphilic structure. It is composed of fine filaments embedded in gelatinous matrix rich in mucopolysaccharides. Fibers consist of a protein similar to epidermal keratin *(see fig. 72, plate II)*.

SPIRAL GANGLION *(see fig. 74, plate II)*

- Is housed in the modiolus.
- Is a craniospinal ganglion by virtue of its lacation on the VIIIth cranial nerve, the **vestibulocochlear nerve**.
- The neurons in it are structurally classified as **bipolar** in structure and **sensory** in nature.
- The afferent fibers from these bipolar neurons make up the cochlear branch of the nerve.



General scheme of the structure of the organ of Corti

Histophysiology of hearing

Sound waves impinging on the tympanic membrane set the auditory ossicles into motion. The large difference in area of the tympanic membrane and the footplate of the stapes ensures the efficient transmission of mechanical motion from air to the fluids of the internal ear. Two striated skeletal muscles are in the middle ear: the **tensor tympani** muscle (attached to the malleus) and the **stapedius** muscle (attached to the stapes). Loud sounds cause reflex contractions of these muscles, which limit excursions of the tympanic membrane and the stapes; this helps prevent damage to the internal ear. These reflexes are too slow, however, to guard against sudden loud sounds, such as gunshots.

The following is a step-by-step explanation of how sound waves are converted to electrical impulses in the internal ear. Sound waves are longitudinal waves, with **compression** and **rarefaction** phases. The compression phase causes the stapes to move inward. Because the fluids of the internal ear are almost incompressible, the pressure change is transmitted across the vestibular membrane and the basilar membrane, causing them to be deflected downward toward the scala tympani. This pressure change also causes the covering of the round window to bulge outward, thereby relieving the pressure. Because the tips of the pillar cells form a pivot, downward deflection of the basilar membrane is converted into lateral shearing of the stereocilia of hair cells against the

tectorial membrane. The tips of the stereocilia are deflected toward the modiolus and away from the basal body.

During the rarefaction phase of the sound wave, everything is reversed: The stapes move outward, the basilar membrane moves upward toward the scala vestibuli, and the stereocilia of the hair cells bend toward the stria vascularis and the basal body. Deflection in this direction sets up depolarizing generator potentials in the hair cells, resulting in the release of a neurotransmitter (whose chemical nature is unknown) that causes the production of action potentials in bipolar neurons of the spiral ganglion (**excitation**).

Path of sound

Discrimination between sound frequencies is based on the response of the basilar membrane. The membrane responds to the frequency of sound with different displacement at different points along its length. High frequencies are detected at the basal end of the membrane, whereas low frequencies are detected in the apex of the organ of Corti. This **tonotopic** localization can be correlated with the width and stiffness of the basilar membrane: The narrow basilar membrane, with greater stiffness at the base, responds best to high-frequency sounds.

SEMICIRCULAR DUCTS

The semicircular ducts (and the semicircular canals through which they run) are oriented in three planes of movement:

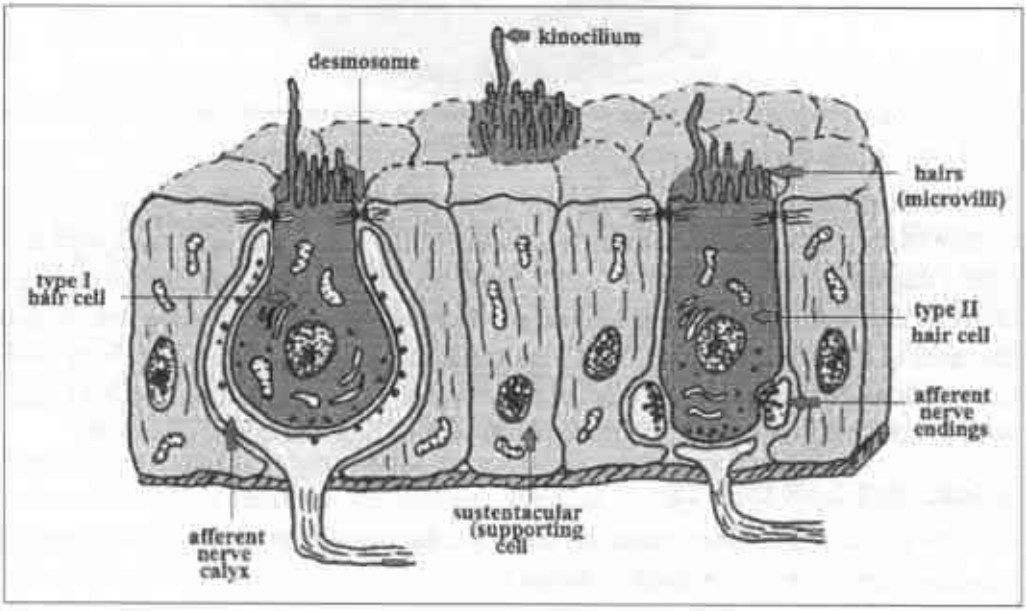
- vertical,
- horizontal,
- anterior-posterior.

Inside the semicircular ducts are elevated areas of epithelium – crista ampullaris.

CRISTA AMPULLARIS

- Resides in the end of each of the semicircular ducts.
- They're bathed in endolymph, and possess 2 types of cell:
  - **Supporting** cells
  - **Hair cells** have on the apical pole: stereocilia and one kinocilium. There are two types of hair cells:

1. **Type I Hair cells** are *flask shaped* cells with a rounded base, narrow neck; form a chalice investing the base. Nucleus is basal, surrounded by mitochondria; with supranuclear *Golgi complex*, occasional *cisternae of RER* & *small vesicles*. These cells have 50-100 sterocilia on free surface. Tallest hair is 10μm near kinocilium & shortest is 1μm on the opposite side. Each *kinocilium* is limited by plasma membrane & with several bundles of *Actin* filaments.
2. **Type II Hair cells** are more columnar; kinocilium, sterocilia, cytoplasmic organelles are similar to type 1. Golgi complex is larger, small vesicles found in great numbers in cytoplasm. The base does not form a calyx but end in small terminal boutons.

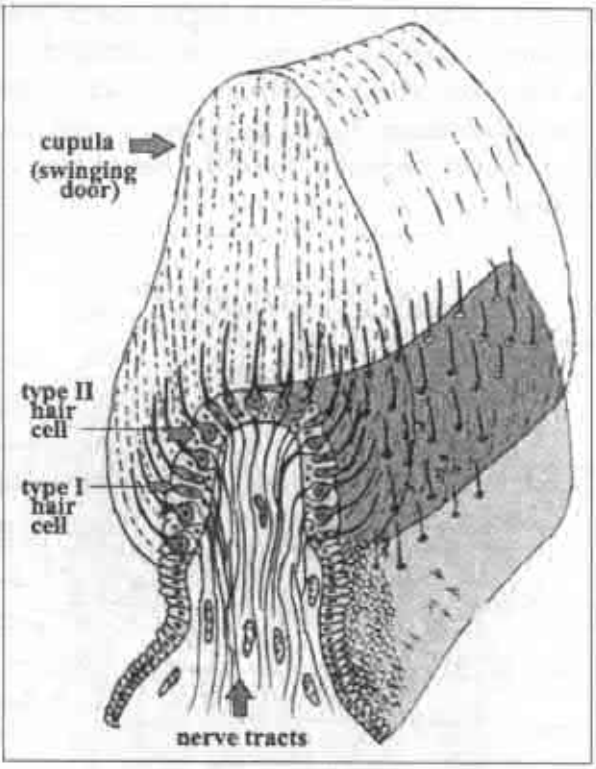


Hair cells of the crista ampullaris

- **CUPULA** is a dome-shaped gelatinous protein-polysaccharide mass. Cupula has a conical form. It swings from side to side in response to

currents in the endolymph that bathes it. Cupula is not covered with otoliths. The cupula extends across the ampullae, establishing contact with its opposite wall.

- Nerve endings surround the cell bodies of hair cells.



Structure of the crista ampullaris

Function: They are structures for the detection of head and body rotational movements (angular accelerations).

Otolith organs of the vestibule: UTRICLE & SACCULE

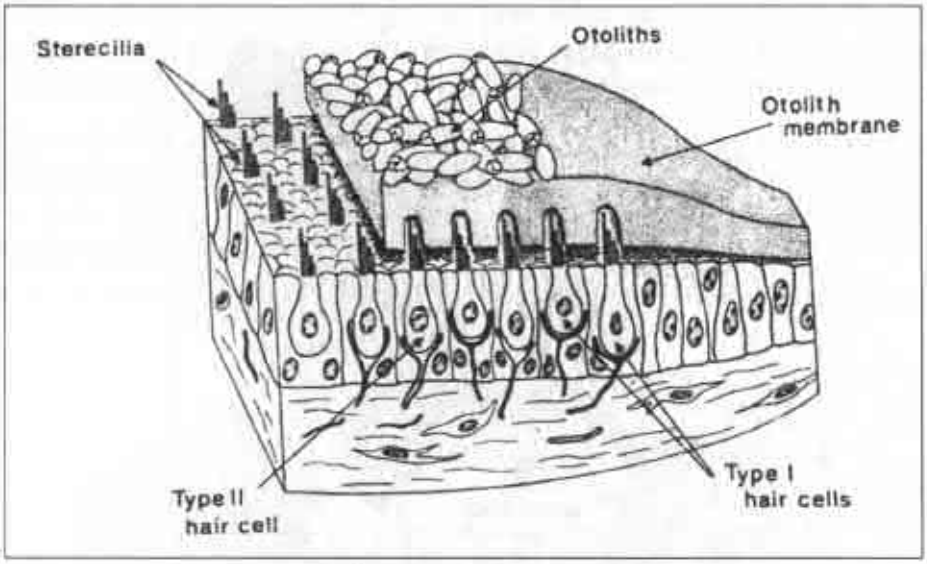
The utricle is a membranous labyrinth, filled with endolymph. The vestibule in which it's suspended is the bony labyrinth, filled with perilymph. The utricle and saccule display a sensory epithelium called the macula. Maculae in both locations have the same basic histological structure.

MACULA is a receptor area designed to detect **Static equilibrium:**

- Gravity
- Linear acceleration

Macula has:

- Supporting cells are disposed between the hair cells; are columnar in shape, with microvilli on the apical surface.
- Hair cells are characterized by the presence of a long, rigid **stereocilia** (are nonmotile), which are actually highly specialized microvilli, and one **kinocilium** (is motile). Stereocilia are arranged in rows of increasing length. There are two types of hair cells, distinguished by the form of their afferent innervation. **Type I cells** have a large, cup-shaped ending surrounding most of the base of the cell, whereas **type II cells** have many afferent endings.



Drawing of a portion of the macula utriculae showing the relation of the otolithic membrane to the hair cells

- Both cell types have efferent nerve endings that are probably inhibitory.
- **OTOLITHIC MEMBRANE** gelatinous glycoprotein layer, probably secreted by the supporting cells, that contains numerous small crystalline bodies:
  - Otoliths (from the Greek "ear stones") – contain calcium carbonate.

Function:

- Mass of otoliths affected by gravity / linear movement
- Bending of hair cells
- Stimulation of nerve endings

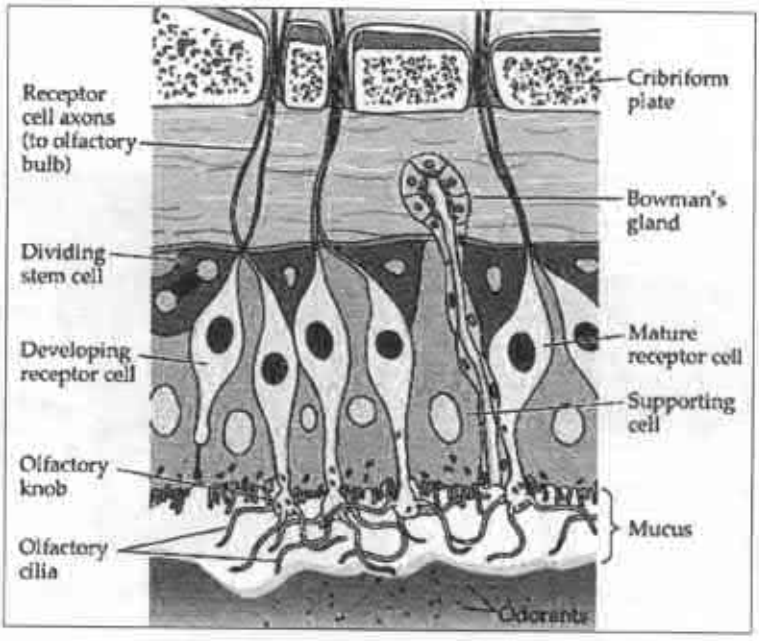
SMELL

The olfactory chemoreceptors are located in the **olfactory epithelium,** a specialized area of the mucous membrane in the superior conchae, located in the roof of the nasal cavity. Axons of sensitive cells pass through cribriform plate to brain.

OLFACTORY EPITHELIUM is pseudostratified columnar ciliated.

Contains 3 types of cells:

- Olfactory receptor cells (bipolar neurons)
- Supporting cells
- Basal cells



Olfactory mucosa showing the three cell types (supporting, olfactory, and basal)and a Bowman's gland

The **supporting cells** have broad, cylindrical apexes and narrower bases. On their free surface are microvilli submerged in a fluid layer. Well-developed junctional complexes bind the supporting cells to the adjacent olfactory cells. The supporting cells contain a light yellow pigment that is responsible for the color of the olfactory mucosa.

The **basal cells** are small; they are spherical or cone-shaped, and form a single layer at the base of the epithelium. They have regenerative function.

Between the basal cells and the supporting cells are the **olfactory cells** distinguished from the supporting cells by the position of their nuclei, which lie below the nuclei of the supporting cells. Their apexes (dendrites) possess elevated and dilated areas from which arise six to eight cilia. These cilia are very long and nonmotile, and respond to odoriferous substances by generating a receptor potential. The cilia increase the receptor surface considerably. The afferent axons of these bipolar neurons unite in small bundles directed toward the brain, where they synapse with neurons of the brain **olfactory lobe.**

The lamina propria of the olfactory epithelium possesses the glands of Bowman. Their secretion produces a fluid environment around the olfactory cilia that may clear the cilia, facilitating the access of new odoriferous substances.

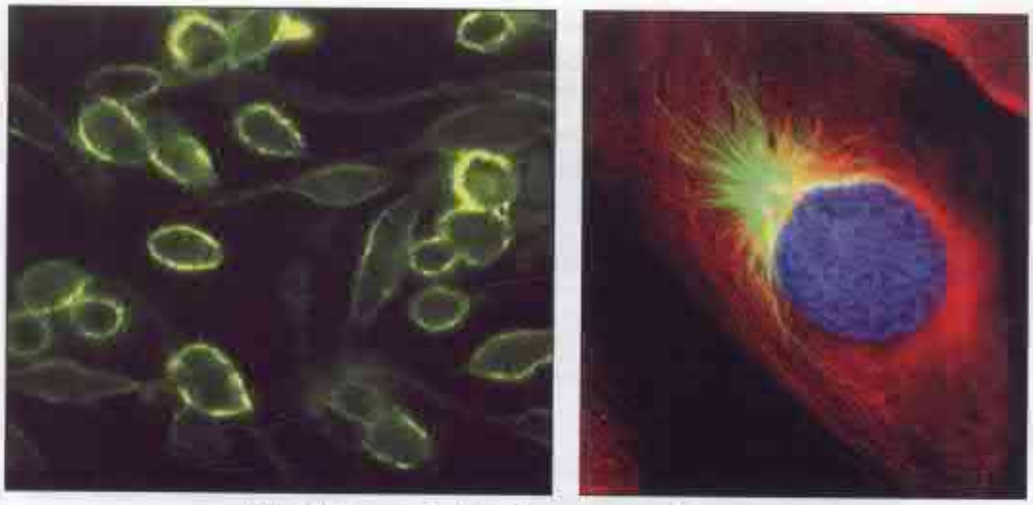


Fig. 1. Images of cells in fluorescence microscopy

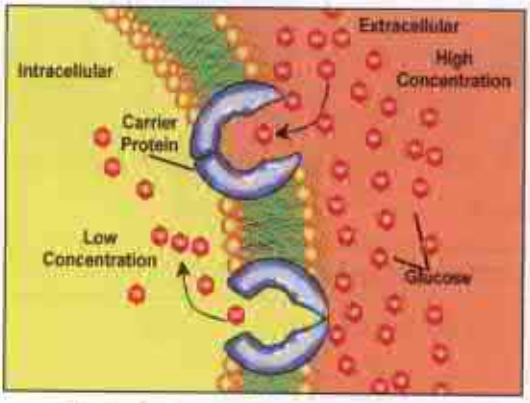


Fig. 2. Facilitated diffusion for glucose

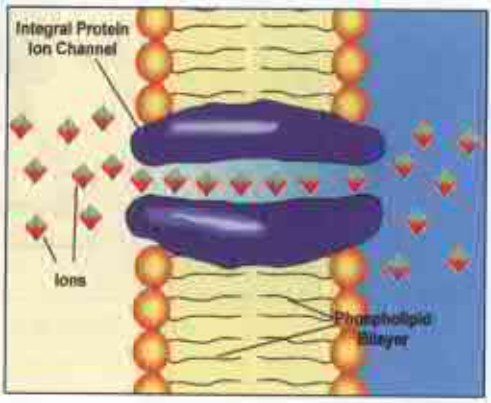


Fig. 3. Diffusion via a channel

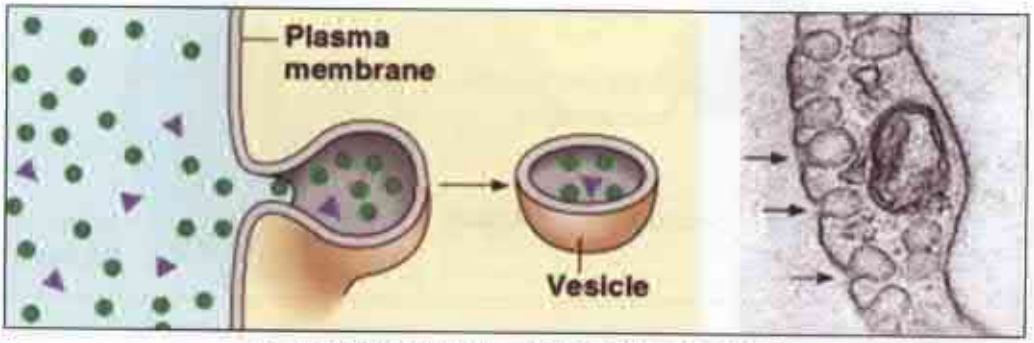


Fig. 4. Schematic representation of the pinocytosis

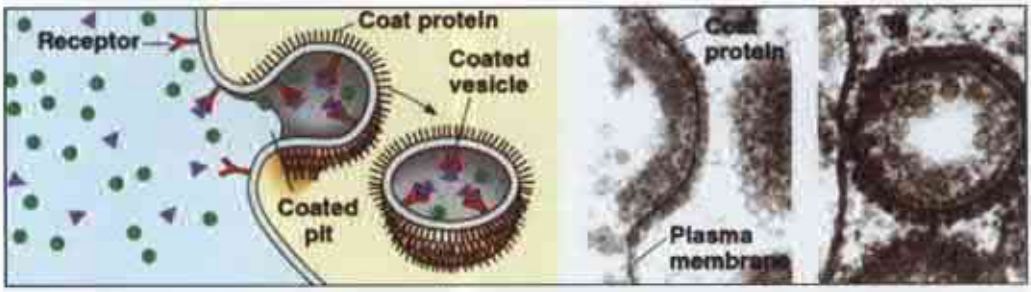


Fig. 5. Schematic representation of the receptor-mediated endocytosis. Ligands, such as hormones and growth factors, bind to specific surface receptors and are internalized in pinocytotic vesicles coated with clathrin and other proteins.

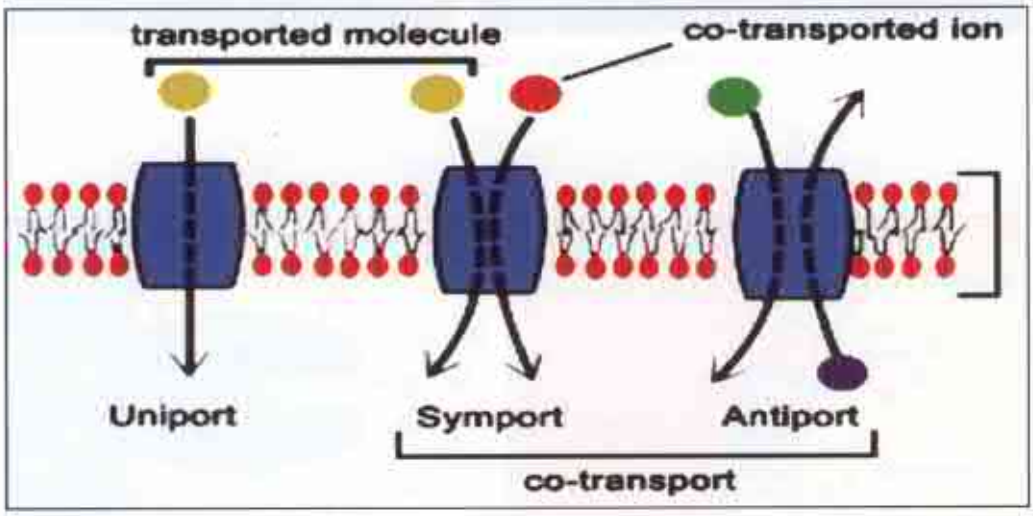


Fig. 6. Scheme of uniport and cotransport

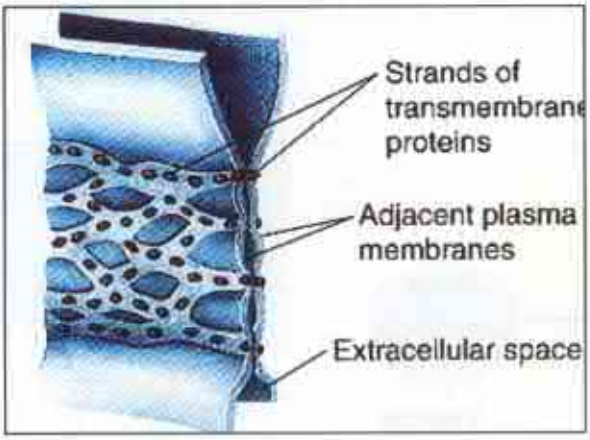


Fig. 7. Schematic drawing of tight junction

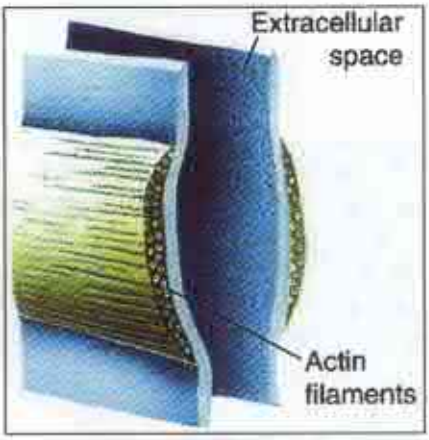


Fig. 8. Schematic drawing of zonula adherens

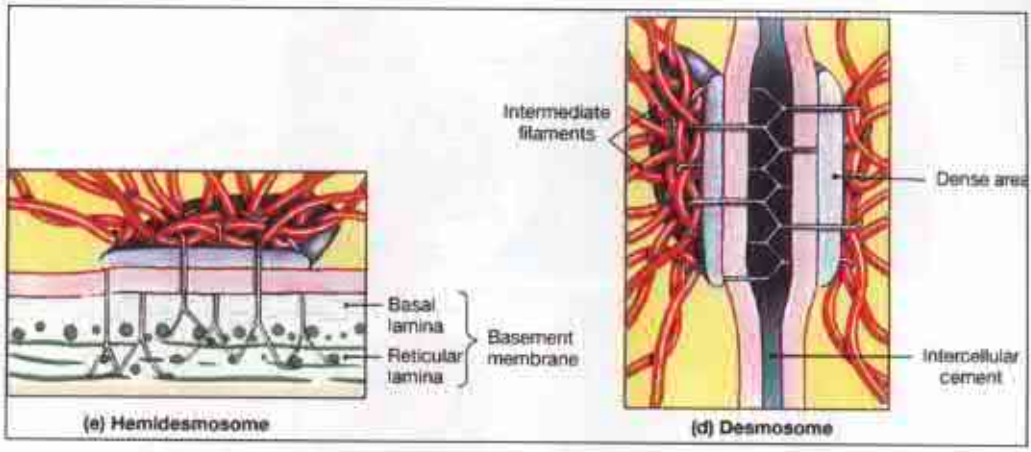


Fig. 9. Schematic drawing of hemidemosome (a) and desmosome (b)

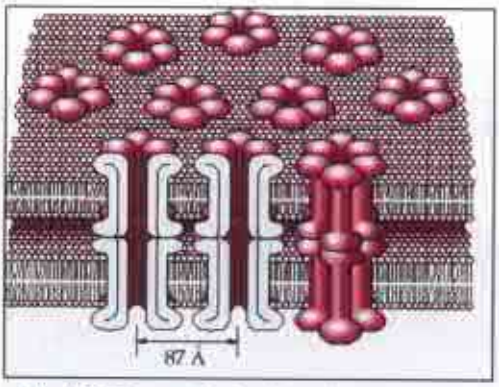


Fig. 10. Schematic drawing of gap junctions

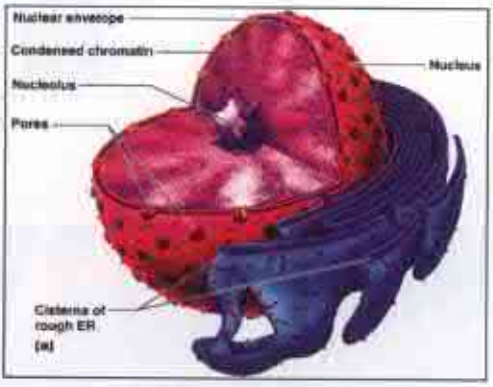


Fig. 11. Schematic representation of a cell nucleus

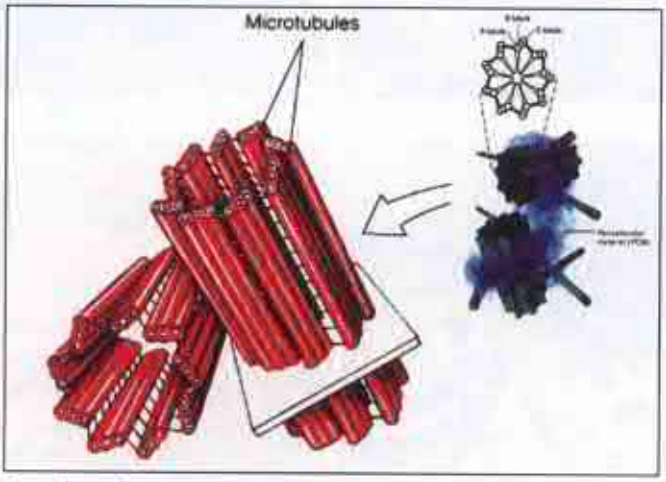


Fig. 12. Drawing of a centrosome with its granular protein material surrounding a pair of centrioles, one shown at a right angle to the other. Each centriole is composed of nine bundles of microtubules, with three microtubules per bundle.

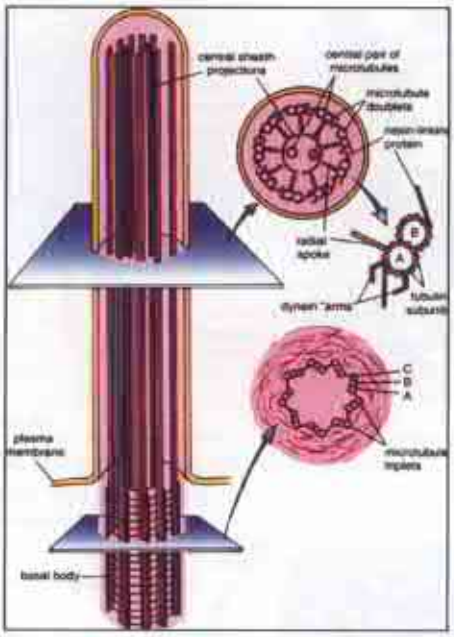


Fig. 13. Schematic representation of ultrastucture of cilia and flagella

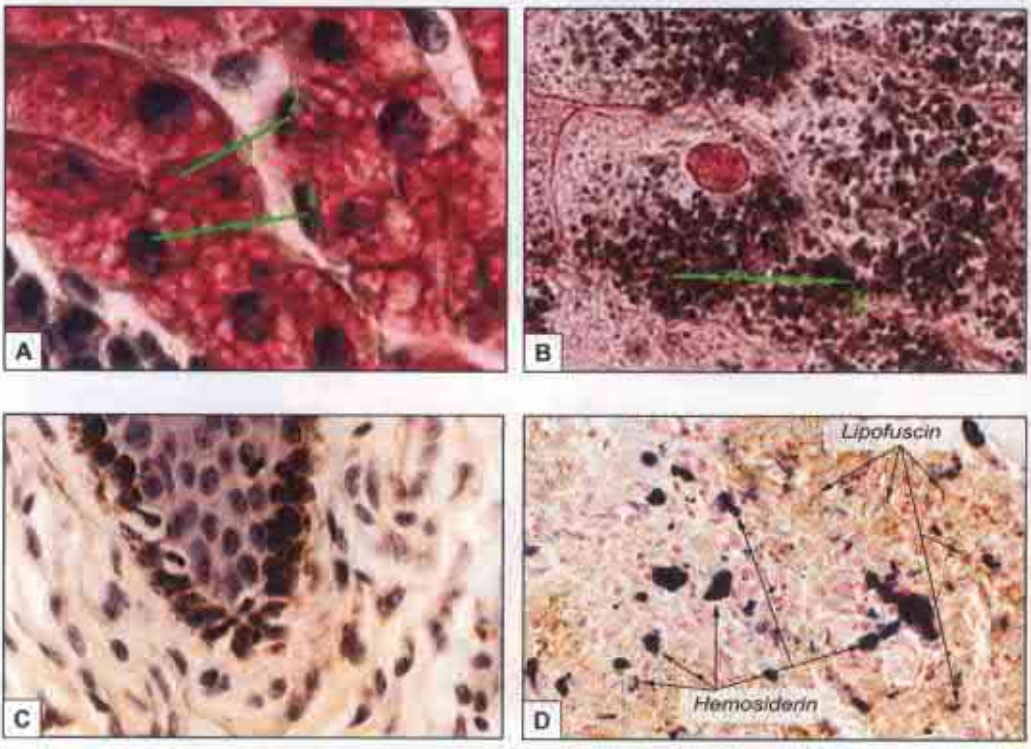


Fig. 14. Light micrographs of cellular inclusions
(A – glycogen inclusion; B – lipid inclusion; C – melanin inclusion; D – lipofuscin and hemosiderin inclusion)

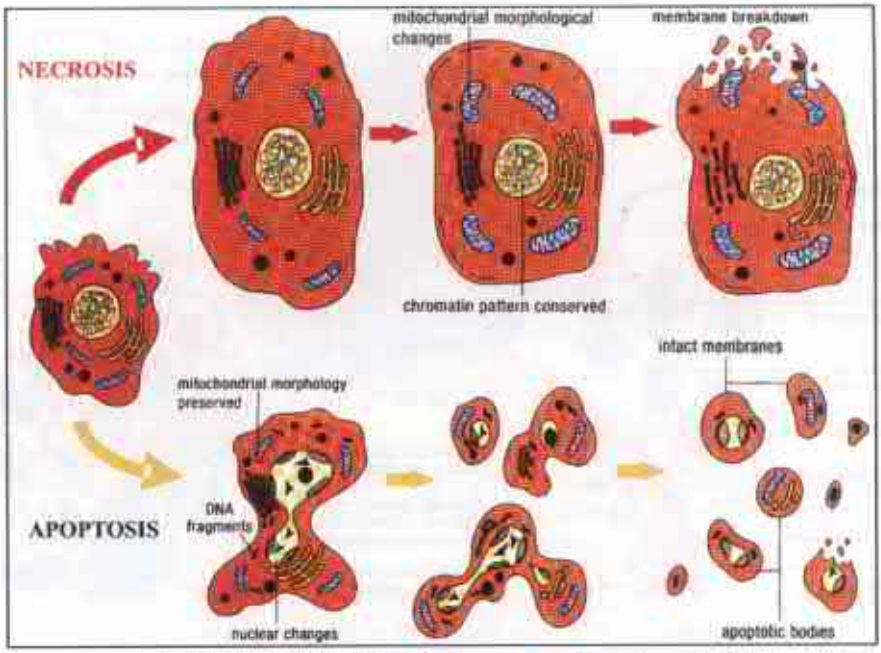


Fig. 15. Two cell death pathways, necrosis and apoptosis

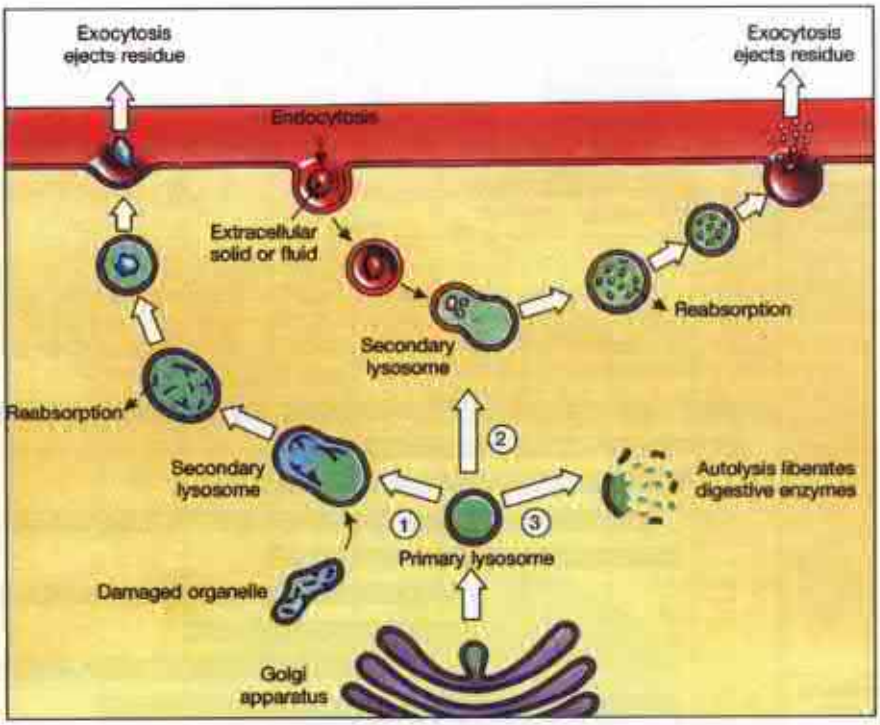


Fig. 16. Current concepts of the functions of lysosomes. Note the heterophagosomes, in which bacteria are being destroyed, the autophagosomes, with mitochondria in the process of digestion, and the autolysis.

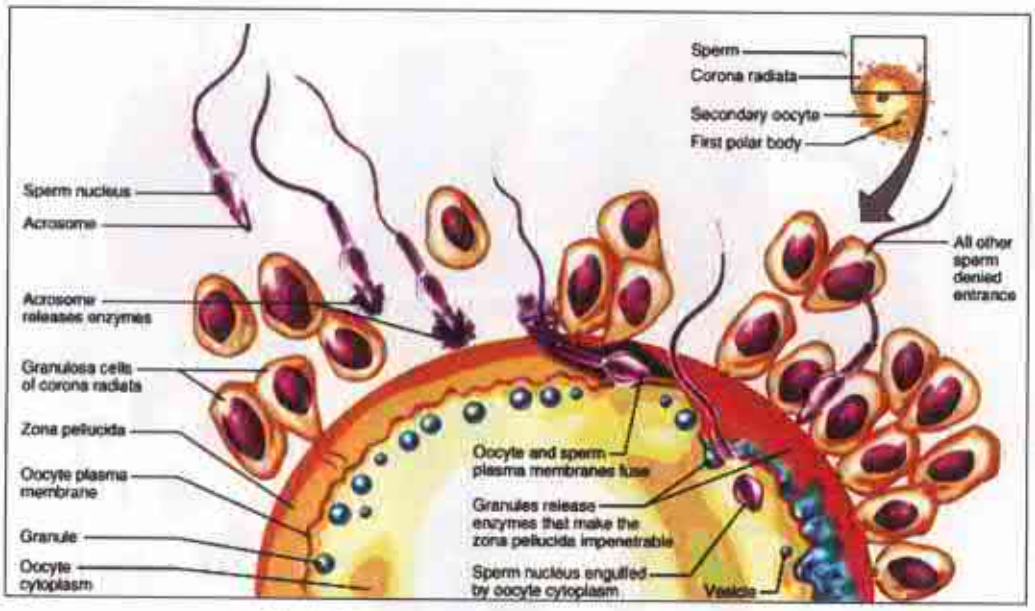


Fig. 17. The three phases of oocyte penetration. In phase 1, spermatozoa pass through the corona radiata barrier; in phase 2, one or more spermatozoa penetrate the zona pellucida; in phase 3, one spermatozoon penetrates the oocyte membrane while losing its own plasma membrane.

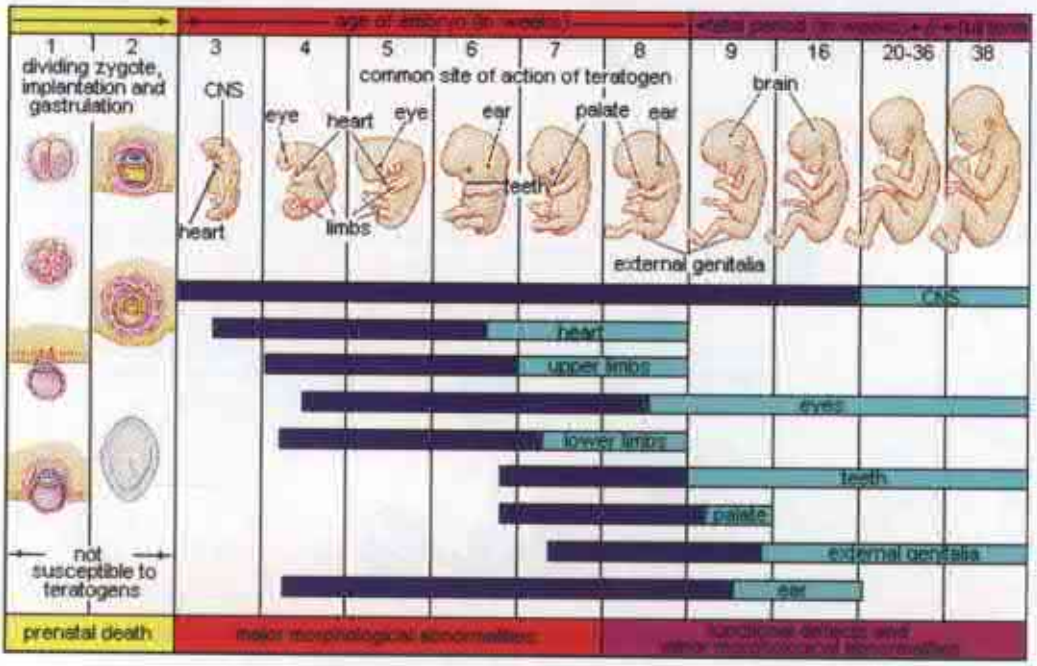


Fig. 18. Critical periods of human development

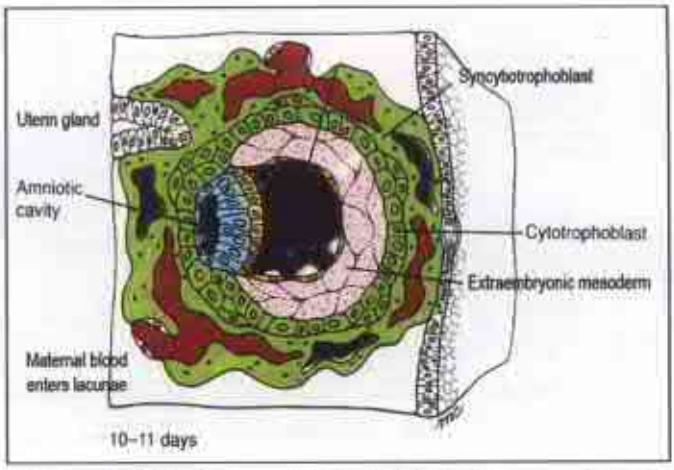


Fig. 19. Human blastocyst of 10 -11 days. The trophoblastic lacunae at the embryonic pole are in open connection with maternal sinusoids in the endometrial stroma. Extraembryonic mesoderm proliferates and fills the space between the exocoelomic membrane and the inner aspect of the trophoblast.

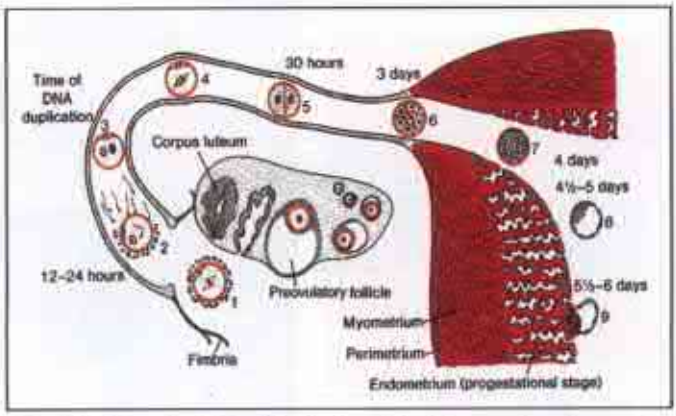


Fig. 20. Events during the first week of human development.
1, Oocyte immediately after ovulation. 2, Fertilization, approximately 12 to 24 hours after ovulation. 3, Stage of the male and female pronuclei. 4, Spindle of the first mitotic division. 5, Twocell stage (approximately 30 hours of age). 6, Morula containing 12 to 16 blastomeres (approximately 3 days of age). 7, Advanced morula stage reaching the uterine lumen (approximately 4 days of age). 8, Early blastocyst stage (approximately 4.5 days of age). The zona pellucida has disappeared. 9, Early phase of implantation (blastocyst approximately 6 days of age). The ovary shows stages of transformation between a primary follicle and a preovulatory follicle as well as a corpus luteum. The uterine endometrium is shown in the progestational stage.

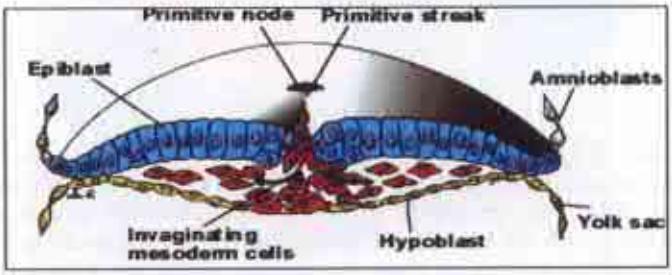


Fig. 21. Cross section of human gastrula during cells migration (late gastrulation)

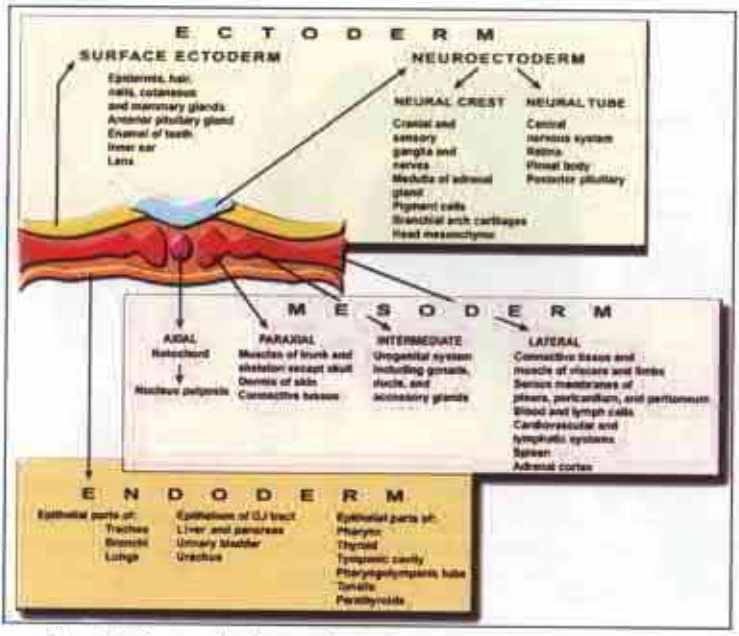


Fig. 22. General scheme of all derivatives of the germ layers

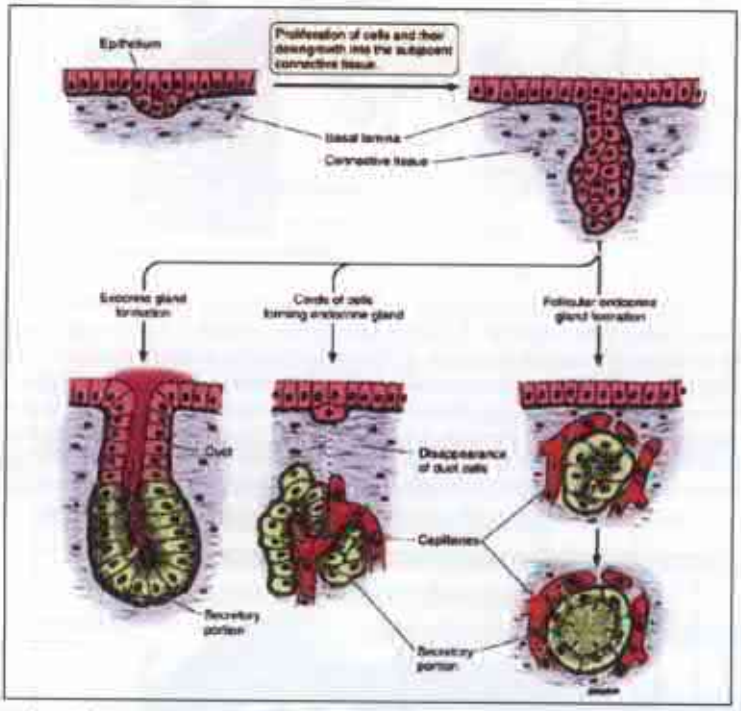


Fig. 23. Formation of glands from covering epithelia. Epithelial cells proliferate and penetrate the connective tissue. They may – or may not – maintain contact with the surface. When contact is maintained, exocrine glands are formed; without contact, endocrine glands are formed. The cells of endocrine glands can be arranged in cords or in follicles. The lumen of the follicles accumulates secretion; cells of the cords store only small quantities of secretions in their cytoplasm.

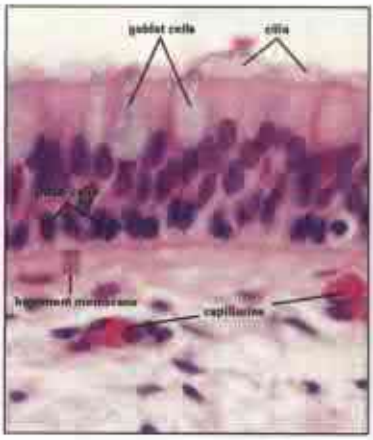


Fig. 24. Light micrograph of a pseudostratified columnar ciliated epithelium

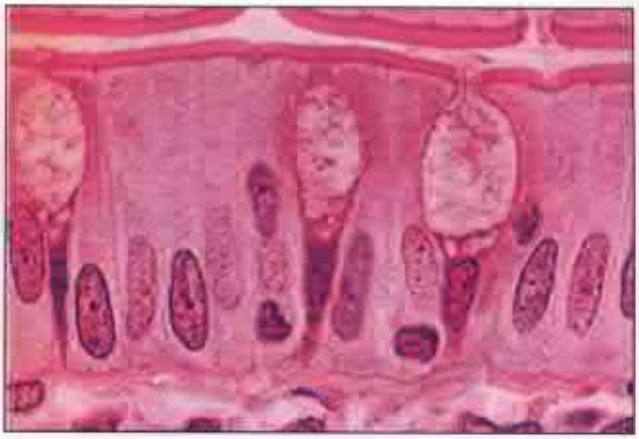


Fig. 25. Light micrograph of a simple columnar epithelium

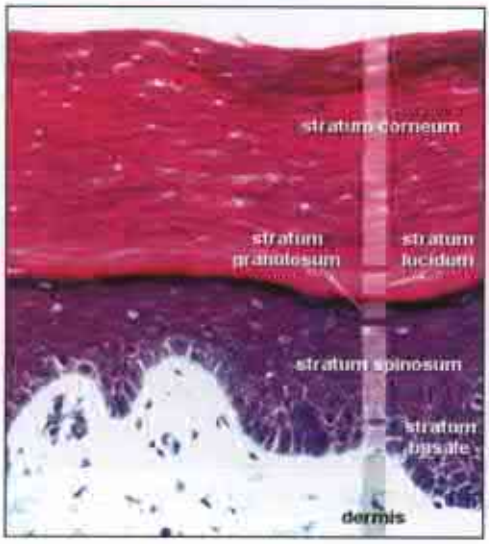


Fig. 26. Light micrograph of a stratified squamous keratinized epithelium

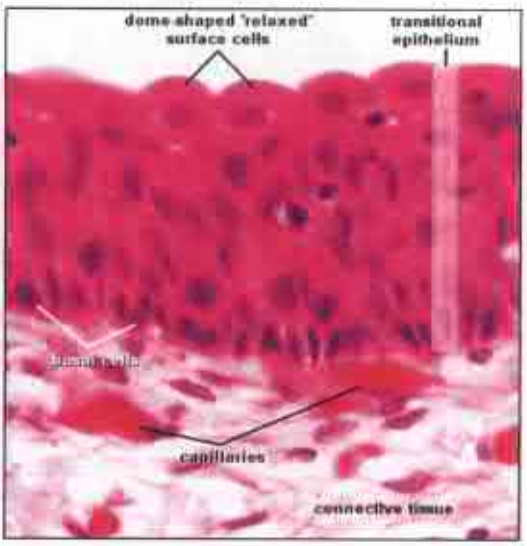


Fig. 27. Light micrograph of a transitional epithelium (urothelium)

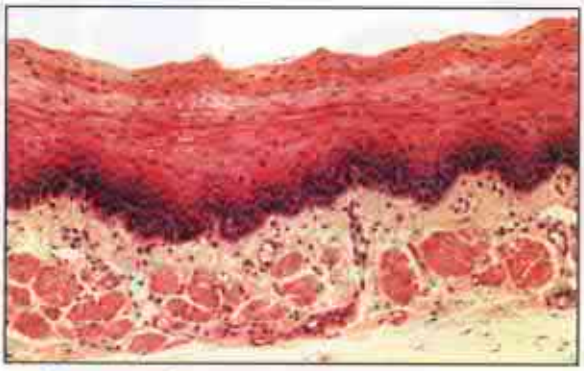


Fig. 28. Light micrograph of a stratified squamous non-keratinized epithelium

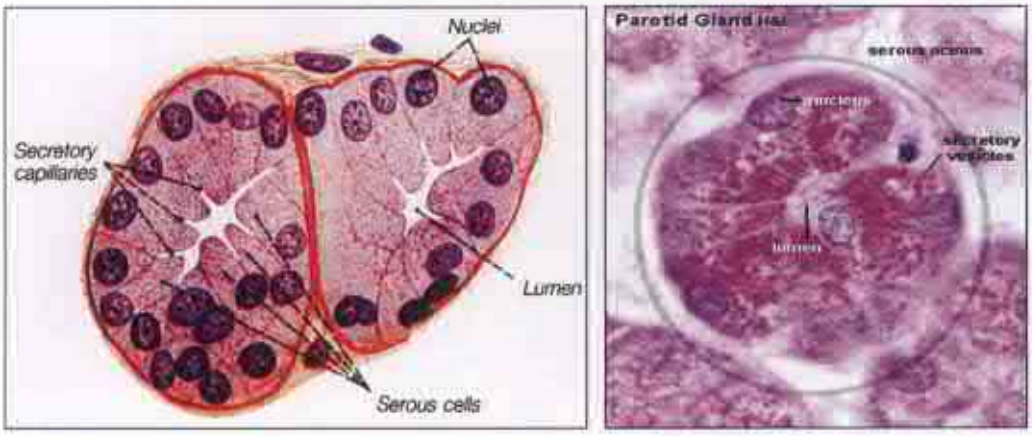


Fig. 29. Schematic drawing and light micrograph of a serous acinus

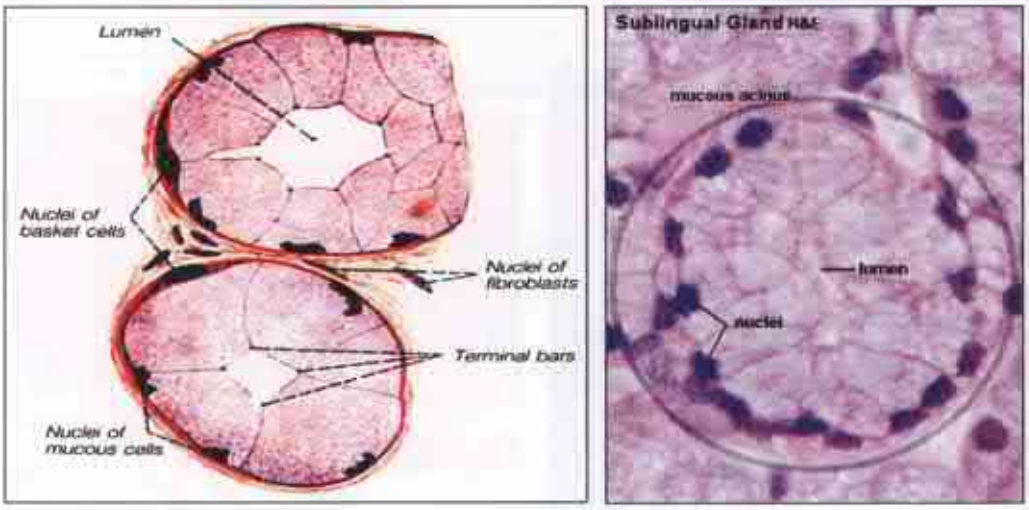


Fig. 30. Schematic drawing and light micrograph of a mucous acinus

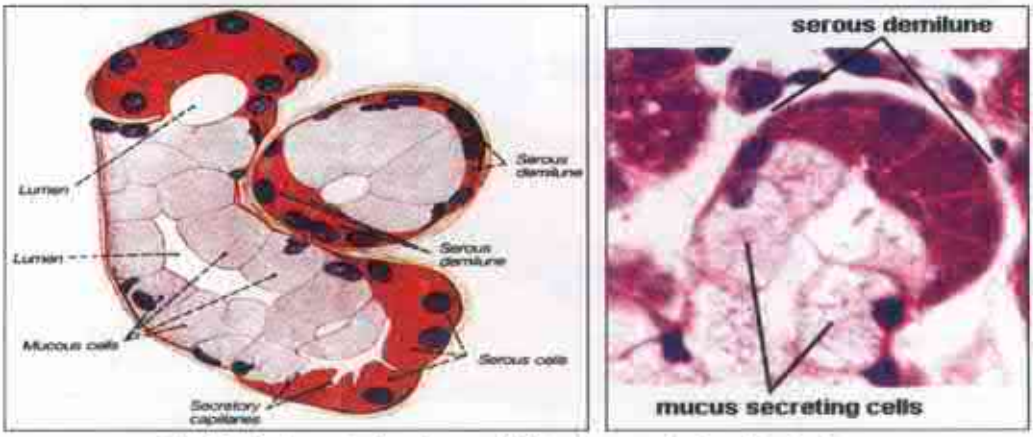


Fig. 31. Schematic drawing and light micrograph of a mixed acinus

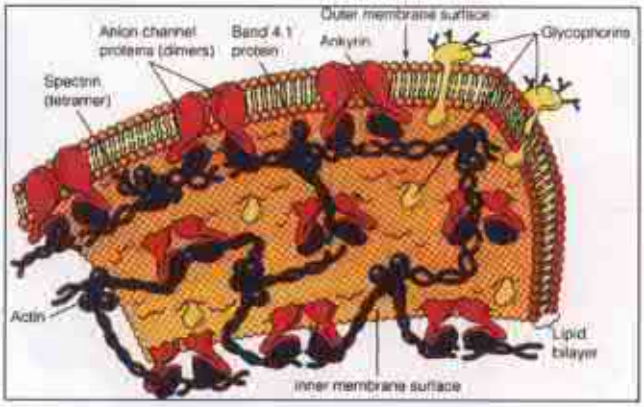


Fig. 32. The structure of the red blood cells membrane

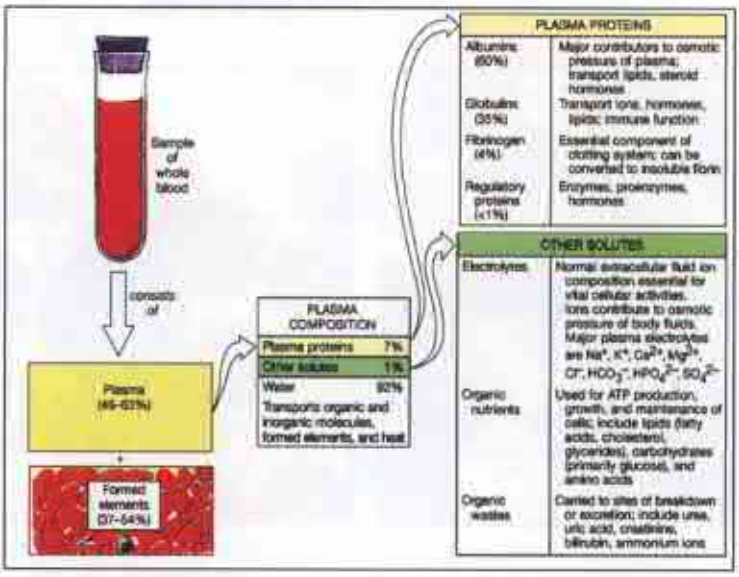


Fig. 33. General scheme of the composition of the blood

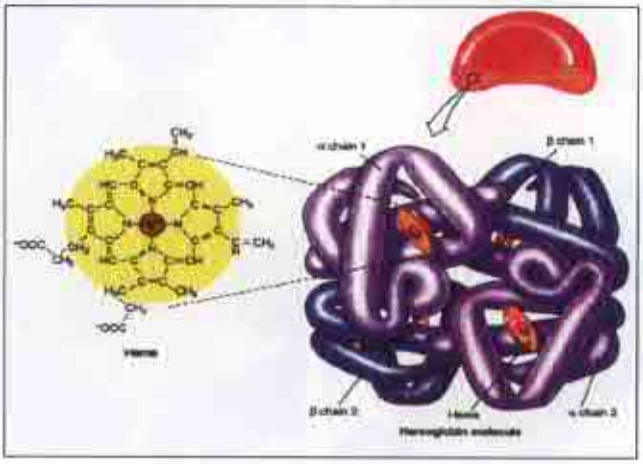


Fig. 34. The structure of hemoglobin

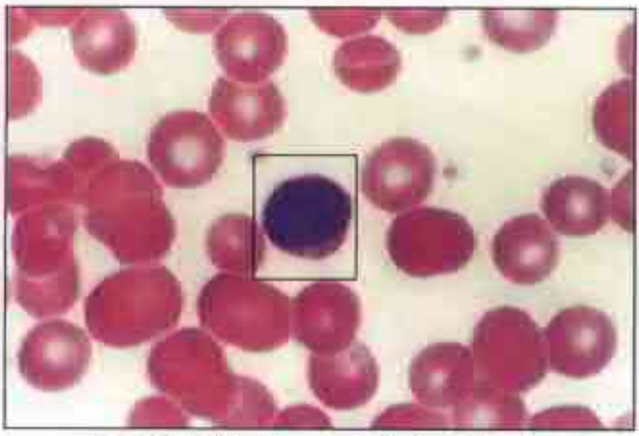


Fig. 35. Light micrograph of a lymphocyte

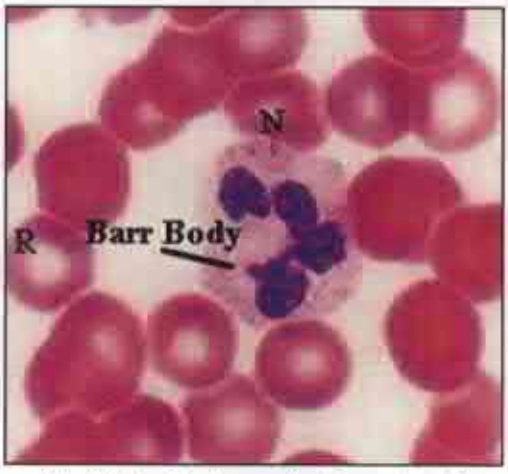


Fig. 36. Light micrograph of a neutrophil

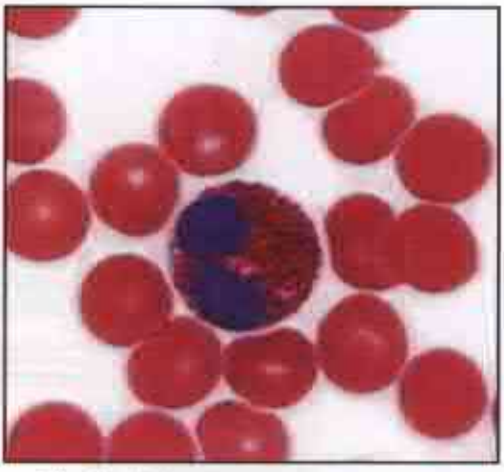


Fig. 37. Light micrograph of a eosinophi

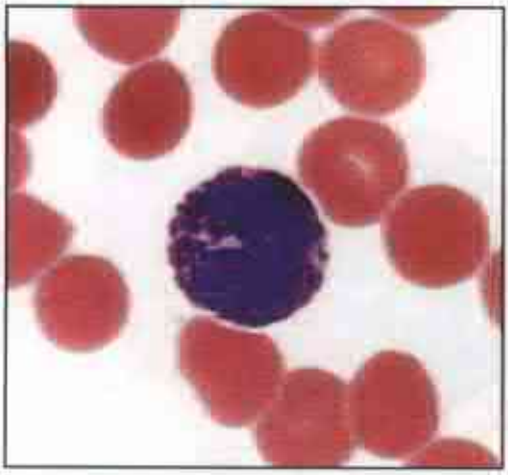


Fig. 38. Light micrograph of a basophil

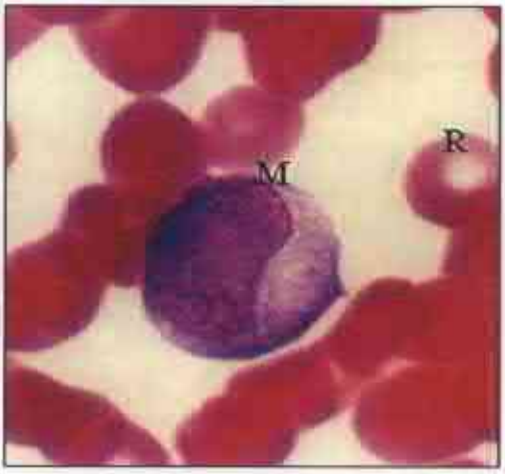


Fig. 39. Llight micrograph of a monocyte

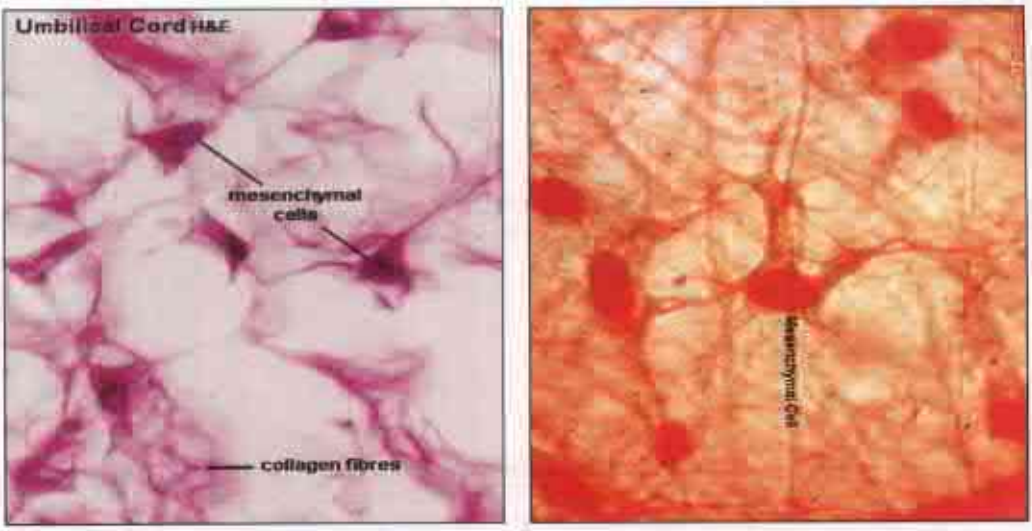


Fig. 40. Light micrographs of a mesenchyme

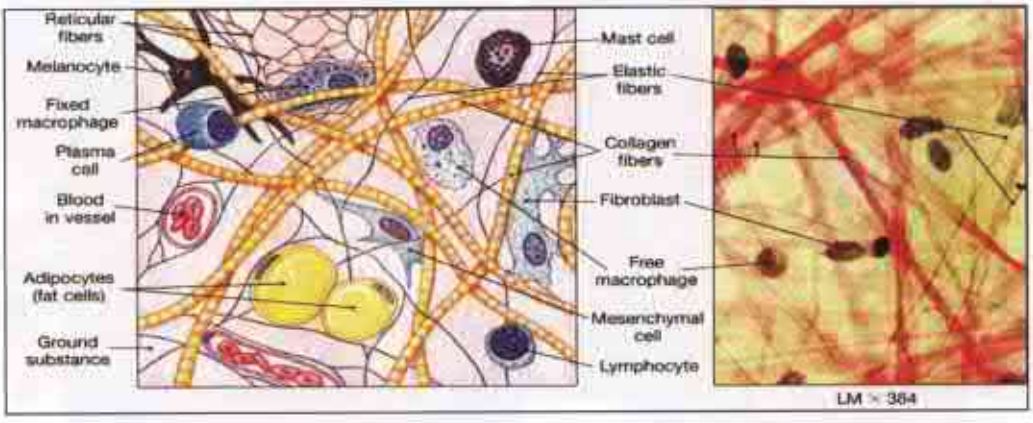


Fig. 41. Scheme and light micrograph of loose connective tissue

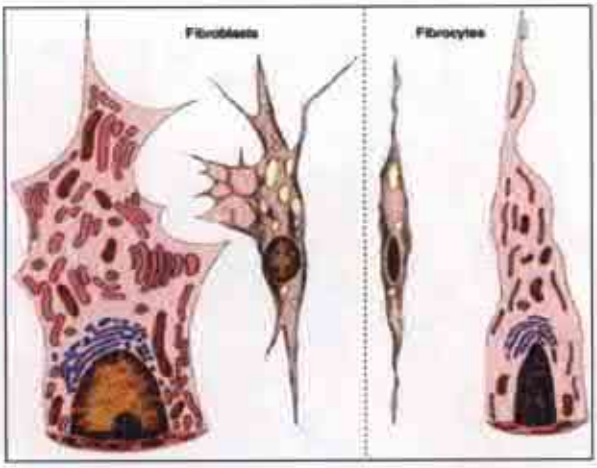


Fig. 42. Scheme of the external morphological characteristics and ultrastructure of the fibroblast and fibrocyte.

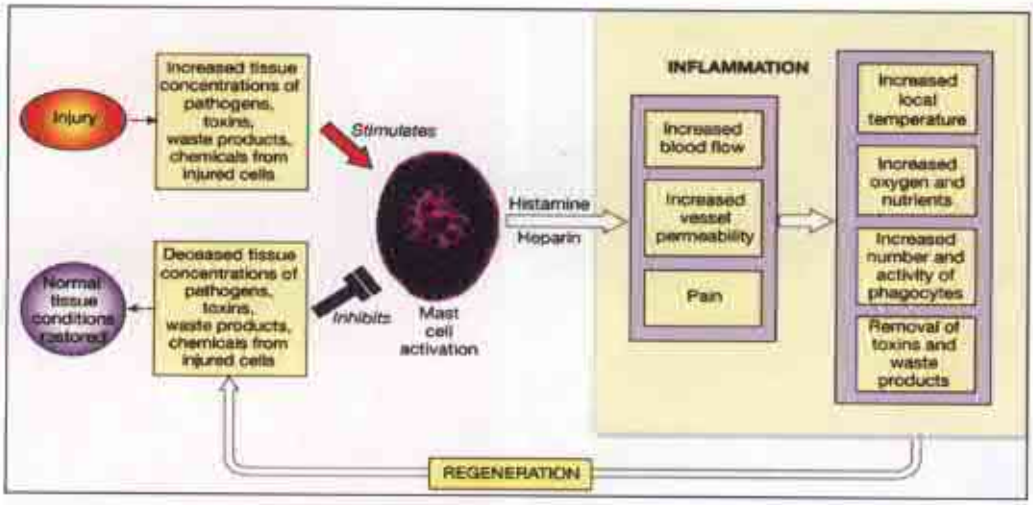


Fig. 43. Schematic diagram of mast cells activity

Fig. 44. Light micrograph of a dense regular connective tissue

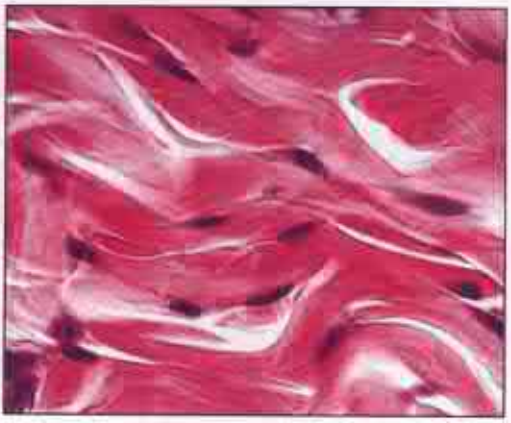


Fig. 45. Light micrograph of a dense irregular connective tissue

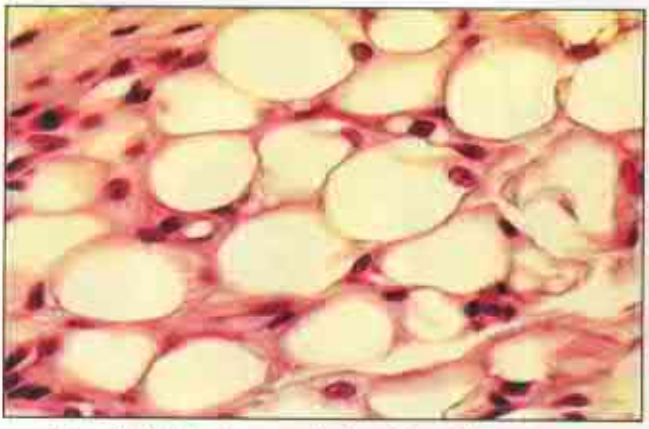


Fig. 46. Light micrograph of a white adipose tissue

Fig. 47. Light micrograph of a brown adipose tissue

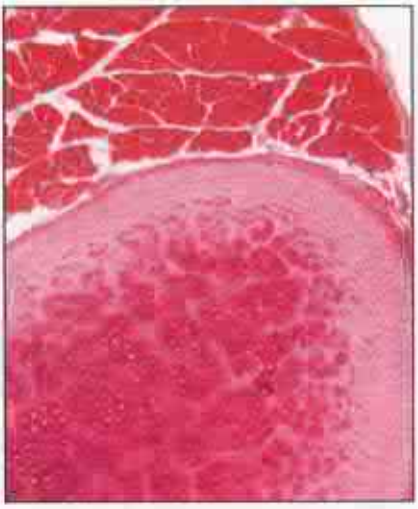


Fig. 48. Light micrograph of a hyaline cartilage

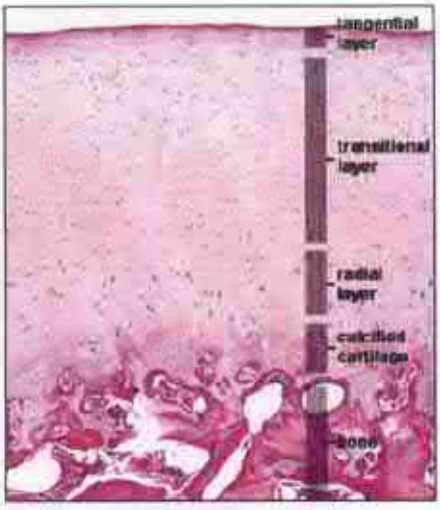


Fig. 49. Light micrograph of a articular cartilage

Fig. 50. Light micrograph of a elastic cartilage

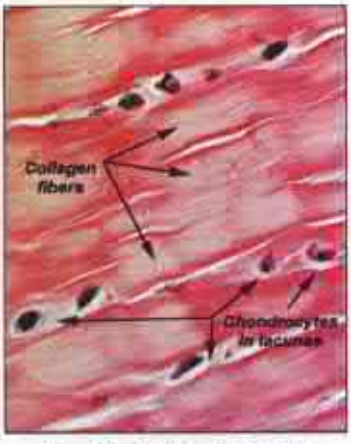


Fig. 51. Light micrograph of a fibrocartilage

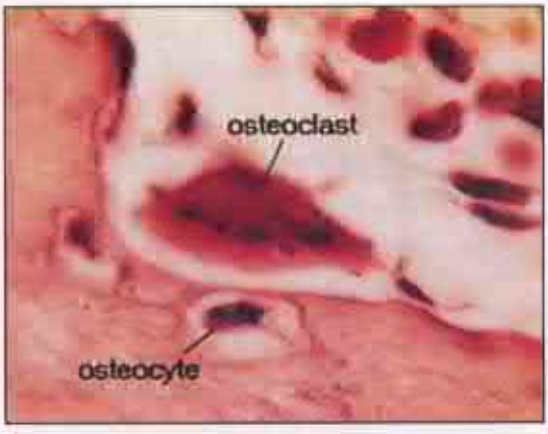


Fig. 52. Light micrograph of osteoclast and osteocyte

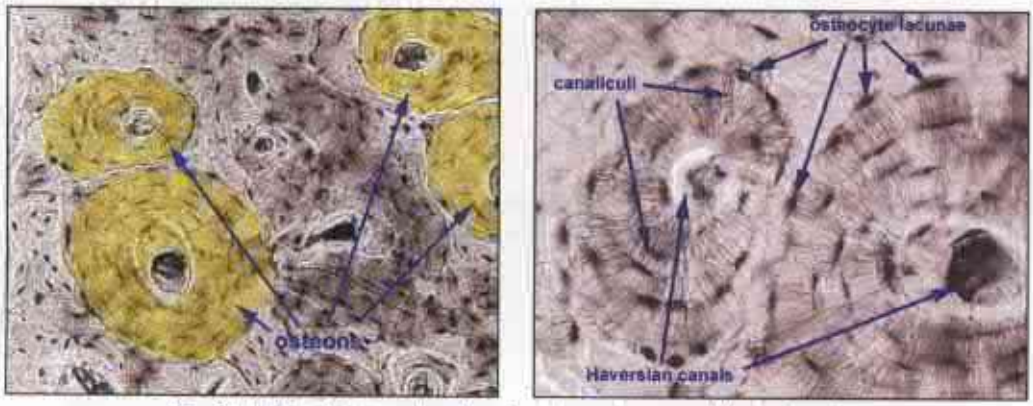


Fig. 53. Light micrographs showing the osteons and their structure

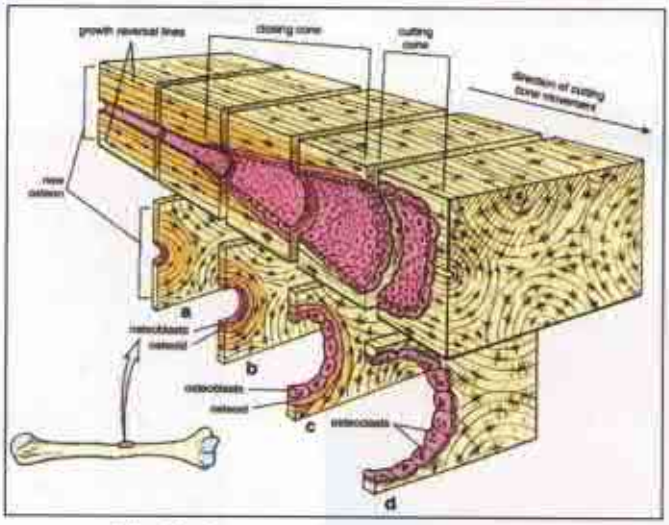


Fig. 54. Diagram of bone-remodeling unit

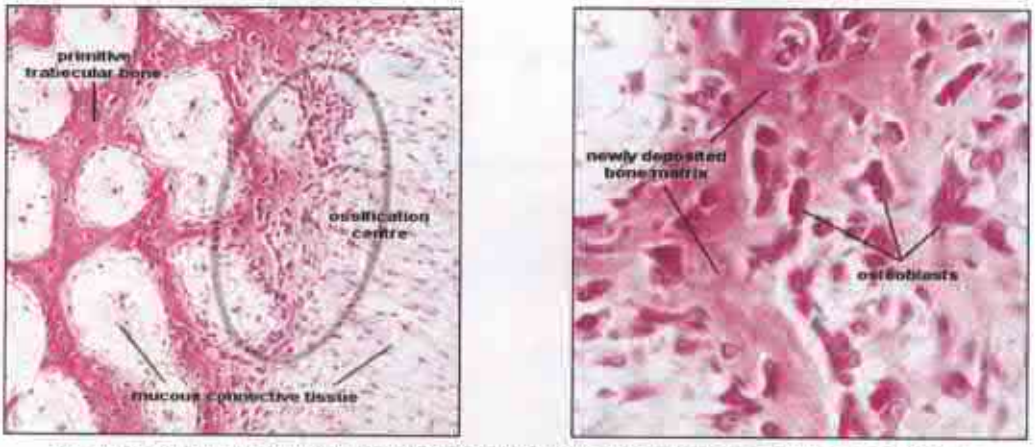


Fig. 55.Sections of mandible developing by the process of intramembranous ossification (low and high magnifications)

CHAPTER III

THE CIRCULATORY SYSTEM

The circulatory system comprises both the blood and lymphatic vascular systems. It consists of:

1. Blood vessels, which circulate blood to and from all parts of the body.
2. The heart, which keep the blood in motion.
3. The lymphatic vessels, in which the lymph circulates.

BLOOD VESSELS

Major types of blood vessels are: **arteries**, **veins**, which form the macrovascular portion of the circulatory system, and microcirculatory or microvascular bed (arterioles, capillaries, postcapillary venules).

- Arteries carry blood AWAY from the heart.
- Veins carry blood TOWARD the heart.
- Capillaries contact tissue cells and directly serve cellular needs.

The wall of all blood vessels, except the smallest, are composed of three layers, or **tunics**. These tunics surround a central blood containing spaces – the vessels lumen. These tunics are:

1. **Tunica intima**
2. **Tunica media**
3. **Tunica adventitia**

The innermost tunic is the **tunica interna or tunica INTIMA**. The second name is easy to remember once you know that this tunic is in intimate contact with the blood in the lumen.

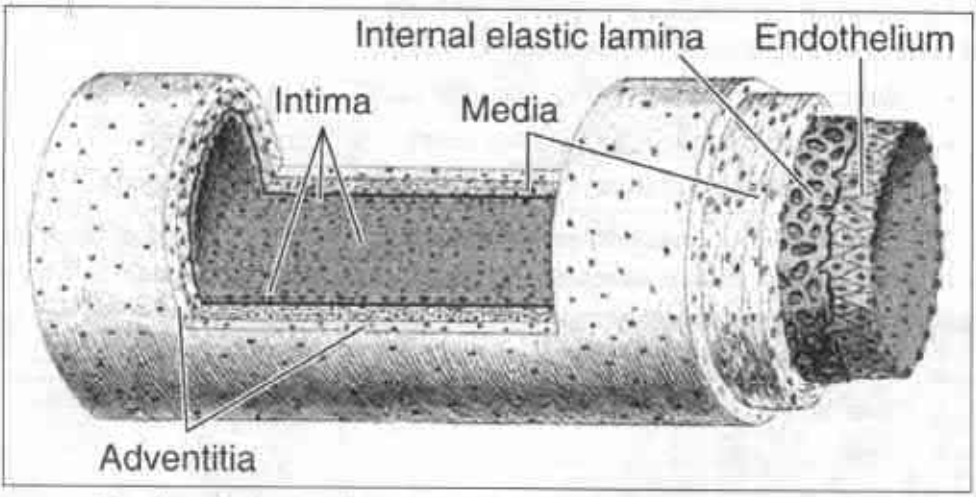
Tunica intima contains:

- **The endothelium** – the simple squamous epithelium, resting on the basal lamina. Endothelium is highly differentiated to actively mediate and

monitor the extensive bidirectional exchange of small molecules and to restrict the transport of some macromolecules. The endothelial cells perform several other functions: **conversion** of angiotensin I to angiotensin II; conversion of bradykinin, serotonin, prostaglandins, norepinephrine, thrombin, etc, to biologically inert compounds; **lipolysis** of lipoproteins by enzymes located on the surface of endothelial cells, to yield triglycerides and cholesterol (substrates for steroid-hormone synthesis and membrane structure); **production of vasoactive factors** that affect the vascular tone, such as endothelins, vasoconstrictive agents, and nitric oxide, a relaxing factor.

- **The subendothelial layer** formed by loose connective tissue, containing occasional smooth muscle cells.
- **The internal elastic membrane** is present in arteries and arterioles. In veins it is absent. It delimits the tunica intima from the tunica media.

Tunica MEDIA is formed by a layer of circumferentially arranged smooth muscle cells and variable amounts of connective tissue. The histological structure is different in different types of blood vessels. In arteries the external elastic membrane is present.



Drawing of a general structure of blood vessels, showing its layers

The outermost layer of a blood vessel's wall is the **tunica externa, or tunica ADVENTITIA**. This tunic is composed of loose connective tissue, adipose tissue. It contains a system of vessels, called **vasa vasorum**, to supply the vessels themselves (supply the more external layer and media, the tunica intima of the

vessels obtains its nutrients directly from blood in the lumen). Vasa vasorum are more frequent in veins than in arteries. Also, the tunica adventitia contains nervi vascularis – a network of autonomic nerves, to control contraction of the smooth muscle in the walls of the vessels.

ARTERIES – conduct blood from the heart to the capillaries.

Arteries are classified into 3 types on the basis of size and the characteristics of the tunica media. There are:

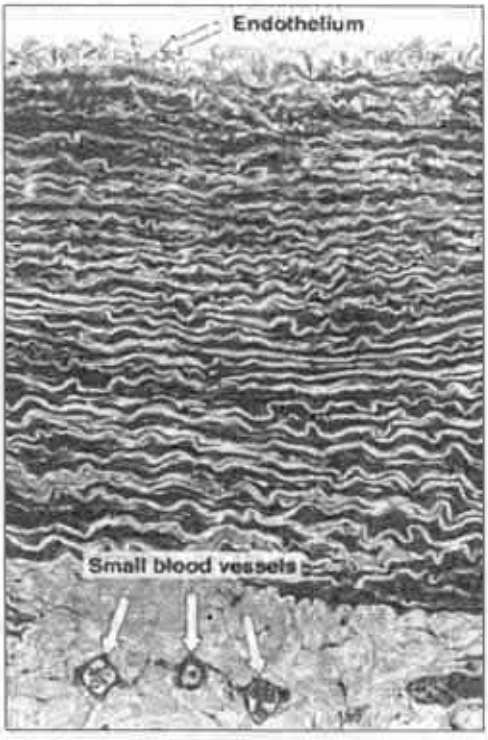
1. Large **ELASTIC arteries** (aorta, pulmonary trunk).
2. Medium sized **MUSCULAR arteries** (ex. medium & small – arteries that carry blood to the organs and extremities).
3. **MIXED arteries** – are arteries that are located between elastic and muscular arteries (subclavian, common carotid, common iliac arteries).

ELASTIC ARTERIES – these arteries are the largest in diameter and the most elastic. They contain more elastin than any other vessel type. It is present in all three tunics, but the tunica media contains the most. Elastic arteries also contain substantial amounts of smooth muscle, but they are relatively inactive in vasoconstriction. Large elastic arteries help to stabilize the blood flow.

Structure:

The **Tunica intima** of elastic arteries consists of:

- The **endothelium.**
- **Subendothelial layer** – consists of loose connective tissue.
- **Smooth muscle cells** that are arranged longitudinally (smooth muscle cell is not only contractile, but also produces the extracellular ground substance and fibers).
- **Plexus fibroelasticus** – is same as internal elastic membrane.



Transverse section showing part of a large elastic artery showing a well-developed tunica media

The **Tunica media** is the thickest of the three layers. It consists of **concentric fenestrated lamellae** of elastic fibers. In adult about 50 – 70 elastic lamellae are found in the tunica media of the aorta. Number of concentric fenestrated lamellae increases with age (there are 40 in the newborn and 70 in the adult). **Smooth muscle**, collagen fibers, fibroblasts and ground substance are arranged between elastic lamellae. The elastic fenestrated lamellae contribute to the important function of making the blood flux more uniform. During ventricular contraction (**systole**), the elastic laminae are stretched and reduce the pressure change. During ventricular relaxation (**diastole**), ventricular pressure drops to a low level, but the elastic rebound of large arteries helps to maintain arterial pressure. As a consequence, arterial pressure and blood velocity decrease and become less variable as the distance from the heart increases.

The **Tunica adventitia** consists of loose connective tissue, which contains vasa vasorum, and nervi vasorum. The tunica adventitia appears thinner than the tunica media.

MUSCULAR ARTERIES have more smooth muscle and less elastic fibers in the tunica media than do elastic arteries *(see fig. 99, plate II)*. Prominent internal and external elastic membranes help to distinguish them from elastic arteries.

Structure:

The tunica intima is thinner than in elastic arteries. It consists of three layers:

1. **The endothelium**
2. **The subendothelium**
3. **The internal elastic lamina**, which is much better developed than external elastic lamina (is thicker).

The tunica media is dominated by numerous concentric layers of smooth muscle cells. Fine elastic fibres and a few collagen fibres are also present. Between tunics media and adventitia is located an external elastic lamina. Smooth muscle cells are arranged in a spiral fashion. Their contraction assists in maintaining the blood pressure.

The thickness and appearance **of tunica adventitia** (consists of loose connective tissue, which contains vasa vasorum, and nervi vasorum) is variable.

MIXED ARTERIES

- Tunica media is composed of 50% of smooth muscle cells & 50% of elastic fibers.
- Are placed between elastic & muscular arteries.

THE MICROCIRCULATORY BED

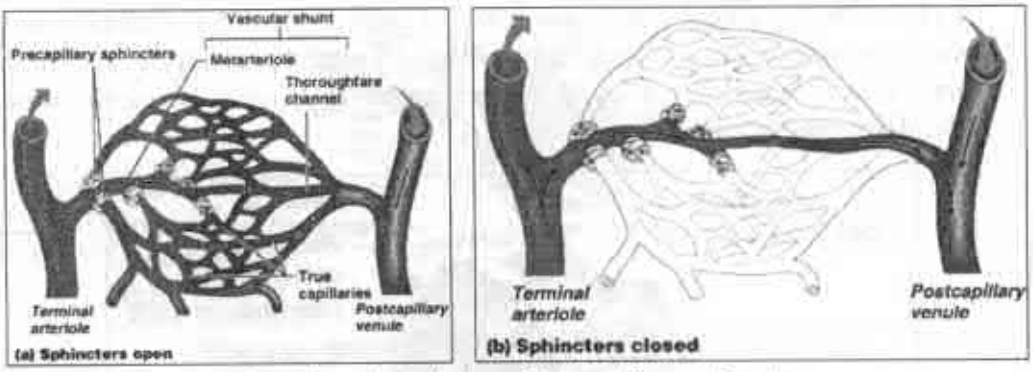
It is that part of circulatory system concerned with the exchange of gases, fluids, nutrients and metabolic products.

There are 4 parts in the microcirculatory bed:

1. **arterioles**
2. **capillaries**
3. **venules**
4. **arteriovenous shunts (anastomosis)** – direct connections between the arterial and venous systems.

ARTERIOLES

- Regulate the distribution of blood to different capillary beds by vasoconstriction and vasodilatation in different regions.
- A slight thickening of the smooth muscle at the origin of a capillary bed from an arteriole is called the **precapillary sphincter**
- The regulation of the blood flow is important in directing blood where it may be needed most. For example: intense physical activity is taking place. Blood flow to the skeletal muscle is increased by the dilation of arterioles. At the same time blood flow to the intestines is reduced by the contraction of arterioles.



Drawing of physiology of precapillary sphincter

The wall of the arteriole consists of 3 tunics also, but they are thinner. There are only 1 or 2 layers of smooth muscle in the tunica media. The internal elastic lamina is absent.

CAPILLARIES

Functions:

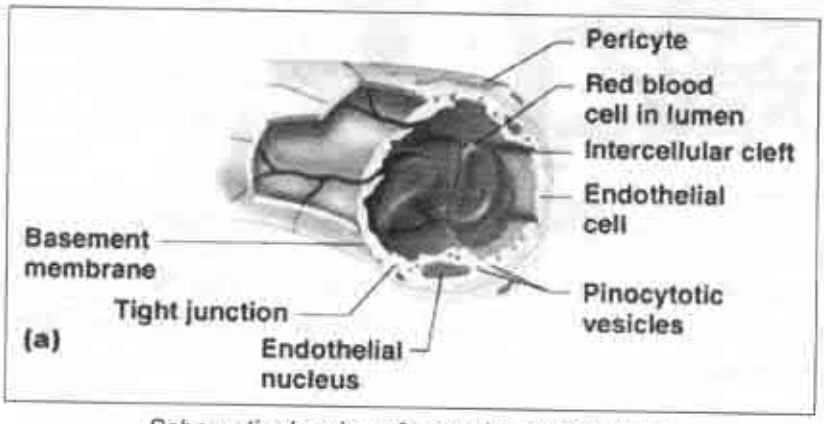
1. Providing nutrients and oxygen to the surrounding tissue.
2. The absorption of nutrients, waste products and carbon dioxide.
3. The excretion of waste products from the body.

Capillary circulation is controlled by neural and hormonal stimulation. The richness of the capillary network is related to the metabolic activity of the tissues. Tissues with high metabolic rates, such as the kidney, liver, and cardiac and skeletal muscle, have an abundant capillary network; the opposite is true for tissues with low metabolic rates, such as smooth muscle and dense connective tissue.

Capillaries are extremely thin tubes formed by a single layer of highly permeable endothelial cells surrounded by a basement membrane and an incomplete layer of cells – the pericytes surrounding the capillary. Pericytes have contractile properties and can regulate blood flow in capillaries, provide support, and have potential for regeneration. Around capillaries is located a loose connective tissue, which contains adventitial cells. Note that the diameter of capillaries is similar to that of the red blood cells contained within them.

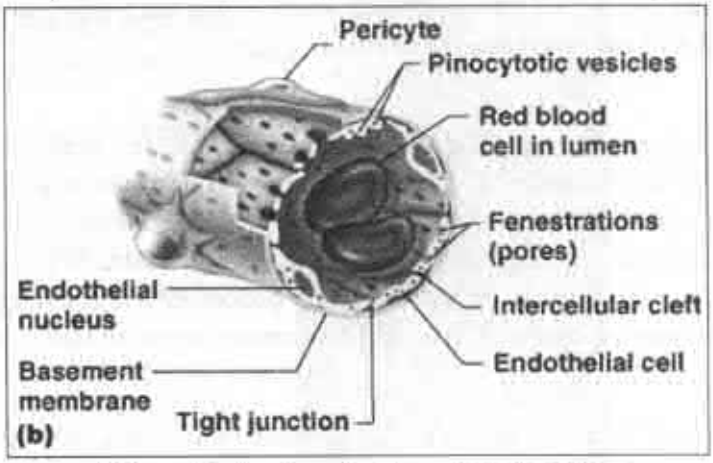
Three types of capillaries can be distinguished based on features of the endothelium:

1. **Continuous capillaries (somatic)** – endothelial cells have a complete cytoplasm, the basement membrane is continuous. Both endothelial cells and the basal lamina can act as selective filters in continuous capillaries. They are found in brain, skin, muscle, connective tissue, thymus, lungs, exocrine glands, nervous tissue.



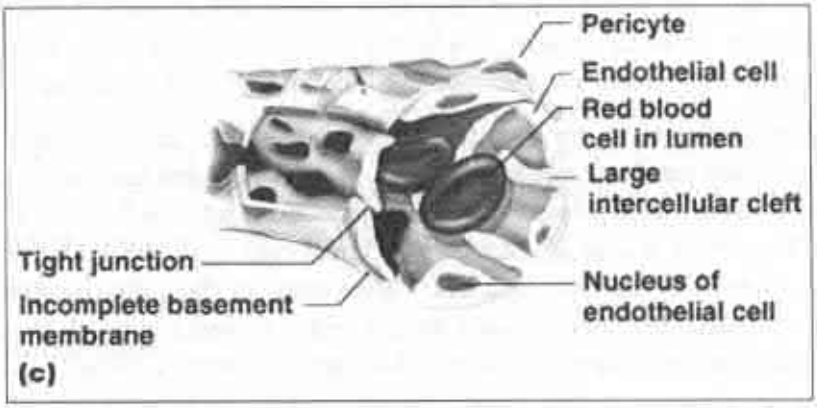
Schematic drawing of a continuous capillary

2. **Fenestrated capillaries (visceral)** – the endothelial cell has many fenestrae (pore, openings), with or without a thin diaphragm. The basal lamina is continuous. They are found in kidney, intestine and endocrine glands.



Schematic drawing of a fenestrated capillary

3. **Discontinuous capillaries (sinusoids)** – Are formed by incomplete endothelial layer and incomplete basement membrane, have enlarged diameter and irregular shape, found in liver, bone marrow, and spleen.



Schematic drawing of a discontinuous sinusoid capillary

ARTERIOVENOUS ANASTOMOSES participate in the regulation of blood flow in certain regions of the body by allowing direct communication between arterioles and venules. The luminal diameters of anastomotic vessels vary with the physiological condition of the organ. Changes in diameter of these vessels regulate blood pressure, flow, and temperature and the conservation of heat in particular areas. In addition to these direct connections, there are more com-

plex structures, the **glomera** (singular, **glomus**), mainly in fingerpads, fingernail beds, and ears. When the arteriole penetrates the connective tissue capsule of the glomus, it loses an internal elastic membrane and develops a thick muscular wall and small lumen. All arteriovenous anastomoses are richly innervated by the sympathetic and parasympathetic nervous systems.

VENULES

They are larger than capillaries. Small venules are surrounded by pericytes. A few smooth muscle cells may surround larger venules. Tunica intima of these vessels is composed of endothelium and a very thin subendothelial layer. They contain prominent adventitia. Venules have several features in common with capillaries, eg, participation in inflammatory processes and exchange of cells and molecules between blood and tissues. The venules merge to form the vein.

VEIN

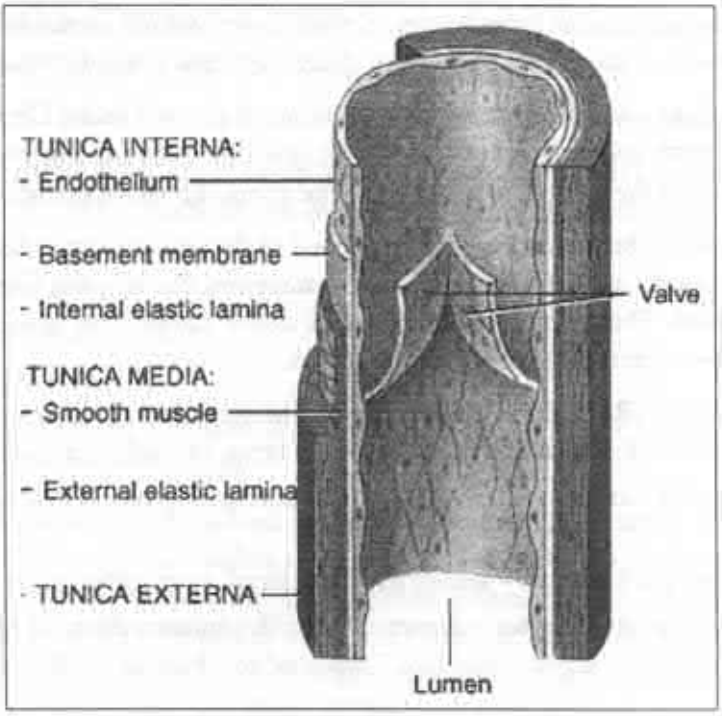
The walls of veins are thinner than the wall of arteries, while their diameter is larger.

Classification of veins

1. **MUSCULAR VEIN** – the intima is composed of endothelium and some subendothelial connective tissue. The intima is very thin. Internal and external elastic laminae are absent or very thin. The media appears thinner than the adventitia. Smooth muscle cells have an irregular orientation. Vaso vasorum are more in the tunica adventitia. The wall has the predisposition to collapse.

The muscular veins are classified in small, medium, and large-sized. Majority of veins are **small** or **medium-sized.** A typical characteristic of small and medium – sized vein is valves. Valves are projections into the lumen of the tunica intima. They have a core of elastic endothelial cells from subendothelial layers and are covered by endothelial cells. Valves prevent the reflux of blood. Large veins have a well-developed tunica intima, but the media is much thinner, with few layers of smooth muscle cells and abundant connective tissue.

2. **UNMUSCULAR (FIBROUS) VEIN** – contains only tunica intima and adventitia. Examples of these types of vessels are:
   - vein of pia and dura mater
   - vein of retina
   - vein of bone
   - vein of spleen
   - vein of placenta



Drawing of a general structure of a muscular vein

HEART

The heart is a muscular organ that contracts rhythmically, pumping the blood through the circulatory system. It is also responsible for producing a hormone called **atrial natriuretic factor.** The cardiac wall consists of three layers:

1. **Endocardium**
2. **Myocardium**
3. **Epicardium**

The fibrous central region of the heart, called, rather inappropriately, the **fibrous skeleton,** serves as the base of the valves as well as the site of origin and insertion of the cardiac muscle cells.

ENDOCARDIUM – the innermost layer of the heart consists of:

- Endothelium (simple squamous epithelium) that rest on the second layer.
- Subendothelial layer (loose connective tissue).
- Fibromuscular plexus – elastic, collagen fibers and smooth muscle cells.

- Subendocardial layer (outer fibrous layer) which contains small blood vessels, collagenous and elastic fibers, but not smooth muscle.

The cardiac valves consist of a central core of dense fibrous connective tissue (containing both collagen and elastic fibers), lined on both sides by endothelial layers. The bases of the valves are attached to the annuli fibrosi of the fibrous skeleton.

The cardiac fibrous skeleton is composed of dense connective tissue. Its principal components are the **septum membranaceum,** the **trigona fibrosa,** and the **annuli fibrosi.** These structures consist of dense connective tissue, with thick collagen fibers oriented in various directions.

The MYOCARDIUM is the thickest of the tunics of the heart and is formed by cardiac striated muscle tissue. There are 3 types of cardiac muscle cells:

a) **Contractile cardiocytes** – which contract to pump blood through the circulation.

b) **Secretory cardiocytes** – which produce atrial natriuretic factor.

c) **Conductive cardiocytes** – present under the endocardium of interatrial and interventricular septa. There are 3 types of conductive cardioctes:

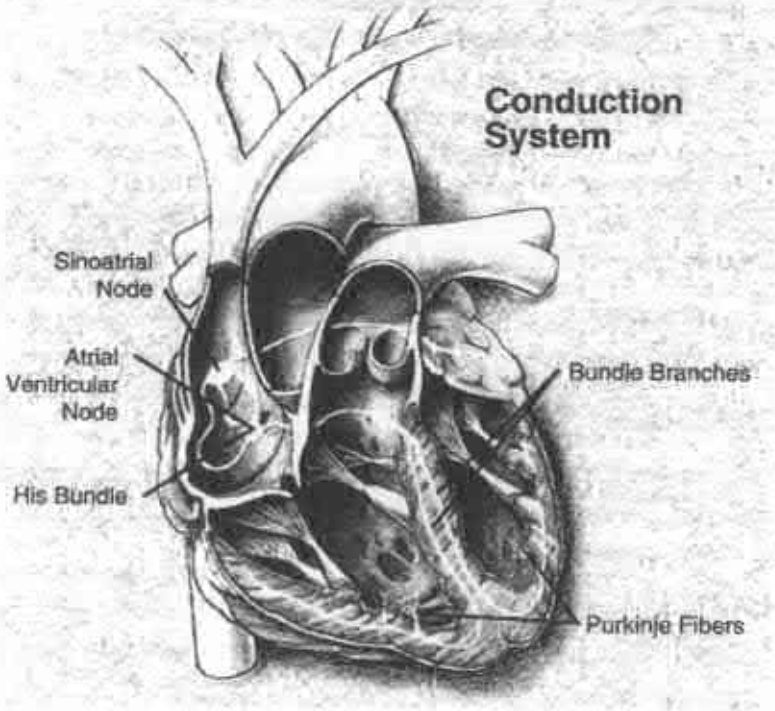


Diagram of the heart, showing the impulse-generating and -conducting system

- Type **I** located in the *sinoatrial node.*
- Type **II** located in the *atrioventricular node.*
- Type **III** located in the *left and right bundles of Purkinje fibers.*

Contraction of heart depends on electrical stimulation of myocardium. Impulse is initiated on the right atrium and spreads throughout the heart. Conduction pathway:

- *sinoatrial* node.
- *atrioventricular* node.
- *Bundle of His.*
- *Purkinje fibers.* Stimulation of Purkinje fibers causes both ventricles to contract simultaneously.

Purkinje fibers can be found beneath interventricular septa. They can be distinguished from regular cardiac muscle cells by their location, their larger size and lighter cytoplasmatic staining. Like cardiac contractile muscle cells, Purkinje fibers are striated and are linked to each other by atypical intercalated disks. They contain many mitochondria and glycogen in the cytoplasm, a reduced number of myofibrils located at the periphery of the fiber. Purkinje cells have one or two central nuclei

Epicardium – the outermost layer, formed by thin layer of loose connective tissue and mesothelium. The epicardium corresponds to the visceral layer of the **pericardium,** the serous membrane in which the heart lies. Between the visceral layer (epicardium) and the parietal layer is a small amount of fluid that facilitates the heart's movements.

The **LYMPHATIC VASCULAR SYSTEM** returns the extracellular liquid to the bloodstream. Parts of blood plasma will exude from the blood vessels into the surrounding tissues because of transport across the endothelium or because of blood pressure and the fenestration of some capillaries. Lymphatic vessels collect fluid from the tissue spaces and return it to the blood. The lymphatic vessels are dedicated to unidirectional flow of liquid – the lymph.

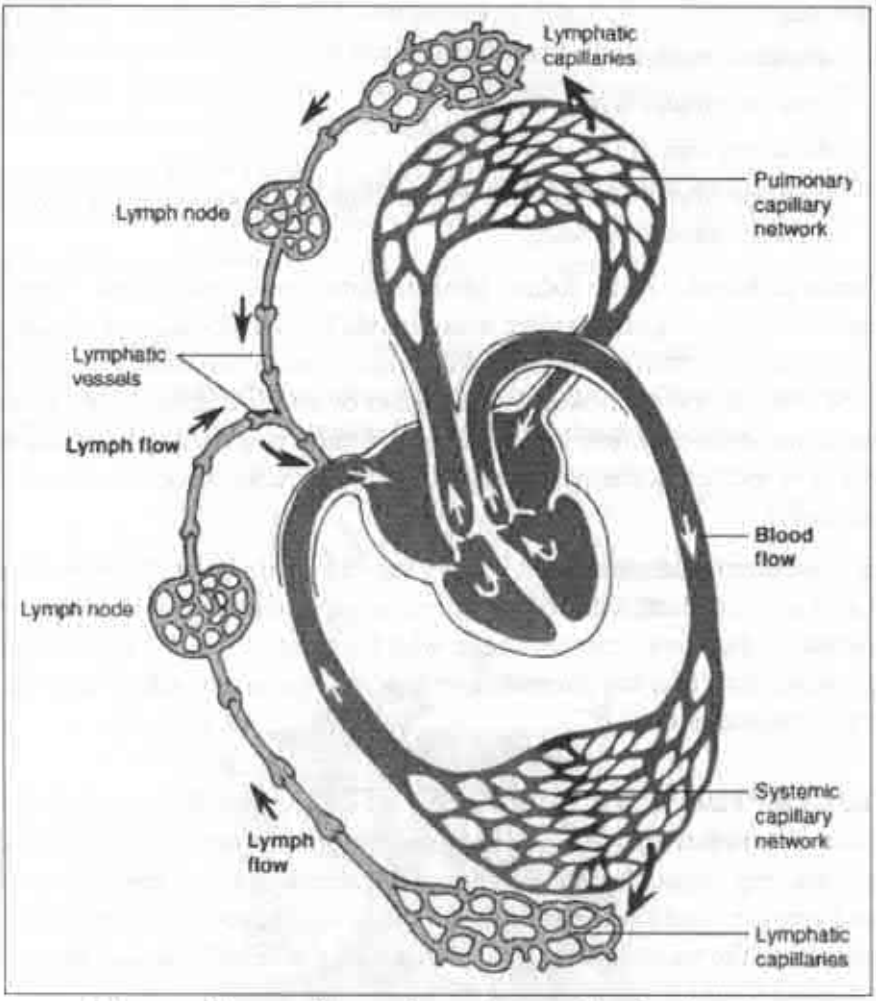
Three types of lymph vessels can be distinguished based on their morphology:

a) **Lymphatic capillaries** consist of a single layer of endothelium and an incomplete basal lamina. Lymphatic capillaries are held open by numerous microfibrils of the elastic fiber system, which also bind them firmly to the surrounding connective tissue.

b) Lymphatic collecting vessels.

c) Lymphatic ducts.

The lymphatic vessels are similar in structure to the veins except they have thinner walls in relation to their diameter. The layers of the wall are indistinct, and **valves** are present in the larger lymphatic vessels.



Scheme of the cardiovascular system and lymphatic system

CHAPTER IV

THE LYMPHATIC (IMMUNE) SYSTEM

The blood-forming tissue of the body (hematopoietic tissue) is a specialized form of connective tissue and is composed of two major divisions:

- **Myeloid tissue** (red bone marrow)
- **Lymphoid (lymphatic) tissue.**

Lymphatic tissue is characterized by an abundance of lymphocytes. There is no lymphatic tissue in myeloid tissue. Red bone marrow should not be regarded as a true lymphatic tissue as it does not contain an abundant population of lymphocytes, but only the stem cells from which lymphocytes develop.

Lymphatic tissue is involved with lymphocyte production and immune responses. Lymphatic tissue has a major function in defending the body from disease and the spread of infection. Traces of foreign proteins (such as from invading microorganisms or cells) can elicit an **immune response**.

There is a constant **recirculation** of lymphocytes (especially T-lymphocytes) between the lymph and blood. The lymphocytes can leave the blood and enter the lymph system in specialized blood vessels (**post-capillary venules**) in lymph nodes. They eventually return to the blood together with the lymph via the thoracic duct. This recirculation involves lymphocytes that have acquired the ability to distinguish which molecules or cells belong to the body ("self") from foreign molecules or cells ("non-self"). These recirculating lymphocytes can be considered as being on protective patrol duties and are described as **immunocompetent**. When they encounter foreign molecules or cells they activate a series of immune responses to neutralize and destroy the invader.

LYMPHATIC ORGANS

The lymphatic organs are divided into two groups: primary and secondary.

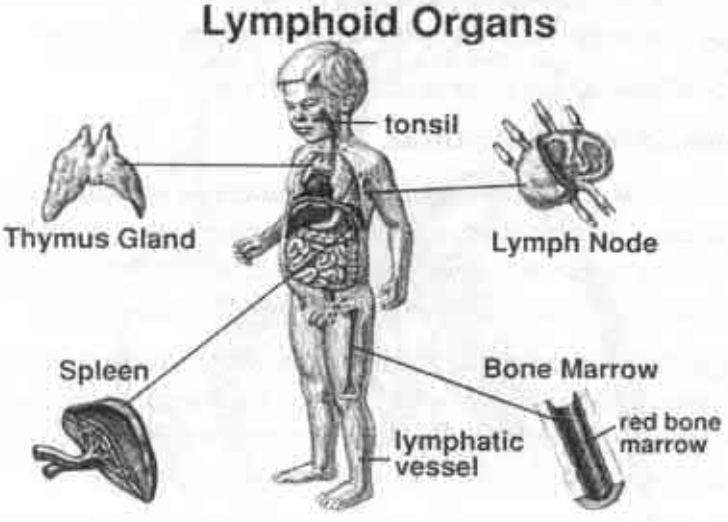
Primary (central) organs – site of antigen independent proliferation and differentiation to T- and B-lymphocytes. There are:

- Red bone marrow
- Thymus gland

Secondary (peripheral) organs – site of Lymphocyte proliferation and exposure to antigens.

Organs:

- Lymph nodes
- Spleen
- MALT – mucosa-associated lymphoid tissue (GALT, BALT).



Primary Lymphoid Organs

Immature lymphocytes generated in hematopoiesis mature and become committed to a particular antigenic specificity within the primary lymphoid organs. Only after a lymphocyte has matured within a primary lymphoid organ is the cell **immunocompetent** (capable of mounting an immune response).

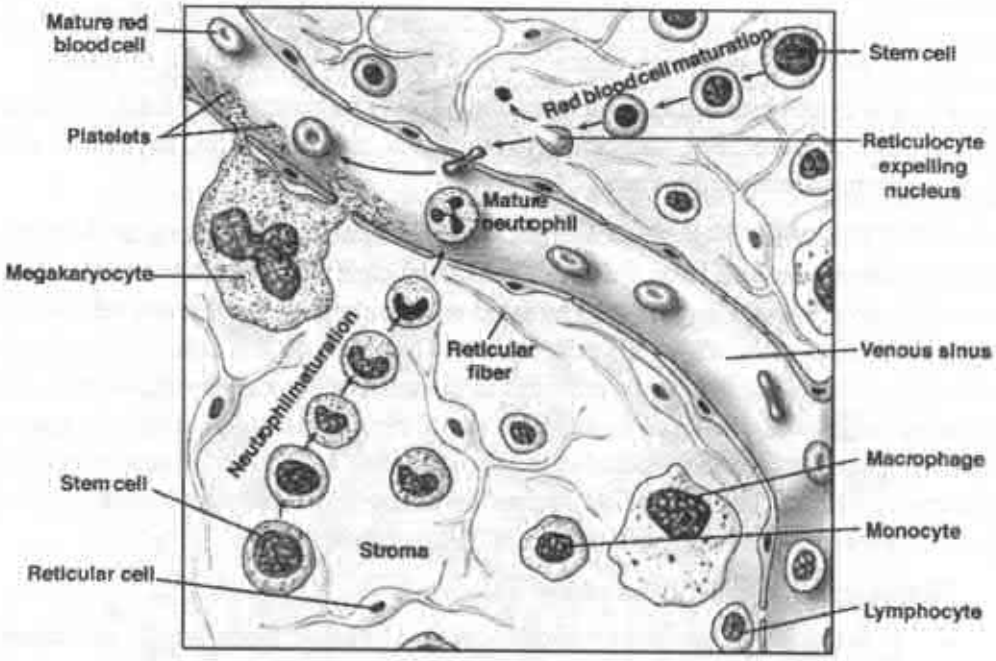
BONE MARROW

Bone marrow is the soft tissue in the hollow shafts of long bones.

- There are two types of bone marrow: red marrow (consisting mainly of myeloid tissue) and yellow marrow (consisting mainly of fat cells).
- Red blood cells, platelets and most white blood cells arise in red marrow;

Most of the red bone marrow changes gradually with ages into the yellow variety. Under certain conditions, such as severe bleeding or hypoxia, yellow bone marrow is replaced by red bone marrow.

Red bone marrow is composed of a **stroma, hematopoietic cords,** and **sinusoidal capillaries.** The stroma is a three-dimensional meshwork of reticular cells and delicate reticular fibers containing hematopoietic cells and macrophages. The stroma of bone marrow contains collagen types III, fibronectin, laminin, and proteoglycans. Laminin, fibronectin, and another cell-binding substance, **hemonectin,** interact with cell receptors to bind cells to the stroma.



Schematic diagram of the red bone marrow

The main functions of red bone marrow are the production of blood cells, destruction of worn-out red blood cells, and storage (in macrophages) of iron derived from the breakdown of hemoglobin.

THYMUS

The thymus is located in the mediastinum above the heart; it attains its peak development during youth.

Functions

- proliferation and maturation of T cells.
- T cell "education". As thymocytes develop, an enormous diversity of T-cell receptors is generated by a random process that produces some T cells with receptors capable of recognizing antigen-MHC complexes. However, most of the T-cell receptors produced by this random process are incapable of recognizing antigen-MHC complexes and a small portion react with combinations of self antigen-MHC complexes. The thymus induces the death of those T cells that cannot recognize antigen-MHC complexes and those that react with self-antigen– MHC and pose a danger of causing autoimmune disease. More than 95% of all thymocytes die by apoptosis in the thymus without ever reaching maturity.
- It secretes hormones which regulate T cell maturation. At least four hormones have been identified: thymosin-α, thymopoietin, thymulin, and thymus humoral factor.

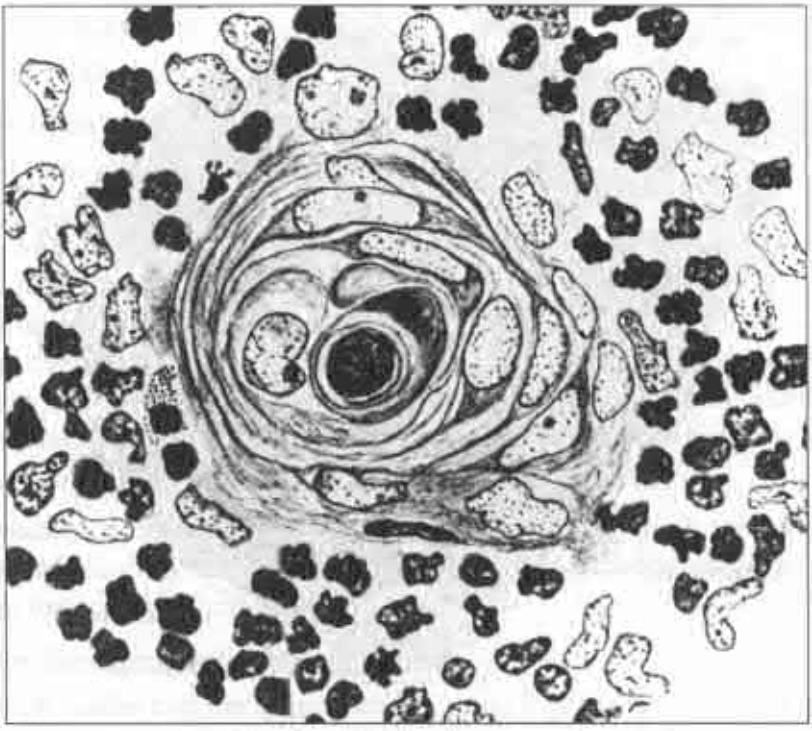
DEVELOPMENT of the thymus. This lymphoepithelial organ develops from ectoderm derived from the third branchial cleft and endoderm of the third branchial pouch including mesenchynmal components derived from cells of the cephalic neutral crest which all migrate from the neck to the anterior mediastinum. The rudimentary thymus enlarges in successive waves as lymphocytes and hematopoietic cells emigrate from embryonal blood vessels to infiltrate spaces between plump thymus epithelial cells. Proliferation and entry of new lymphoid precursors distend interepithelial spaces, resulting in densely packed clusters of lymphocytes in a desomsome-linked epithelial cell reticulum.

Histological organization of the Thymus

- It is enclosed by thin connective tissue capsule from which numerous septa extend into the thymus subdividing the parenchyme into numerous lobules (has lobular organization).
- Blood vessels enter and leave the thymus via the connective tissue septa.
- Stroma is formed by the epithelial reticular cells. The thymus stroma plays a decisive role in the further development of the thymocytes into T cells. The epithelial cells of the thymus medulla secrete chemokines (MDC = macrophage-derived chemokines) that chemotactically attract the immature thymocytes, the thymocytes express binding sites that are highly specific for these MDC. These MDC are responsible for the maturation of the thymocytes. The stromal epithelial cells also produce hormones.

Each lobule has: **cortex** (greater cell density) with many T lymphocytes surrounding **medulla**.

- **Cortex** contains many lymphocytes, macrophages, epithelial reticular cells. Because the cortex is richer in small lymphocytes than the medulla, it stains more darkly.
- **Medulla** contains more epithelial reticular cells and fewer differentiated lymphocytes.
  - Mature T lymphocytes leave from here and from cortex to go to spleen and lymph nodes.
  - **Hassal's corpuscles (thymic corpuscles):** are rounded eosinophilic structures, located in the medulla. They are characteristic of this region, although their function is unknown. Thymic corpuscles consist of concentrically arranged, flattened epithelial reticular cells. The size of these structures varies from 20 μm to more than 100 μm in diameter. Thymic corpuscles may calcify, and their core may "dissolve" (degenerate) leading to the formation of a cyst. Thymic corpuscles increase in size and numbers with age.

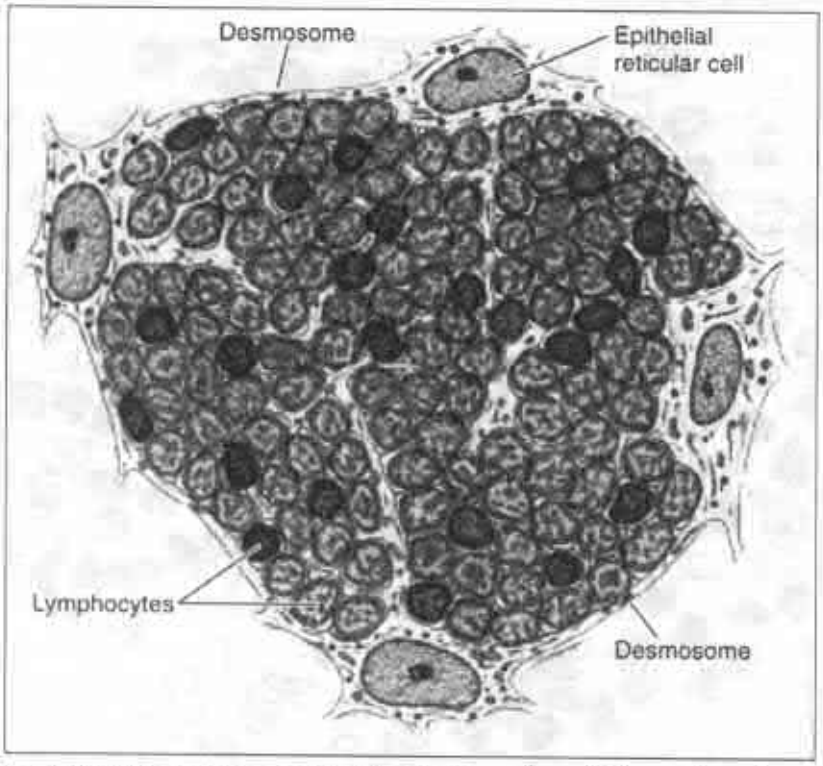


Drawing of a thymic corpuscle

The functional thymus consists of two cell populations: **the stromal (epithelial) cells** and the **thymocytes (T-lymphocytes)**.

A. The epithelial reticular cells are stellate cells with light-staining oval nuclei. They are usually joined to similar adjacent cells by desmosomes. Bundles of intermediate keratin filaments (tonofibrils) in their cytoplasm are evidence of the epithelial origin of these cells. The stromal cells include:
   1. the **subcapsular epithelial cells** (lining the trabeculae and perivascular spaces);
   2. the **cortical epithelial cells** of ectodermic origin;
   3. the **medullary epithelial cells** of endodermic origin that give rise to Hassall's corpuscle
   4. **macrophages** present in both cortex and medulla, involved in the removal of apoptotic thymocytes eliminated during clonal selection.
   5. Langerhans-like **dendritic cells**, confined to the medulla.

B. Thymocytes include T cells at different stages of maturation.



The relationship between epithelial reticular cells and thymus lymphocytes

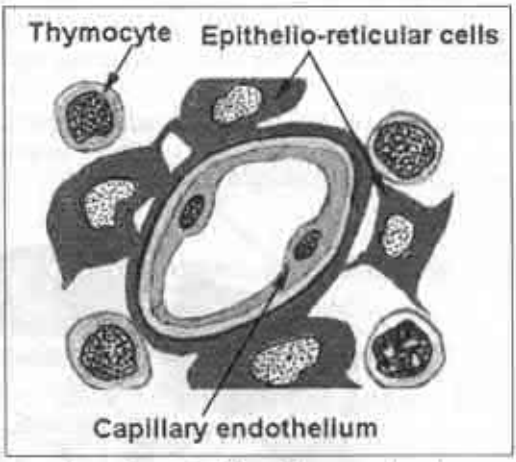
Vascularization of the thymus

Arterioles and capillaries in the thymus are surrounded by processes of epithelial reticular cells. The thymus has no afferent lymphatic vessels and does not constitute a filter for the lymph. The few lymphatic vessels encountered in the thymus are all efferent; they are located in the walls of blood vessels and in the connective tissue of the septa and the capsule.

Thymus capillaries have a nonfenestrated endothelium and a very thick basal lamina, making these blood vessels particularly impermeable to proteins. This prevents most circulating antigens from reaching the thymus cortex, thus creating the so-called **thymic–blood barrier.**

BLOOD-THYMUS BARRIER consists of:

- Capillary endothelium
- Endothelial basal lamina
- Thin perivascular connective tissue sheath containing many macrophages
- Basal lamina of the epithelial reticular cell
- Epithelial reticular cell



Drawing of a blood-thymus barrier

Involution of the thymus

After puberty much of the parenchyma of the thymus, in particular cortical lymphoid tissue, is replaced by adipose tissue. The process, which is called **involution**, initially proceeds rapidly but slows down in adulthood. Whereas the average weight of the thymus is 70 g in infants, its age-dependent involution leaves an organ with an average weight of only 3 g in the elderly. Aging is accompanied by a decline in thymic function. This decline may play some role in the decline in immune function during aging in humans.

Involution is under the control of steroid hormones (both sexual hormones and stress hormones). Although most pronounced in the thymus, involution is a common feature of all lymphoid tissues.

Secondary Lymphoid Organs

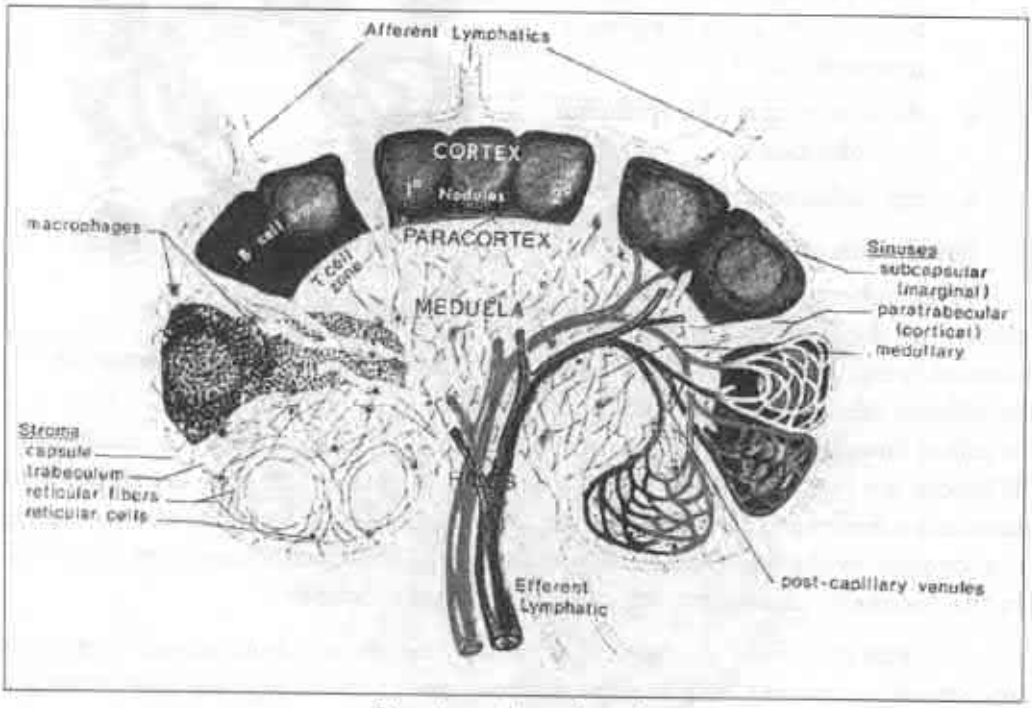
Lymph nodes and the **spleen** are the most highly organized of the secondary lymphoid organs; they comprise not only lymphoid follicles, but additional

distinct regions of Tcell and B-cell activity, and they are surrounded by a fibrous capsule.

Less-organized lymphoid tissue, collectively called mucosal-associated lymphoid tissue (MALT), is found in various body sites. MALT includes Peyer's patches (in the small intestine), the tonsils, and the appendix, as well as numerous lymphoid follicles within the lamina propria of the intestines and in the mucous membranes lining the upper airways, bronchi, and genital tract.

LYMPH NODES

Lymph nodes are distributed throughout the body along the course of the lymphatic vessels. The nodes are found in the axilla and the groin, along the great vessels of the neck, and in large numbers in the thorax and abdomen, especially in mesenteries. Lymph nodes constitute a series of in-line filters that are important in the body's defense against microorganisms and the spread of tumor cells.



Structure of lymph node

Functions:

- Filtration of particulate matter and bacteria from lymph by phagocytic activity of macrophages.

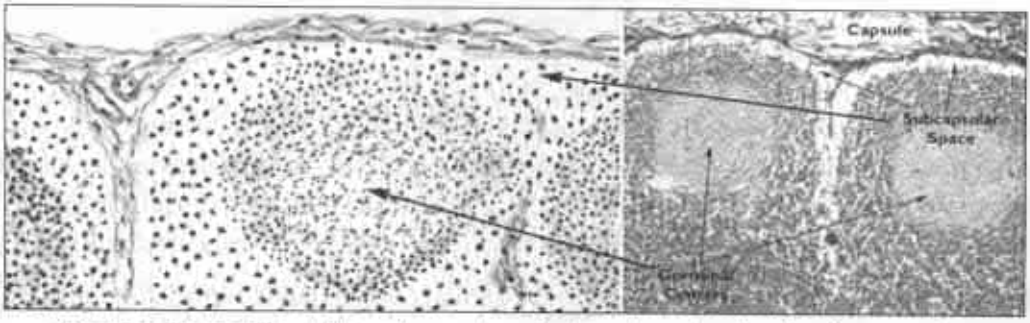
- Activation and proliferation of B cells leading to plasma cell formation and antibody production.
- Activation and proliferation of T cells.
- Exchange of lymphocytes with the blood allowing for generalized systemic responses to localized infections.

The lymph node is the most organized of the lymphatic organs. They are oval or kidney-shaped organs that have a convex surface that is the entrance site of lymphatic vessels and a concave depression, the **hilum** through which afferent arteries and nerves enter and veins and efferent lymphatic vessels leave the organ.

The lymph node is surrounded by a connective tissue capsule with **trabeculae** extending from **cortex** to **medulla**. The stroma is formed by reticular tissue. The parenchyma of lymph node is subdivided in 3 regions: cortex (outer cortex), paracortex (inner cortex), and medulla.

CORTEX consists of the following components:

1. A diffuse population of cells composed mainly of T lymphocytes and reticular cells; macrophages.
2. Lymphoid nodules, with or without germinative centers, formed mainly by B lymphocytes, embedded in the diffuse population of cortical cells.
3. Areas of loose lymphoid tissue (whose reticular fibril meshes are wide) situated immediately beneath the capsule, called the **subcapsular sinuses**. They are composed of a loose network of reticular cells and fibers. Lymph, containing antigens, lymphocytes, circulates around the wide spaces of these sinuses after being delivered into these channels by the afferent lymphatic vessels.
4. **Trabecular sinuses** that run between lymphoid nodules.



Section of a portion of the outer cortex of a lymph node showing the capsule, subcapsular sinuses, diffuse lymphoid tissue, and lymphatic nodules

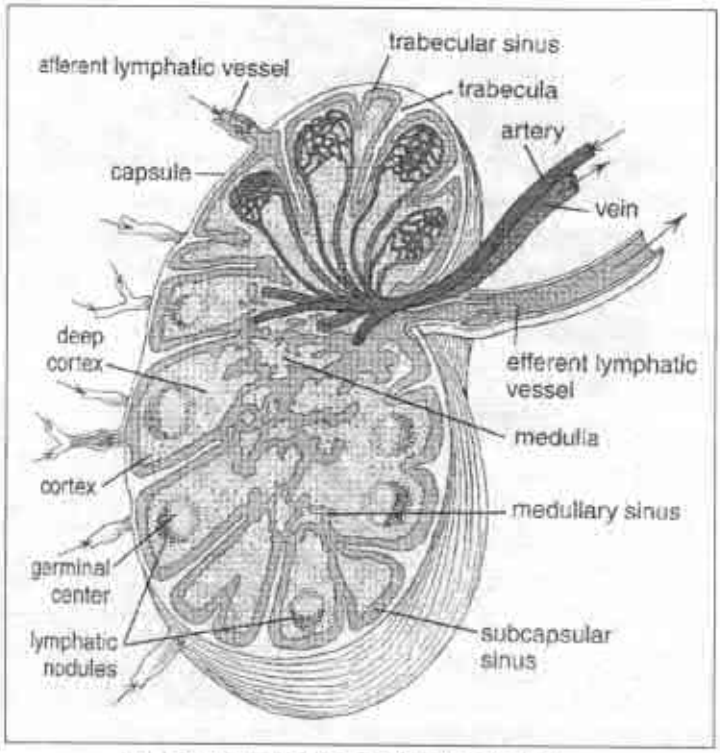
The INNER CORTEX or paracortical region does not have precise boundaries with the outer cortex and contains many T lymphocytes.

MEDULLA has two components: medullary cords and medullary sinuses.

- medullary cords are ramifying processes of lymphoid tissues which contain lymphocytes, macrophages, and plasma cells held in a loose framework of reticular tissue and fibers.
- medullary sinuses – join at the hilum delivering the lymph to the efferent lymph vessel of the lymph node. **Subcapsular sinus communicates with medullary sinuses through trabecular sinuses.**

Lymph Circulation

Afferent lymphatic vessels cross the capsule and pour lymph into the subcapsular sinus. From there, lymph passes through the intermediate sinuses and, finally, into the medullary sinuses. During this passage, the lymph infiltrates the cortex and the medullary cords. The lymph is finally collected by efferent lymphatic vessels at the hilum. Valves in both the afferent and efferent vessels aid the unidirectional flow of lymph.



Schematic diagram of lymph circulation

Because all lymph formed in the body drains back into the blood, lymphocytes that leave the lymph nodes by efferent lymphatic vessels eventually reach the bloodstream. They may then leave the blood vessels by entering the tissues and return to another lymph node by a lymph vessel. They may also return to a lymph node by crossing the walls of specific blood vessels, the **high endothelial venules (HEVs)**, present in lymph nodes.

These venules have an unusual endothelium with tall cuboidal cells. L-selectin present on the lymphocyte surface recognizes sugar-rich ligands of the endothelial cell surface, and as a consequence, the lymphocyte stops in the internal wall of the vein.

High endothelial venules are also present in other lymphoid organs, such as the appendix, tonsils, and Peyer's patches, but not in the spleen.

SPLEEN

The spleen represents the largest lymphoid organ. It is located in the upper left quadrant of abdomen. Because of its abundance of phagocytic cells, the spleen is an important defense against antigens that reach the blood circulation.

Functions:

- Blood formation
  - All blood cells in fetus
  - Only lymphocytes after birth
- Blood filtration
  - Removes bacteria, particles, worn out RBCs and platelets (recycles iron)
- Blood storage
  - Can contain over one pint of blood
- Represents the site of destruction of aged erythrocytes.

Structure:

The spleen is surrounded by a capsule of connective tissue from which trabeculae extend into the organ. Capsule and trabeculae contain fibroblasts (cells of loose connective tissue) and smooth muscle cells. The stroma is formed by reticular tissue.

Splenic pulp (parenchyma) has two components: **the white pulp & the red pulp.**

WHITE PULP:

Is represented by **lymphatic nodules** (little islands), which contain mostly B cells, but also T cells and macrophages. Each lymphatic nodule has 4 zones:

1. GERMINAL CENTER contains proliferating B-lymphocytes, differentiating plasma cells, dendritic cells, macrophages, and supporting reticular cells.
2. PALS (periarterial lymphatic sheath): mostly T cells.
3. MANTLE ZONE – B-cells, few T-cells, plasma cells, macrophages.
4. MARGINAL ZONE – T, B-cells, macrophages.

RED PULP contains:

- **Venous sinuses** are surrounded by an incomplete basal lamina. Their lining consists of long endothelial cells oriented along the longitudinal axis of the vessel. Because the spaces between the endothelial cells of the splenic sinusoids are very small, only flexible cells are able to pass easily from the red pulp cords to the lumen of the sinusoids.
- **Splenic cords** (*Billroth's cords*) contain a network of reticular cells supported by reticular fibers. The splenic cords contain T and B lymphocytes, macrophages, plasma cells, and many blood cells (erythrocytes, platelets, and granulocytes).

Blood circulation

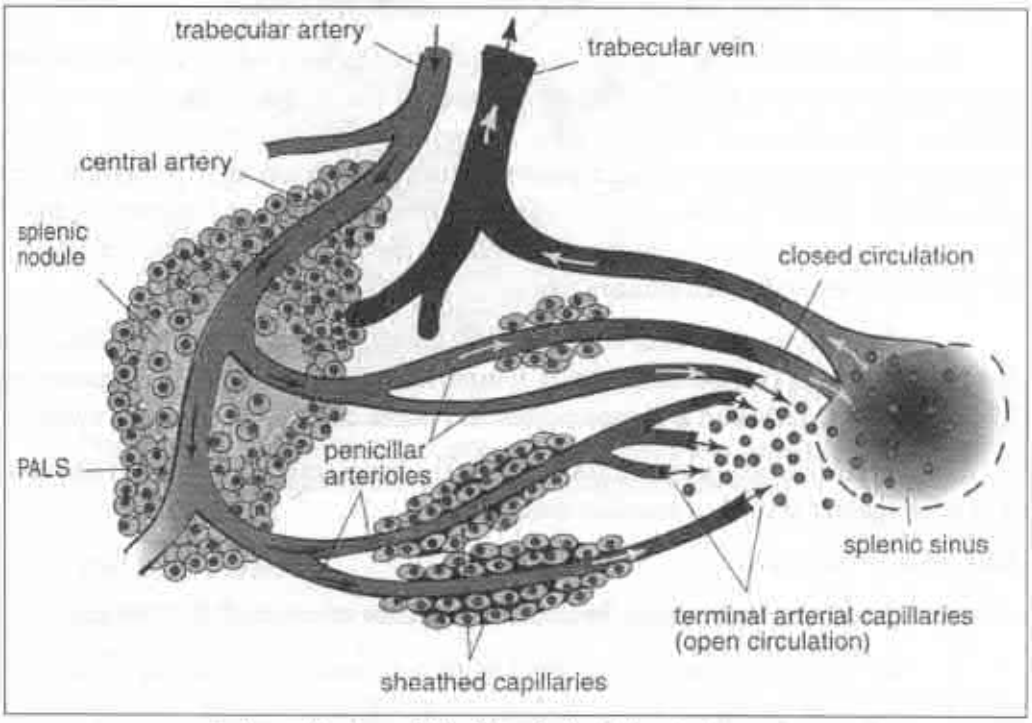
The splenic artery divides as it penetrates the hilum, branching into **trabecular arteries** of various sizes that follow the course of the connective tissue trabeculae. When they leave the trabeculae to enter the parenchyma, the arteries are immediately enveloped by a sheath of lymphocytes. These vessels are known as **central arteries** or **white pulp arteries.** In these nodules the artery, which has now turned into an arteriole, occupies an eccentric position but is still called the central artery.

After leaving the white pulp, the central artery (arteriole) subdivides to form straight **penicillar arterioles**. Near their termination, some of the penicillar arterioles are surrounded by a thick sheath of reticular cells, lymphoid cells, and macrophages.

The manner in which blood flows from the arterial capillaries of the red pulp to the interior of the sinusoids has not yet been completely explained. Some investigators suggest that the capillaries open directly into the sinusoids, forming a **closed circulation** in which the blood always remains inside the vessels. Others maintain that the prolongations of the penicillar arteries open into the splenic

cords, and the blood passes through the space between the cells to reach the sinusoids (**open circulation**).

From the sinusoids, blood proceeds to the red pulp veins that join together and enter the trabeculae, forming the **trabecular veins**. The splenic vein originates from these vessels and emerges from the hilum of the spleen. The trabecular veins do not have individual muscle walls. They can be considered channels hollowed out in the trabecular connective tissue and lined by endothelium. Trabecular veins form the **splenic vein.**



Schematic view of the blood circulation in the spleen

TONSILS

Tonsils belong to the MALT, but because they are incompletely encapsulated, they are considered organs and will be studied apart from the MALT. The tonsils are accumulations of lymphoid tissue surrounding the openings of the digestive and respiratory tracts. They form a ring around the opening to these tracts called Waldeyer's ring (**lymphatic tissue ring**).

Depending on their localization we distinguish: **palatine tonsils** (the tonsils), which are located in the lateral wall of the oropharynx and covered by a stratified

squamous epithelium, **lingual tonsils** which are situated in the lamina propria at the root of the tongue and also covered by a stratified squamous epithelium, and **pharyngeal tonsils** (also called nasopharyngeal tonsils or adenoids) which are located in the upper posterior part of the throat (nasopharynx) and covered by a pseudostratified ciliated epithelium with goblet cells, and **tubal tonsil** which is located posterior to the opening of the pharyngotympanic tube into the nasopharynx.

The tonsils do not have afferent lymph vessels. Efferent lymph vessels are present. Exposure to antigens relies on the contact of antigens with cells of the immune system across the epithelium which covers the tonsils.

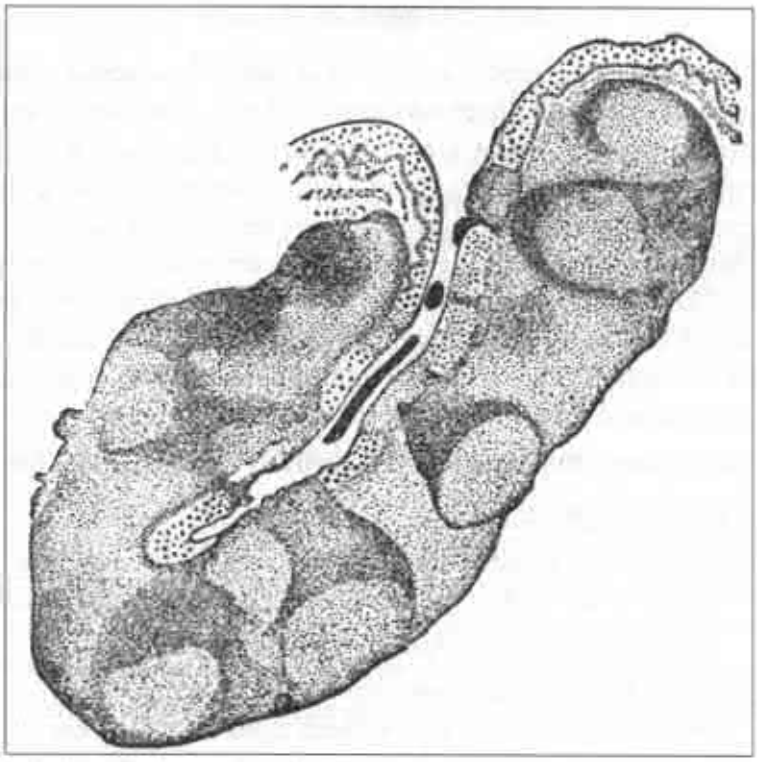
The epithelium of the palatine and lingual tonsils forms deep crypts into the lymphoid tissue, and the consequent increase of the surface area is one way to facilitate the contact of antigens with the cells of the immune system. Crypts trap and destroy bacteria and particulate matter. In addition, the epithelium may specialize in places to form an open meshwork of cells with an incomplete basal lamina (a reticulated epithelium) which allows the infiltration of the epithelium by lymphocytes and macrophages.

Lymphoid tissue of tonsils contains follicles with germinal centres. Tonsillar lymphoid nodules consist mainly of B-lymphocytes. Other areas are occupied by T-lymphocytes, activated B-lymphocytes and other cells of the immune system.

The capsule (when present) lies between the tonsil and the subjacent tissue as a barrier against spread of tonsillar infections.

PALATINE TONSIL

- The surface is irregular because of presence of crypts (10-12 cripts).
- The surface is lined by stratified squamous nonkeratinized epithelium,
- The wall of the crypts is represented by stratified squamous nonkeratinized epithelium that can be infiltrated with leukocytes.
- The lamina propria contains lymphatic nodules. Each nodule has 2 regions: germinative centre and marginal zone.
- Tunica submucosa forms the hemicapsule of the tonsil which delimits them from the pharyngeal muscle and facilitates their removal in tonsillitis. Submucosa is represented by a connective tissue, contains blood vessels, nerves and mucous minor salivary glands, which are drained by ducts directly on the outer surface of tonsil.



The palatine tonsil consists of diffuse lymphocytes and lymphoid nodules disposed under a stratified squamous epithelium

LINGUAL TONSILS

The lingual tonsils are rounded masses of lymphatic tissue that cover the posterior region of the tongue.

They are on the dorsal surface at the base of the tongue. Their lymphatic tissue is dense and nodular, their surface is covered with stratified squamous epithelium which invaginates as a crypts into each lingual tonsil. They are partially surrounded by connective tissue placing them in the group of Partially-Encapsulated Lymphatic Organs. They have associated mucous glands in the submucosa which are drained by ducts directly into the tonsillar crypt.

PHARYNGEAL TONSILS, unlike other types of tonsils, have pseudostratified columnar epithelium. They also differ from the other tonsil types by lacking crypts. The adenoids are often removed along with the tonsils. This can cause a very sore throat for about a week and rather unpleasant breath. Most people's adenoids are not even in use after a person's third year, but if they cause problems they must be removed or they may otherwise shrink.

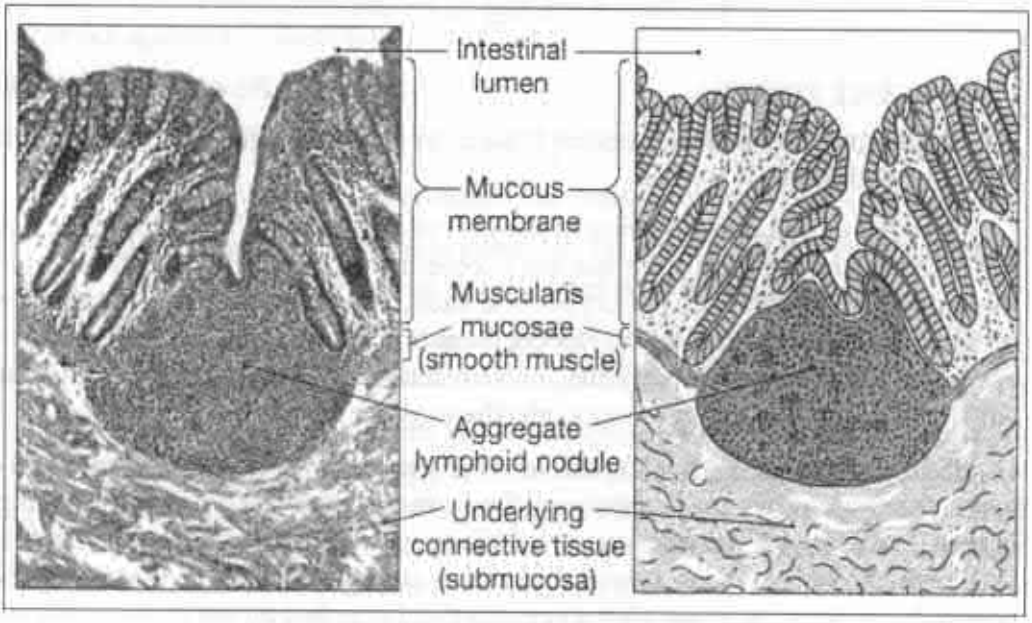
MALT

MALT is really lymphatic tissue located beneath mucous membranes in which the lymphocyte is the predominant cell type. Examples occur in the respiratory, gastrointestinal, urinary and reproductive tracts. The exact extent of these aggregations of lymphocytes is not easily discernible because they have no distinct capsule like that of lymph nodes. However, they are like lymph nodes in that they often have a pale-staining germinal center containing actively dividing lymphocytes like the germinal centers in lymph nodes. The larger aggregations contain B and T cell zones and antigen processing cells; the smaller, more scattered MALT components are mostly T lymphocytes. Some B cells and plasma cells are also present.

- MALT protects the digestive and respiratory systems from foreign matter.

Aggregates of Lymphoid Follicles

- Peyer's patches – isolated clusters of lymphoid tissue, similar to tonsils.
  - Found in the wall of the distal portion of the small intestine.
  - Similar structures are found in the appendix.
- Peyer's patches and the appendix:
  - Destroy bacteria, preventing them from breaching the intestinal wall.
  - Generate "memory" lymphocytes for long-term immunity.



The lymphoid nodule of the large intestinum

THE IMMUNE SYSTEM

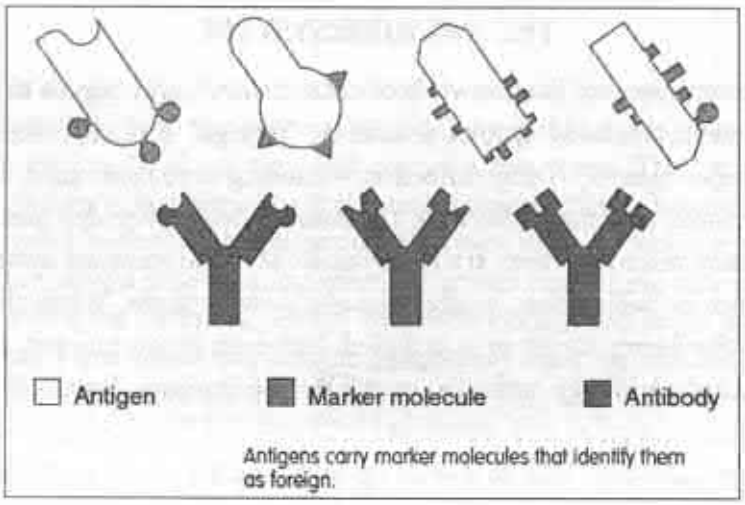
The immune system is a network of cells, *tissues**, and organs that worktogether to defend the body against attacks by "foreign" invaders. These are primarily *microbes* (germs) – tiny, infection – causing *organisms* such as *bacteria, viruses, parasites,* and *fungi.* Because the human body provides ideal environment for many microbes, they try to break in. It is the immune system's job to keep them out or, failing that, to seek out and destroy them. When the immune system hits the wrong target or is crippled, however, it can unleash a torrent of diseases, including *allergy,* arthritis, or *AIDS.* The immune system is amazingly complex. It can recognize and remember millions of different enemies, and it can produce secretions and cells to match up with and wipe out each one of them. The secret to its success is an elaborate and dynamic communications network. Millions and millions of cells, organized into sets and subsets, gather like clouds of bees swarming around a hive and pass information back and forth. Once immune cells receive the alarm, they undergo tactical changes and begin to produce powerful chemicals. These substances allow the cells to regulate their own growth and behavior, enlist their fellows, and direct new recruits to trouble spots.

Self and Nonself

The key to a healthy immune system is its remarkable ability to distinguish between the body's own cells–self and foreign cells–nonself. The body's immune defenses normally coexist peacefully with cells that carry distinctive "self" marker *molecules.* But when immune defenders encounter cells or organisms carrying markers that say "foreign," they quickly launch an attack.

Anything that can trigger this *immune response* is called an *antigen.* An antigen can be a microbe such as a virus, or even a part of a microbe. Tissues or cells from another person (except an identical twin) also carry nonself markers and act as antigens. This explains why tissue transplants may be rejected.

In abnormal situations, the immune system can mistake self for nonself and launch an attack against the body's own cells or tissues. The result is called an *autoimmune disease.* Some forms of arthritis and diabetes are autoimmune diseases. In other cases, the immune system responds to a seemingly harmless foreign substance such as ragweed pollen. The result is allergy, and this kind of antigen is called an *allergen.*



Antigens carry marker molecules that identify them as foreign.

Immune Cells and Their Products

The immune system stockpiles a huge arsenal of cells, not only lymphocytes but also cell-devouring *phagocytes* and their relatives. Some immune cells take on all comers, while others are trained on highly specific targets. To work effectively, most immune cells need the cooperation of their comrades. Sometimes immune cells communicate by direct physical contact, sometimes by releasing chemical messengers.

The immune system stores just a few of each kind of the different cells needed to recognize millions of possible enemies.

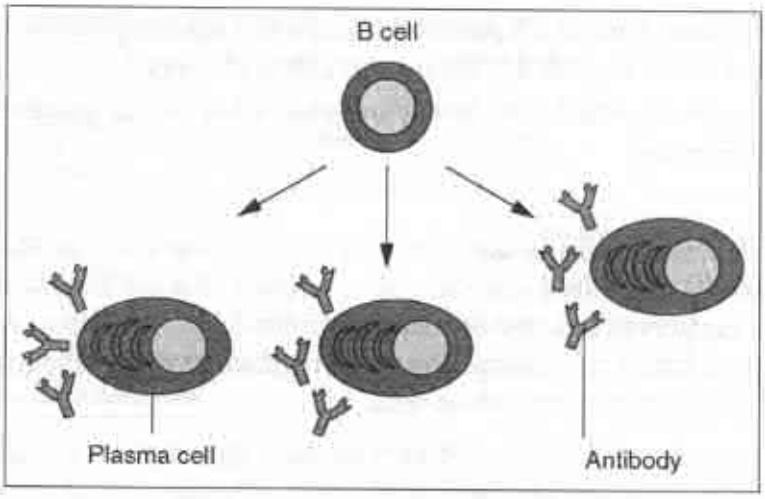
When an antigen appears, those few matching cells multiply into a full-scale army. After their job is done, they fade away, leaving sentries behind to watch for future attacks.

All immune cells begin as immature *stem cells* in the bone marrow. They respond to different *cytokines* and other signals to grow into specific immune cell types, such as T cells, *B cells*, or phagocytes. Because stem cells have not yet committed to a particular future, they are an interesting possibility for treating some immune system disorders. Researchers currently are investigating if a person's own stem cells can be used to regenerate damaged immune responses in autoimmune diseases and immune deficiency diseases.

B cells and T cells are the main types of lymphocytes.

B Lymphocytes

B cells work chiefly by secreting substances called *antibodies* into the body's fluids. Antibodies ambush antigens circulating the bloodstream.



They are powerless, however, to penetrate cells. The job of attacking target cells – either cells that have been infected by viruses or cells that have been distorted by cancer–is left to T cells or other immune cells.

Each B cell is programmed to make one specific antibody. For example, one B cell will make an antibody that blocks a virus that causes the common cold, while another produces an antibody that attacks a bacterium that causes pneumonia.

When a B cell encounters its triggering antigen, it gives rise to many large cells known as *plasma cells*. Every plasma cell is essentially a factory for producing an antibody. Each of the plasma cells descended from a given B cell manufactures millions of identical antibody molecules and pours them into the bloodstream.

An antigen matches an antibody much as a key matches a lock. Some match exactly; others fit more like a skeleton key. But whenever antigen and antibody interlock, the antibody marks the antigen for destruction.

Antibodies belong to a family of large molecules known as *immunoglobulins*. Different types play different roles in the immune defense strategy.

- Immunoglobulin G, or IgG, works efficiently to coat microbes, speeding their uptake by other cells in the immune system.
- IgM is very effective at killing bacteria.
- IgA concentrates in body fluids – tears, saliva, the secretions of the respiratory tract and the digestive tract – guarding the entrances to the body.

- IgE, whose natural job probably is to protect against parasitic infections, is the villain responsible for the symptoms of allergy.
- IgD remains attached to B cells and plays a key role in initiating early B-cell response.

T Cells

Unlike B cells, T cells do not recognize free-floating antigens. Rather, their surfaces contain specialized antibody-like receptors that see fragments of antigens on the surfaces of infected or cancerous cells. T cells contribute to immune defenses in two major ways: some direct and regulate immune responses; others directly attack infected or cancerous cells.

Helper T cells, or *Th cells*, coordinate immune responses by communicating with other cells. Some stimulate nearby B cells to produce antibody, others call in microbe-gobbling cells called phagocytes, still others activate other T cells.

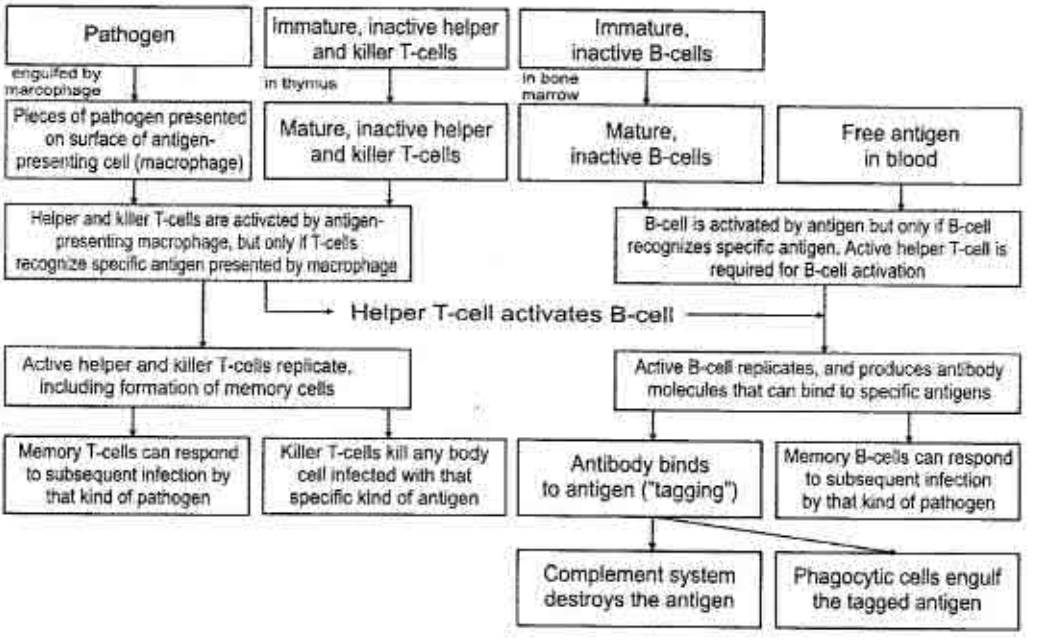
Killer T cells – also called *cytotoxic T lymphocytes* or *CTLs* – perform a different function. These cells directly attack other cells carrying certain foreign or abnormal molecules on their surfaces. CTLs are especially useful for attacking viruses because viruses often hide from other parts of the immune system while they grow inside infected cells. CTLs recognize small fragments of these viruses peeking out from the cell membrane and launch an attack to kill the cell.

In most cases, T cells only recognize an antigen if it is carried on the surface of a cell by one of the body's own *MHC*, or *major histocompatibility complex*, molecules. MHC molecules are proteins recognized by T cells when distinguishing between self and nonself. A self MHC molecule provides a recognizable scaffolding to present a foreign antigen to the T cell.

Natural killer (NK) cells are another kind of lethal white cell, or lymphocyte. Like killer T cells, NK cells are armed with *granules* filled with potent chemicals. But while killer T cells look for antigen fragments bound to self-MHC molecules, NK cells recognize cells lacking self-MHC molecules. Thus NK cells have the potential to attack many types of foreign cells.

Phagocytes and Their Relatives

Phagocytes are large white cells that can swallow and digest microbes and other foreign particles. *Monocytes* are phagocytes that circulate in the blood. When monocytes migrate into tissues, they develop into *macrophages*. Specialized types of macrophages can be found in many organs, including lungs, kidneys, brain, and liver.



Macrophages play many roles. As scavengers, they rid the body of worn-out cells and other debris. They display bits of foreign antigen in a way that draws the attention of matching lymphocytes. And they churn out an amazing variety of powerful chemical signals, known as *monokines*, which are vital to the immune responses.

Granulocytes are another kind of immune cell. They contain granules filled with potent chemicals, which allow the granulocytes to destroy *microorganisms*. Some of these chemicals, such as histamine, also contribute to inflammation and allergy.

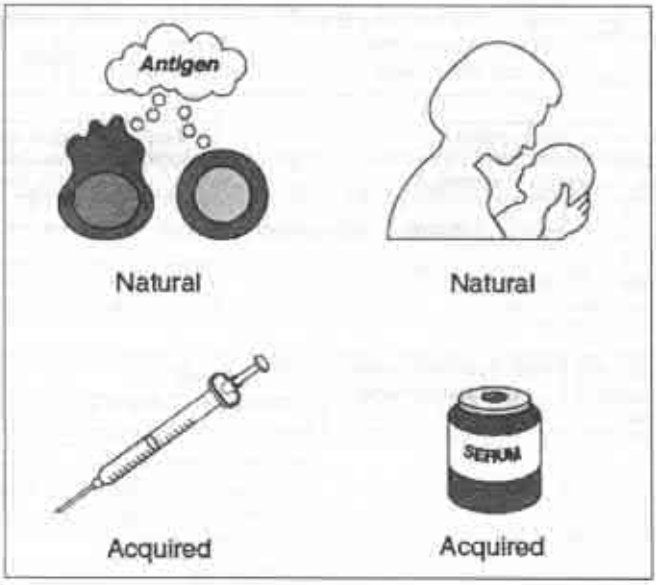
One type of granulocyte, the *neutrophil*, is also a phagocyte; it uses its prepackaged chemicals to break down the microbes it ingests. *Eosinophils* and *basophils* are granulocytes that "degranulate," spraying their chemicals onto harmful cells or microbes nearby.

The *mast cell* is a twin of the basophil, except that it is not a blood cell. Rather, it is found in the lungs, skin, tongue, and linings of the nose and intestinal tract, where it is responsible for the symptoms of allergy.

Complement

The *complement* system is made up of about 25 proteins that work together to "complement" the action of antibodies in destroying bacteria. Complement also helps to rid the body of antibody-coated antigens (antigen-antibody complexes). Complement proteins, which cause blood vessels to become dilated and then

leaky, contribute to the redness, warmth, swelling, pain, and loss of function that characterize an *inflammatory response.*



Complement proteins circulate in the blood in an inactive form. When the first protein in the complement series is activated– typically by antibody that has locked onto an antigen–it sets in motion a domino effect. Each component takes its turn in a precise chain of steps known as the *complement cascade.* The end product is a cylinder inserted into–and puncturing a hole in–the cell's wall. With fluids and molecules flowing in and out, the cell swells and bursts. Other components of the complement system make bacteria more susceptible to *phagocytosis* or beckon other cells to the area.

Immunity: Natural and Acquired

Immunity can be strong or weak, short-lived or long-lasting, depending on the type of antigen, the amount of antigen, and the route by which it enters the body.

Immunity can also be influenced by inherited *genes.* When faced with the same antigen, some individuals will respond forcefully, others feebly, and some not at all.

An immune response can be sparked not only by infection but also by immunization with *vaccines.* Vaccines contain microorganisms–or parts of microorganisms–that have been treated so they can provoke an immune response but not full-blown disease.

Immunity can also be transferred from one individual to another by injections of *serum* rich in antibodies against a particular microbe (*antiserum*).

For example, immune serum is sometimes given to protect travelers to countries where hepatitis A is widespread. Such *passive immunity* typically lasts only a few weeks or months.

Infants are born with weak immune responses but are protected for the first few months of life by antibodies received from their mothers before birth. Babies who are nursed can also receive some antibodies from breast milk that help to protect their digestive tracts.

Immune Tolerance

Immune *tolerance* is the tendency of T or B lymphocytes to ignore the body's own tissues. Maintaining tolerance is important because it prevents the immune system from attacking its fellow cells. Scientists are hard at work trying to understand how the immune system knows when to respond and when to ignore.

Tolerance occurs in at least two ways. Central tolerance occurs during lymphocyte development. Very early in each immune cell's life, it is exposed to many of the self molecules in the body. If it encounters these molecules before it has fully matured, the encounter activates an internal self-destruct pathway and the immune cell dies. This process, called clonal deletion, helps ensure that self-reactive T cells and B cells do not mature and attack healthy tissues.

Because maturing lymphocytes do not encounter every molecule in the body, they must also learn to ignore mature cells and tissues. In peripheral tolerance, circulating lymphocytes might recognize a self molecule but cannot respond because some of the chemical signals required to activate the T or B cell are absent. So-called clonal anergy, therefore, keeps potentially harmful lymphocytes switched off. Peripheral tolerance may also be imposed by a special class of regulatory T cells that inhibits helper or cytotoxic T-cell activation by self antigens.

Vaccines

Medical workers have long helped the body's immune system prepare for future attacks through vaccination. Vaccines consist of killed or modified microbes, components of microbes, or microbial *DNA* that trick the body into thinking an infection has occurred. An immunized person's immune system attacks the harmless vaccine and prepares for subsequent invasions. Vaccines remain one of the best ways to prevent infectious diseases and have an excellent safety record. Previously devastating diseases such as smallpox, polio, and whooping cough have been greatly controlled or eliminated through worldwide vaccination programs.

CHAPTER V

FORMATION OF BLOOD CELLS
(HEMATOPOIESIS)

Mature blood cells have a relatively short life span (die within hours, days or weeks) and consequently the population must be continuously replaced with the progeny of stem cells **Hematopoiesis** is the process by which immature precursor cells develop into mature blood cells. There are two types of hematopoiesis: prenatal (embryonic) and post-natal.

PRENATAL (embryonic) HEMATOPOIESIS represents formation of blood as tissue. This process takes place in the embryo, in: **yolk sac**, **liver**, **thymus**, **lymph nodes, spleen, bone marrow.**

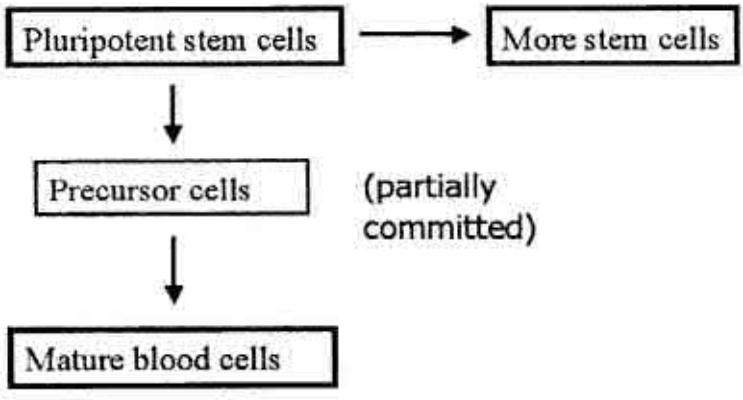
In the fetus, hematopoiesis starts during the first trimester in islands of hematopoiesis found in the wall of the the yolk sac (*the first phase of hematopoiesis*). The islands develop from hemangioblasts, the progenitors of both hematopoietic and endothelial cells. Hemangioblasts give rise to primary red blood cells, which are biggest in size than normocytes (*megaloblastic type of hemopoiesis*). The first site of red blood cells formation occurs inside of the first blood vessels. This type of hematopoiesis is called the **intravascular hemopoiesis.**

The same time begins formation of the granulocytes (*especially neutrophils and eosinophils*, but in comparison with formation of red blood cells this process take place extravascular (outside of the blood vessel).

Fetal hematopoiesis continues after the second trimester in the liver (*the second or hepatic phase of hematopoiesis*) and then in the spleen. Hemopoiesis in the liver and other organs occurs only extravascular. Hematopoiesis gives rise to all blood cell types. Hemopoiesis in the liver takes place until birth.

During the seventh month of intrauterine life, the bone marrow becomes the primary site of hematopoiesis, where it remains during adulthood (*the third phase of hematopoiesis*).

The currently accepted theory on how the hematopoiesis works is called the monophyletic theory which simply means that a single type of stem cell gives rise to all the mature blood cells in the body. This stem cell is called the **pluripotential (pluripotent) stem cell**.



Schematic diagram of the monophyletic theory

PLURIPOTENT CELLS (stem cells) are undifferentiated cells. Pluripotent stem cells can self-renew and produce two other types of cells: the **myeloid** and **lymphoid** progenitor stem cells that develop into distinct cell progenies. They are located in bone marrow.

PROGENITOR CELLS (hemistem cells or multipotential cells) have reduced potentiality. They proliferate and differentiate into unipotential stem cells (colony forming units [CFU]) in the presence of appropriate growth factors. Four colony forming units derive from the myeloid progenitor: the erythroid CFU, the megakaryocyte CFU, the granulocyte CFU, and the monocyte-macrophage CFU.

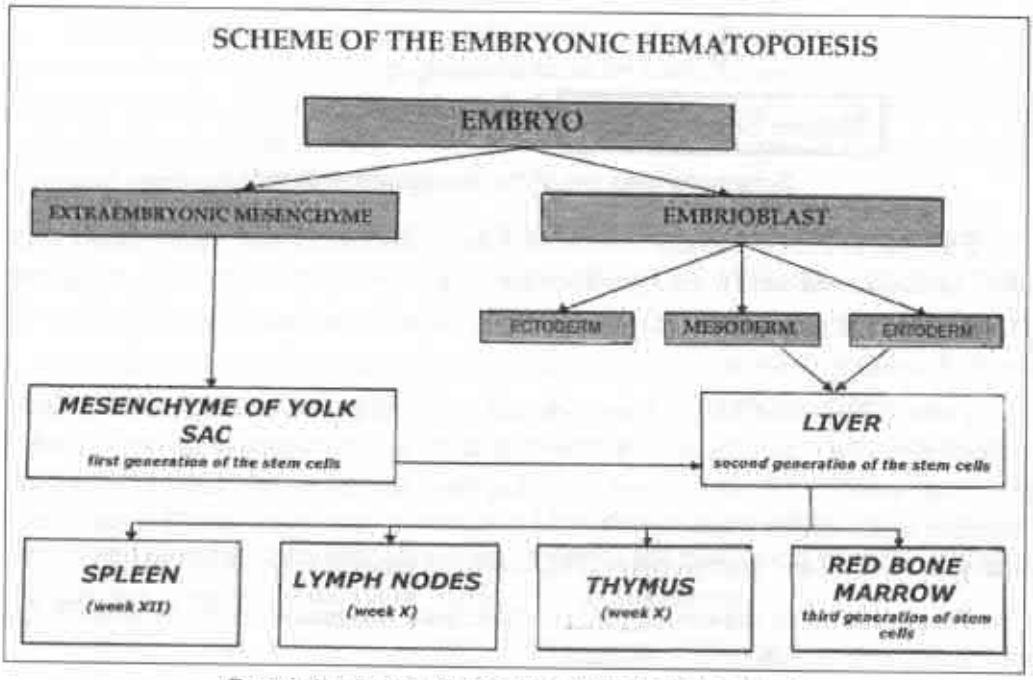
Progenitor cells can divide and produce both progenitor and precursor cells, precursor cells produce only mature blood cells.

PRECURSOR CELLS are all the cells in each lineage that display distinct morphologic characteristics for the first time, in contrast, stem and progenitor cells cannot be morphologically distinguished and resemble large lymphocytes.

Although the pluripotent stem cells and the unipotential stem cells cannot be distinguished from one another histologically, the precursor cells can be distinguished with a trained and practiced eye.

Hematopoiesis depends on favorable microenvironmental conditions and the presence of growth factors. The microenvironmental conditions are furnished by cells of the stroma of hematopoietic organs, which produce an ad-

equate extracellular matrix. A general view of hematopoiesis shows that as this process takes place, both the potential for differentiation and the self-renewing capacity of the initial cells gradually decrease. In contrast, the mitotic response to growth factors gradually increases, attaining its maximum in the middle of the process. From that point on, mitotic activity decreases, morphological characteristics and functional activity develop, and mature cells are formed. Once the necessary environmental conditions are present, the development of blood cells depends on factors that affect cell proliferation and differentiation. These substances are called **growth factors, colony-stimulating factors** (CSF), or **hematopoietins (poietins)**.



General scheme of the embryonic hematopoiesis

POSTNATAL HEMATOPOIESIS is a physiological regeneration of the blood.

- occurs only in red marrow of flat bones like sternum, ribs, skull and pelvis and ends of long bones (myeloid tissue) and in lymphatic tissue.

ERYTHROPOIESIS (development of erythrocytes)

The basic process in maturation of the red blood cells is the synthesis of hemoglobin and the formation of an enucleated, biconcave, small corpuscle. The development of an erythrocyte from the first recognizable cell of the series to the

release of reticulocytes into the blood takes approximately 7 days. The hormone erythropoietin and substances such as iron, folic acid, and cyanocobalamin (vitamin B_{12}) are essential for the production of erythrocytes. **Erythropoietin** is a glycoprotein produced mainly in the kidneys that stimulates the production of mRNA for **globin,** the protein component of the hemoglobin molecule.

Erythropoiesis includes the following sequence:

1. Erythroblast
2. Pronormocyte
3. Basophilic normocyte
4. Polychromatophilic normocyte
5. Orthochromatophilic normocyte
6. Reticulocyte
7. Erythrocyte

The first recognizable cell in the erythroid series is the **pronormocyte.** It is a large cell with loose, lacy chromatin and clearly visible nucleoli; its cytoplasm is basophilic. The next stage is represented by the **basophilic normocyte** with a strongly basophilic cytoplasm and a condensed nucleus that has no visible nucleolus. The basophilia of these two cell types is caused by the large number of polyribosomes involved in the synthesis of hemoglobin. During the next stage, polyribosomes decrease, and areas of the cytoplasm begin to be filled with hemoglobin. At this stage, staining causes several colors to appear in the cell – the **polychromatophilic normocyte.** In the next stage, the nucleus continues to condense and no cytoplasmic basophilia is evident, resulting in a uniformly acidophilic cytoplasm – the **orthochromatophilic normocyte.**

At a given moment, this cell puts forth a series of cytoplasmic protrusions and expels its nucleus, encased in a thin layer of cytoplasm. The expelled nucleus is engulfed by macrophages. The remaining cell still has a small number of polyribosomes that, when treated with the dye brilliant cresyl blue, aggregate to form a stained network. This cell is the **reticulocyte,** which soon loses its polyribosomes and becomes a mature erythrocyte *(see fig. 79, plate II).*

GRANULOPOIESIS (development of granulocytes)

The maturation process of granulocytes takes place with cytoplasmic changes characterized by the synthesis of a number of proteins that are packed in two organelles: the **azurophilic** and **specific granules.** These proteins are produced in

the rough endoplasmic reticulum and the Golgi complex in two successive stages. The first stage results in the production of the **azurophilic granules,** which stain with basic dyes in the Wright or Giemsa methods and contain enzymes of the lysosomal system. In the second stage, a change in synthetic activity takes place with the production of several proteins that are packed in the **specific granules.** These granules contain different proteins in each of the three types of granulocytes and are utilized for the various activities of each type of granulocyte. Evidently, a shift in gene expression occurs in this process, permitting neutrophils to specialize in bacterial destruction and eosinophils and basophils to become involved in the regulation of inflammation. The different stages of maturation and the morphological changes that occur during this process are:

The **myeloblast** is the most immature recognizable cell in the myeloid series. It has a finely dispersed chromatin, and nucleoli can be seen. In the next stage, the **promyelocyte** is characterized by its basophilic cytoplasm and azurophilic granules. These granules contain lysosomal enzymes and myeloperoxidase. The promyelocyte gives rise to the three known types of granulocyte. The first sign of differentiation appears in the myelocytes, in which specific granules gradually increase in quantity and eventually occupy most of the cytoplasm. These **neutrophilic, basophilic,** and **eosinophilic metamyelocytes** mature with further condensation of the nucleus and a considerable increase in their specific granule content. Before its complete maturation, the neutrophilic granulocyte passes through an intermediate stage in which its nucleus has the form of a curved rod (**band cell**). This cell appears in quantity in the blood after strong stimulation of hematopoiesis *(see fig. 81, plate II).*

The total time taken for a myeloblast to emerge as a mature neutrophil in the circulation is about 11 days.

DEVELOPMENT OF MONOCYTES

The **monoblast** is a committed progenitor cell that is almost identical to the **myeloblast** in its morphological characteristics. Further differentiation leads to the **promonocyte,** a large cell with a basophilic cytoplasm and a large, slightly indented nucleus. The chromatin is lacy, and nucleoli are evident. Promonocytes divide twice in the course of their development into **monocytes.** A large amount of rough endoplasmic reticulum is present, as is an extensive Golgi complex in which granule condensation can be seen to be taking place. These granules are **primary lysosomes,** which are observed as fine **azurophilic granules** in blood monocytes. Mature monocytes enter the bloodstream, circulate for about 8 h, and then enter the connective tissues, where they mature into **macrophages** and function for several months *(see fig. 83, plate II).*

DEVELOPMENT OF PLATELETS

In adults, platelets originate in the red bone marrow by fragmentation of the cytoplasm of mature **megakaryocytes**, which, arise by differentiation of **megakaryoblasts.** Megakaryocytes release platelets in at least two ways:

- extension of pseudopodia through the wall of the sinuses; pseudopodia contain "strings" of platelets that are pinched off and released into the circulation.
- passage of mature megakaryocyte into circulation and fragmenation in the pulmonary vascular bed.

MEGAKARYOCYTES

Megakaryocytes are very large cell. They have a multilobed nucleus with numerous nucleoli. The nucleus becomes highly polyploid before platelets begin to form. The cytoplasm of this cell is homogeneous and intensely basophilic; contains numerous mitochondria, a well-developed rough endoplasmic reticulum, and an extensive Golgi complex *(see fig. 82, plate II).*

DEVELOPMENT OF LYMPHOCYTE

Study of the precursor cells of lymphocytes is difficult, because these cells do not contain specific cytoplasmic granules or nuclear lobulation, both of which facilitate the distinction between young and mature forms of granulocytes. Lymphocytes are distinguished mainly on the basis of size, chromatin structure, and the presence of nucleoli in smear preparations. As lymphocyte cells mature, their chromatin becomes more compact, nucleoli become less visible, and the cells decrease in size. In addition, subsets of the lymphocyte series acquire distinctive cell-surface receptors during differentiation that can be detected by immunocytochemical techniques.

All lymphocyte progenitor cells originate in the bone marrow. Some of these lymphocytes migrate to the thymus, where they acquire the full attributes of T lymphocytes. Subsequently, T lymphocytes populate specific regions of peripheral lymphoid organs. Other bone marrow lymphocytes differentiate into B lymphocytes in the bone marrow and then migrate to peripheral lymphoid organs, where they inhabit and multiply in their own special compartments.

The first identifiable progenitor of lymphoid cells is the **lymphoblast,** a large cell capable of incorporating and dividing two or three times to form **prolymphocytes.** Prolymphocytes are smaller and have relatively more condensed chromatin but none of the cell-surface antigens that mark prolymphocytes as T or B lymphocytes .

In the bone marrow and in the thymus, these cells synthesize cell-surface receptors characteristic of their lineage *(see fig. 80, plate II).*

CHAPTER VI

ENDOCRINE SYSTEM

Cell communication is vital for any multicellular organism to function efficiently. At a local level, cells communicate via cell surface molecules and gap junctions, while remote communication is mediated by the secretion of chemical messengers, which activate cells by interacting with specific receptors.

There are three types of secretions:

1. **Autocrine secretion**
   - occurs when a cell secretes a chemical messenger to act on its own receptors.
   - is particularly evident in the local control of cell growth by growth factors such as epidermal growth factor.
2. **Paracrine secretion**
   - is the secretion of chemical messengers to act on adjacent cells.
   - is also mainly concerned with the local control of cell growth.
   - is a mode of action of many of the cells of the diffuse neuroendocrine system within the respiratory and digestive tracts.

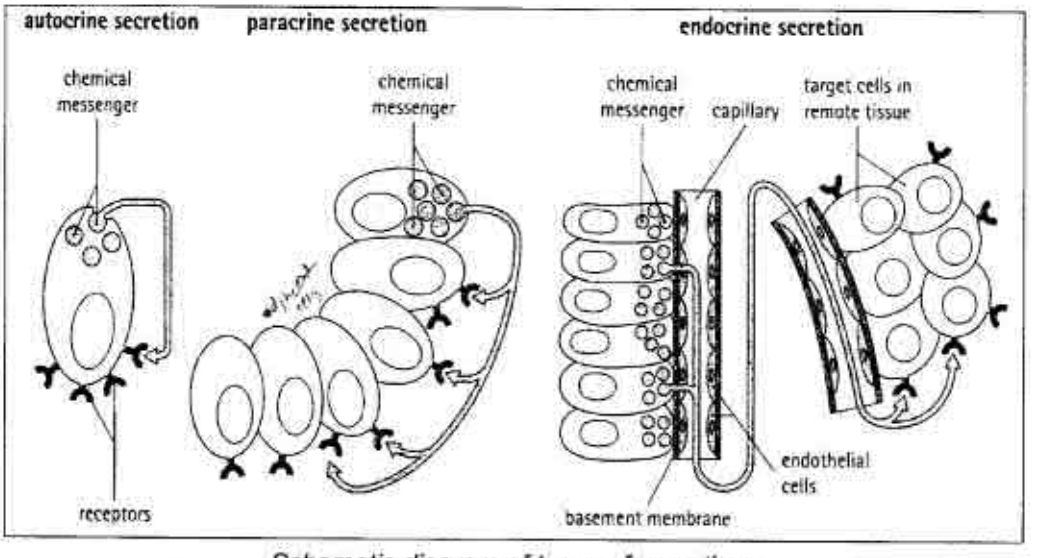
   Other common features of autocrine and paracrine secretion
   - Secretions do not enter the circulatory system but act on local receptors.
   - Chemical messengers are rapidly destroyed after secretion thereby limiting their activity and region of influence.
3. **Endocrine secretion**
   - is the secretion of chemical messengers called hormones into the blood stream to act on distant tissues. Secretory pole of endocrine cells is adjacent to the capillary vessel wall, while the nucleus is found at the opposite pole unlike other types of secretory cells.

a. **Endocrine tissues**

- are usually highly vascular to facilitate rapid dissemination of secreted products into the blood stream.

b. **Hormones**

- act on receptors of distant cells to cause an intracellular response.
- act slowly and are diffused throughout the body via the circulatory system.



Schematic diagram of types of secretions

ENDOCRINE SYSTEM

- Includes the group of organs, parts of some organs and single cells that produce hormones into the blood or lymph.
- Hormones act to the target organs or target cells.
- Hormones have properties of tropism (like a key of a lock).
- Target cell has specific receptors for the hormone.

Endocrine cells and tissue specialization

A. **Distribution of endocrine cells:**

1. **Endocrine gland**

- is a specialized organ composed of endocrine cells (e.g. adrenal, pituitary, and thyroid glands).

2. **Discrete clusters of endocrine cells**
   - located in a specialized organ which also has other non-endocrine functions (e.g. ovarian follicle secretes estrogen, cells in testis secrete testosterone, islets of Langerhans in pancreas secrete insulin).
3. **Diffuse neuroendocrine cells**
   - are dispersed singly amongst other cells in epithelial tissues, particularly in the gut and respiratory tract. Example: Granule cells of respiratory epithelium secrete chemicals that affect goblet cell secretion.

APUD cells (**a**mine **p**recursor **u**ptake and **d**ecarboxylation)

- are neuroendocrine cells that secrete amines or peptides.
- take up amine precursors, which then undergo decarboxylation in the process of hormone synthesis.

B. **Neuroendocrine cells**
   - have chemical messengers within membrane-bound vesicles, which are called granules.
   - can transiently store the granules, which can be identified in cells using immunohistochemical staining techniques.
   - Secretion is achieved by exocytosis in which the membrane of the vesicle fuses with the cell membrane, thereby discharging the chemical messenger outside the cell.

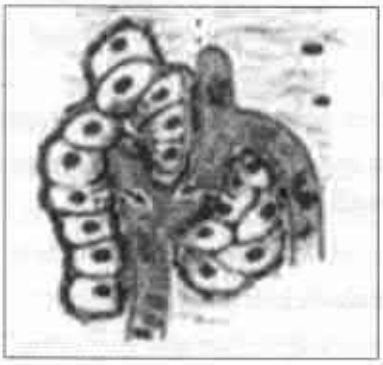
Functions:

- Metabolism and tissue maturation
- Ion regulation
- Water balance
- Immune system regulation
- Heart rate and blood pressure regulation
- Control of blood glucose and other nutrients
- Control of reproductive functions
- Uterine contractions and milk release
- Coordinate the function of the organs, system of organs of the human body

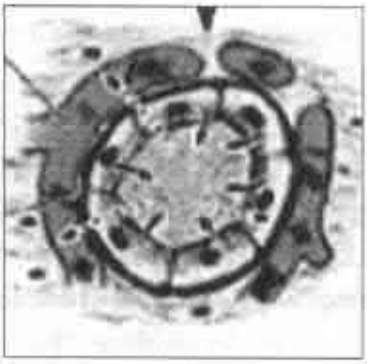
GENERAL CHARACTERISTIC OF ENDOCRINE ORGANS

- Secretory cell is called **endocrinocyte.**
- Endocrine organs have a **great blood supply** (a network of blood capillaries are surrounded cords or follicles of cells).

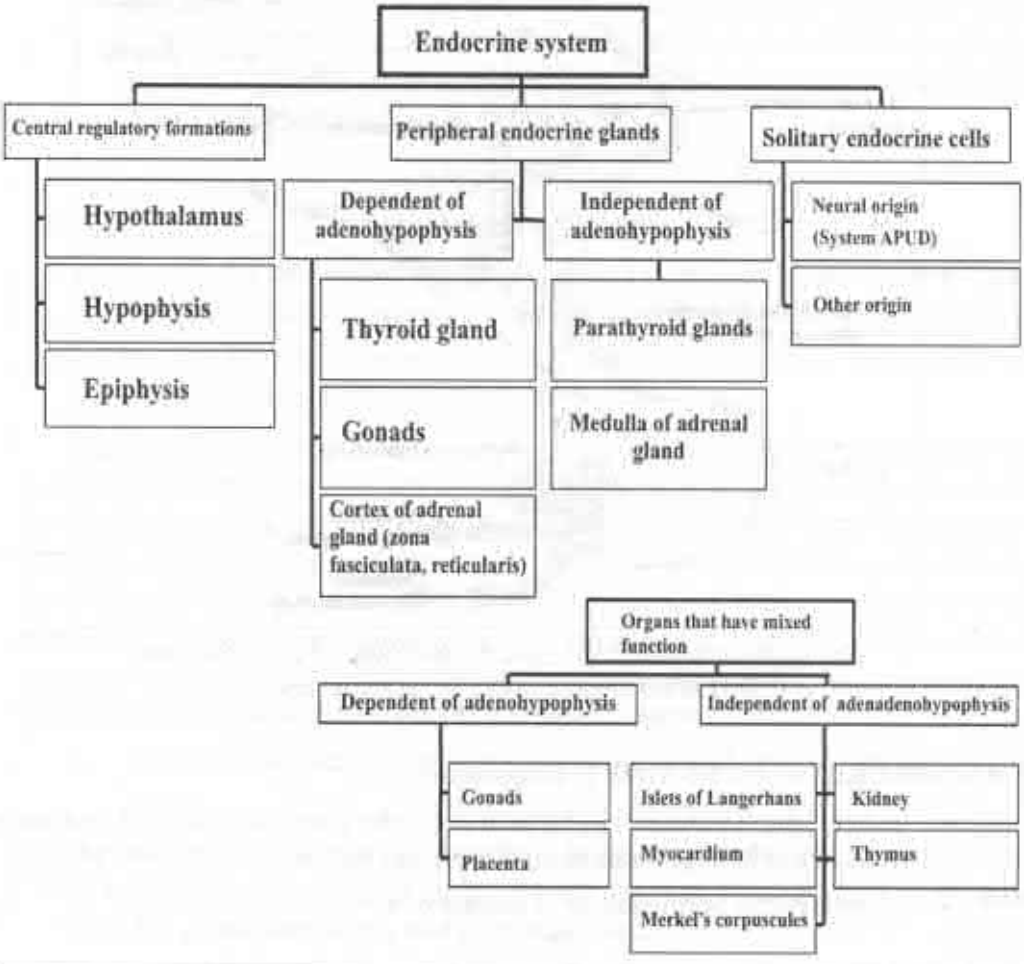
- Type of **capillaries: fenestrated** sinusoid.
- The arrangement of cells can be into **cord** or into **follicle.**



cord



follicle



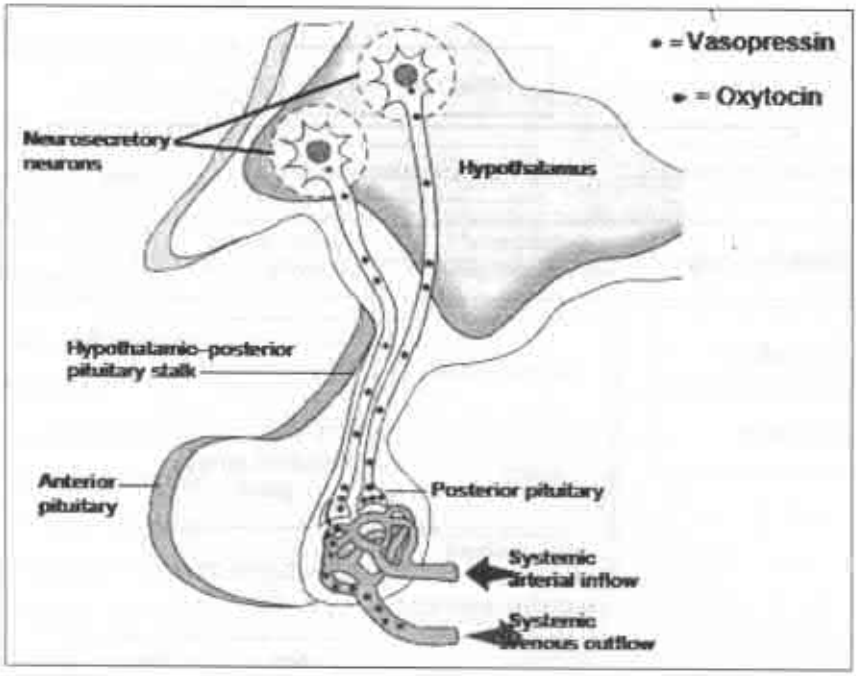
CENTRAL ENDOCRINE REGULATORY FORMATIONS

HYPOTHALAMUS

- Neurons of the grey matter of hypothalamus are formed of 32 pairs of nuclei.
- These nuclei are situated in 3 zones: anterior, medium and posterior.
- According to the sizes of neurons and their functions these nuclei can be divided into: **large-celled and small-celled**

LARGE-CELLED NUCLEI are:

- **Supraoptic and paraventricular nuclei.**
- These nuclei produce 2 hormones: vasopressin and oxytocin.



The hypothalamo-hypophyseal system, with its vascularization and sites of hormone production, storage, and release

ANTIDIURETIC HORMONE (vasopressin)

- acts on the tubules of nephron in the kidney and provides the reabsorption of the 99% of water from the primary urine;
- increases the permeability of nephrons for urea.

- increases blood pressure by promoting the contraction of smooth muscles (vasoconstriction) in small arteries and arterioles.
- Lack of ADH secretion is a cause of diabetes insipidus.

OXYTOCIN

- causes the contraction of uterine smooth muscle during copulation and delivery;
- causes **involution** of the uterus after delivery.
- causes the contraction of the myoepithelial cells of the secretory alveoli and alveolar ducts of the breast at time of the breast-feeding;
- causes the contraction of the myoepithelial cells of the seminiferous tubules of testis at time of orgasm and ejaculation.

SMALL-CELLED NUCLEI

- Produce the proteins that promote or inhibit the secretion and excretion of adenohypophyseal hormones. For example: growth-hormone-releasing factor, growth-hormone-inhibiting factor, thyrotropin-releasing factor, gonadotropin-releasing factor & other.

HYPOPHYSIS
(pituitary gland)

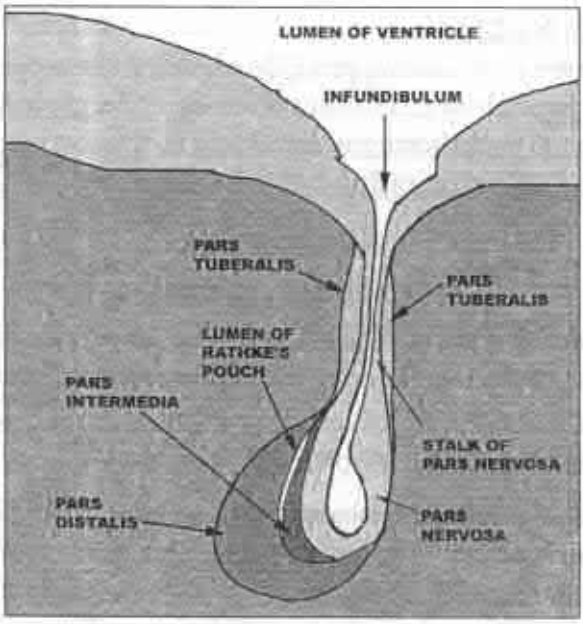
- is an endocrine gland producing several hormones that have important functions in the regulation of metabolism, growth, and reproduction.
- is a small complex organ located at the base of the brain in the sella turcica (small depression of sphenoid bone).

During embryogenesis, the hypophysis develops partly from oral ectoderm and partly from nerve tissue. The neural component arises as an evagination from the floor of the diencephalon and grows caudally as a stalk without detaching itself from the brain. The oral component arises as an outpocketing of ectoderm from the roof of the primitive mouth of the embryo and grows cranially, forming a structure called **Rathke's pouch.** Later, a constriction at the base of this pouch separates it from the oral cavity. At the same time, its anterior wall thickens, reducing the lumen of Rathke's pouch to a small fissure.

- Because of its dual origin, hypophysis is divided into two major components:

1. **Adenohypophysis** (anterior pituitary) that has epithelial origin and consists of epithelial secretory cells (endocrinocytes). It is divided into 3 parts: **pars distalis, pars intermedia and pars tuberalis.**

2. **Neurohypophysis** (posterior pituitary) consists of neuroglial cells (**pituicytes**); has neural origin; is formed embryologically as the part of a down growth (infundibulum) from the floor of the hypothalamus. This connection to the hypothalamus is maintained by the definitive posterior pituitary. It form three definitive structures:
   a. **Pars nervosa** – is the large inferior portion adjacent to the adenohypophysis.
   b. **Infundibular stem (stalk)** – is the intermediate connecting piece joining the pars nervosa to the floor of the hypothalamus (median eminence).
   c. **Median eminence** – is the upper most part of the neurohypophysis. It forms part of the floor of the third ventricle of the hypothalamus.

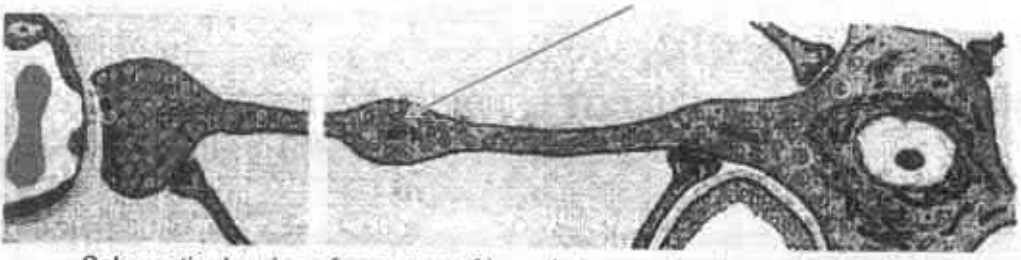


The schematic structure of the hypophysis

PARS NERVOSA OF THE NEUROHYPOPHYSIS is not an endocrine gland but rather a storage site for the neurosecretions of hormones by the large-sized cells (magnocellular) of the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus.

- axons constitute 75% of the pars nervosa.
- forms a direct neuronal link that conveys neurosecretory hypothalamic hormones from the neurons of the hypothalamus to the pars nervosa of the posterior pituitary.

- unmyelinated axons course from **Supraoptic and paraventricular** nuclei through the median eminence, down the infundibular stem, and into the pars nervosa.
- axon terminals end in close proximity to fenestrated capillary network of the pars nervosa.
- Hormones travel down the axons within membrane-bound neurosecretory granules. These are stored in dilated axonal terminals called **Herring bodies**.



Schematic drawing of a neuron of hypothalamus showing a Herring body

The Hypothalamo-Hypophyseal System

Because of its embryological origin, the hypophysis is connected to the hypothalamus, with which it has important anatomic and functional relationships.

In the hypothalamo-hypophyseal system there are three known sites of production of hormones that liberate three groups of hormones:

1. The first group consists of peptides produced by aggregates (nuclei) of secretory neurons in the hypothalamus: the supraoptic and the paraventricular nuclei. The hormones are transported along the axons of these neurons and accumulate in the ends of these axons, which are situated in the neurohypophysis. These hormones are released by exocytosis, enter capillaries of the neurohypophysis, and are distributed by the blood.
2. The second group of peptide hormones is produced by neurons of the dorsal medial, ventral medial, and infundibular nuclei of the hypothalamus. These hormones are carried along axons that end in the median eminence where the hormones are stored. After being released these hormones enter the blood capillaries of the median eminence and are transported to the adenohypophysis through the first stretch of the hypophyseal portal system.

3. The third group of hormones consists of proteins and glycoproteins produced by cells of the pars distalis and liberated into blood capillaries of the second stretch of the portal system. These capillaries surround the secretory cells and distribute the hormones to the general circulation.

ADENOHYPOPHYSIS

- is the master gland of the endocrine system.
- produces small protein or glycoprotein hormones which act as follows: ***1. Stimulate or inhibit the secretions of other endocrine glands (e.g. thyroid gland) in the body*** and ***2. Stimulate or inhibit non-glandular tissues (e.g. epiphyseal plate of long bones for growth).***

HISTOLOGY OF PARS DISTALIS OF ADENOHYPOPHYSIS

- Pars distalis contains a large collection of hormone producing epithelial cells that are arranged in cords or clusters.
- Pars distalis has a rich network of large fenestrated capillaries (sinusoids) surrounding the clusters and cords of epithelial cells. This allows the released hormones to easily enter the blood.

CELLS OF PARS DISTALIS OF ADENOHYPOPHYSIS (*classification based on histological dye staining*) may be classified in:

1. **Chromophobes** (stain poorly)
2. **Chromophils** (stain well) *(see fig. 108, plate II)*
   - **Acidophils** (stain with acid dyes into pink color)
   - **Basophils** (stain with basic dyes into blue color)

CHROMOPHOBES form groups of small cells in the interior of the cords.

They are represented by:

1. **cambial reserve** (undifferentiated endocrinocytes)
2. **partially degranulated acidophils or basophils**
3. **old cells**
4. **supporting cells**

CHROMOPHILS

ACIDOPHILS produce the following hormones:

- **somatotrophs** – somatotropin (growth hormone, GH)
- **lactotrophs** – prolactin (mammotropin)

BASOPHILS

- **thyrotrophs** – thyroid stimulating hormone (TSH)
- **gonadotrophs** – follicle-stimulating hormone (FSH)
- luteinizing hormone (LH)
- **adrenocorticotrophs** – adrenocorticotropic hormone (ACTH)

HORMONES	**ACTION** in the human body
Somatotropin (growth hormone, GH)	Provides the growth of all tissues practically. The insufficiency of this hormone reduces growth (dwarf). The surplus of this hormone: giantism, acromegaly).
Prolactin (mammotropin)	Stimulates the growth of the mammary glands and secretion of the milk.
Thyroid stimulating hormone (TSH)	Stimulates the thyroid gland to secrete thyroxine (tetraiodthyronine, T4) and triiodthyronine (T3).
Adrenocorticotropic hormone (ACTH)	Stimulates the cortex of the adrenal gland to produce glucocorticoids and gonadocorticoids.
Luteinizing hormone (LH)	- Provides the ovulation. - Provides the secretion of the female sex hormones in ovaries and testosterone in testis.
Follicle-stimulating hormone (FSH)	- Stimulates the growth of the follicles in ovaries. - Stimulates the spermatogenesis.

PARS INTERMEDIA OF ADENOHYPOPHYSIS

- Is poorly developed
- Consists of cords of cells that resemble basophils, except they are smaller and chromophobes. They sometimes form vesicles – **pseudofollicles.**
- Cells produce: **melanocyte-stimulating hormone** (MSH) that stimulates pigmentation within the melanocytes of the skin and **lipotropin** that increases the lipid metabolism.

Blood Supply

To understand the function of the hypophysis, it is important to first study its blood supply. The blood supply of the hypophysis derives from the internal carotid artery. From above, the **superior hypophyseal arteries** supply the median eminence and the neural stalk; from below, **inferior hypophyseal arteries** provide blood mainly for the neurohypophysis, with a small supply to the stalk.

The superior hypophyseal arteries divide into a plexus of fenestrated capillaries that irrigates the stalk and median eminence. These capillaries then rejoin to form veins that develop into a secondary capillary plexus in the adenohypophysis. This **hypophyseal portal system** is of outmost importance because it carries neurohormones from the median eminence to the adenohypophysis where they control the function of the cells of this part of the hypophysis.

PINEAL GLAND
(epiphysis cerebri or pineal body)

Development

- is derived from the neuroectoderm of the posterior portion of the roof of the diencephalon and remains attached to the brain by a short stalk.
- Maximal growth is attained at about seven years of age and thereafter shows retrogressive changes. This regression includes an increase in the amount of connective tissue and the formation of *corpora arenacea* (brain sand), which is comprised of *calcium carbonate and phosphates* within an organic matrix, and their functional significance is unknown.

Predominant cell types are *pinealocytes and astrocytes* (glial cells).

1. PINEALOCYTES
   - comprise *95% of the parenchymal cells.*
   - are arranged in clumps or cords within the lobules formed by connective tissue septa that extend into the gland from the pia mater.
   - appear (silver stain preparations) as irregularly shaped cells with long branching processes that terminate in bulbous endings near blood vessels.
   - produce *melatonin*, which enters the blood vessels for distribution.
2. ASTROCYTES
   - serve as supporting elements and form an interwoven network around and between the cords and clumps of pinealocytes.

Innervation:

- is exclusively via postganglionic autonomic fibers from the *superior cervical sympathetic ganglion.*
- controls *secretory activity of pinealocytes.*

Functions of pineal gland:

- Contain numerous neurotransmitters and neuroendocrine regulatory peptides including norepinephrine, dopamine, serotonin, histamine, somatostatin, and melatonin.

- Acts as a *neuroendocrine transducer*, converting a neural input into a hormonal output that modifies the functional activity of other endocrine organs.
- has a role in adjusting to sudden changes in day length, such as those experienced by travelers who suffer jet lag, and in regulating emotional responses to reduced day length during winter in temperate and subarctic regions.

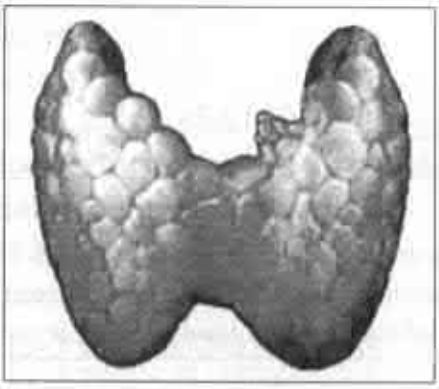
MELATONIN

- functions in regulating reproductive cycles.
- Light ultimately inhibits the pineal gland from secreting melatonin; plasma levels rise during darkness and falls during light in a daily 24 hour *(circadian) rhythm*. Light entering the eye entrains (sets) the endogenous clock within the suprachiasmatic nucleus (SCN) of the hypothalamus, which effects various biological functions in a circadian (24 hour) manner. Suprachiasmatic nucleus ultimately innervates the superior cervical ganglion, which innervates the pineal gland.

PERIPHERAL ENDOCRINE SYSTEM

THYROID GLAND

- is the largest endocrine gland
- consists of right and left lobes connected by a narrow isthmus. A sheath, derived from the pretracheal layer of deep fascia, surrounds the gland and attaches it to the larynx and trachea.
- Thin connective tissue capsule surrounds the thyroid gland and trabeculae penetrate the parenchyma to partially outline irregular lobes and lobules.

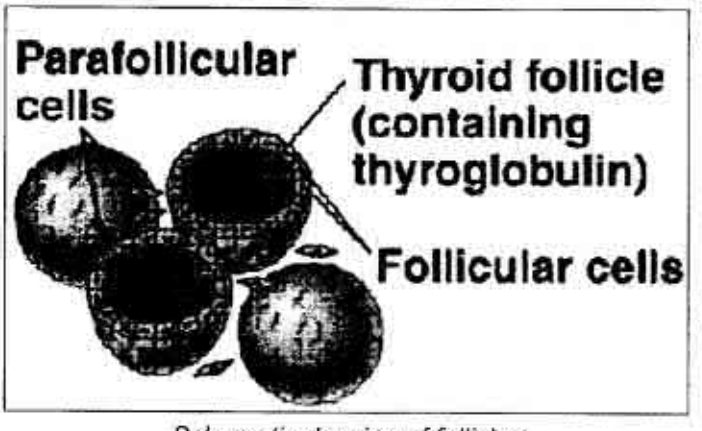


Anatomy of the human thyroid

FOLLICLES

- are the **structural (morpho-functional) units** of the gland *(see fig. 109, plate II)*.
- are roughly spherical cyst-like structures with a wall formed by ***simple cuboidal epithelium***. Epithelial cells can range from squamous (hypofunction) to columnar (hyperfunction) depending upon their activity.

- are filled with a gel-like mass called **colloid**.
- Apical surface of the follicular epithelial cells are in contact with the colloid and the basal surfaces rest on a typical basement membrane.
- vary in diameter from about 0.2 mm to 1.0 mm.
- **each follicle is surrounded by the capillary network.** Type of capillaries is fenestrated sinusoid.
- Two types of follicular epithelia cells are the ***follicular (principal) cells*** and ***parafollicular cells (C-cells).***



Schematic drawing of follicles

FOLLICULAR CELL

- has a free border that faces the lumen of the follicle.
- has small microvilli, which are characteristic of both absorptive and secretory cells, projecting into the lumen.
- secrete **T3 (triiodthyronine), T4 (tetraiodthyronine, thyroxine)**

PARAFOLLICULAR or C-CELLS

- are derived from neural crest cells.
- are *pale staining cells* that most frequently occur in small clusters between follicles.
- do not extend to the follicular lumen to contact the colloid.
- are characterized by the presence of *numerous membrane-bound granules* throughout the cytoplasm.
- **secrete *calcitonin*** in response to high blood calcium levels. Calcitonin ***lowers blood calcium* levels** by: 1) *Suppressing bone resorption* by the

osteoclasts and 2) Increasing the rate of *osteoid calcification*. Low levels of blood calcium inhibit calcitonin release. Calcitonin is an antagonist of parathyroid hormone.

COLLOID OF FOLLICULAR LUMEN

- is mostly formed by a large glycoprotein called ***thyroglobulin***, which is iodinated.
- is synthesized by the follicular cells.

a. **THYROGLOBULIN**

- has a protein portion which is synthesized in the RER of the follicular epithelial cells and glycosylated there and in the Golgi apparatus.
- is secreted by exocytosis into the lumen of the follicle.
- **is NOT a hormone**, but is an *inactive precursor storage form of thyroid hormone*. The thyroid is unique among endocrine glands in that it *stores large amounts of its secretory product extracellularly*.

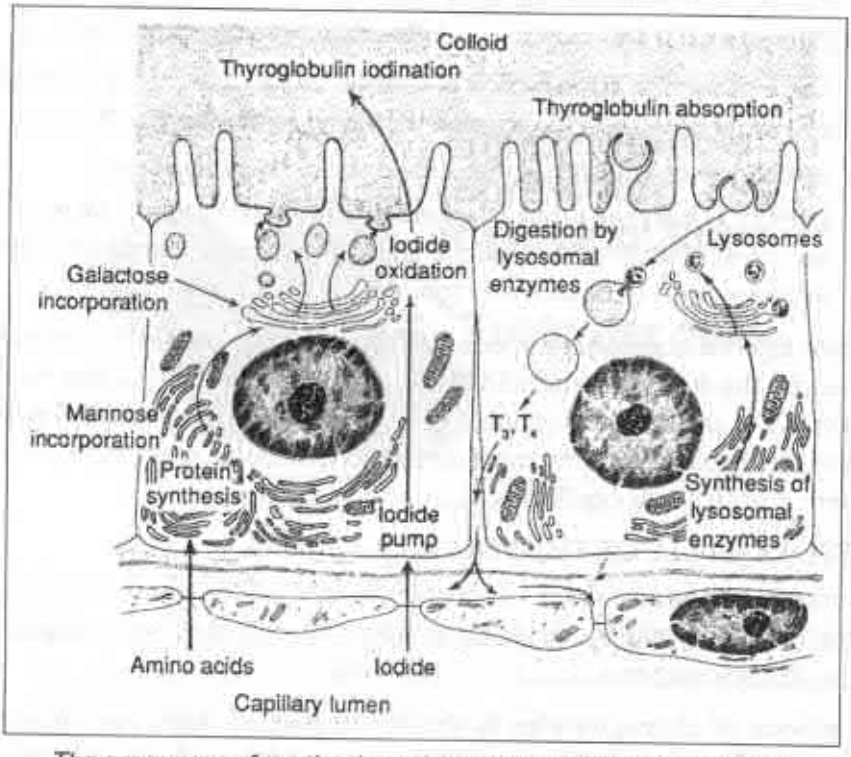
b. When thyroid hormones are needed thyroglobulin in the colloid is endocytosed by the follicular epithelial cells, processed into the active forms thyroxine (T4) and triiodothyronine (T3). T3 and T4 (lipid-soluble) pass freely through the basolateral membrane into extracellular space between follicles where it passes into capillaries.

SECRETORY CYCLE OF THE FOLLICULAR CELL

Synthesis and accumulation of hormones take place in four stages: synthesis of thyroglobulin, uptake of iodide from the blood, activation of iodide, and iodination of the tyrosine residues of thyroglobulin.

1. **Synthesis of thyroglobulin** is similar to that in other protein-exporting cells. Briefly, the secretory pathway consists of the synthesis of protein in the rough endoplasmic reticulum, the addition of carbohydrate in the endoplasmic reticulum and the Golgi complex, and the release of thyroglobulin from formed vesicles at the apical surface of the cell into the lumen of the follicle.
2. The **uptake of circulating iodide** is accomplished in the thyroid follicular cells by a membrane transport protein. This protein, called the Na/I symporter (NIS), is located in the basolateral membrane of the follicular cells and simultaneously carries two molecules, sodium and iodide. Serum iodine plays an important role in regulating thyroid function because low iodine levels increase the amount of NIS and thus increase the uptake, compensating for the lower serum concentration.

3. Iodide is **oxidized** by thyroid peroxidase and is transported into the follicle cavity by an anion transporter called pendrin.
4. Within the colloid occurs the **iodination of tyrosine residues** of thyroglobulin, also catalyzed by thyroid peroxidase, resulting in the formation of monoiodotyrosine and diiodotyrosine. The coupling of these molecules produces the hormones T_3 and T_4, which become part of the much larger thyroglobulin molecule.



The processes of synthesis and secretion of thyroid hormones. These events may occur simultaneously in the same cell

Function of thyroid hormone:

a. Regulates carbohydrate, lipid, and protein metabolism.
b. Primary effect of thyroid hormones is to modulate the activity of the sodium and other ion pumps of the plasma membrane, thereby, regulating the entry of metabolites into the cell.
c. Fetal thyroid begins to function during the tenth week of gestation and its secretions are *essential for normal growth and development*. Thyroid hormone deficiency during fetal development results in *irreversible damage to the CNS*.

Control of the Thyroid

The major regulator of the anatomic and functional state of the thyroid is thyroid-stimulating hormone (TSH; thyrotropin), secreted by the anterior pituitary. TSH stimulates all stages of production and release of thyroid hormones. Thyroid hormones inhibit the synthesis of TSH maintaining an adequate quantity of T_4 and T_3 in the organism. TSH increases the height of the follicular epithelium and decreases the quantity of the colloid and the size of the follicles. The cell membrane of the basal portion of follicular cells is rich in receptors for thyrotropin. Secretion of thyrotropin is also increased by exposure to cold and decreased by heat and stressful stimuli.

When stimulated by TSH, thyroid follicular cells take up colloid by endocytosis. The colloid within the endocytic vesicles is then digested by lysosomal enzymes. Hydrolysis of thyroglobulin results in T_4, T_3, diiodotyrosine, and monoiodotyrosine, which are liberated into the cytoplasm. The free T_4 and T_3 cross the basolateral cell membrane and are discharged into the capillaries. Monoiodotyrosine and diiodotyrosine are not secreted into the blood, and their iodine is removed by a deiodinase. The products of this enzymatic reaction, iodine and tyrosine, are reused by the follicular cells. T_4 is the more abundant compound, constituting 90% of the circulating thyroid hormone, although T_3 acts more rapidly and is more potent.

Clinical consideration:

- Thyroid hormone deficiency during fetal development gives rise to ***cretinism.***
- In adults: ***endemic goiter, myxedema.***

PARATHYROID GLANDS

- are small endocrine glands usually located in the connective tissue on the posterior surface of the thyroid.
- are ovoid and a few millimetres in diameter.
- are arranged in two pairs, the superior and inferior parathyroid glands. However, the number and location may vary. In some individuals (2% to 10%) additional parathyroid glands are associated with the thymus.

STRUCTURE

- Thin connective tissue capsule surrounds the parathyroid gland and separates it from the thyroid. Septa extend from the capsule into the gland to divide it into poorly defined lobules.

- Connective tissue is more evident in the adult.
- *Fat cells increase with age*, ultimately reaching as much as 60-70% of the glandular mass.
- Endocrine cells are arranged into cords. In the parenchyma are distinguished 2 types of cells:

1. PRINCIPAL or CHIEF CELLS
   - secrete parathyroid hormone (PTH).
   - are more numerous than the oxyphil cells.
   - are small cells with a centrally located nucleus and *very pale staining cytoplasm* (in H&E) so nuclei appear to be densely and evenly packed similar to lymphatic tissue.
   - Small, *dense membrane-limited granules* seen with TEM (transmission electron microscopy), or after using special LM (light microscopy) stains, are thought to be the storage form of PTH.
2. OXYPHIL CELLS
   - constitute a *minor portion* of the parenchymal cells.
   - are a **population of old principal cells** & their function is unknown.
   - do not have a secretory role. Secretory granules are not present, and little RER exists.
   - are more rounded and *considerably larger* than the principal cells.
   - have a lightly acidophilic cytoplasm due to abundant mitochondria *(see fig. 110, plate II)*.
   - are found singly or in clusters and *increase in number with age*. Oxyphils are not present in children.

Function of parathyroid hormone

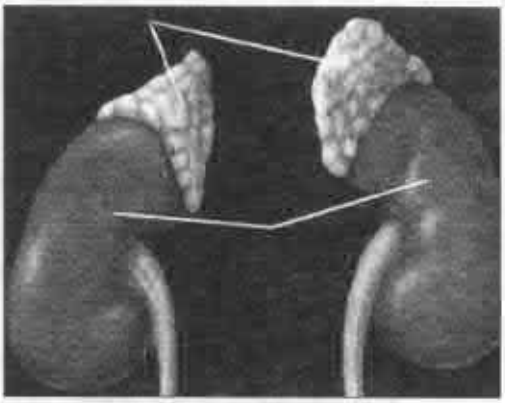
- ***Increases blood levels of calcium ions by three ways:***

1. Bone: **Stimulates *osteoclasts*** *to release stored calcium from bone.*
2. Kidney: ***Stimulates calcium resorption by kidney tubule cells*** *but increases the excretion of phosphate (reduces phosphate concentration in the blood).*
3. Intestine: ***Increased absorption of calcium***.

PTH is essential for life; therefore, care must be taken during thyroidectomy to leave some functioning parathyroid tissue. If the glands are totally removed, death will ensue because muscles, including the laryngeal and other respiratory muscles go into tetanic contraction as the blood calcium levels fall.

THE ADRENAL (suprarenal) GLANDS

The adrenal glands are located in the retroperitoneal tissue near the superior poles of the kidneys. They are covered by a thick connective tissue capsule. Thin collagenous trabeculae extend from the capsule to penetrate the interior of the gland. Trabeculae carry blood vessels, lymphatics, and nerves.



The location of the adrenal glands at the superior pole of each kidney

HISTOLOGIC APPEARANCE AND EMBRYOLOGY

Cells are arranged in cords along capillaries. Gland is composed of two concentric layers.

1. **Adrenal cortex** – is the peripheral layer.
2. **Adrenal medulla** – is the central inner layer.

Embryology

Cortex and medulla are derived from different germ layers and can be distinguished via functional and morphological characteristics, thus they can be considered as two organs which became united during embryonic development.

a. **Adrenal cortex**
   - is derived from mesoderm.

b. **Adrenal medulla**
   - is derived from neural crest neuroectoderm, which also gives rise to sympathetic ganglia. Both adrenal medulla and sympathetic ganglia cells synthesize norepinephrine.
   - can be considered a collection of modified sympathetic ganglia cells that have lost their axonal processes.

ADRENAL CORTEX

The adrenal cortex is essential for life. It is comprised of cells which synthesize and secrete steroid hormones (e.g. corticosteroids). Steroid hormones are low-molecular weight and *lipid soluble*. Steroids do not require exocytosis for release from the cell because they freely diffuse through the plasma membrane.

Secretion is on demand and cells *do not store their product*. Parenchyme of the adrenal gland can be subdivided into 3 concentric layers based on the arrangement and appearance of the cells *(see fig. 111, plate II)*.

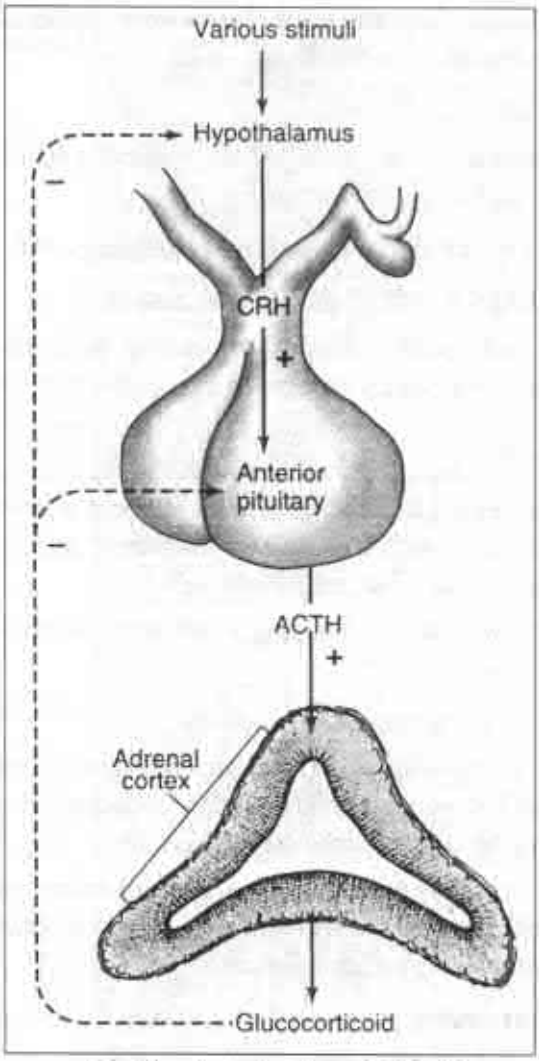
These zones are (from outermost to innermost):

1. **Zona glomerulosa**
   - is associated with *mineralocorticoid* (primarily *aldosterone*) production.
   - is located just underneath the capsule.
   - consists of cells arranged in glomi (L. sphere, sing. glomus), which are oriented around capillaries.

STEROID SECRETING CELLS of glomi
- Cytoplasm is eosinophilic due to abundant membranes of SER. Cytoplasm may appear as either homogenous or as vacuolated (foamy) due to the loss of abundant lipid droplets during histologic preparation.
- Lipid droplets contain cholesterol and other precursors of active hormones.
- Steroid synthesis requires collaboration of SER and mitochondria.

Function: Aldosterone is responsible for sodium and water absorption in distal convoluted tubules of kidney. Angiotensin in blood stimulates release of aldosterone.

2. **Zona fasciculata**
   - Is mainly associated with production of *glucocorticoids* (*cortisol, corticosterone, hydrocortisone*) production.
   - Is located below zona glomerulosa and forms the majority of the cortex.
   - Consists of cells arranged in *straight cords* (fascicles) one or two cells thick, that run toward the medulla with capillaries between them.
   - Cells are referred to as *spongiocytes* because they are much more vacuolated (spongy) than cells of glomerulosa after histologic preparation, due to large amount of lipid droplets.
   - Cellular organelles are similar, but more abundant, than those found in the zona glomerulosa.
   - Glucocorticoid secretion is regulated by the anterior pituitary hormone adrenocorticotropic hormone (ACTH), which is regulated by the hypothalamic hormone corticotropin-releasing hormone (CRH). Glucocorticoid hormones have negative effects on secretion of CRH from the hypothalamus and ACTH from the anterior pituitary.



Feedback mechanism of ACTH and glucocorticoid secretion

Function: They promote normal metabolism of carbohydrates; provide resistance to stress; suppress inflammatory response and some allergic reactions.

3. **Zona reticularis**
   - is associated with *androgen steroid* production and to a lesser extent with estrogen and progesterone production.
   - cells are arranged in *irregular cords and clumps* that form an anastomosing network (*reticulum*).

- cells are smaller than those of the more peripheral zones and can be spongy, but less so than fasciculata cells.

ADRENAL MEDULLA

- Is predominantly under sympathetic control and its hormones contribute to the "fight or flight" response.
- Cells are arranged in *clumps and anastomosing cords around capillaries*:

CHROMAFFIN CELLS – cells of the adrenal medulla

- Synthesize and secrete *catecholamines* (e.g. epinephrine and norepinephrine). Catecholamines are stored in secretory granules (unlike cortical cells).
- Are named for the chromaffin reaction, a staining technique in which catecholamines bind chromium salts to form a brown color. There are two types of chromaffin cells: *norepinephrine secreting cells* (dark cells) *and epinephrine secreting cells* (light cells).
- Are oriented with one end facing a capillary and the opposite end facing a venule.

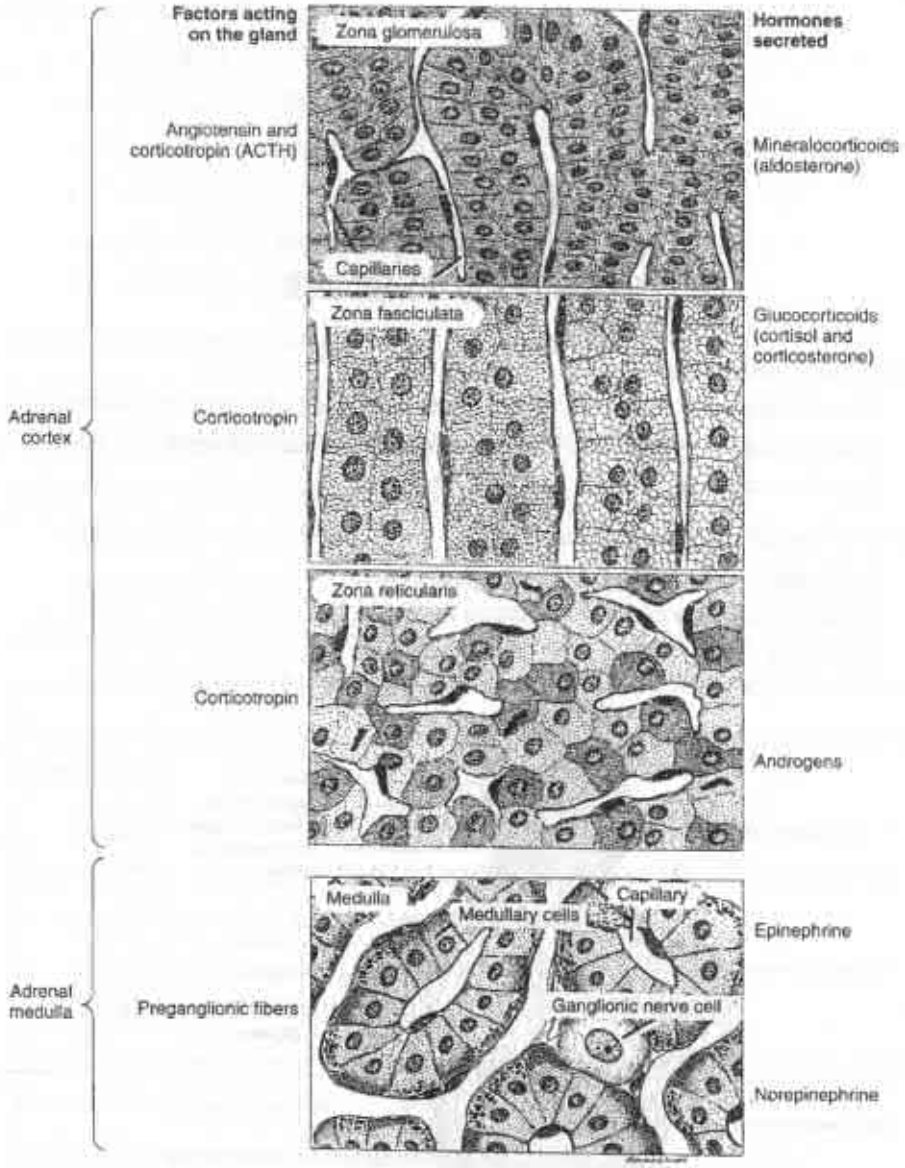
Preganglionic cholinergic sympathetic fibers:

- innervate each chromaffin cell at the capillary end. Chromaffin cells can be regarded as modified sympathetic postganglionic neurons that have lost their axons and dendrites and became secretory cells.
- stimulate the chromaffin cells to release catecholamines into the blood stream at the venule end. Catecholamine release is due to *receptor mediated exocytosis* (receptors activated by acetylcholine).

EPINEPHRINE (adrenaline)

- Adrenal medulla is the major source of epinephrine in the body.
- Is converted from norepinephrine by an enzyme phenylethanolamine-N-methyltransferase (PNMT). Glucocorticoids regulate the synthesis of this enzyme required for the conversion. Glucocorticoids secreted by cortical cells are brought to the medulla via cortical capillaries.

Function: Increase heart rate, blood pressure, sweating, and rate of respiration, induce dilation of bronchioles, decrease urine production, and reduce blood flow to viscera and skin.



Structure and physiology of the adrenal gland

CHAPTER VII

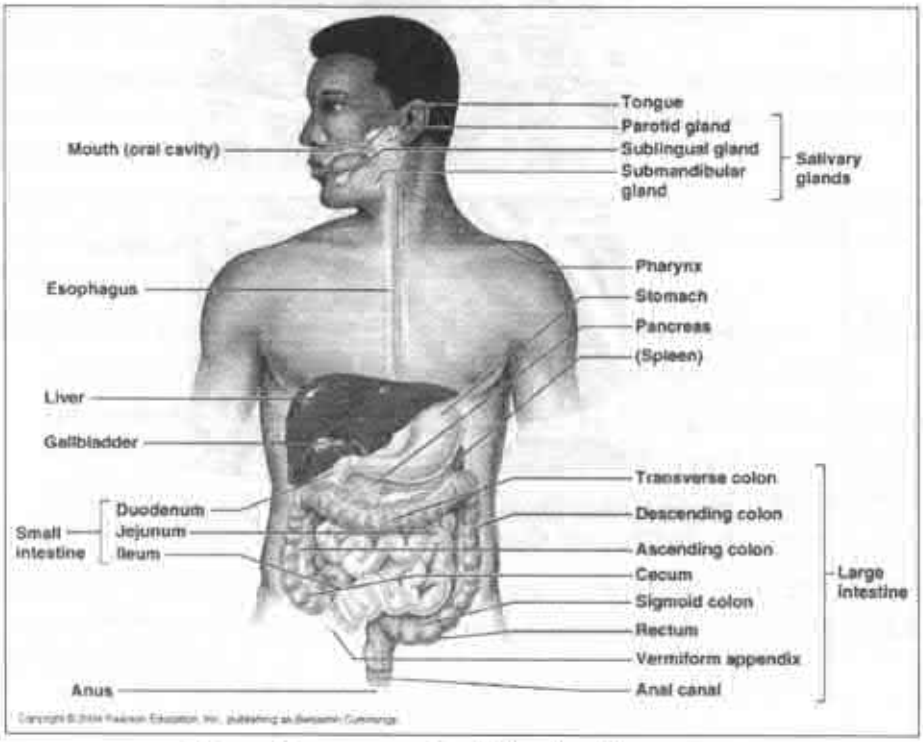
DIGESTIVE SYSTEM

Digestive system consists of:

- The **alimentary canal** (three regions: anterior, medium & posterior)
- & **associated organs of the alimentary canal** (tongue, teeth, salivary glands, pancreas, liver & gallbladder)

Parts of the alimentary canal

- Anterior (oral cavity, pharynx, esophagus)
- Medium (stomach, small & large intestine)
- Posterior (rectum & anus)



The main components of the digestive system

Main functions of the digestive system

- ingestion & propulsion
- mechanical digestion
- enzymatic digestion
- absorption
- compaction (formation of feces)
- elimination (defecation)

Digestive process involves 6 essential activities:

1. **ingestion** – taking food in
   - bolus- an amount of food passing through the tract
2. **propulsion** – moves food through the tract
   - swallowing – voluntary.
   - peristalsis – involuntary muscular waves moves food forward.
3. **mechanical digestion** – physical chewing, mixing, churning
   - segmentation – rhythmic local constrictions of intestine for mixing.
4. **chemical digestion** – catabolism/ breakdown of polymers into monomers by enzymes:
   - begins in mouth, essentially complete in small intestine.
5. **absorption** – passage of digested end products across wall into blood:
   - monomers, vitamins, minerals and water.
   - pass through mucosal cells lining tract.
   - small intestine major absorption site.
6. **defecation** – eliminates indigestible substances and other wastes from body via anus in form of feces.

Embryonic sources of the digestive system:

- The epithelium of the anterior & posterior parts of the alimentary canal is of ectodermal origin.
- The epithelium of the medium part is of endodermal origin.
- Liver, pancreas, gallbladder are of endodermal origin.
- The muscular & peritoneal components are of mesodermal origin.

ORAL CAVITY

The oral cavity is divided in a vestibule, the area "outside" the teeth, and an oral cavity proper. The entire oral cavity is lined by a MUCOSA, which consists of 2 structural components:

- *lining epithelium* – is an epithelium of the oral cavity.
- *lamina propria mucosae* – represented by loose connective tissue.

Epithelium of the oral cavity is stratified squamous:

1. orthokeratinized (keratinized)
2. parakeratinized gum soft palate
3. nonkeratinized

The **ortokeratinized** (keratinized) epithelium consists of four layers: stratum basale, stratum spinosum, stratum granulosum, and stratum corneum. The cells in the stratum corneum (superficial) have no nuclei.

The parakeratinized epithelium is similar histologically to keratinized, except that the cells in the stratum corneum retain their nuclei (cells display flat, pyknotic nuclei), and the cytoplasm of the surface cells stain intensely with eosin.

In **nonkeratinized epithelium** the surface cells retain nuclei, but the cytoplasm of the surface cells does not stain intensely with eosin (no keratin).

According to functional proprieties oral mucosa is classified into 3 types:

- *Lining mucosa*
- *Masticatory mucosa*
- *Specialized mucosa*

1. **Lining mucosa** is found on the:
   - inner surface of the lips
   - ventral surface of the tongue
   - soft palate
   - floor of the oral cavity
   - alveolar mucosal surface
   - cheeks

   Characteristics:

   – Epithelium is stratified squamous nonkeratinized, in some places it may be parakeratinized.

- Covers no stress area.
- Consists of a thin lamina propria mucosa that is very vascular.
- Brighter red in color than the masticatory mucosa.
- Have submucosa that contains bands of collagen and elastic fibers that bind the mucosa to the underlining muscle. The submucosa contains the minor salivary glands (at the border between the submucosa and lamina propria); the larger blood vessels, lymphatic vessels and nerves, all of which, in turn, supply the subepithelial neurovascular networks of the lamina propria.

2. **Masticatory mucosa** is found on the:
   - gums
   - hard palate

   Characteristics:
   - Epithelium is stratified squamous orthkeratinized or parakeratinized.
   - Lamina propria mucosae is thick, it forms the prominent papillae.
   - Have no submucosa.
   - It adapts for mastication and friction.

3. **Specialized mucosa** is restricted to the:
   - dorsal surface of the tongue (papillae & taste buds)

   Characteristics:
   - Epithelium is stratified squamous nonkeratinized or parakeratinized.
   - Epithelium and lamina propria mucosae form specialized structures known as the lingual papillae which contain taste buds.
   - Have no submucosa.
   - It adapts for taste.

THE LIPS AND THE CHEEKS

- The lips and the cheeks have a core of skeletal muscle covered externally by skin.
- The lips and cheeks help keep food between the teeth when we chew and play a small role in speech.

LIPS

- Aid in obtaining food and placing it in the mouth so that the teeth and tongue can manipulate it and begin fragmenting it.

- Lips are much larger than people think, anatomically they extend from the interior margin of the nose to the superior boundary of the chin. The lips are composed of 3 parts:

1. **cutaneous area** *(external surface).*
2. **"red" area** *(transition zone or vermillion border).*
3. **inner** *(mucosal)* **surface.**

Part of lips	Type of epithelium	Glands	Hair
cutaneous area	Stratified squamous keratinized (skin)	Sebaceous and sweat glands	Present
"red" area	Stratified squamous keratinized **BUT** thinner than the cutaneous area. Lamina propria mucosa forms the prominent papillae which extend deep into the epithelium. Lamina propria contains rich capillary networks which determine the red color of the transition zone.	Sebaceous glands may occasionally be present, but they are not active **(so "red" area must be moistened with saliva periodically to prevent it from becoming dry and cracked).**	Absent
inner surface	Stratified squamous nonkeratinized (is thicker than keratinized epithelium)	Minor salivary (labial) glands	Absent

- This transitional zone, where keratinized skin meets the oral mucosa is poorly keratinized and translucent, allowing the red colour of blood in the underlying capillaries to show through *(see fig. 113, plate II).*

CHEEKS

- Have a core of skeletal muscles: buccinators.
- **The inner epithelial surface** – is represented by the stratified squamous nonkeratinized epithelium.
- **There is tunica submucosa,** that provides strong attachment to muscles and that contains mixed & mucous small salivary glands.
- **The outer surface is lined by skin** – stratified squamous keratinized epithelium.

THE PALATE

- forms the roof of the oral cavity, has 2 parts: the **hard palate** anteriorly and the **soft palate** posterioly.

The HARD PALATE

- Has a nasal and oral surface.
- Is firmly adhered to the palatine process (bone), and it forms a rigid surface against which the tongue forces food during chewing.
- Its color is usually pale pink.
- **Epithelium is stratified squamous parakeratinized** (is similar to keratinized except that the cells of the stratum corneum do not lose their nuclei).
- **Lamina propria mucosa is thick** and is firmly attached to the underlying bone without an intervening submucosa. This attachment is so firm that the lamina propria of the oral mucosa serves partly as a periosteum for the bone underneath. This arrangement is called a mucoperiosteum. Lamina propria is formed by dense irregular connective tissue.
- **Tunica submucosa is absent** in the area adjacent to the gums and in the midline *(is present in the lateral part of the hard palate)*. The submucosa in the anterolateral zone contains a variable amount of fat tissue, and also is rich in blood vessels and nerves.
- The mucosa on either side of its raphe, a middle ridge, is slightly corrugated, which helps to create friction.

The SOFT PALATE

- Is a mobile fold formed mostly of skeletal muscle.
- The soft palate rises reflexively to close off the nasopharynx when we swallow.
- **Epithelium:** stratified squamous nonkeratinized in the anterior part and simple pseudostratified columnar ciliated in the posterior one. The lamina propria is separated from the submucosa by a layer containing numerous elastic fibers.
- The submucosa contains the numerous mucous and mixed minor salivary glands.

TONGUE

- occupies the floor of the mouth and fills most of the oral cavity when the mouth is closed.
- freely moving the muscular organ attached to the floor of the pharynx.
- anterior and posterior portions have different embryonic origins.

- boundary between two regions is V-shaped.
- boundary is location of principal gustatory receptors, the **circumvallate papillae.**
- posterior portion contains the lingual tonsils.
- **The core of the tongue is formed by** skeletal muscle. Skeletal (striated) bundles cross one another in four layers: superior longitudinal, vertical, inferior longitudinal, and horizontal. Arrangement of muscle bundles allows the delicate voluntary movement of the tongue. Skeletal (striated) bundles are separated by connective tissue septae which extends into the lamina propria of the overlying mucous membrane. **Between the muscle bundles are located serous, mucous, and seromucous small salivary glands; blood vessels and nerves** (lingual branches of hypoglossal nerve). Lingual muscles are innervated by lingual branches of hypoglossal nerve.

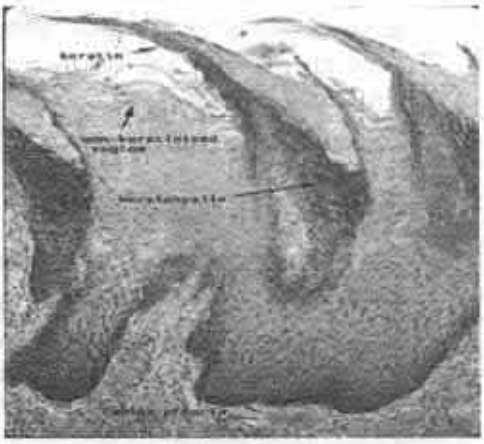
Functions include:

- Gripping and repositioning food during chewing
- Mixing food with saliva and forming the bolus
- Initiation of swallowing, and speech

Tongue has 4 surfaces:

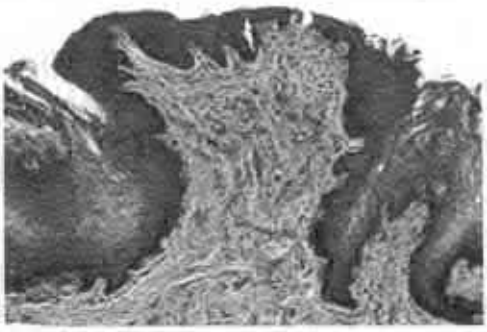
- Dorsal
- Ventral
- Lateral (2)
- The dorsal surface of the tongue is divided by the sulcus terminalis into an oral part, the anterior two-thirds, and a pharyngeal part, the posterior one-third. The epithelium of the pharyngeal part forms a somewhat irregular surface which covers the lingual tonsils. The velvety dorsal surface of the anterior two thirds of the tongue is created by mucosal projections called **lingual papillae**. There are four types of papillae: filiform, fungiform, foliate, and circumvallate. Each papilla consists of a connective tissue core (primary and secondary papillae) covered with a stratified squamous epithelium.

FILIFORM PAPILLAE



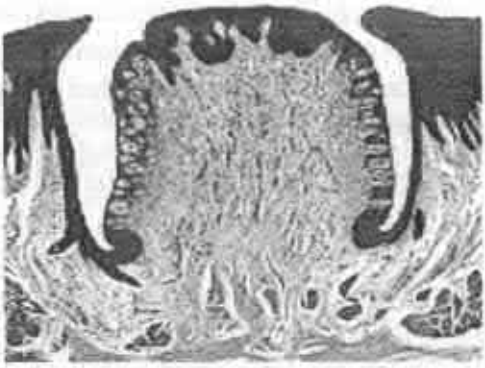
- **Are** the smallest and **the most numerous** papillae.
- **Have the conical shape.**
- **Do not contain taste buds.**
- **Epithelium is stratified squamous nonkeratinized, but on the tips of papillae it becomes keratinized.**
- By providing the tongue with a rough surface they aid in the manipulation and processing of foods – **mechanical function**.

FUNGIFORM PAPILLAE



- Resemble **mushrooms.**
- **Contain taste buds** along their upper surface.
- Are relatively **few in number** and are distributed among the filiform papillae.
- Their connective tissue core is richly vascularised. The epithelium is slightly thinner than on the remaining surface of the tongue.

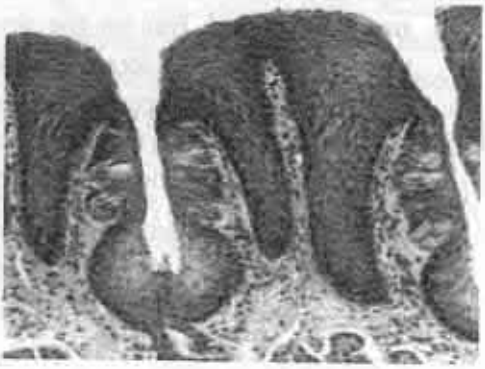
CIRCUMVALLATE PAPILLAE



- **Are the largest and least numerous papillae,** there are between 8 and 12 of them.
- **Do not rise above the surface of the tongue.**
- **Contain about 2500 taste buds.** The taste buds are particularly numerous on the lateral surfaces of these papillae.
- **Main function** – taste perception.

- **Papilla is surrounded by moat** (deep groove) **with numerous taste buds** along stratified squamous nonkeratinizing epithelial wall of groove. This deep groove is a site where the excretory ducts of serous salivary gland (glands of von Ebner) are opening.
- The connective tissue core is richly vascularised and innervated.

FOLIATE PAPILLAE



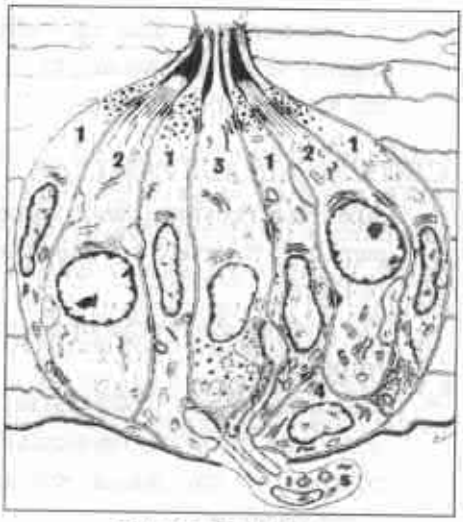
- **Are not highly developed in adults.**
- Are well developed **in newborns.**
- Their shape is **as parallel folds.**
- Are placed along the posterolateral border of the tongue.
- Also contain taste buds that **are functionally active in the childhood.** Taste buds degenerate at an early age.

TASTE BUDS

Taste buds are most numerous in the fungiform, circumvallate and foliate papillae. In addition, taste buds are found in the palate, palatoglossal and palatopharyngeal arches and in the pharynx and larynx.

In histological sections they appear as ovoid lightly stained bodies, which extend perpendicular from the basement membrane to a little opening formed in the epithelium, **the taste pore**. The taste buds are surrounded by a thin connective tissue capsule. Each taste bud consists of 50 to 150 cells. The cells that form the taste bud can functionally be divided into three groups:

1. **sensory** or neuroepithelial **cells,** make contact with nerve fibers. These nerve fibers are contained in the fascial (VII) nerve, glossopharyngeal (IX) nerve, and vagus (X) nerve. Near the taste pore the cells taper, and each sends microvilli through the taste pore.



Schematic diagram of the taste bud
(1 – dark supporting sells; 2 – light supporting sells; 3 – sensory cells; 4 – basal cells)

2. **supporting cells** – secrete an amorphous, dense substance. There are two types of supporting cells: **dark** *(contain granules in their apical cytoplasm)* and **light** *(do not contain granules).*

Sensory and supporting cells are elongate and extend from the basal lamina to the taste pore.

3. **basal cells** – are located at the periphery of the taste bud near the basal lamina.

Microvilli of sensory cells contain the receptors for the different basic taste modalities (sweet, salty, bitter and acid). Basal cells regenerate the two other cell types. Cell turnover is quite high, and it is thought that the cells of the taste buds are replaced (on average) every 10 to 14 days.

Sweet, sour, bitter, and salty are four classic taste sensations. A fifth taste is umami (the taste of monosodium glutamate). A specific taste sensation is generated by specific taste receptor cells. Some taste receptor cells respond to only one of the basic taste substances. Others are sensitive to more than one taste substance.

TOOTH AND SUPPORTING STRUCTURES

A tooth is divided into three parts: the **crown**, one or more **roots**, and the **cervix**

The Crown

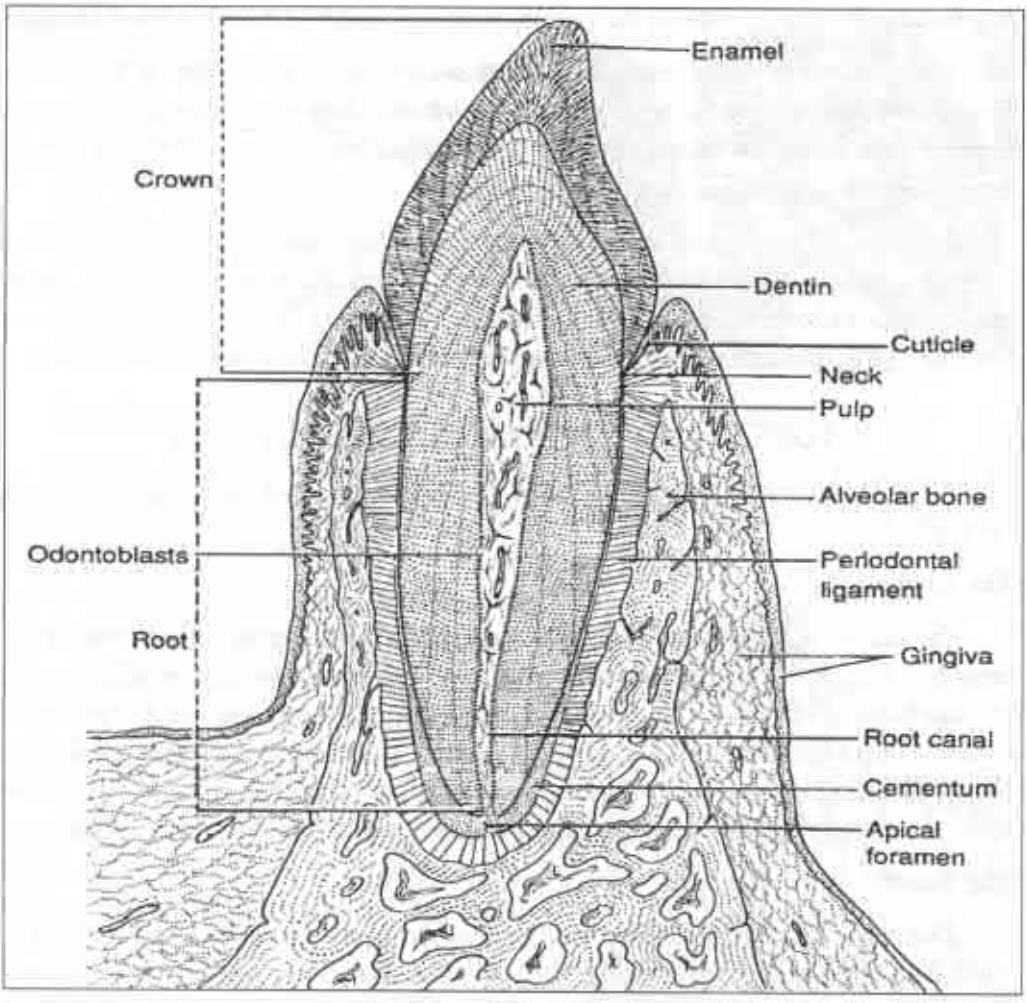
The crown is divided into the **anatomic** and **clinical crown**. The **anatomical crown** is that portion of the tooth encased in enamel. In young people, areas of the anatomical crown are frequently buried in gingival tissue. As a person gets older, it becomes common for a tooth's enamel to be completely exposed above the gingiva and to have root surface showing (gingival recession). The term ***clinical crown*** is applied to the part of the crown exposed (visible) in the mouth.

The Root

The **root** of a tooth is embedded in alveolar bone and is covered by cementurn. The tooth may have a single root or it may have two or three roots. When teeth have more than one root, the region where the roots separate is called the *furcation.* When a tooth has two roots, the root portion is said to be bifurcated. When it has three roots, the root portion is said to be trifurcated. If a tooth has four or more roots, it is said to be multirooted. The tip of each root is called apex. On the apex of each root, there is a small opening that allows for the passage of blood vessels and nerves into the tooth. This opening is called the apical foramen.

The Cervix (the neck)

The ***cervix*** or cervical line is a slight indentation that encircles the tooth and marks the junction of the crown with the root. The cementum joins the enamel at the cervix of the tooth. The point at which they join is called the cementoenamel junction or cervical line.



Schematic diagram of a tooth embedded in the jaw

TOOTH DEVELOPMENT

Tooth development is the complex process by which teeth form from embryonic cells, grow, and erupt into the mouth. **Tooth development begins from the 6th week of the intrauterine development from 2 embryonic origins:**

I. **Ectoderm** – oral epithelium – **enamel.**

II. **Ectomesenchyme** (neural crests) – dentin, cement, dental pulp, periodontal ligament.

Teeth are organs which develop primarily through inductive interactions between dental epithelium and surrounding ectomesenchyme.

Primary (baby) teeth start to form between the sixth and the eighth week in the uterus, and permanent teeth begin to form in the twentieth week in uterus. If teeth do not start to develop at or near these times, they will not develop at all. Dental development includes the formation of specific number of teeth which in humans have different shapes and are located in defined positions in the maxillary and mandibular dental arches. The number, shaper, size and position of teeth are determined by the expression and interaction of numerous genes.

There are 2 main processes in the tooth development:

- Crown formation
- Root formation (is usually completed approximately 2 -3 years after the tooth erupts)

I. Crown formation

FUNCTIONAL STAGES

- Initiation
- Proliferation
- Morpho-differentiation and histo-differentiation
- Apposition (formation of dentin & enamel)

MORPHOLOGICAL STAGES

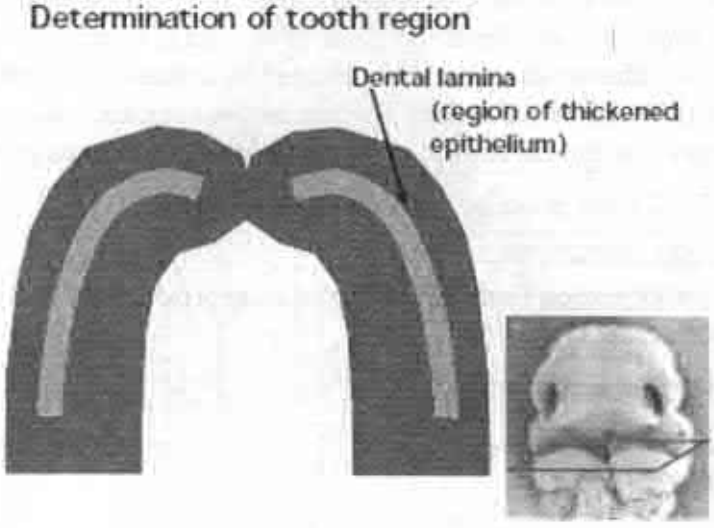
- Bud stage
- Cap stage
- Bell stage (early & late)
- Early & late crown

One of the earliest steps in the development of a tooth is the formation of the **PRIMITIVE EPITHELIAL BAND** or **LAMINA,** which is subdivided into:

1. **VESTIBULAR LAMINA** – it is an ectodermal epithelial extension from the lining of the oral cavity that proliferates into the underlying ectomesenchyme of the maxilla and the mandible lateral to the dental lamina. The central cells of this lamina disintegrate to form the sulcus which is

the space between the maxilla and the mandible on one side and the lips and the cheeks on the other side.

2. **DENTAL LAMINA** – it is derived from the ectodermal epithelial lining of the oral cavity. These epithelial cells form a sheet-like structure that extends into specific areas of the ectomesenchymal tissues within the alveolar processes of the maxilla and mandible. These areas are parts of the developing dental arch regions where teeth form and eventually erupt.



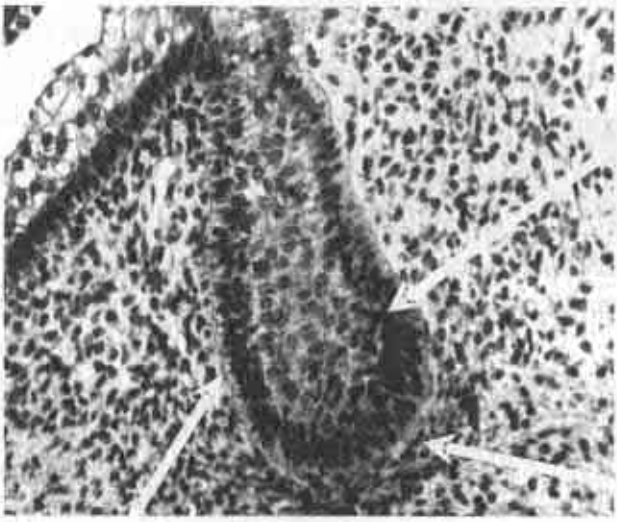
The position of the dental lamina

- The dental lamina connects the developing tooth bud to the epithelium of the oral cavity.
- The entire primary dentition is derived from the original dental lamina. Permanent successors to the primary teeth are derived from extensions of the dental lamina of each primary predecessor, namely **SUCCESSIONAL LAMINAE**. The permanent molars which have no primary predecessors are derived from a posterior extension of the original dental lamina.

THE INITIATION stage. BUD stage.

- **The progressive proliferation of the dental lamina into the ectomesenchyme results in the formation of a DENTAL BUD (also known as the TOOTH GERM) at the distal end of the dental lamina.**

- The dental bud looks like a round mass of proliferating epithelial cells and it is surrounded by condensation of ectomesenchymal tissues which form the dental papilla and dental sac.



Dental germ in the bud stage

THE PROLIFERATION stage. CAP stage

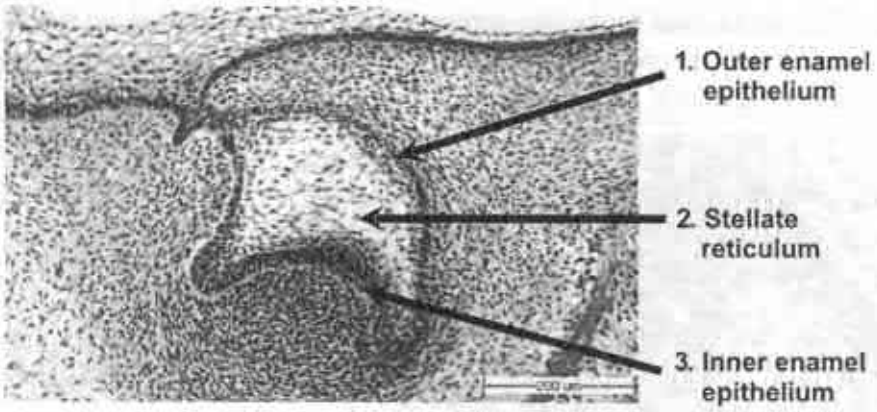
During the cap stage, an unequal growth of epithelial cells grows down to form a concavity around the mesenchyme. The dental bud differentiates into an **enamel organ** extending from the dental lamina.

- The epithelial dental organ looks like a head cap with a round convex side and a straight or slightly concave one.
- During the **cap stage** are formed the **dental papilla** & **dental sac (follicle)** *(see fig. 119, plate II).*

The ENAMEL ORGAN consists of 3 epithelia:

1. Outer enamel epithelium
2. Stellate reticulum
3. Inner enamel epithelium

Outer enamel epithelium (also known as the external enamel epithelium) – is a layer of cuboidal cells located on the periphery of the enamel organ in a developing tooth.



The structure of the enamel organ

Stellate reticulum (also known as a enamel pulp) – is a group of cells located in the center of the enamel organ of a developing tooth. These cells are star shaped and synthesize glycosaminoglycans. As glycosamingoglycans are produced, water is drawn in between the cells and stretches them apart. As they are moved further away from one another, the stellate reticulum maintain contact with one another through desmosomes, resulting in their unique appearance.

Inner enamel epithelium – is a layer of columnar cells located on the periphery of the enamel organ of a developing tooth. The cells of the inner enamel epithelium will become ameloblasts.

The rim of the dental organ where the outer and inner enamel epithelia join is called the **cervical loop**. The growth of cervical loop cells into the deeper tissues forms Hertwig's Epithelial Root Sheath, which determines the root shape of the tooth.

DENTAL PAPILLA:

The concentration of ectomesenchyme, which is in part enveloped by the invaginated inner enamel epithelium, is named the **DENTAL PAPILLA**. Mesenchymal cells within the dental papilla are responsible for formation of tooth pulp. The dental papilla contains cells that develop into **ODONTOBLASTS**, which are dentin-forming cells.

DENTAL SACK:

The dental sack is a concentration of ectomesenchyme that encircles the enamel organ and the dental papilla. The *dental sack* gives rise to three important entities: **cementoblasts, osteoblasts, and fibroblasts**. Cementoblasts form the cementum of a tooth. Osteoblasts give rise to the alveolar bone around the roots

of teeth. Fibroblasts develop the periodontal ligaments which connect teeth to the alveolar bone through cementum.

HISTO-DIFFERENTIATION & MORPHO-DIFFERNTIATION stage.

BELL stage.

The bell stage is known for the histodifferentiation and morphodifferentiation that takes place.

The ENAMEL ORGAN (bell stage) – is bell-shaped during this stage.

The characteristics of the stage:

- Cellular differentiation
- Morphological specialization, both with alternative, inductive and receptive role.

We recognized two different processes during this stage:

1. **dentinogenesis** – which precedes and follows what comes next, that is
2. **amelogenesis**

The ENAMEL ORGAN consists of 4 epithelia:

- **Outer enamel epithelium** – the flattened shaped cells with protective role.
- **Stellate epithelium** – the star shaped cells with protective and nutritive roles.
- **Stratum intermedium** – is a layer of two or three cells between the inner enamel epithelium and the newly forming cells of the stellate reticulum. The stratum intermedium has a notably high alkaline phosphatase activity. This layer, along with the inner enamel epithelium, is responsible for the tooth enamel formation and will become important in transporting nutrients to the future ameloblasts.
- **Inner enamel epithelium – high columnar cells** – preameloblasts which becomes ameloblasts – surrounds the dental papilla with a role in the:
  - Formation of enamel matrix.
  - Induction of: – appearance of odontoblasts
    – differentiation of mesenchymal cells for the appearance of dental sack:
      - Fibroblasts
      - Cementoblasts
      - Osteoblasts

Preameloblasts initiate the differentiation of **odontoblasts** which arise from cells in the dental papilla. The odontoblasts are called **preodontoblasts** before they begin the production of dentin.

All of the inner enamel epithelium cells divide to increase the overall size of the tooth bud, but rapid dividing, stops during the crown stage at the location where the cusps of the teeth form. The first mineralized hard tissues are formed at this location. At the same time, the inner enamel epithelial cells change in shape from cuboidal to columnar. The nuclei of these cells move closer to the stratum intermedium and away from the dental papilla. The basal membrane of this epithelium starts to disappear.

The adjacent layer of cells in the dental papilla suddenly increases in size and differentiates into odontoblasts, which are the cells that form dentin. Researchers believe that the odontoblasts would not form if it were not for the changes occurring in the inner enamel epithelium. As the changes to the inner enamel epithelium and the formation of odontoblasts continue from the tips of the cusps, the odontoblasts secrete a substance, an organic matrix, into their immediate surrounding. The organic matrix contains the material needed for dentin formation. As odontoblasts deposit organic matrix, they migrate toward the center of the dental papilla. Thus, unlike enamel, dentin starts forming in the surface closest to the outside of the tooth and proceeds inward. Cytoplasmic extensions are left behind as the odontoblasts move inward. The unique, tubular microscopic appearance of dentin is a result of the formation of dentin around these extensions.

After dentin formation begins, the cells of the inner enamel epithelium secrete an organic matrix against the dentin. This matrix immediately mineralizes and becomes the tooth' s enamel. Outside the dentin are ameloblasts, which are cells that continue the process of enamel formation; therefore, enamel formation moves outwards, adding new material to the outer surface of the developing tooth.

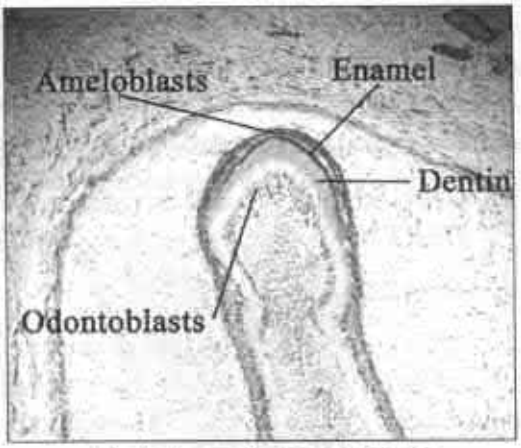
Formation of the PREDENTIN – DENTIN initiates the formation of the ENAMEL.

Other events occur during the bell stage:

The **dental lamina disintegrates**, leaving the developing teeth completely separated from the epithelium of the oral cavity; the two will not join again until the final eruption of the tooth into the mouth. The dental lamina disintegrates into small clusters of epithelium and is resorbed. In situations when the clusters are not resorbed, eruption cysts are formed over the developing tooth and delay its eruption into the oral cavity.

LATE BELL stage is characterized by:

- Appearance of dentin.
- Appearance of enamel.
- Transformation of the dental papilla into **DENTAL PULP.**
- Morphological changes appear in the dental sac.



Dental germ in the bell stage

THE APPOSITION stage. CROWN stage.

The appositional stage occurs when the tissue matrix of the tooth is formed. The cells having the potential for deposition of the extracellular matrix fulfil the plan of the tooth germ established by previous stages. Deposition of the dentin & enamel occurs by regular apposition with alternation of active & resting states *(see fig. 121, plate II).*

Outer enamel epithelium, intermedium epithelium and stellate epithelium become thinner and together form **STRATIFIED EPITHELIUM** of developing tooth.

Following the formation of the crown, the enamel organ collapses to form the reduced enamel epithelium which covers the tooth through eruption. The **REDUCED ENAMEL EPITHELIUM** consists of the mature/protective ameloblasts and remnants of the outer layers of the enamel organ **(STRATIFIED EPITHELIUM** of developing tooth). Numerous capillaries, which had formed to supply oxygen and nutrients to the ameloblasts following dentin formation, surround the reduced enamel epithelium.

The **REDUCED ENAMEL EPITHELIUM:**

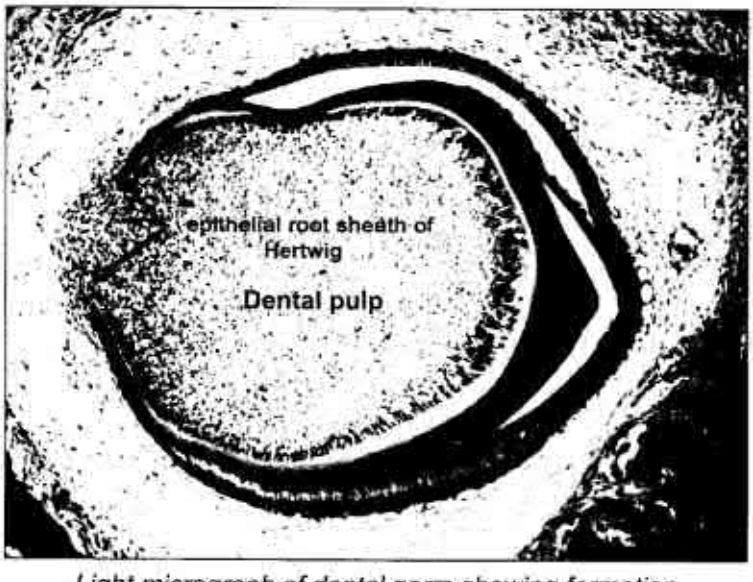
- Takes part in the crown modeling.
- Induces the dentinogenesis of the crown and root.
- Assists in enamel formation and in the formation of dentin-gum connection.
- Isolates the enamel from the connective tissue of the dental sac.
- Takes part in the tooth eruption.

ROOT FORMATION

- Begins after complete formation of the tooth crown and continues after the eruption.
- Key elements, that take part in the root formation, are:

1. **Cervical loop** – that is transformed into **EPITHELIAL ROOT SHEATH OF HERTWIG,** that differentiates into **EPITHELIAL DIAPHRAGM.**
2. **Dental sac.**

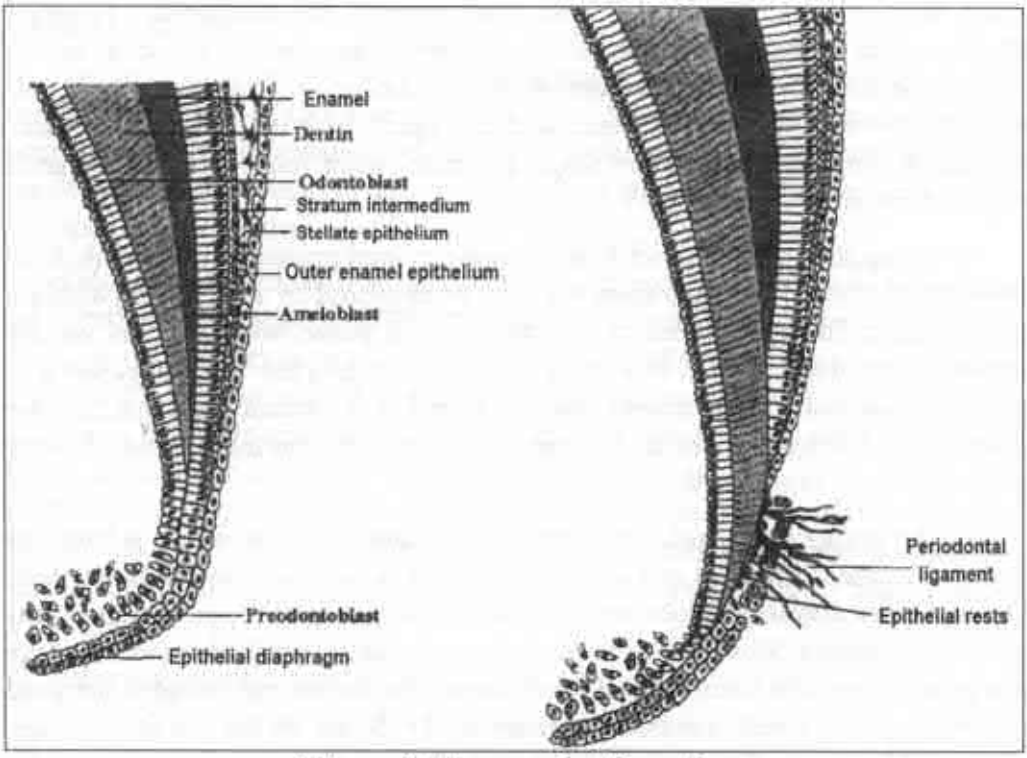
After complete formation of the crown of the tooth, the **root** begins to form. An extension of the enamel organ, called the **epithelial root sheath of Hertwig**, continues to grow apically. The epithelial root sheath is made up of inner and outer enamel epithelial layers without the other two interposing layers. The epithelial root sheath induces the differentiation of odontoblasts which form root dentin. The apical most portion of the root sheath turns inward toward the **radicular pulp cavity** (that portion of the pulp cavity inside the root) and is called the **epithelial diaphragm.**



Light micrograph of dental germ showing formation of the epithelial root sheath of Hertwig

During the stage of root development the epithelial root sheath of Hertwig induces the differentiation of adiajent cells of the ectomesenchymal dental papilla into odontoblasts which form radicular (root) dentin. Once the first layer of

root dentin is formed, the epithelial root sheath disintegrates forming **epithelial rests of Malassez**, small groups of epithelial cells that can remain around the root. The adjacent cells of the ectomesenchymal dental sac differentiate into cementoblasts which form cementum. The timing of these interactions is precise because the target tissue and/or cell must be competent to receive and respond to the inductive message.



Schematic diagram of root formation

Hertwig's root sheath determines the number, size and shape of roots and is presumably the inductor of dentin formation in the developing root.

Tissues of the teeth can be divided into 2 groups: HARD and SOFT tissues.

Hard tissues of the tooth:

ENAMEL

- Has epithelial origin (ectodermal).
- Is the most highly mineralized tissue.
- Is absolutely noncellular tissue of the human.

- Degree of its mineralization is about 96–98%.
- Organic matter (protein – enamelin) & water are 2–4%.
- The morpho-functional unit of enamel is ENAMEL ROD.

Enamel formation

Enamel formation is called amelogenesis and occurs in the crown stage of tooth development. "Reciprocal induction" governs the relationship between the formation of dentin and enamel; dentin formation must always occur before enamel formation. Generally, enamel formation occurs in two stages: the secretory and maturation stages. Proteins and an organic matrix form partially mineralized enamel in the secretory stage; the maturation stage completes enamel mineralization.

In the secretory stage, ameloblasts release enamel proteins that contribute to the enamel matrix, which is then partially mineralized by the enzyme alkaline phosphatase. The appearance of this mineralized tissue, which occurs usually around the third or fourth month of pregnancy, marks the first appearance of enamel in the body. Ameloblasts deposit enamel at the location of what become cusps of teeth alongside dentin. Enamel formation then continues outward, away from the center of the tooth.

In the maturation stage, the ameloblasts transport some of the substances used in enamel formation out of the enamel. Thus, the function of ameloblasts changes from enamel production, as occurs in the secretory stage, to transportation of substances. Most of the materials transported by ameloblasts in this stage are proteins used to complete mineralization. The important proteins involved are amelogenins, ameloblastins, enamelins, and tuftelins. By the end of this stage, the enamel has completed its mineralization.

DENTIN

Dentin is the light yellow substance that is more radiolucent than enamel and is very porous; it constitutes the largest portion of the tooth. The pulp chamber is located on the internal surface of the dentin walls. Dentin is harder than bone but softer than enamel. Dentin consists of approximately 72% inorganic matter and 28% organic matter and water. Calcium and phosphorus are its chief inorganic components.

Dentin is a living tissue and must be protected during operative or prosthetic procedures from dehydration (drying) and thermal shock. The dentin is perforated by tubules (similar to tiny straws) that run between the cementoenamel

junction and the pulp. The dentinal tubules are the morpho-functional unit of the dentin. Cell processes from the pulp reach part way into the tubules like fingers. These cell processes create new dentin and mineralize it. Dentin transmits pain stimuli by the way of dentinal fibers. Because dentin is a living tissue, it has the ability for constant growth and repair that reacts to physiologic (functional) and pathologic (disease) stimuli.

Dentin formation

Dentin formation, known as dentinogenesis, is the first identifiable feature in the crown stage of tooth development. The formation of dentin must always occur before the formation of enamel. The different stages of dentin formation result in different types of dentin: mantle dentin, primary dentin, secondary dentin, and tertiary dentin.

Odontoblasts, the dentin-forming cells, differentiate from cells of the dental papilla. They begin secreting an organic matrix around the area directly adjacent to the inner enamel epithelium, closest to the area of the future cusp of a tooth. The organic matrix contains collagen fibers with large diameters (0.1-0.2 μm in diameter). The odontoblasts begin to move toward the center of the tooth, forming an extension called the odontoblast process. Thus, dentin formation proceeds toward the inside of the tooth. The odontoblast process causes the secretion of hydroxyapatite crystals and mineralization of the matrix. This area of mineralization is known as mantle dentin and is a layer usually about 150 μm thick.

Whereas mantle dentin forms from the preexisting ground substance of the dental papilla, primary dentin forms through a different process. Odontoblasts increase in size, eliminating the availability of any extracellular resources to contribute to an organic matrix for mineralization. Additionally, the larger odontoblasts cause collagen to be secreted in smaller amounts, which results in more tightly arranged, heterogeneous nucleation that is used for mineralization. Other materials (such as lipids, phosphoproteins, and phospholipids) are also secreted.

Secondary dentin is formed after root formation is finished and occurs at a much slower rate. It is not formed at a uniform rate along the tooth, but instead forms faster along sections closer to the crown of a tooth. This development continues throughout life and accounts for the smaller areas of pulp found in older individuals. Tertiary dentin, also known as reparative dentin, forms in reaction to stimuli, such as attrition or dental caries.

CEMENTUM

- Covers the root of the tooth in a thin layer.
- Is the mineralized connective tissue.
- Is avascular.
- Its chemical composition is actually quite similar to that of woven bone:
  - Mineral content – 45-50%.
  - Organic matter (55%): glycosaminoglycans, collagen type I, [illegible]
- Cells of the cementum are:
  - **Cementocytes** that are located in lacunae.
  - **Cementoblasts** that are located on the outer surface of the cementum, adjacent to the periodontal ligament.
- Cementum is capable of formation, destruction and repair and remodels continually throughout life. It is nourished from vessels within the periodontal ligament.
- **Functions:**
  - It protects the dentin (occludes the dentinal tubules).
  - It provides attachment of the periodontal fibers.
  - It reverses tooth resorption.

Cementum formation

Cementum is similar to bone, but there are no Haversian systems.

Cementum formation is called cementogenesis and occurs late in the development of teeth. Cementoblasts are the cells responsible for cementogenesis. Two types of cementum form: cellular and acellular.

Acellular cementum forms first. The cementoblasts differentiate from follicular cells, which can only reach the surface of the tooth's root once Hertwig's Epithelial Root Sheath (HERS) has begun to deteriorate. The cementoblasts secrete fine collagen fibrils along the root surface at right angles before migrating away from the tooth. As the cementoblasts move, more collagen is deposited to lengthen and thicken the bundles of fibers. Noncollagenous proteins, such as bone sialoprotein and osteocalcin, are also secreted. Acellular cementum contains a secreted matrix of proteins and fibers. As mineralization takes place, the cementoblasts move away from the cementum, and the fibers left along the surface eventually join the forming periodontal ligaments.

Cellular cementum develops after most of the tooth formation is complete and after the tooth occludes (in contact) with a tooth in the opposite arch. This type of cementum forms around the fiber bundles of the periodontal ligaments. The cementoblasts forming cellular cementum become trapped in the cementum they produce.

Cellular cementum is usually not found in teeth with one root. In premolars and molars, cellular cementum is found only in the part of the root closest to the apex and in interradicular areas between multiple roots.

Soft tissue of the tooth:

DENTAL PULP consists of loose connective tissue, contains blood vessels and nerve fibers, cellular content: fibroblasts, fibrocytes, macrophages, lymphocytes, mast cells, plasma cells and others.

- It decreases in size with age
- It is **subdivided** into:
  1. **PULP OF THE CROWN** (occupies and resembles the crown).
  2. **PULP OF THE ROOT CANAL** (occupies roots, contains the apical foramen).

A histological section of the pulp reveals four cellular zones:

1. **ODONTOBLASTIC layer**
2. **CELL-FREE ZONE of WEIL**
3. **CELL-RICH ZONE**
4. **MIDDLE layer (Pulp core)**

Functions of the dental pulp:

- The primary function of the dental pulp is to **form dentin** (by the odontoblasts).

Other functions include

- **Nutritive**: the pulp keeps the organic components of the surrounding mineralized tissue supplied with moisture and nutrients;
- **Sensory**: extremes in temperature, pressure, or trauma to the dentin or pulp are perceived as pain;
- **Protective**: the formation of reparative or secondary dentin (by the odontoblasts).

PERIODONTAL LIGAMENT

- It is the fibrous dense regular connective tissue joining the tooth to its surrounding bone (*is anchored in cementum of the tooth and the bundle bone layer of the cribriform plate*).
- It provides for:
  - Attachment
  - Support
  - Bone remodeling (during movement of a tooth)
  - Nutrition of adjacent structures
  - Proprioception
  - Tooth eruption
- **Interstitial spaces** are regions of loose connective tissue located between periodontal fiber bundles. These regions contain fibroblasts, blood vessels, and nerves and are responsible for providing nutrients to the periodontal ligament and cells of the cementum.

Cells of the periodontal ligament

The main types of cell in the periodontal ligament are the fibroblasts, fibrocytes, macrophages, mast cells, plasma cells, epithelial rests of Malassez. Adjacent to the cementum are cementoblasts, and adjacent to the cribriform bone are osteoblasts. **Epithelial rests of Malassez in the periodontal ligament** are cellular residues of the embryonic structure known as Hertwig's epithelial root sheath These are located in the periodontal ligament adjacent to the surface of cementum.

Extracellular matrix of the periodontal ligament

Extracellular matrix of the periodontal ligament consists of ground substance (proteoglicans, glicosaminoglycans) and fibers (collagen type I and III, elastic fibers: oxytalan & elaunin). The periodontal ligament is made of large collagen fibers that course between the cementum and the alveolar bone. These fibers are embedded in the outer layer of cementum and periosteum of alveolar bone and are called Sharpey's fibers. They project into the cementum between groups of cementoblasts, and lie perpendicular to the surfaces of the cementum and bone.

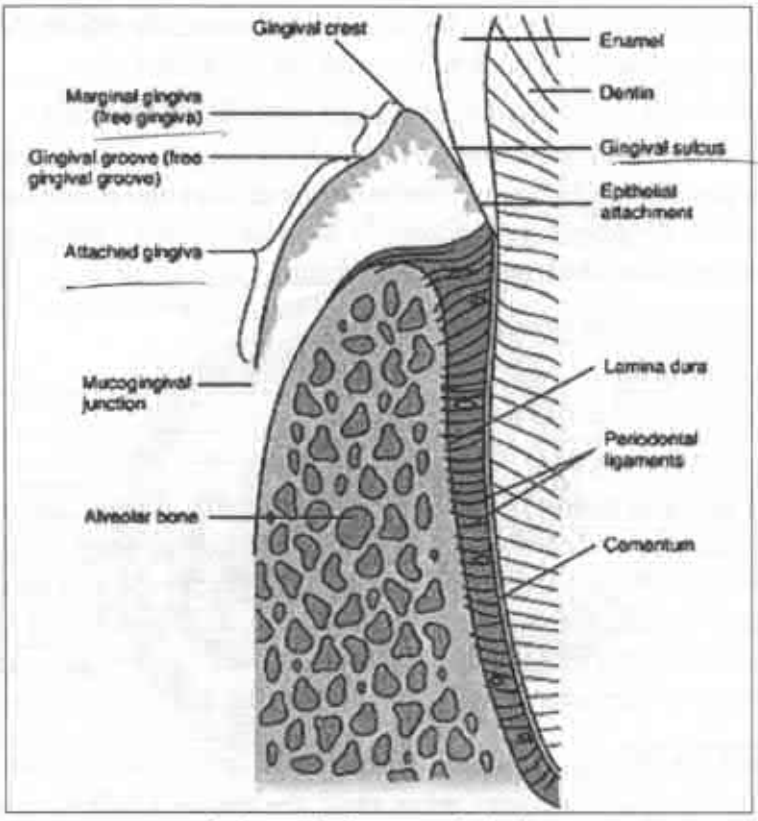
GINGIVA

- Is the part of the mucous membrane commonly called the gums.
- It is firmly attached to the teeth and underlying bony tissue.

- The color of healthy gingiva can range from pale pink to darker shades (purple to black) depending on each individual's pigmentation.
- Gingiva is highly vascular.
- The gingival mucosa is synonymous with masticatory mucosa that consists of:
  - Stratified squamous orthokeratinized & parakeratinized (in some areas).
  - Lamina propria mucosae with two layers: papillary (is formed by loose connective tissue) and reticular (is formed by dense irregular connective tissue).

Zones of gingiva

- Free gingiva
- Attached gingiva
- Interdental gingiva



Schematic diagram of gingiva

Territories of gingiva

- **Free (or marginal) gingiva** – forms the gingival margin which is visible during examination. It surrounds the crown of the tooth.
- **Gingival crest.**
- **Free gingival groove.**
- **Attached gingiva** – it tightly adheres to the subgingival connective tissue and bone via deep rete pegs. It is keratinised to withstand the stress of ripping and tearing food.
- **Muco-gingival junction** (MGJ) – is the junction between the soft, fleshy mucus membrane of the oral cavity and the tough, collagen rich gingiva.
- **Interdental papilla.**

Gingival sulcus – located between the tooth and the free gingival margin and is the crevice that surrounds the tooth.

ALVEOLAR PROCESS (BONE)

- Is that bony portion of the maxilla and mandible where the teeth are embedded and by which tooth roots are supported.
- Is the bone that contains the sockets (alveoli) for the teeth.
- The alveolar socket is the cavity in which the root of the tooth is held by the periodontal ligament. The bone that divides one socket from another is called the interdental septum. When multirooted teeth are present, the bone is called the interradicular septum.
- Alveolar process consists of:

1. **Supporting bone**:
   a) outer cortical plates (compact bone)
   b) a central spongiosa
2. **Proper alveolar bone** (bone lining the alveolus) – a thin layer of compact bone, forms the wall of alveolus. It is the bone to which the principal fibers of the periodontal ligamentum are attached. The proper alveolar bone is mainly lamellar bone, but the surface of the bone also contains areas of nonlamellar ***bundle bone***, especially where new bone has been formed and where the collagen fibers of the periodontal ligament are attached.

PERIODONTIUM

- The term periodontium refers to all the tissues involved in the attachment of a tooth to the jaw.

- Their main functions of the periodontum are to support, protect, and provide nourishment to the teeth.

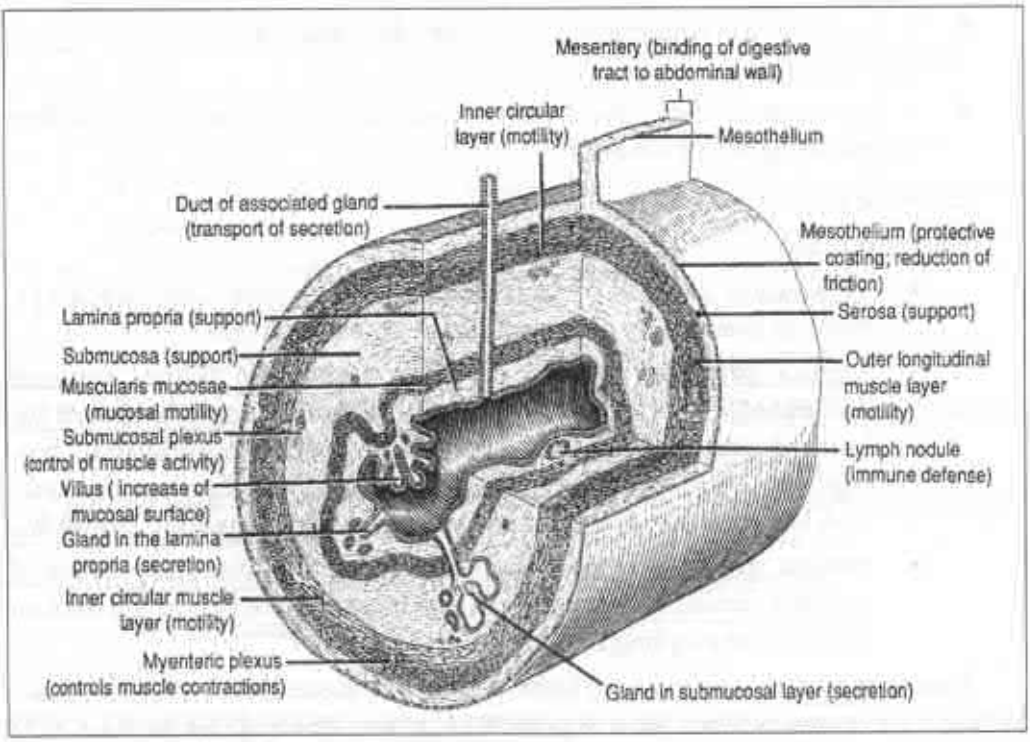
Periodontum consists of:

1. **Cementum**
2. **Periodontal ligament**
3. **Alveolar processes of the maxilla and mandible**
4. **Gums (gingiva)**

DIGESTIVE TUBE

BASIC PLAN OF THE DIGESTIVE TUBE

Wall structure of the GI (gastrointestinal) tract (from esophagus to rectum) has a consistent layered pattern. Specializations of the layers will help you identify the different regions. The layers from inside out consist of the *mucosa, submucosa, muscularis externa, and serosa / adventitia.*



Scheme of general organization of the digestive tube

- **Tunica mucosa**: This layer is composed of epithelium, connective tissue and muscle. These tissues can usually be found in distinct layers as follows:
  - epithelium
  - lamina propria mucosae: consists of loose connective tissue
  - lamina muscularis mucosae: consists of smooth muscle
- **Tunica submucosa**: consists of loose connective tissue; contains nerves, blood vessels, and glands in some organs (**esophagus, duodenum**)
- **Muscularis externa** (tunica muscularis): consists of at least two layers, an **inner (circular)** and an **outer (longitudinal)** with parasympathetic ganglia located between the layers
- **Tunica adventitia** consists of loose connective tissue or **tunica serosa** consists of loose connective tissue and mesothelium (simple squamous epithelium).

ESOPHAGUS

- The esophagus connects the oral cavity with the stomach allowing and aiding in the movement of food particles to the stomach.
- It is a muscular tube having the layers described above for the typical tubular organ.
- In the esophagus the layers are specialized for the function of further fragmenting food particles.

Layers of the esophagus

Tunica mucosa:

- **epithelium** consists of **stratified squamous non-keratinized** epithelium that can be highly folded in an empty organ;
- **lamina propria**: consists of loose connective tissue, contains ***ESOPHAGEAL CARDIAC GLANDS*** that are simple branched tubular glands, they produce mucus, mucin, chlorides and some biologically active substances. These glands are located only at the junction between the pharynx and esophagus, esophagus and stomach.
- **lamina muscularis mucosae** consists of longitudinally oriented smooth muscle fibers that form one layer (can be two in the bottom third of the esophagus).

Tunica submucosa consists of loose connective tissue that is very elastic allowing for expansion when food is present; contains ***ESOPHAGEAL GLANDS PROPER***; they are compound tubuloalveolar glands, which produce mucous.

Tunica submucosa and Tunica mucosa form longitudinal folds (elevations), which fill the lumen of the esophagus, that's why the **lumen** in cross section is asterisk shaped.

Tunica Muscularis externa consists of smooth and/or skeletal muscles:

Proximal end – **skeletal muscle cells** (voluntary for swallowing)

Middle region – **skeletal plus smooth muscle**

Distal end – **smooth muscle cells** (involuntary for peristalsis)

Muscle tissue is arranged in 2 layers:

- inner circular layer
- outer longitudinal layer

Tunica adventitia/serosa. Adventitia consists of typical loose connective tissue that blends into the connective tissue of surrounding tissues. Serosa is presented only at the distal end that enters peritoneal cavity.

STOMACH

Functions:

- Continues the digestion of carbohydrates that started in the mouth
- Adds acidic fluid to the bolus
- Transform food into chyme (mechanical & chemical breakdown)
- Promotes initial digestion of proteins (via pepsin) and triglycerides (via lipase)
- Absorption (water, salts, alcohol, glucose and some drugs)
- Excretion (ammonium, etc)
- Antianaemic function (secrete intrinsic factor Castle)
- Barrier (separates the lumen of the digestive tract, which is continuous with the environment, from the body of the organism)

The stomach is an expanded part of the digestive tube located directly under the diaphragm. The stomach is divided into four distinct parts:

- Cardia (*cardiac region*), near the esophageal orifice
- Fundus (*fundic region*)
- Body
- Pyloris (*pyloric region*) – is continuous with the duodenum through the pyloric sphincter.

The **inner surface** of the stomach is irregular. There are:

- **Rugae** – are longitudinally oriented folds or ridges, composed of the mucosa and underlying submucosa, which serve to accommodate the filling and expanding of the stomach. Rugae are more prominent in the lower, narrow region of the stomach.
- **Gastric (mamillated) areas** – bulging irregular areas formed by grooves or shallow trenches.
- **Gastric pits** – funnel-shaped depressions. Gastric glands empty into the bottom of the gastric pits.

The stomach has the same general structural plan, consisting of a mucosa, submucosa, muscularis externa, and a serosa.

TUNICA MUCOSA

- **Epithelium** consists of **simple columnar glandular epithelium** that forms branched, tubular glands; organized into gastric pits that open into the lumen and gastric glands that empty into the base of the gastric pits.

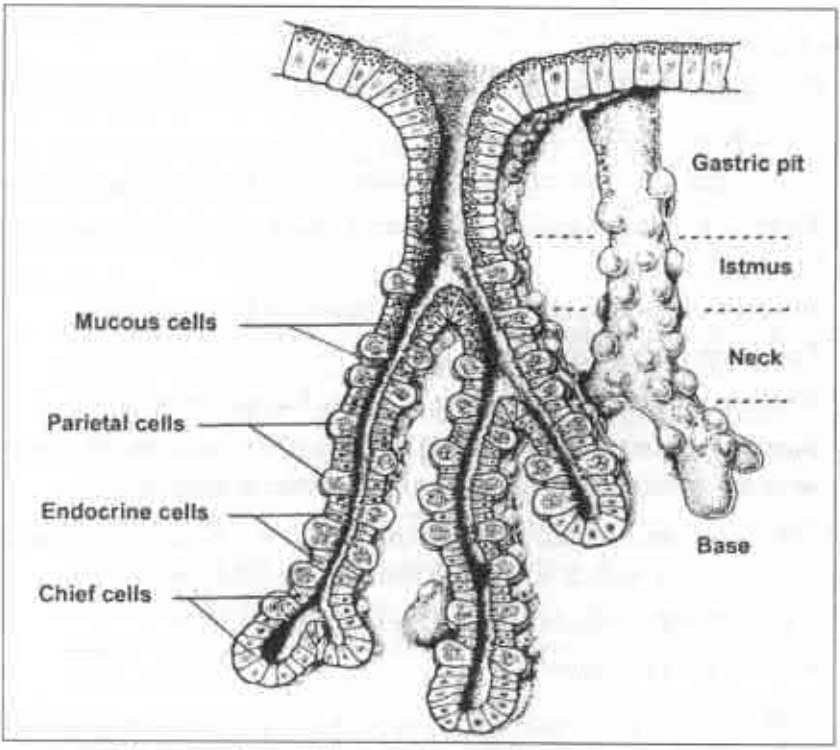


Diagram of a gastric gland illustrating the relationship of the gland to the gastric pit

- **Lamina propria** consists of loose connective tissue that in the glandular stomach is minimal between gastric glands and difficult to see in sections; highly vascular containing many blood and lymphatic capillaries.
- **Lamina muscularis mucosae** consists of three layers of smooth muscle oriented both longitudinally and circularly; usually not very thick.

TUNICA SUBMUCOSA: typical loose connective tissue contains submucosal plexuses also known as Meissner's plexuses.

MUSCULARIS EXTERNA: has 3 layers of smooth muscle:

- Inner oblique
- Middle circular (has high development in the pyloric region and form **pyloric sphincter**)
- Outer longitudinal
  - between the muscle layers is located the myenteric or Auerbach's plexus.

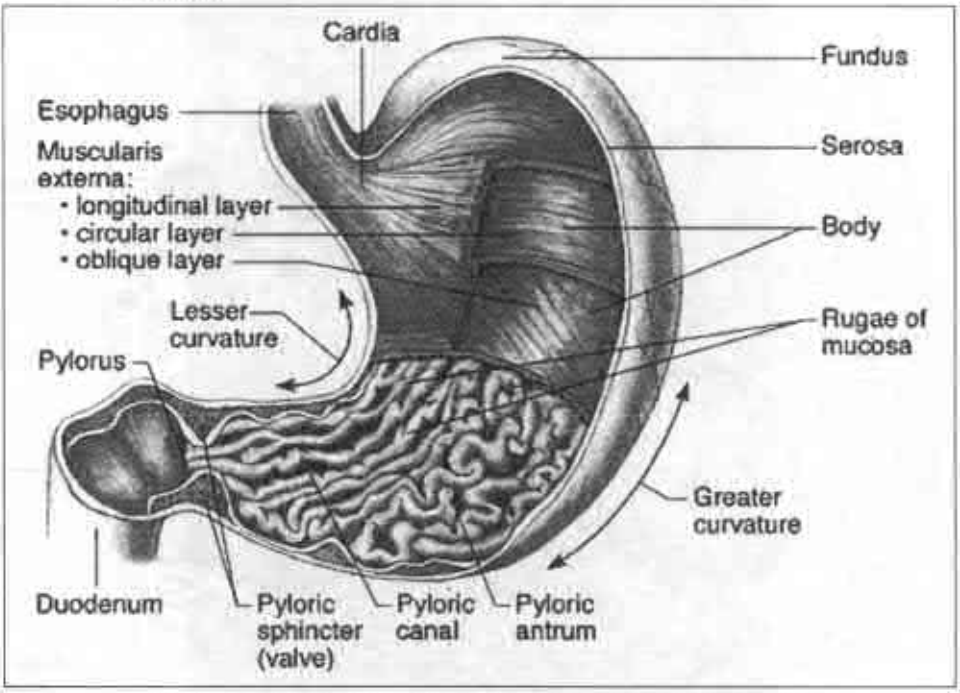


Diagram showing the structure of the muscularis externa

TUNICA SEROSA contains small amount of loose connective tissue with overlying simple squamous epithelium or mesothelium.

Proper Gastric glands (from fundus and body of the stomach) **contain 5 types of cells:**

1) **Chief cells** (zymogenic cells) – found mostly near the base of the gastric glands; very basophilic (purple) containing basally positioned nucleus and prominent basophilic apical cytoplasm filled with many ribosomes; the apical pole has few microvilli, secretes **pepsinogen**, which is activated to pepsin by HCl in the stomach. Pepsin is an enzyme which is able to break down proteins. Also secretes chymosin (breakdown the proteins of milk).

2) **Parietal cells** (oxyntic cells) – found throughout the gastric gland; round cells that contain distinct eosinophilic (pink) cytoplasm and round, prominent nucleus; demonstrate deep surface invaginations, called intracellular canaliculi, that are lined with microvilli as seen in EM. This great expansion of surface area facilitates active transport of hydrogen and chloride ions against a strong concentration gradient. Secretes **HCl** and the **intrinsic factor**, needed for absorption of vitamin B12 in the ileum.

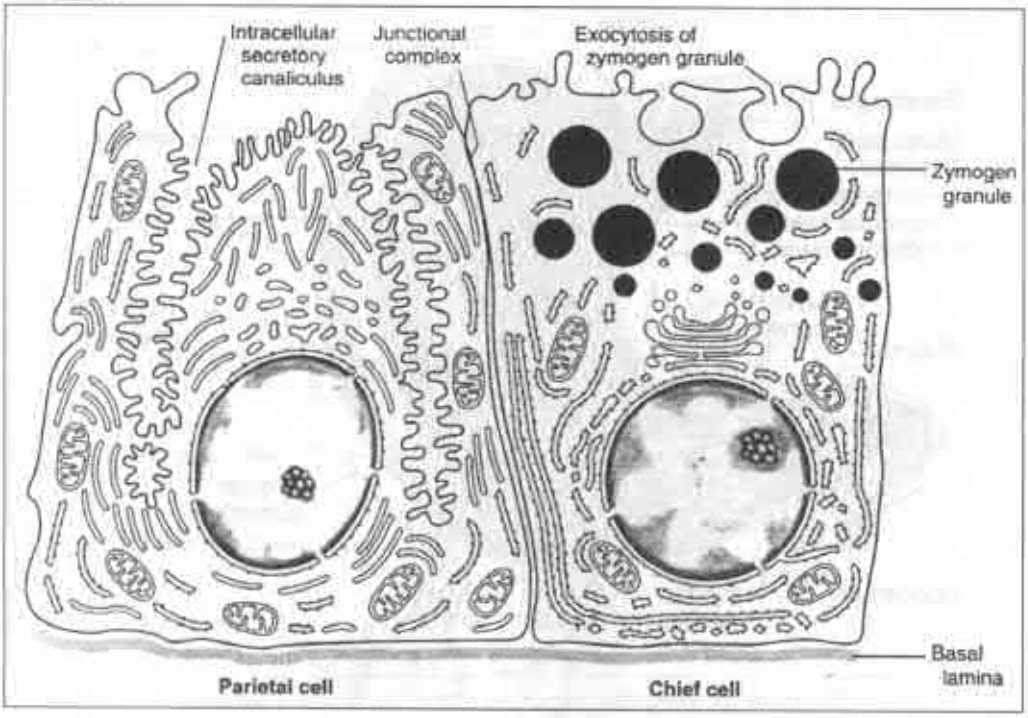


Diagram of parietal and chief cells

3) **Mucous neck cells (not goblet cells)** – found dispersed between the parietal cells; are found near the border of the pit and its gland secrets a **mucus** that is thinner than that secreted by the surface mucous cells; the mucus protects other glandular cells from action of proteases and HCl.

4) **Endocrine cells** – difficult to distinguish by conventional light microscopy; are found primarily in the bottom of fundic glands, make hormones that are released into the lamina propria. These substances act locally on other cell types (paracrine and endocrine mecanism). Several types are present; some secrete **gastrin, glucagon** and **somatostatin, histamine, endorphins, serotonin, cholecystokinin (CCK)** among other hormones. They regulate gastrointestinal motility and functions.

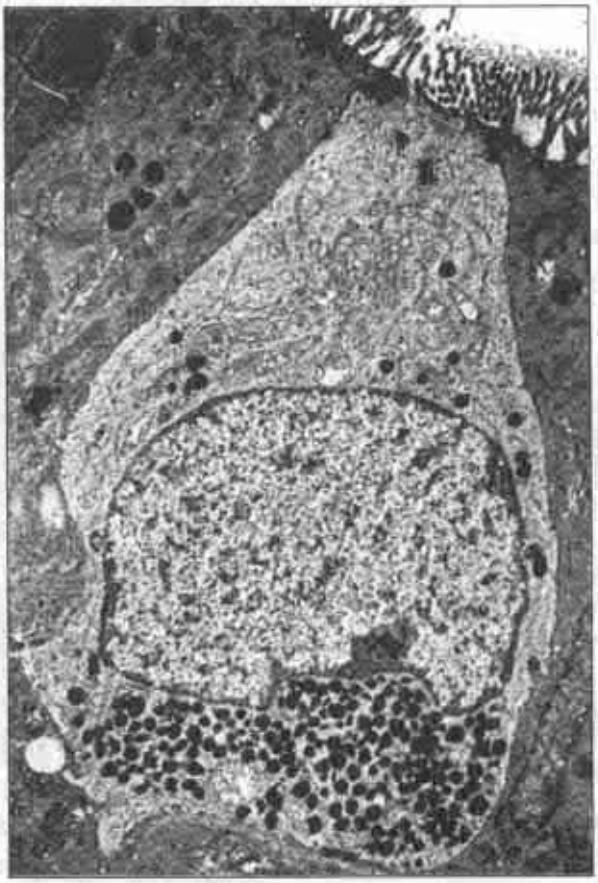


Diagram of an APUD cell (open type)

5) **Undifferentiated cells** – located primarily in the neck region; difficult to identify in routine H&E sections; undergo mitosis to form more cells (stem

cells) then differentiate into the other cell types present in the gland. Surface and pit cells are replaced every 2-6 days. When damaged, the surface epithelia can be replaced in less than 24 hours by migration and spreading of existing cells, and production of new cells. Glandular cells are replaced at a much slower rate.

The stomach is divided into three histological regions based on the nature of the glands.

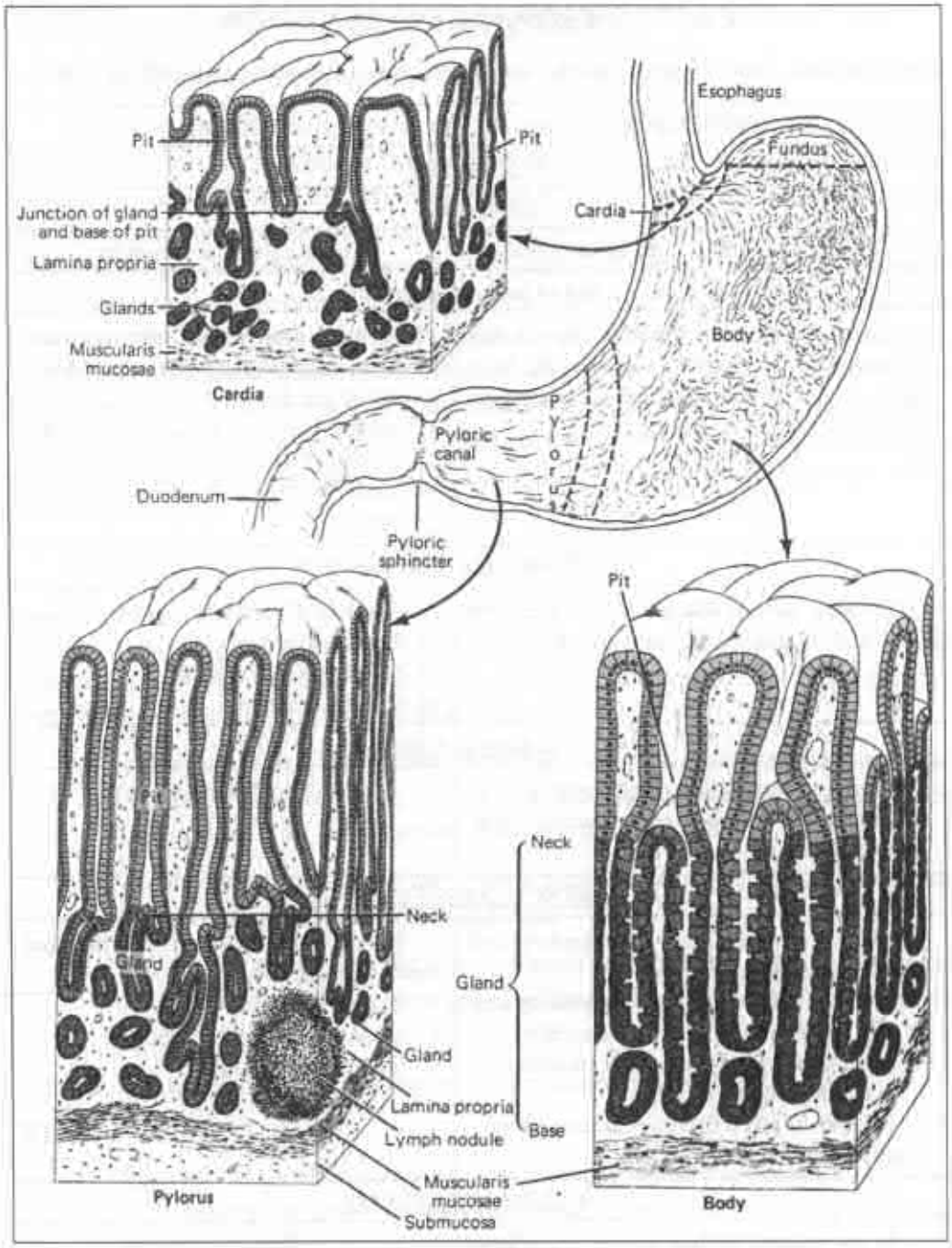
CARDIAC REGION

- is the region surrounding the esophageal opening into the stomach.
- Cardiac glands are tubular, occasionally branched glands (similar to the cardiac glands of the esophagus) and frequently coil at their terminal part, which contain mainly mucus-producing cells. Their secretion protects the esophagus against gastric reflux. A few of the secretory cells characteristic for the corpus-fundic glands (chief and parietal cells) may be present.
- Surface epithelium, pits, and glands are all lined with cells that secrete mucus and small amounts of lysozyme *(attacks bacterial membranes)*.
- Gastric pits in the cardiac region are fairly shallow.

The BODY and FUNDIC REGION constitute the majority of the stomach. The glands in this region are known as gastric or fundic glands and extend all the way to the muscularis mucosae. From three to seven glands open into the base of each gastric pit. Each gland has a fairly long, narrow neck and a short, wider base. At their base, the glands may divide into two or three branches which become slightly coiled. In the fundic region, almost the entire lamina propria is occupied by glands. The lumen of the glands is usually not identifiable and they usually appear more like cords of cells. The only "typical" lamina propria can be seen in the areas between the foveolae and around the bases of the glands.

PYLORIC REGION

- mostly contains mucous pits and glands (looks very similar to the cardiac region).
- Pyloric glands are more coiled than corpus-fundic glands, and they may be more branched. The lumen is relatively wide. A few parietal cells may be present but chief cells are usually absent. Cells primarily secrete mucus.
- The gastric pits in this region are very deep, going about halfway down to the muscularis mucosae.



Schematic diagram of three histological regions of the stomach based on the nature of the glands

GASTRO-ESOPHAGEAL JUNCTION

Comparative characteristic of the wall structure of esophagus and stomach

ESOPHAGUS	STOMACH
1. MUCOSA	
Epithelium	
Stratified squamous non-keratinized	Simple columnar glandular
Lamina propria of mucosa	
Contains esophageal cardiac glands that are simple branched tubular glands. They produce mucus, mucin, chlorides and some biologically active substances.	Contains gastric glands that are simple branched tubular. Are observed 3 groups of glands: 1) cardiac glands *in the cardiac region* 2) pyloric glands *in the pyloric region* 3) fundic or gastric glands *in the fundic region.*
Muscularis mucosae	
Consists of longitudinally oriented smooth muscle fibers that form 1 layer (can be 2).	Consists of 3 layers of smooth muscle: 1) Inner – circular 2) Middle – longitudinal 3) Outer – circular
2. SUBMUCOSA	
Contains esophageal glands proper. They are compound tubuloalveolar glands, which produce mucous.	Glands are absent.
3. MUSCULARIS EXTERNA	
• In the upper one-third – is present striated skeletal muscle. • In the middle one-third – is present striated (50%) and smooth muscle (50%). • In the distal third – is present smooth as in rest of the digestive tract. It forms 2 layers: inner – circular; outer – longitudinal	Present only smooth muscle that forms 3 layers: • Inner – obligue • Middle – circular • Outer – longitudinal
4. TUNICA EXTERNA	
In the thoracic cavity is present adventitia. After entering the abdominal cavity is present serosa.	Serosa is present.

SMALL INTESTINE

Small intestine is the longest component of the digestive tract.

The small intestine is a typical tubular organ in that it has all of the typical tunics and layers. However, the tunica mucosa is especially modified to fulfill the function of absorption. The small intestine has three regions: **the duodenum, the jejunum, and the ileum**, each have special modifications to the wall to enable each region to better perform its particular function. In the small intestine digestion occurs in the lumen as well as at the surface of the lining epithelial cells. Pancreatic enzymes such as trypsin, chymotrypsin, elastase, carboxypeptidases, peptide hydrolases, amylase and lipases are adsorbed onto the membrane surface of the epithelial cells where they mix with the chyme present in the lumen catalyzing the breakdown of proteins, carbohydrates and lipids. The smaller breakdown products are then absorbed by the lining epithelial cells that are called enterocytes.

Specializations to enhance absorption ability

The small intestine has all of the "layers" of a typical tubular organ but the tunica mucosa is highly specialized to perform the function of absorption. To fulfill this function it uses several strategies to increase the surface area of the plasma membrane of the absorptive epithelial cells.

- Individual cells have numerous projections of their apical plasma membranes called **microvilli,** which give the apical region of the cell a striated appearance, called ***striated border (brush border).***
- The epithelium and lamina propria together form finger-like folds that project out into the lumen called **villi.**
- The tunica mucosa and tunica submucosa together form large transverse folds into the lumen called **plicae circulares**, most abundant in jejunum.
- The small intestine is extremely long (usually several meters).

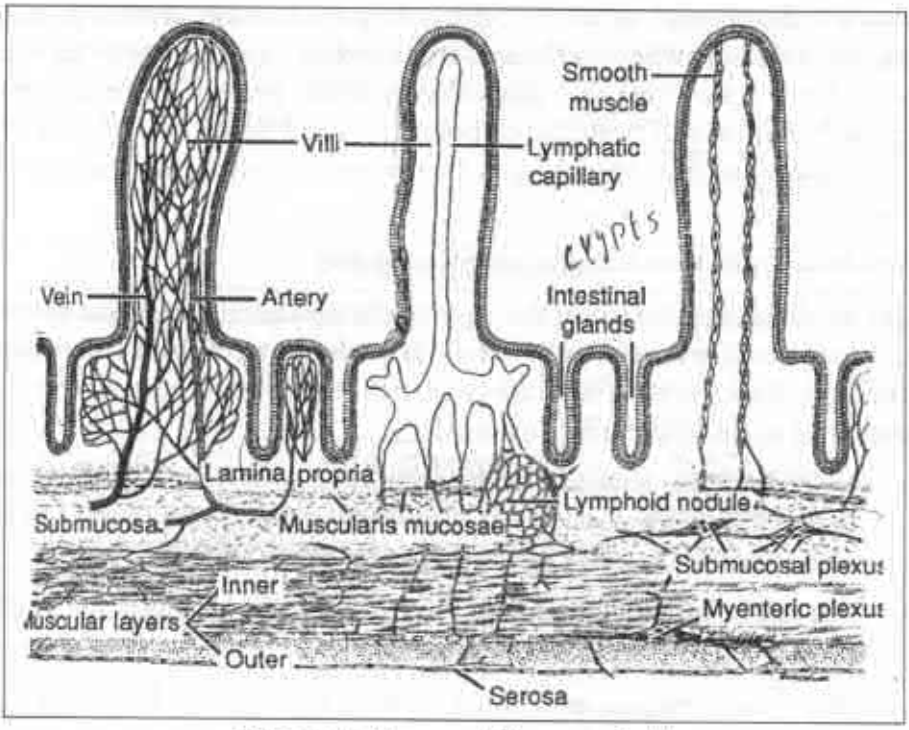
VILLI

– represent finger-like projections of mucosa. Villi are approximately 1 mm high, giving the inside lining of the small intestine a velvety appearance. The large number of villi (20-40 villi per square millimeter) increases the surface area for nutrient absorption. The Villi **DECREASE** in size and height as your approach the large intestine.

The core of the villus is formed by lamina propria mucosa, which **contain:**

- a central, blind-ending lymphatic capillary (lacteal)
- fenestrated blood capillary network
- few smooth muscle cells derived from muscularis mucosae
- myofibroblasts

The core of villus is covered by intestinal epithelium – **simple columnar epithelium.**



Schematic diagram of the core of villi

Layers of the wall of the Small Intestine

I. **Tunica mucosa:**

- **Epithelium** is simple columnar.
- **Lamina propria** formed by loose connective tissue rich in blood and lymphatic vessels present in the core of the villi and between crypts.
- **Lamina muscularis mucosae** formed by thin layer of smooth muscle located at the base of the crypts.

The epithelium of the villus contains 3 types of cells:

1. Enterocytes (absorptive cells) – are tall columnar cells that perform the main function of the small intestine, the absorption of the products of digestion.
   - enterocytes have *microvilli* on their surface for absorption and *junctional complexes* between them to seal the surface.
   - enterocytes have *disaccharidase* in glycocalyx, and hydrolyze disaccharides in the intestinal contents to easily absorb monosaccharides.
   - *Lateral and basal infoldings of enterocytes* – is the site of passage, via exocytosis, of absorbed food stuffs from the cell into the loose connective tissue of the lamina propria of the villi.
   - Exocytosed digestive products in the lamina propria of the villi are picked up by capillaries and lymph vessels (*lacteals*) present in the villi.

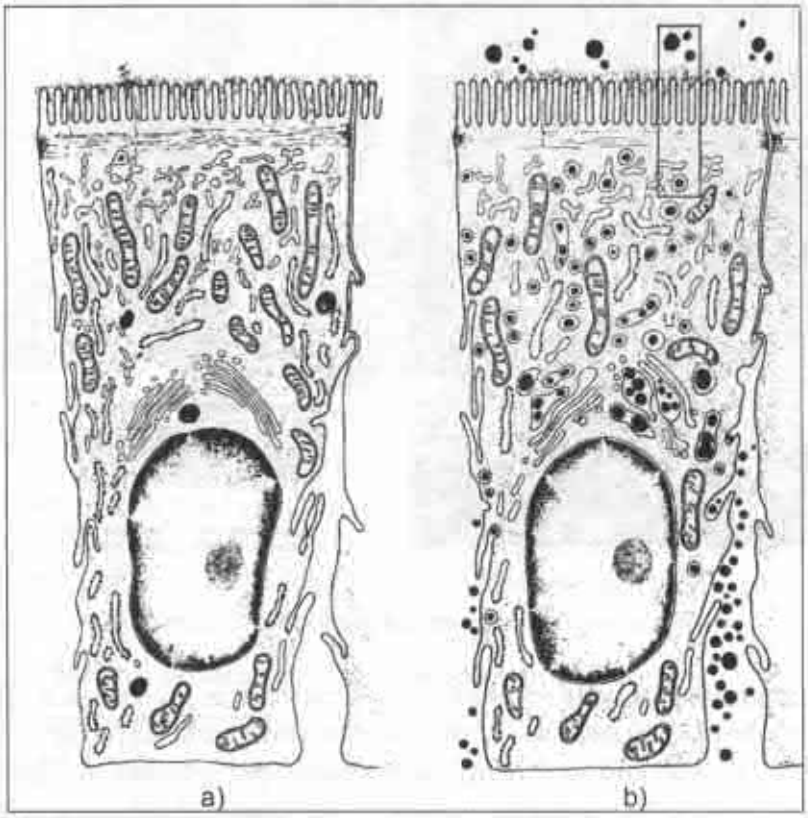


Diagram of an enterocyte (a) showing a striated border on its apical surface and junctional complexes that seal the lumen of the intestine from the lateral intercellular space. The cell in (b) shows the distribution of lipid during fat absorption as seen with the transmission electron microscope.

2. **Goblet cells** – unicellular mucus-secreting glands, increase in number from the proximal to the distal small intestine; are scattered among enterocytes and are joined to them by junctional complexes; are easy to recognize because of their lighter staining quality (water soluble mucinogen is lost during preparation of a routine H&E section) and goblet shape. Goblet cells have two domains: a cup or goblet-shaped apical domain (*containing large mucus granules that are discharged on the surface of the epithelium*) and a narrow basal domain (*containing the nucleus, most of rER, free ribosomes, and mitochondria*).

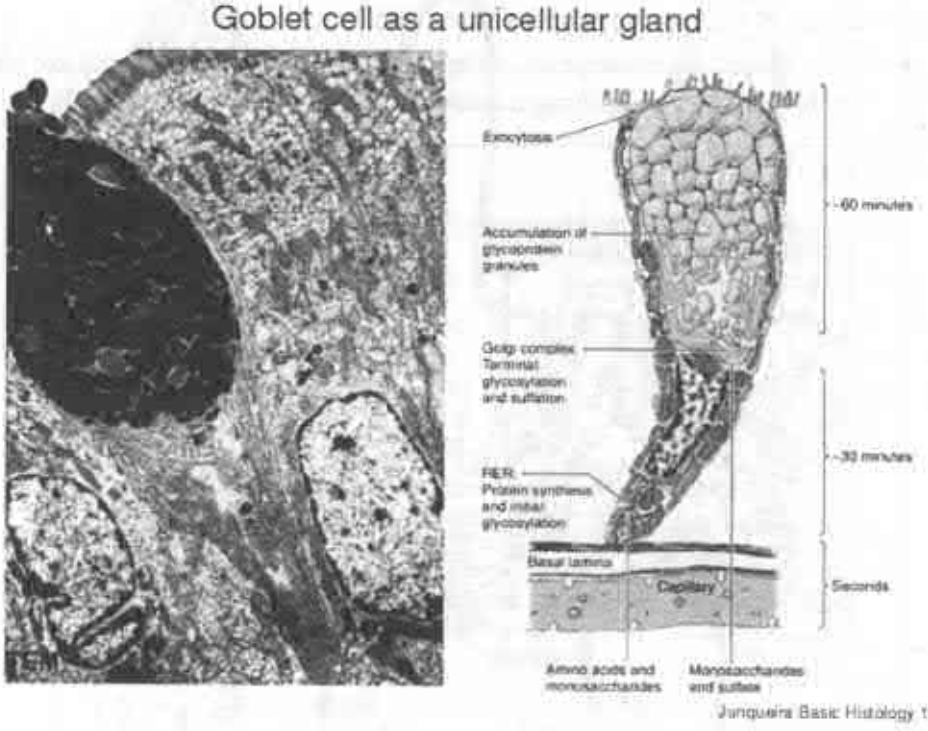


Diagram of a goblet cell

3. **Enteroendocrine cells** resemble those described in the stomach. These cells secrete hormones such as secretin, somatostatin, enteroglucagon, cholecystokinin, gastrin and serotonin; one hormone per type of cell. They are controlled either by the synaptic activity of the autonomic nervous system or by response to hormonal signals.

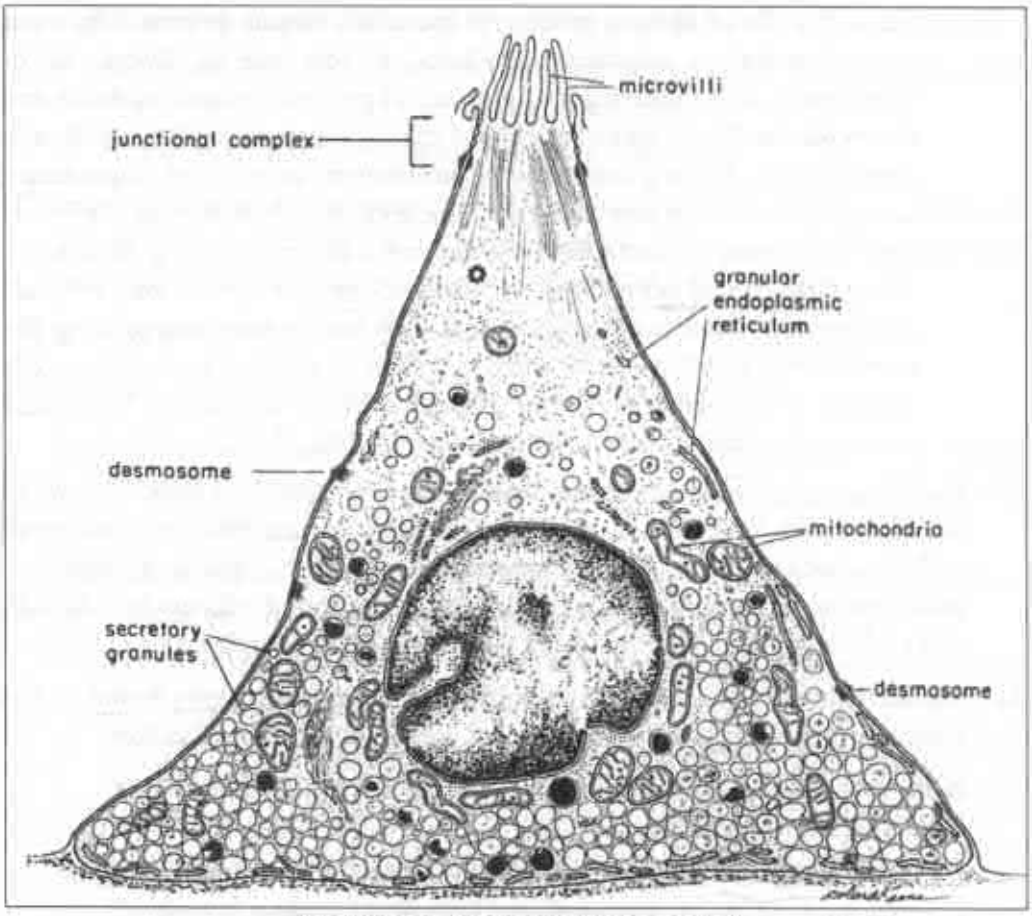


Diagram of an APUD cell (close type)

The intestinal mucosa contains glands throughout its length. The **intestinal crypts** (glands of Lieberkühn) are simple tubular glands; located between the villi at their bases and project into the lamina propria (away from lumen); provide *mucus, digestive enzymes, hormones, and bacteriocidal substances.*

The epithelium of the crypt contains 5 types of cells:

1. **Enterocytes** (absorptive cells)
2. **Goblet cells**
3. **Enteroendocrine cells**
4. **Paneth cells** – are found in the bases of the glands. They have a basophilic basal cytoplasm and large, intensely acidophilic apical secretory granules. These granules contain: the antibacterial enzyme **lysozyme** (digests

the cell walls of certain groups of bacteria), **tumor necrosis factor-α** (proinflammatory substance produced in response to diverse infectious agents and tissue injury), a group of proteins known as **defensins** or **cryptidins** (have an antimicrobial effect by increasing the membrane permeability of a target organism – *parasites or bacteria*), **glycoproteins**, an arginine-rich protein and zinc. These chemicals represent the "first-line" of defence against microbes that enter through the digestive tract. The **antibacterial action and the phagocytosis** of certain bacteria and protozoa by Paneth cells suggest that they have a role in regulating the normal bacterial flora of the small intestine. Compared to the other cells present in the epithelial lining, Paneth cells are long-lived, i.e., weeks versus a few days for the other cells *(see fig. 127, plate II)*.

5. **Undifferentiated cells** renew intestinal epithelia in much the same manner as in the stomach. New cells are produced in the crypts and either migrate up to replace villous epithelia, or move down to renew crypt epithelia. Epithelia replacement in villi results from a continuous migration of cells up the villi with older cells sloughed off at the tips of villi. Turnover is completed in 3-5 days.

II. **Tunica submucosa**: This layer is formed by loose connective tissue. It has glands (in the duodenum), submucosal plexus, and **Peyer's Patches**.

In the duodenum submucosa has coiled branched glands known as **BRUNNER'S GLANDS**:

- Exocrine glands: deposit secretion into base of the intestinal crypts.
- The ducts of glands open into the base of the crypts.
- Produce a clear, viscous alkaline solution.
- Protect small intestine from acidic gastric secretion.
- Raise pH towards neutrality to allow pancreatic enzyme action.

Submucosal Plexus (Meissner): (control of muscle activity

- These are localized on the boarder between the submucosa and the muscularis externa.
- Remember that these are neuron cell bodies; the nucleus has an owl' s eye appearance.

Peyer's Patches

- Present in the **ILEUM**.
- Extensive lymphoid tissue (e.g. nodules). Appears as a clear center surrounded by maturing lymphocytes.

- There can also be diffuse lymphoid tissue in the small intestine as well.
- Of course lymphoid tissue can exist anywhere in the small intestine, but the Peyer's Patches are prevalent in the ileum.
- They can extend to the lamina propria of mucosa.

III. **Tunica Muscularis externa**: typically consisting of an inner circular layer and an outer longitudinal layer. Between layers are septae of loose connective tissue which contain the myenteric or Auerbach's plexus.

IV. **Tunica serosa:** typical (*loose connective tissue and mesothelium*).

REGIONAL VARIATIONS IN THE SMALL INTESTINE

Each of the three major anatomic portions of the small intestine – the duodenum, jejunum and ileum – has distinctive features that allow recognition under the light microscope.

DUODENUM – extends from the pyloric region of the stomach to the junction with the jejunum and has the following characteristics:

- **presence of Brunner's glands in the submucosa** – compound tubuloalveolar branched glands, mixed glands.
- presence of chyme in the small intestine induces cells of Brunner's glands to secrete alkaline mucus that neutralizes gastric acid and pepsin and further promotes digestion.
- **has large and short, branched villi.**
- **the dorsal surface is covered by adventitia.**
- The duodenum collects bile and pancreatic secretions transported by the common bile duct and pancreatic duct.

JEJUNUM has the following characteristics:

- does **not contain glands** in the submucosa.
- it has longest villi of all three regions, correspondence villi/crypts = 5:1.
- Peyer's patches in the lamina propria may be present but they are not predominant in the jejunum.

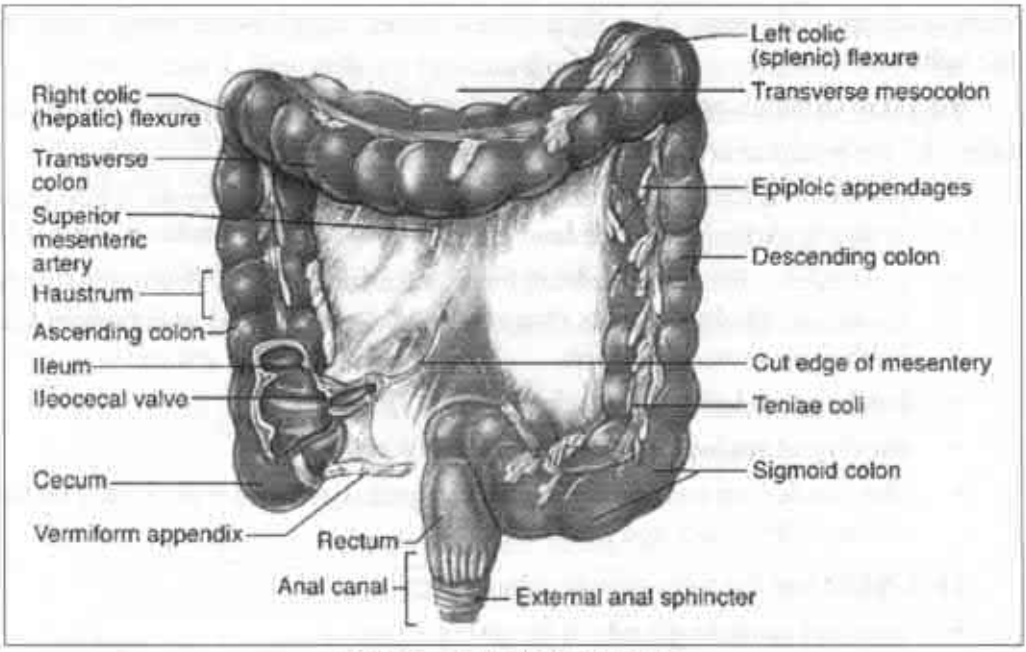
ILEUM has a prominent diagnostic feature:

- permanent presence of aggregated lymphoid nodules – **Peyer's patches** – in the submucosa.
- presence of shortest villi.
- presence of highest number of goblet cells.

LARGE INTESTINE

The large intestine extends from the ileocecal junction to the anus and is about 1.5m long. The large intestine has the following regions:

- **Cecum** with Appendix
- **Colon**
  - Ascending
  - Transverse
  - Descending
- **Rectum**
- **Anal canal**



Scheme of the large intestine

Functions:

- **Reabsorption of electrolytes and water** – its **PRIMARY FUNCTION.**
- **Formation of waste.**
- **B vitamins & vitamin K synthesized** (by the bacteria inhabiting the colon). The bacterial flora is also important for normal large intestine function by creating gas that aids peristalsis and by aiding in the digestion of certain materials. Certain consumed foods may create a healthy

bacterial flora, which can help overwhelm "bad" bacteria that try to populate the gut.

Unlike the small intestine, there are no villi in the large intestine so the surface of the tunica mucosa is more uniform and flatter than that of the small intestine.

Layers of the wall of the Large Intestine

I. **Tunica mucosa:**

- **epithelium** – simple columnar epithelium that forms straight tubular glands (crypts)

The epithelium of the crypt contains four types of cells:

1. **Goblet cells** – are more numerous than in the small intestine, their mucus lubricates passage of feces.
2. **Enterocytes** (absorptive cells) – few cells; remove residual water in feces.
3. **Enteroendocrine cells** – only few cells.
4. **Undifferentiated cells**

! Paneth cells are absent (present only in crypts of the appendix).

- **lamina propria**- loose connective tissue that contains numerous blood and lymphatic vessels, collagen, lymphocytes and plasma cells.
- **muscualris mucosae** – present beneath the base of the crypts and prominent; undergoes rhythmic contractions.

II. **Tunica submucosa**: forms by loose connective tissue, contains: blood vessels, nerves plexus, Peyer's patches which are aggregations of solitary follicles or groups of lymph nodules. Each pach contains from 10 to 70 nodules.

III. **Muscularis externa** is different from that of the small intestine. It contains 2 layers: inner circular and outer longitudinal. The inner circular layer of muscle forms the usual sheath around the large intestine, but the outer longitudinal muscle layer forms three flattened strands, **the taenia coli**. Only a thin layer of longitudinal muscle surrounds the inner circular muscle layer between the taenia coli.

IV. **Tunica serosa** is typical.

Specialized Sections of the Large Intestine

APPENDIX

1. Blind evagination of the cecum.
2. Has **extensive lymphoid tissue** that is both diffuse and nodular. This lymphoid tissue is located in the lamina propria as well as the submucosa.
3. Intestinal glands are short and irregular.
4. Only a few Goblet cells, Paneth cells are present.
5. No Villi, but glands are present that are filled with mucous-producing cells.
6. There is often fatty tissue in the submucosa.
7. The muscularis externa is thinner than in the remainder of the large intestine and, the outer, longitudinal smooth muscle layer of the muscularis externa does not aggregate into taenia coli.
8. An extreme proliferation of lymphocytes (lymphoid hyperplasia) as a consequence of bacterial or viral stimulation may lead to the obstruction of the lumen of the appendix and thereby cause appendicitis, but this is only one of many possible causes.

RECTUM/ANAL CANAL

1. Is the 2.5-4 cm long terminal part of the digestive tract. The mucosa has a characteristic surface relief of 5-10 longitudinal folds, the anal columns.
2. Each column contains a terminal branch of the superior rectal artery and vein.
3. Small mucosal folds between the anal columns (anal valves) form the pectinate line. This line defines sections of the anal canal with different arterial and nerve supplies, different venous and lymphatic drainages and different embryological origins.
4. Crypts disappear below the pectinate line and the epithelium changes from the tall, columnar type seen in other parts of the large intestine to a stratified squamous epithelium. Contains **sebaceous glands** that go into the lamina propria. They resemble the sebaceous glands in normal skin, and can get infected easily and cysts can develop within them.
5. The upper portion of the anal canal has mucosa with simple columnar epithelium (**colorectal zone**). The lower portion of the anal canal is

surfaced by stratified squamous nonkeratinized epithelium (**squamous zone**). The stratified squamous epithelium is continuous with that of the skin (stratified squamous keratinized). Between the upper portion and lower portion of the anal canal there is a variable amount of stratified columnar epithelium (**anal transition zone**).

6. In the skin of the circumanal region there are **circumanal glands** (large apocrine glands).
7. The muscularis externa gradually becomes thicker and forms the involuntary internal anal sphincter.

The anal canal is surrounded by a complex group of sphincter muscles.

- ***Internal anal sphincter*** is formed from a thickening of the circular layer of the muscularis externae (smooth muscle) and is the involuntary sphincter of the anal canal.
- ***External anal sphincter*** is circular skeletal muscle (continuous with levator ani muscle) and is the voluntary anal sphincter. It is located external to the internal anal sphincter.

DIGESTIVE GLANDS

SALIVARY GLANDS

The salivary glands all empty their secretions into the oral cavity. They vary as to their distance from the buccal cavity, their size and the nature of their secretory products. They can also be divided into major and minor glands.

1. **Major** salivary glands
   - **parotid**
   - **sublingual**
   - **submandibular**
2. **Minor** salivary glands
   - **lingual**
   - **labial**
   - **alveolar**
   - **buccal**
   - **palatine**

Development of Salivary Glands:

Primordia of the major salivary gland appear in the embryo during the 6th-8th week. The primordium of the parotid gland appears at the beginning of the 6th week that of the submandibular appears at the end of the 6th week and the sublingual primordium at the 7th-8th weeks. Primordia of the minor salivary glands appear after the third month in utero. Acini and ductal cells differentiate during the last 2 months of gestation and in general the glands continue to grow after birth up to 2 years.

All these primordia are derived from the ectodermal lining of the stomodeum and these epithelial strands develop into the parenchyma of the glands i.e. the acini and the ducts. The surrounding ectomesenchymal cells form the stroma that is the connective tissue septa that divide the major glands into lobes and lobules. Also in the parotid and submandibular glands a connective tissue capsule surrounds the gland. During the development of salivary glands, epithelial/mesenchymal interactions play a significant role in guiding the organization and the differentiation of different components of the gland. Fibroblast growth factors (FGFs) and transforming growth factor J3 (TGFJ3) are the major factors involved in sequential development of major salivary glands. Evidence indicates that ectomesenchyme is the determining factor in formation of the different parts of the epithelial parenchyma and deciding which will differentiate into acinar cells or various ductal cells. This role is similar to the one played by the dental papilla which determines the type of the developing tooth and whether it will be an incisor, canine, premolar or a molar.

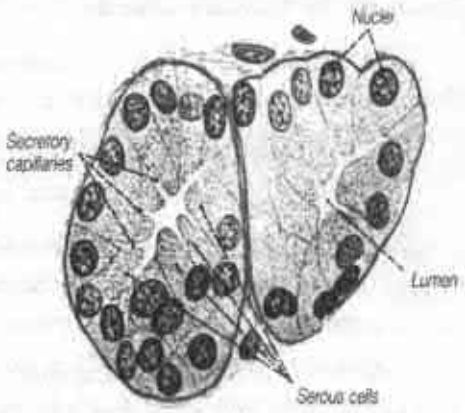
Major salivary glands are tubuloacinar glands, they have secretory acini but the first part of the duct system originating from the acini also participates in the secretory process. The salivary glands are divided by connective tissue septa into lobes, which are further subdivided into lobules.

Functionally the secretory acini can be divided into three groups:

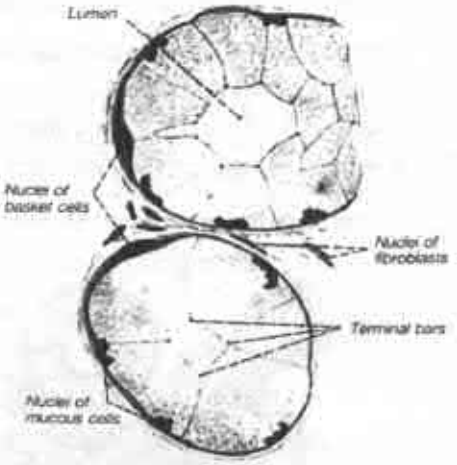
- those that secrete a rather liquid product – **serous acini,**
- those that secrete a very viscous product – **mucous acini,**
- and those that secrete a mixed product – **mixed acini.**

This functional differentiation is reflected in the appearance of these acini in histological sections.

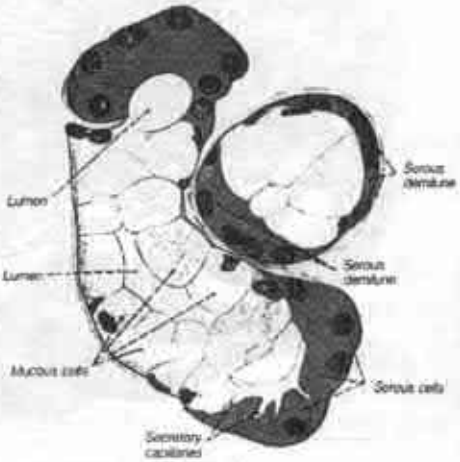
a) **Serous acinar cells**: These are pyramidal shaped cells with round nuclei located in the basal third of the cell, that is the part adjacent to the connective tissue surrounding the acinar cells but separated from them by a basement membrane. The cytoplasm appears basophilic in H&E sections reflecting the presence of abundant granular endoplasmic reticulum cisternae. The apical cytoplasm contains numerous secretory granules.



b) **Mucous acinar cells**: These pyramidal shaped cells have flattened nuclei which are located at the basal cell membrane. The cytoplasm appears lightly stained in H&E stained sections.



c) **Mixed acini**: consist of mucous acinar cells that line the lumen. At their outer periphery at their basal ends crescent-shaped caps of serous acinar cells are present which are called ***serous demilunes***. Intercellular canaliculi delivers the serous secretion of these demilunes to the lumen of the mixed acinus since the canaliculi are actually extensions of the lumen.



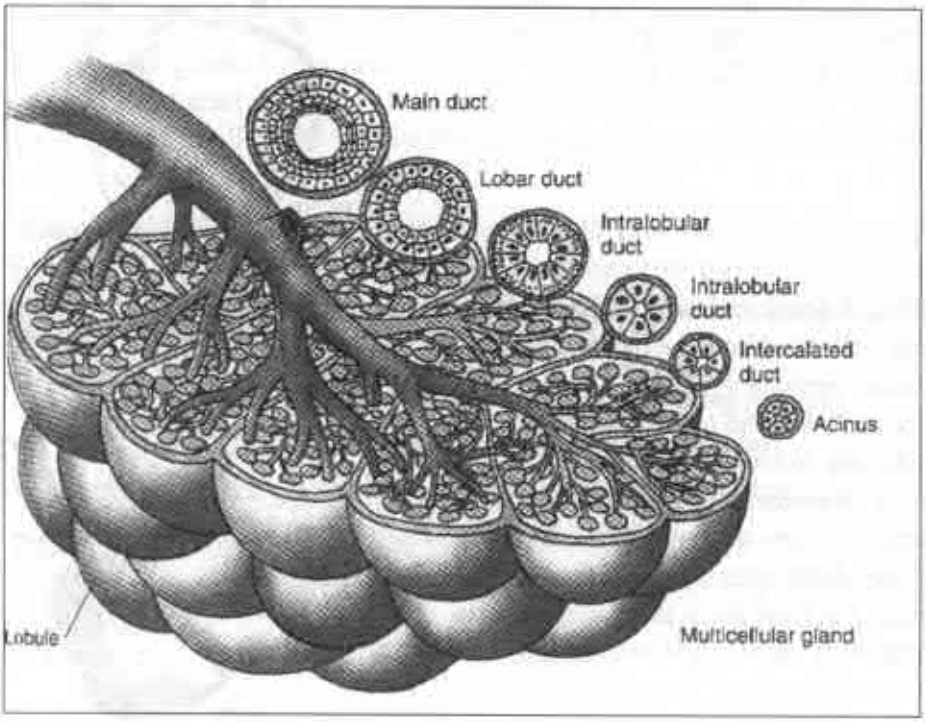
Ducts of the Salivary Glands

The ducts of the salivary glands can, according to their position in relation to the lobes and lobules of the glands, be divided into two parts.

1. **Interlobular or interlobar** ducts are embedded in the connective tissue surrounding the lobes and lobules of the glands.
2. **Intralobular** ducts are located in between the secretory acini within the lobules and, consequently, only surrounded by scant, if any visible connective tissue.

Interlobar and interlobular ducts function mainly in the conduit of the saliva and are formed by a **stratified cuboidal or stratified columnar epithelium**. The epithelium is replaced by the **stratified squamous nonkeratinized epithelium** as they approach the opening into the oral cavity.

The product of serous glands is extensively modified by the initial part of the duct system. Intralobular ducts can on the basis of their function be divided into intercalated ducts and striated ducts. The secretory acini empty into intercalated ducts which merge into the striated ducts.



Schematic diagram of ducts of the salivary glands

Cells forming the intercalated ducts add bicarbonate ions to the saliva (buffering function) and absorb chloride from the saliva. They are typically formed by **simple cuboidal epithelium**.

Striated ducts are formed by **simple columnar epithelium.** In contrast to many other columnar epithelia, the nucleus of these cells is located approximately midways between the apical and basal cell surfaces. The striations of the striated duct are found in the basal part of the cytoplasm of the cells where numerous mitochondria are found between folds of the basal cell membrane. This specialization provides the cell with the necessary energy and surface area to perform its task in the modification of the saliva – the secretion of potassium and the absorption of sodium. Cells of the striated ducts also take up different forms of antibodies and release them into the saliva.

Each major salivary gland consists of:

1. Capsule – represented by dense fibrous connective tissue.
2. Stroma – loose connective tissue stroma forming the septa between lobes and lobules also extend as thin strands supporting individual acini and ducts. This connective tissue contains the following cells: fibroblasts, macrophages, dendritic cells, mast cells, plasma cells as well as fat cells. The extracellular compartment contains collgen and elastic fibers as well as glycoproteins and proteoglycans.
3. Parenchyma – is represented by secretory acini and ducts.

PAROTID GLAND

Parotid gland is the largest salivary gland. It is compound alveolar branched gland.

This salivary gland is composed primarily of serous secretory units. These units are organized into lobules that are separated and defined by loose connective septa containing nerves, blood vessels and the larger secretory ducts. Parotid gland is purely serous in the adult but it contains few mucous acini in the newborn.

Serous cells secrete their watery product directly into intercalated ducts lined with low cuboidal epithelium that is continuous with the cells of the secretory units, called acini. From the intercalated duct the watery secretion passes through the larger striated ducts that are lined with a simple columnar epithelium. Parotid gland has long and branched intercalated ducts, numerous well developed striated and excretory ducts.

Its main duct, Stenon's duct opens in the mouth opposite the second maxillary molar. Parotid gland's connective tissue stroma consists of well developed capsule and septa with numerous fat cells especially in old persons.

SUBMANDIBULAR GLAND

Submandibular gland is compound tubulo-alveolar branched gland. The submandibular salivary gland is a mixed gland, but predominantly serous (90%) as it contains mixed acini but the serous acini are more numerous than mucous ones. The overall organization of the gland is the same as the parotid, lobules of secretory units surrounded and separated from each other by loose connective septa containing nerves, blood vessels and the secretory ducts. The intercalated ducts are shorter than those in the parotid but the striated ducts are longer. Excretory ducts are well developed and the main duct (Wharton's) opens in the floor of the mouth. Prominent septa and connective tissue capsule are present. Submandibular gland produces about 60% of saliva volume.

SUBLINGUAL GLAND

Sublingual gland is compound tubulo-alveolar branched gland; is mixed but predominantly mucous as pure serous acini are rare or absent but serous acinar cells are present as demilunes in mixed acini. The intercalated and striated ducts are poorly developed. Several small terminal ducts as well as the main duct (Bartholin's) open into the floor of the mouth. Prominent connective tissue septa are present but only a poorly developed capsule exists. Sublingual gland produces only 5% of saliva volume.

So, in conclusion:

- Glands located close to the oral cavity have mainly mucous secretions, whereas glands located further away from the oral cavity have mainly serous secretions. Following this general rule, the parotid glands contain almost exclusively serous acini, the submandibular glands contain both serous and mucous acini, and the sublingual glands contain mainly mucous acini or mucous acini with serous demilunes.

SALIVA consists of:

- proteins (emzymes, mucoproteins, glycoproteins, secretory IgA)
- carbohydtares
- lipids
- Inorganic substances

Saliva also contains:

- lymphocytes
- granular leukocytes
- detached epithelial cells

The composition of primary saliva collected from the intercalated ducts is modified while it passes through the striated and excretory ducts, mainly by reabsorption and secretion of electrolytes and possibly by addition of other constituents. The primary saliva is isotonic, with sodium and chloride concentrations higher than potassium while the saliva reaching the oral cavity is hypotonic with low sodium and cloride but high potassium concentrations.

Functions of Saliva:

1. **Protection**: The fluid nature and the components of saliva protect the oral cavity in several ways. Saliva provides a washing action that clears non-adherent potentially harmful substances in the oral cavity. Viscous components e.g. mucins lubricate oral tissues and form a barrier against microbial products. Bicarbonate, phosphate ions as well as basic proteins in saliva maintain the near neutral pH in the oral cavity which prevents demineralization of enamel that would otherwise occur due to acids produced by sugar metabolizing bacteria. Salivary proteins form a thin coating on tooth surfaces, the salivary pellicle which contributes to protecting these surfaces. Saliva is supersaturated with calcium and phosphate ions and this state is maintained by certain calcium binding proteins, notably acidic proline-rich proteins and statherin. This leads to posteruptive maturation of enamel surfaces which increases their hardness and resistance to demineralization. Further, such environment favors remineralization of the beginning of carious lesions (white spots) provided that cavitation did not occur.
2. **Digestion**: Saliva contains two digestive enzymes, namely amylase which breaks down complex carbohydrates such as starch into glucose and maltose and lipase (a product of lingual glands) which hydrolyzes triglycerides into mono- and diglycerides.
3. **Defense**: Saliva plays a major bacteriostatic role in the oral cavity. It interferes with microbial colonization and mucin form a physical anti-microbial barrier. Salivary IgA (sIgA) is an important factor in oral immune defense, together with salivary agglutinins (glycoproteins) sIgA causes clumping of certain microorganisms thus preventing them from adhering to oral and dental surfaces. Other components namely histatins, lysozyme, lactoferrin and peroxidase inhibit bacterial growth.

Humans who suffer from a decreased salivary secretion due to diseases affecting acini or as a consequence of other systemic diseases or as side effect of certain medications have difficulty in eating, swallowing or speaking. These persons are susceptible to mucosal infections and high incidence of dental caries.

4. **Taste**: The solubilization of ingested material by saliva is essential for the functioning of taste buds which act as chemoreceptors when they are in contact with the dissolved material and initiate taste sensation

LIVER

- Largest gland of the body
- Two principal **lobes: right** and **left**
- Right lobe further subdivided:
  - **Quadrate lobe** and **caudate lobe**

Liver is surrounded by a thin capsule of connective tissue (Glisson's capsule). Glisson's capsule is covered by peritoneum except at the bare area (superior & posterior surfaces).

In the porta hepatis the fibrous tissue thickens and follows as supportive tissue the blood and lymph vessels and the bile ducts to their termination or origin in the portal spaces between the liver lobules.

FUNCTIONS OF THE LIVER

Digestive and Metabolic Functions

- synthesis and secretion of bile.
- storage of glycogen and lipid reserves.
- maintaining normal blood glucose, amino acid and fatty acid concentrations.
- synthesis and release of cholesterol bound to transport proteins.
- inactivation of toxins.
- storage of iron reserves.
- storage of fat-soluble vitamins.

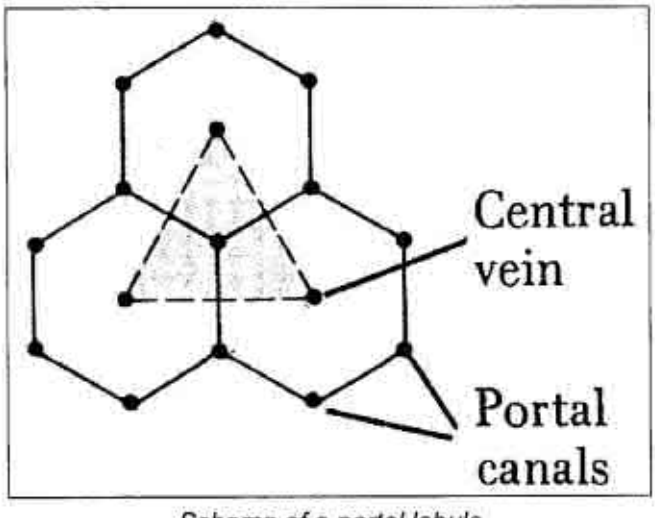
Non-Digestive Functions

- synthesis of plasma proteins.
- synthesis of clotting factors.
- synthesis of the inactive angiotensinogen.

- breakdown of circulating hormones (insulin and epinephrine) and immunoglobulins.
- inactivation of lipid-soluble drugs.

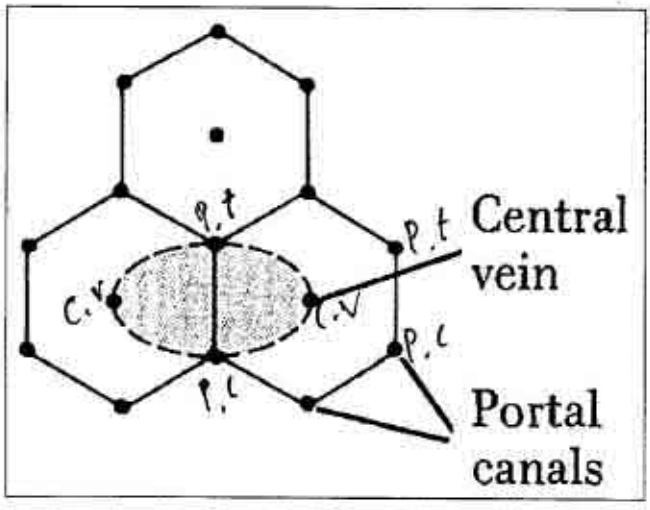
The morpho-functional units of the liver

- **"Classical liver lobule"** – is a six-sided prism about 2 mm long and 1 mm in diameter. It is delimited by interlobular connective tissue (slightly visible in humans). In its corners we find the portal triads. In cross sections, the lobule is filled by cords of hepatic parenchymal cells, hepatocytes, which radiate from the central vein and are separated by vascular sinusoids.
- **Portal lobule** – is another way of looking at the organization of the liver; is a classic lobule in reverse. *Central veins are on the outside of lobule* and *portal triad is centrally located.* Blood flows from the center (portal triad) to the periphery (central vein) of the lobule, and bile and lymph flow from the periphery to the portal triad in the center of the lobule. ***The portal lobule emphasizes the exocrine gland function (bile formation) of the liver.***



Scheme of a portal lobule

- **Hepatic (liver) acinus (of Rappoport)** – **Acini** are smaller units than portal or "classical" liver lobules; – is yet another conception of a lobule; – is shaped like a square or diamond with a pair of *central veins* on two opposite corners, and *two portal triads* on the other two opposite corners; – was conceived to help study zones, based on proximity to fresh incoming blood, that appear to be involved in different processes.



Scheme of the hepatic (liver) acinus

1. **Zone I**
   - is closest to the two portal triads and receives fresh blood (blood that contains the highest levels of oxygen and nutrients (glucose)).
   - hepatocytes will be the first to **store glycogen** and **break it down** when necessary *(blood glucose regulation)*.
2. **Zone II**
   - the hepatocytes further away from the vessels will receive blood that has already been altered by cells in Zone I; following a meal, they will pick up any glucose or nutrients that were not taken up by Zone I cells.
   - *contain waste products in higher concentration* than in zone I.
3. **Zone III**
   - the hepatocytes most distant from the distributing vessels will receive blood that has the least amount of oxygen, glucose and other nutrients. These cells are rich in enzymes of **glycolysis** and in enzymes involved in **drug and alcohol detoxification**.
   - is *most at risk for damage* and *degenerative changes* because of low oxygen tension, and other factors.

The hepatic acinus provides a way of understanding that all hepatocytes are <u>not</u> undergoing similar physiological processes; their particular activity depends in large measure upon their location relative to the blood vessels and their enzyme content. Furthermore, Zone III generally suffers most from injury,

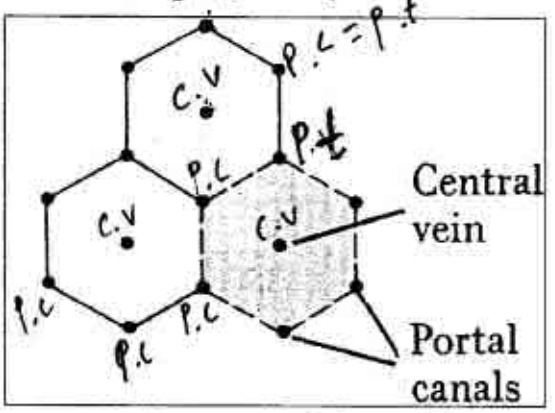
whether viral, toxic, or anoxic. Zone I usually survive longer and may serve as the core from which **regeneration** will proceed, although it suffers the most when obstruction of bile outflow occurs (bile stasis).

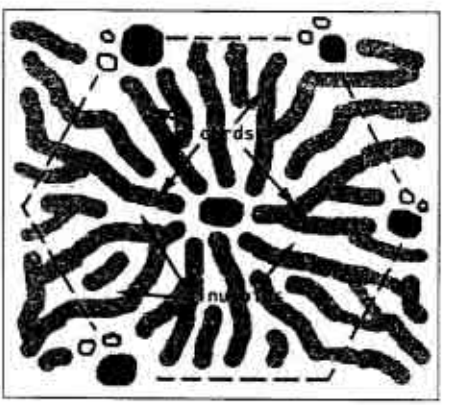
- When hepatocytes are repeatedly damaged by toxins, they undergo fatty degeneration and an excess of connective tissue (from Ito cells) is produced in the damaged area, resulting in disorganization of liver structure **(cirrhosis)**. Cirrhosis compromises blood flow through the sinusoids and can lead to portal hypertension, which can lead to venous swelling at the esophagus, hemorrhage, and death.

CLASSICAL LIVER LOBULE

Hexagonal-shaped liver lobule (classical lobule) is the traditional description of the liver parenchyma organization.

- Each **CLASSICAL HEPATIC LOBULE** is made up of interconnecting plates of epithelial cells called **hepatocytes,** which are radially arranged around a **central vein**. The plates of hepatocytes are usually one cell thick and are separated from each other by sinusoidal capillaries.
- The epithelial cells that make up the bulk of the liver – **hepatocytes** [***parenchyma***] – are structurally supported by **reticular fibers** – type III collagen [***stroma***].





Schemes of the classical liver lobule

- The lobules are generally considered to be roughly hexagonal, although there are no clear boundaries between lobules in the human.
- The lobules are **three dimensional**, and are approximately 0.7 mm in diameter and 2 mm in length, with the central vein running vertically through the center of the lobule. Central veins drain into sublobular

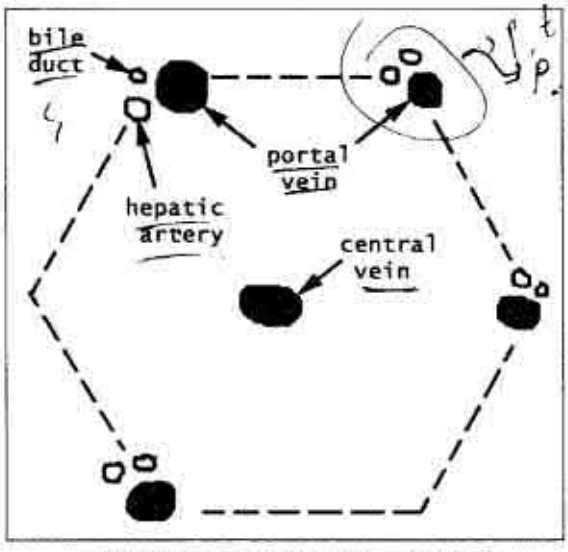
veins. Sublobular veins gradually unite and ultimately form 2 or more **hepatic veins**, which drain into the **inferior vena cava.**

- Portal triads are found at each of the six corners of each liver lobule

PORTAL TRIADS

Portal triads (also called ***portal areas*** or ***portal canals***) are located at the corners of liver **lobules**. Each **portal area** contains *three* (hence the term portal *triad*) more-or-less conspicuous tubular structures all wrapped together in **connective tissue.**

- a branch of the **bile duct** (lined by simple cuboidal epithelium).
- a branch of the **portal vein** – an interlobular branch of the portal vein.
- a branch of the **hepatic artery** – an interlobular branch of the hepatic artery.
- also, **Lymphatic vessels** (do not penetrate into the lobules; lymph derived from the space of Mall).



Schematic diagram of a portal triad

Cells of the liver: The main cell type constituting 60% of all liver cells is the hepatocyte. It is the most versatile cell type in the human body and carries out all the main liver functions:

- **Absorption, Processing and Storage** of nutrients transported from the intestine. During a single passage through the lobule 60-100% of most metabolites are removed.

- **Secretion**. The liver produces most of the proteins found in the blood plasma including albumin, lipoproteins, transferrin, clotting proteins, growth factors (100 g/day).
- **Production of Bile**. Hepatocytes continuously synthesize bile acids from cholic acid, a metabolite of cholesterol.

HEPATOCYTES

- have one or two large euchromatic nuclei.
- are **polyhedral** epithelial cells; have **6 or more surfaces**.
- a typical hepatocyte has ***two*** **surfaces with microvilli** (As in other areas of the body, these structures serve to increase the surface area of the cell membrane that comes in contact with the blood facilitating exchange of molecules between hepatocytes and the blood).

Three types of surfaces:

1. Exposed to space of Disse (corresponds to basal surface of a typical epithelial cell) – microvillus border; exchange of metabolites to and from blood occurs here.
2. Exposed to bile canaliculus (corresponds to apical surface of a typical epithelial cell) – microvilli; secretion of bile from hepatocyte occurs here.

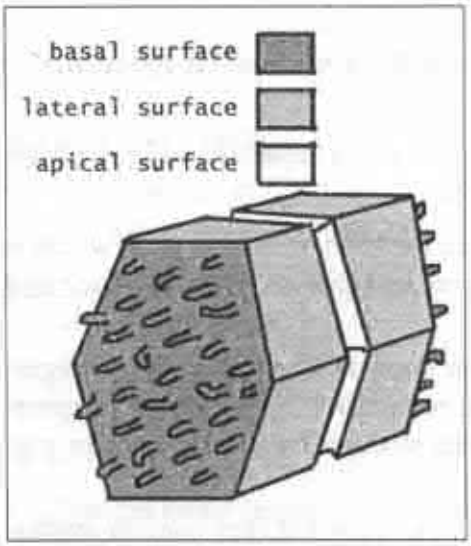
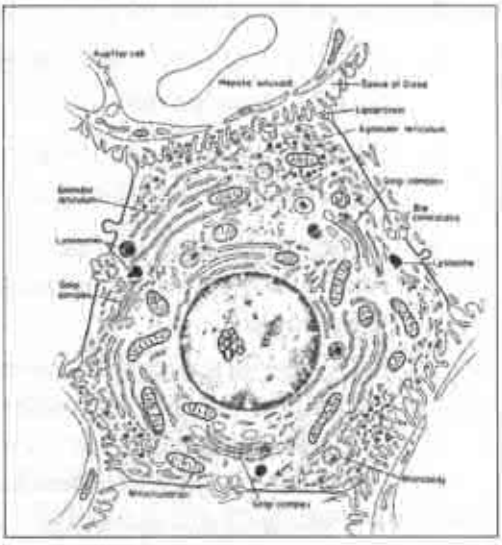


Diagram of the hepatocyte showing the hepatocyte's surfaces



Schematic diagram of a hepatocyte showing representative cytoplasmic constituents

3. In contact with adjacent hepatocytes (corresponds to lateral surface of a typical epithelial cell) – gap junctions allow communication to coordinate physiologic activity. Desmosomes and zonulae adherentes junctions attach hepatocytes to their neighbors.

- **Hepatocytes** have abundant, grainy cytoplasm that stains well with both acid and basic dyes (reflecting the abundance of various cellular constituents). Hepatocytes are richly supplied with **mitochondria** (2,000/cell). They have abundant **Golgi apparatus**, **endoplasmic reticulum**, both smooth (SER) and rough (RER):
  1. RER synthesizes proteins (e.g. albumin, fibrinogen, prothrombin, the protein portion of lipoproteins) which are not stored in granules – they are immediately released into the blood.
  2. SER is distributed diffusely through the cytoplasm – inactivation or detoxification of various substances occurs in SER, so the amount of SER increases when there is an increase in circulating drugs or toxins. It also synthesizes the cholesterol and lipid portions of very low density lipoprotein (VLDL). Glycogen is closely associated with the SER.

Carbohydrate Metabolism – The liver plays an important role in maintaining the normal blood glucose concentration:

1. Glucose enters hepatocytes from sinusoids via **insulin-independent GLUT-2 transporters.**
2. Glucose is polymerized by a series of enzymatic reactions to form glycogen, usually in the vicinity of the SER.
3. If the need arises, glycogen is broken down by phosphorylase which is activated by epinephrine and glucagon, and glucose is released back into the sinusoids.

- Other organelles: **Lysosomes** are important in turnover and degradation of other organelles; also participate in destroying receptor-macromolecular complexes; iron storage; frequently contain pigment granules (**lipofuschin**).
- **Peroxisomes** are lysosome-like structures, but they contain **oxidases** and **catalase.** The action of the oxidases (with O_2) causes oxidization of very long chain fatty acids and detoxification of alcohol to produce toxic H_2O_2. The H_2O_2 is then degraded by catalase to form O_2 and H_2O.

- Hepatocytes may accumulate abundant **lipofuscin** (yellow-brown "wear-and-tear" pigment), especially with advancing age.

Hepatocytes are located in flat irregular plates (cords) that are arranged radially like the spokes of a wheel around a branch of the hepatic vein, called the **central vein** (**a fibrous or amuscular vein**). Each hepatic plate contains 2 rows of hepatocytes. Between 2 rows of hepatocytes of the plate is bile canaliculus. The canaliculi, formed by the plasma membranes of adjacent hepatocytes, have specialized functions related to bile secretion. The canaliculi form a belt-like network around individual hepatocytes. **Bile canaliculi** drain bile into ***Herring canals*** which are located at the periphery of the lobule. Herring canals drain to bile ducts in the portal triad. Bile ducts drain ultimately into the right and left hepatic ducts. Herring canals and bile ducts are lined by a simple cuboidal epithelium.

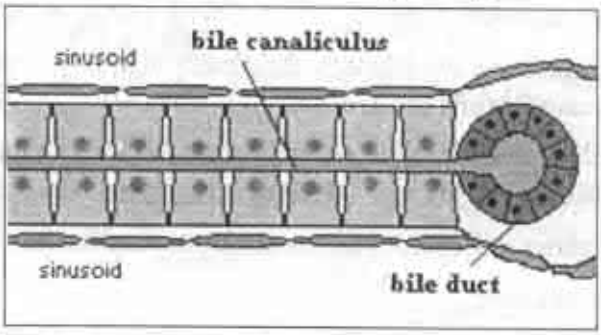


Diagram of the plate of hepatocytes

BILE FORMATION

Bile consists of:

- bile acids (a.k.a. bile salts)
- bilirubin
- cholesterol
- phospholipids
- electrolytes
- water

- Besides being important for **fat emulsification** in the small intestine, bile is also a means of **excretion of unwanted, detoxified substances.** ~One liter/day is produced.
- **Bile acids** are the components that are important for **fat emulsification** in the gut; 90% of the bile acids secreted into the canaliculi have been recircu-

lated from the intestine; 10% have been synthesized in the SER by conjugation of cholic acid (derived from cholesterol) with glycine or taurine → glycocholic and taurocholic acids.

- **Bilirubin** is **derived from hemoglobin** of aged erythrocytes that have been broken down by macrophages in the spleen and, to a lesser extent, by Kupffer cells. It is not water soluble, but is taken up by the hepatocytes and is detoxified by conjugation with glucoronide in the SER. This substance (**bilirubin glucuronide**) is now non-toxic and water soluble; it is excreted into the bile canaliculi. When bilirubin accumulates in the blood (**hyperbilirubinemia**), the person becomes jaundiced. This may result from:

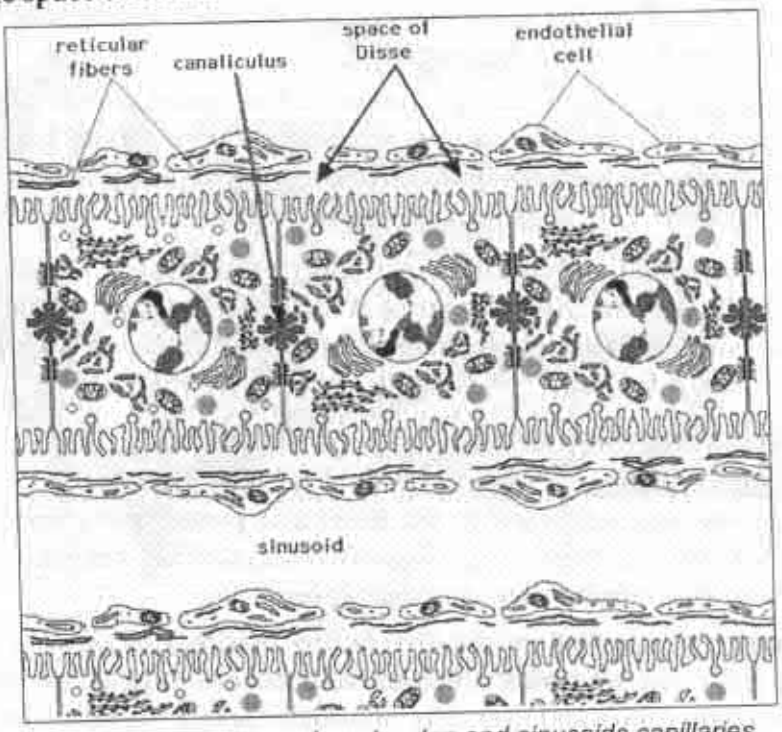
1. Decreased uptake of bilirubin by hepatocytes (**Gilbert syndrome**);
2. Inability to conjugate bilirubin (**Crigler-Najjar syndrome**; **neonatal hyperbilirubinemia**);
3. Inability to excrete the bilirubin glucuronide into the canaliculi (**Dubin-Johnson** and **Rotor's syndromes**);
4. Increased production of bilirubin beyond the liver's capacity to excrete it (**hemolytic jaundice**), e.g. following an internal hemorrhage.

Between the plates of hepatocytes are sinusoids capillaries.

Sinusoids capillaries:

- are larger than conventional capillaries and less regular in shape. They are lined by thin **endothelial cells and lacks a basement membrane** (is absent over large areas except the periphery and center of the hepatic lobule). Endothelial cells are small, elongated with dark-staining nucleus; have few organelles, but numerous micropinocytotic vesicles.
- Also residing on the sinusoidal walls are macrophages called **Kupffer cells**. Kupffer cells are members of the mononuclear phagocyte system. This particular population of macrophages are especially significant, with responsibility not only for cleaning bacteria out of the portal blood stream (the "dirty" blood" from the intestine), but also for removing worn-out red blood cells and recycling hemoglobin – metabolize hemoglobin to **bilirubin** (a job shared with macrophages of the spleen). Kupffer cells synthesize and secrete proteins related to immunologic processes. Kupffer cells are closely associated with the endothelial lining of the liver. Lying along side or draped across the liver sinusoids, the Kupffer cells are not easily distinguished from the endothelial cells. They are numerous in the periportal area where they are most active.

- Pit cells are attached to the Kupffer's cells. These cells contain granules and they resemble large lymphocytes, killer cells. They have an anticancer effect.
- The space between the fenestrated endothelium and the cords is named the **space of Disse.**



Relationship between hepatocytes and sinusoids capillaries

Perisinusoidal space (space of Disse) contains:

- **type I, III and IV collagen fibers** to maintain the architecture of sinusoids;
- **short microvilli of hepatocytes** to provide large surface area for absorption & secretion;
- **blood plasma;**
- **processes of the Kupffer's cells;**
- **lipocytes (adipose cells, commonly called an Ito cells).** They are located between some hepatocytes. These cells contain multiple lipid droplets; have been shown to be the primary storage site for vitamin A. Exogenous **vitamin A** is taken up by the cells and stored in the lipid droplets; it is released into the blood as **retinol** – important for rhodopsin formation in

rods and cones of retina. Under some pathological conditions, they become **myofibroblasts** – synthesize and secrete in the large amount type I and III collagen. This leads to fibrosis (cirrhosis), which can increase vascular resistance in the sinusoids → **portal hypertension** → **esophageal varices;**

- In the fetal liver, the space between blood vessels and hepatocytes contains islands of blood-forming cells;
- Blood can percolate through the sieve plates and discontinuities where it can contact the hepatocytes, whose absorptive surface has been expanded by the presence of microvilli; this permits an efficient exchange of metabolites between the liver and the bloodstream.

The space of Mall – found at the periphery of the hepatic lobule – is continuous with the space of Disse. The space of Mall is drained by lymphatic vessels piercing the limiting plate. Lymphatic vessels surround the blood vessels and bile ductules in the portal space.

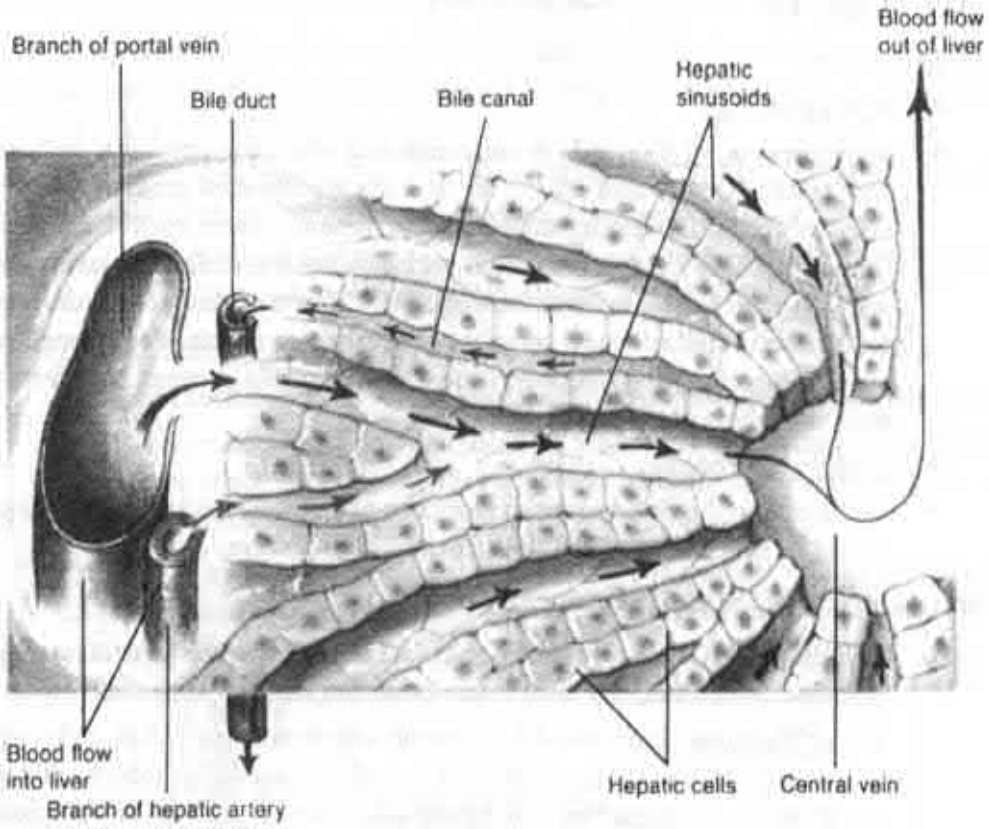
The blood circulation through the liver is subdivided in:

- System of inflow: the liver receives blood from the ***hepatic artery*** (supplies oxygen-rich blood to the liver) and ***portal vein*** (carries venous blood with nutrients from digestive viscera). They branch into ***lobar, segmental, interlobular, distributing branches.***
- System of circulation: the distributing branches of vessels contribute blood to the ***sinusoids*** which provide the exchange of substances between the blood and liver cells. Sinusoids contain the mixed blood.
- System of outflow: sinusoids drain blood from the periphery of the classical hepatic lobule toward its center, into the ***central vein***. Outside hepatic lobules central veins drain into the ***sublobular*** (intercalated) ***veins***, which join 3-4 together and drain into the ***hepatic vein***. It drains into the ***inferior vena cava.***

In conclusion, in the classic hepatic lobule fluids flow in two, opposite, directions.

a. Blood originating in portal triads flows from outside of a lobule toward its center to exit in the central vein.

b. Lymph and bile is generated inside of the lobule, and flows toward the periphery to exit in the lymphatic vessel and bile duct of the triad.



Scheme of the blood circulation through the liver

Clinical Correlation

- Liver fibrosis is defined as an excessive accumulation of connective tissue in the liver following repeated or chronic insult (alcohol, viruses, iron or copper overload, cholestasis, hepatic blood congestion) that triggers a "wound-healing" like reaction. Liver fibrosis can progress into liver sclerosis and eventually liver cirrhosis.
- Liver cirrhosis is marked by an extensive loss of liver cells and disorganization of the liver lobules. Fibrous tissue surrounds nodules of regenerated remaining liver cells. Cirrhosis *starts in zone III and spreads toward zone I.*

GALL BLADDER

Functions:

- storages of bile.
- concentration of bile. **Bile is concentrated** when the epithelial cells absorb water. Na^+, Cl^- and HCO_3^- are actively transported across the lateral cell membranes, creating an osmotic gradient. Water moves from the lumen through the epithelial cells and into the intercellular space, causing it to widen. The water is subsequently absorbed into the underlying fenestrated capillaries and venules of the lamina propria. Tight junctions (zonulae occludentes) between the epithelial cells prevent backflow of fluid into the gall bladder lumen.
- acidification of bile.
- sends bile to the duodenum in response to cholecystokinin, which is secreted by enteroendocrine cells from small intestine.

Tunics (layers) of the Gall Bladder

1. **TUNICA MUCOSA:** When the gall bladder is empty, this layer is extremely folded. When full, this layer is smoother but still has some short folds.
   - **epithelium:** composed by simple columnar epithelial cells with numerous microvilli on their luminal surfaces. **No goblet cells** are present. Epithelial cells have well-developed junctional complexes and numerous lateral folds.
   - **lamina propria**: composed of loose connective tissue rich in reticular and elastic fibers to support the large shape changes that occur in the epithelium; lamina propria may contain compound tubuloalveolar glands. May be mucous or serous.
   - There is **no muscularis mucosa**
2. **TUNICA SUBMUCOSA:** composed of loose connective tissue.
3. **MUSCULARIS EXTERNA:** contains much smooth muscle, poorly organized; and which forms an irregular network of longitudinal, transverse and oblique fibers interspersed with collagenous and elastic fibers; contracts to release bile into the duodenum when cholecystokinin is present in the blood.
4. **TUNICA SEROSA:** composed of loose connective tissue and mesothelium. Where the gall bladder attaches to the liver, **adventitia** is present.

PANCREAS

It is a retroperitoneal organ and is covered with a layer of loose connective tissue and mesothelium on its anterior aspect that do not form a well-defined capsule. The pancreas has 2 parts:

- Exocrine gland (97%)– produce **PROENZYMES** for digestion of carbohydrates, proteins & fats **(amylase, trypsin, lipases).**
- Endocrine gland (3%)– secrete hormones, such as: **INSULIN** and **GLUCAGON (carbohydrate metabolism)** and others.

THE EXOCRINE PANCREAS

- The exocrine portion of the pancreas is a **compound tubuloacinar gland.**
- It has many small lobules, each of which is surrounded by connective tissue septa through which run blood vessels, nerves, lymphatics, and interlobular ducts.

Acini: The acini are round or elongated and consist of 8 – 12 pyramidal cells in a single layer around a narrow lumen. The lumen becomes larger when the gland is actively secreting.

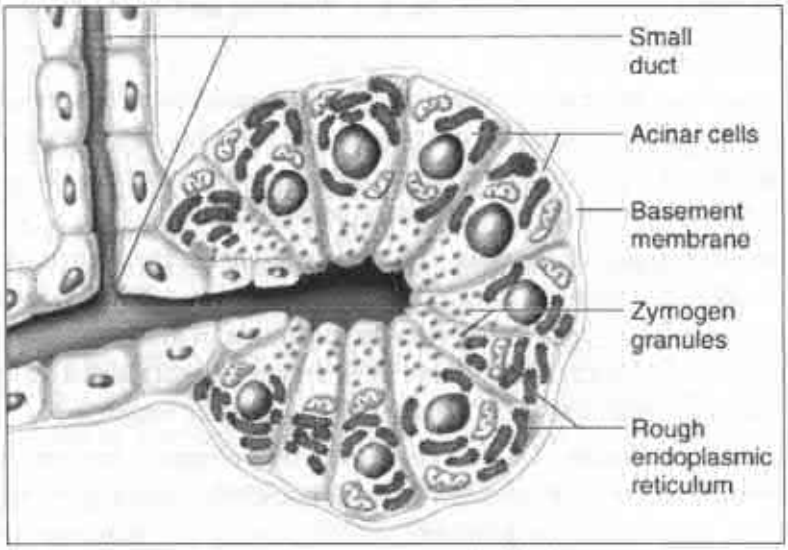


Diagram of the pancreatic acinus

- The **pancreatic acinar cells** are highly active in protein synthesis for export and this high activity is reflected in their **bizonal staining** properties. The basal region of these secretory cells usually stains intensely with hematoxylin reflecting the presence of large amounts of endoplasmic

reticulum where the protein is being synthesized on ribosomes – **homogen zone**. The presence of numerous zymogen granules containing high concentrations of protein is reflected in the intense eosin staining in the apical region of the secretory cells – **zymogen zone**. These granules are most abundant during fasting or between meals and least abundant after a meal has been ingested.

Ducts: The secretory product of the acinar cells is carried out of the pancreas by a duct system as in other exocrine glands.

- The first part of the duct system is called the ***intercalated duct***. It is lined with cuboidal epithelial cells that secrete bicarbonate ion into the secretory product. This duct actually extends into the acinar lumen, where its walls consist of the pale staining ***centroacinar cells***.
- *Intercalated ducts* have very little connective tissue around them but they lead into larger ***intralobular ducts*** and after ***into interlobular ducts*** which are surrounded by large amounts of interlobular connective tissue. Intralobular and Interlobular ducts are lined with a low columnar epithelium that may contain goblet cells. Interlobular ducts empty into the main pancreatic ducts **(duct of Wirsung and duct of Santorini)** that exit the pancreas.

The exocrine pancreas has a low basal rate of continuous secretion, which is periodically greatly increased by nervous (vagal) and hormonal stimulation following the ingestion of food. The hormones derived from the enteroendocrine cells of the GI tract stimulate the secretion of pancreatic juice:

1. **Secretin** is released when chyme is present in the gastric antrum and duodenum. Secretin does not stimulate acinar cells, but does cause intercalated duct cells to secrete a large volume of fluid with a high concentration of sodium bicarbonate ions; serves to neutralize acid in intestine – creates optimum pH for activity of pancreatic enzymes.
2. **Cholecystokinin** (CCK) is released when chyme is present in the duodenum and upper jejunum. Cholecystokinin causes acinar cells to release their zymogen granules, which contain the **inactive proenzymes** that will be converted in the small intestine to their active forms.

There are ***molecular safeguards*** to prevent early activation of digestive enzymes within the pancreas or duct system:

- **Trypsinogen** is cleaved by **enterokinase** in the small intestine to form **trypsin**.

- **Trypsin** cleaves the other proenzymes, resulting in their active forms.
- **Trypsin inhibitor** is secreted with proenzymes. It helps to prevent premature activation of proenzymes – if these intrinsic safeguards are overwhelmed, **pancreatitis** will occur.

Pancreatic juice contains the following enzymes:

- Trypsin, Chymotrypsin, Elastase and Carboxypeptidase hydrolyze proteins into smaller peptides or amino acids;
- Ribonuclease and deoxyribonuclease digest the corresponding nucleic acids;
- Pancreatic amylase digests carbohydrates (glycogen) and starch;
- Triacylglycerol lipase and Phospholipase A_2 digest lipids = **lipolysis** (triglycerides → monoglycerides and free fatty acids);
- Cholesterol esterase breaks down cholesterol esters into cholesterol and a fatty acid.

THE ENDOCRINE PANCREAS

- The cells of the endocrine portion of the pancreas are arranged either in round-to-oval shaped areas rich in blood vessels (fenestrated capillaries) known as the **islets of Langerhans** or they may be scattered throughout the exocrine portions of the pancreas near the acini or ducts.

Islets of Langerhans have 100 – 200 microns in diameter *(see fig. 132, plate II)*. **The number of islets of Langerhans increases from head to tail of pancreas.** Each islet is surrounded by a thin layer of reticular fibers. **Three principle cell types** are present in the islets that are ***not detectable with H & E staining***; all contain membrane-bound secretory granules:

1. ***β-cells*** (75%) which secrete insulin (stimulates the synthesis of glycogen, protein and fatty acids; facilitates the uptake of glucose into cells; activates glucokinase in liver cells). They are located in the central part of the island.
2. ***α-cells*** (20%) which secrete glucagon (effects opposite to those of insulin). They are generally located peripherally in the islets.
3. ***δ-cells*** (5%) are scattered throughout the Islets; secrete somatostatin, which modulates the activity of A and B cells to maintain normal glucose levels.

- a few other endocrine cells:
  - ***PP-cells*** secrete pancreatic polypeptide, which stimulates chief cells in gastric glands, inhibits the bile and bicarbonate secretion.

- ***δ1-cells*** secrete vasoactive intestinal peptide (VIP), which has effects similar to glucagon, but also stimulates the exocrine function of the pancreas and decrease the ***arterial blood pressure.***
- ***EC-cells*** (enterochomaffin cells) secrete secretin, which stimulates the exocrine pancreas, and motilin, which increases GIT motility.

Clinical Correlation:

Diabetes:

Type 1 – juvenile onset. No insulin is produced because the **B cells have been destroyed** by an autoimmune disorder or viral infection.

Type 2 – adult onset. **Insulin resistance** occurs in the peripheral cells because of a decrease in number of insulin receptors, defective receptors, or defective signal transduction, such that glucose is not sufficiently taken up by the cells. There can also be a reduction in insulin production.

CHAPTER VIII

RESPIRATORY SYSTEM

The complex of organs and tissues which are necessary to exchange the carbon dioxide from blood (CO_2) with oxygen (O_2) from air is called the respiratory system.

Functions:

- Provides gas exchange.
- Olfaction.
- Blood storage (good blood supply).
- Blood clotting (synthesis in the lungs of thromboplastin and heparin).
- Endocrine (synthesis of biologically active substances which act on bronchi tonus).
- Immune or protective (presence of lymphocytes, macrophages).

There are two main subdivisions of the respiratory system:

1. **AIR CONDUCTING PORTION consists:**
   a. Nasal cavity, oral cavity.
   b. Nasopharynx, oropharynx, and larynx.
   c. TRACHEA.
   d. Bronchi (main, lobar, segmental, and subsegmental).
   e. Terminal bronchioles.

   These provide a passageway to and from lungs.

   These components also "condition" the inspired air (i.e. moistens, removes particles and some noxious gases, and warms air).

2. **RESPIRATORY PORTION** – for gas exchanges between blood and air.

GENERAL STRUCTURE and FUNCTION of the airways

Most airways are lined with **tunica mucosa** that consists of an *epithelium* overlying a loose irregular CT *lamina propria*. In bronchi and bronchioles the base of the lamina propria contains a layer of smooth muscle, called the *muscularis mucosa*.

Below the mucosa is a loose irregular CT – **submucosa** – that contains glands (serous, mucous, and seromucous) and blood vessels. The next layer of organs of the respiratory system – **tunica fibro-cartilaginous** is constituted by a hyaline (trachea, bronchi, etc) or elastic (epiglottis) cartilage, in the form of rings and plates, which form a rigid but flexible structure that keeps the lumen open under the vacuum of a strong inspiration and when it is bent during movements of the head. In addition, elastic fibers are oriented longitudinally to allow stretch during body movements or when the diaphragm pulls down during inspiration. The outermost layer is the CT (usually loose) **adventitia**.

In order to protect the lungs, the entering air is conditioned and checked for antigens. Hairs in the nose filter out large foreign particles while goblet cells, and mucous and serous glands produce a coating to trap smaller particles, water-soluble gases, and moisten the air. Walls of the airways are very vascularized and this warms the incoming air. Immune tissue, that protects the body from invading organisms, is found in the form of loose, nodulated, and encapsulated (tonsilar) lymphatic tissue (MALT and BALT, mucosa and bronchi associated lymphatic tissue) around the airways. There are also individual motile immune cells (mast cells, lymphocytes, plasma cells, macrophages and a few granular leukocytes) within the CT lamina propria.

Epithelia in the airways primarily consist of pseudostratified respiratory epithelium, but regions of stratified squamous and the specialized pseudostratified olfactory epithelium are also in the airways.

Respiratory epithelium is pseudostratified columnar epithelia with cilia and goblet cells and is found throughout the airways. In bronchioles nears the lungs it gradually becomes simplified as it transforms from respiratory epithelium to simple ciliated columnar to simple ciliated cuboidal and is finally replaced by the simple squamous epithelium of lung tissue. There are several cell types present in respiratory epithelium that are connected together by junctions and fastened to a basement membrane so that they form a continuous sheet that separates the lumen of the airways from the interior of the body:

1. **Ciliated cells** *(see trachea)*
2. **Goblet cells** *(see trachea)*

3. **Granule cells (endocrine cells)** *(see trachea)*
4. **Basal cells** *(see trachea)*
5. **Dendritic cells (Langerhan's)** – antigen-presentation cells, stimulate proliferation of T-lymphocytes.
6. **Brush cells** – are columnar cells with a few microvilli whose function is disputed, but some of them apparently have a sensory function because they synapse on neurons that carry information to the CNS. They are located in the distal portion of bronchial tree.

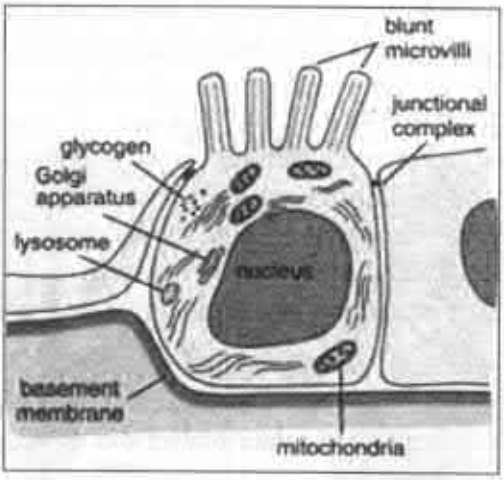
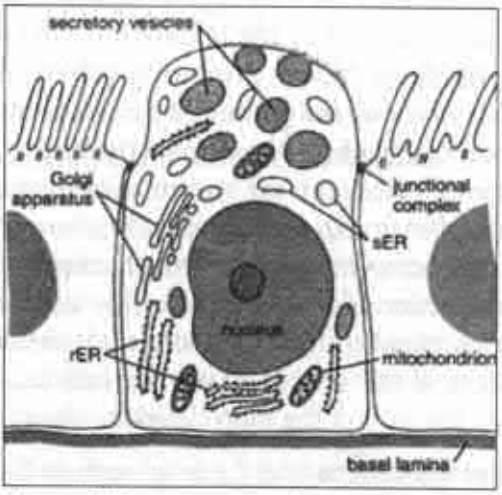


Diagram of a brush cell

7. **Non-ciliated Clara cells** are present in terminal and respiratory bronchioles – possibly produce surfactant.



A Clara cell between bronchiolar epithelial cells

Stratified squamous nonkeratinizing epithelium is found in regions such as the nasopharynx where high air flow causes drying, the epiglottis where there is a lot of abrasion from the passage of food, or portions of the larynx where the vibrations of voice production would disrupt the more delicate respiratory epithelium. The mouth and oropharynx both serve as airways, but are covered with stratified squamous nonkeratinizing epithelium because of food handling. Furthermore regions of respiratory epithelium that is subjected to unusual wear (e.g. heavy smoking) will change into stratified squamous epithelium. This reversible change is called metaplasia.

I. **Conducting portion:**

NASAL CAVITY

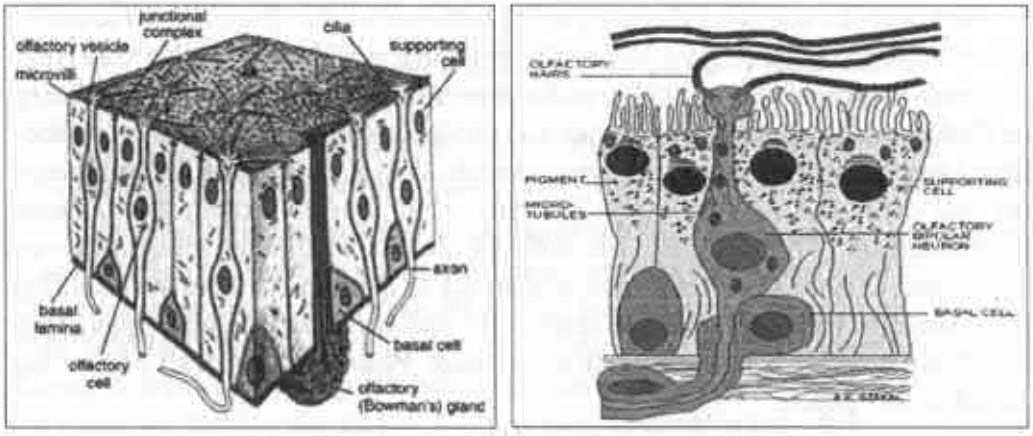
The nasal cavities are paired chambers separated by a bony and cartilaginous septum. Each cavity communicates anteriorly with the external environment through the nares and posteriorly with the nasopharynx through the choanae. The Nasal cavity is divided into three structurally and functionally different parts: the **vestibule**, the **respiratory portion**, and the **olfactory area**.

The vestibules of the nasal cavity (the first ~1.5 cm of the conductive portion following the nostrils) are lined with a stratified squamous keratinized epithelium. Hairs, which filter large particulate matter out of the airstream, and sebaceous glands, are also present.

At the transition from the vestibule to the respiratory region of the nasal cavity the epithelium becomes first stratified squamous nonkeratinized and then pseudostratified columnar ciliated. This type of epithelium is characteristic for all conductive passages dedicated to the respiratory system and therefore also called **respiratory epithelium**. Mucus producing goblet cells are present in the epithelium. The surface of the lateral parts of the nasal cavity is thrown into folds by bony projections called **conchae**. These folds increase the surface area of the nasal cavity and create turbulence in the stream of passing air, both of which facilitate the conditioning (warming, cooling and filtration) of the air. Mucous and serous glands in the connective tissue underlying the epithelium, the lamina propria, supplement the secretion of the goblet cells. The lamina propria of the respiratory portion has a rich vascular network that includes a complex set of capillary loops. The arrangement of the vessels allows the inhaled ait to be warmed by blood flowing through the part of the loop closest to the surface.

Tissues on the superior concha and the nasal septum form the **olfactory area** of the nasal cavity. This region is lined by a tall columnar pseudostratified olfactory

epithelium with cilia. The olfactory epithelium is composed by the following cell types: olfactory cells, supporting cells, and basal cells. ***Olfactory cells (modified neurons)*** span the entire thickness of the epithelium. The apical pole of each olfactory cell is a dendritic process that projects above the epithelial surface as a knoblike structure called the olfactory vesicle. A number of cilia (10 to 20) with typical basal bodies arise from the olfactory vesicle. The internal structure of cilia resembles largely that of normal cilia. But the cilia of olfactory cells do not move, because they lack dynein arms which are necessary for cilial motility. The cell membrane, covering the surface of the cilia contains olfactory receptors, which respond to odour-producing substances, odorants, dissolved in the serous covering the epithelium.



Schematic diagrams of the olfactory epithelium

The axons of the olfactory cells collect into bundles in the lamina propria. The axon' s bundles penetrate the cribriform plate of the ethmoid bone to synapse in the olfactory bulb.

The olfactory system is unusual among sensory systems in that the receptor cell axon enters the CNS directly without synapse in a peripheral ganglia. The olfactory cells and their processes receive mechanical and metabolic support from ***supporting cells*** (or sustentacular cells). Supporting cells are the most numerous cells in the olfactory epithelium. The nuclei of these tall columnar cells occupy a more apical position in the epithelium. They have numerous microvilli on their apical surface and abundant mitochondria. ***Basal cells*** are small, rounded cells located close to the basal lamina. Basal cells can divide and differentiate into either olfactory or supporting cells.

Bowman's glands (olfactory glands) in the lamina propria produce a special serous secretion which dissolves odorous substances, maintains the proper media

to support and protect the epithelia, and cleans the olfactory surface. Olfactory glands are branched tubuloalveolar serous glands. The supporting cells and the secretion of the serous glands contain lipofuscin granules, which give a yellow-brown colour to the surface of the olfactory region.

The **PARANASAL SINUSES** are extensions of the respiratory portion of the nasal cavity and are lined by respiratory epithelium which contains numerous goblet cells. The sinuses are often subject to acute infection after viral infection of the upper respiratory tract.

PHARYNX

The pharynx connects the nasal cavity with the larynx. The pharynx is divided into nasopharynx, oropharynx and laryngopharynx. Depending on the extent of abrasive forces on the epithelium, the pharynx is either lined with **respiratory epithelium** (nasopharynx or epipharynx) or with a **stratified squamous epithelium** (oropharynx or meso- and hypopharynx), which also covers the surfaces of the oral cavity and the esophagus. Lymphocytes frequently accumulate beneath the epithelium of the pharynx. The auditory (Eustachian) tubes connect the nasopharynx to each middle ear. Accumulations of lymphoid tissues surrounding the openings of the digestive and respiratory passages form the pharyngeal tonsils. If the tonsil swells it can block air passage. **Pharynx** provides a resonating chamber for speech.

LARYNX

The two main functions of the larynx are to produce sound, and to close the trachea during swallowing to prevent food and saliva from entering the airway.

The larynx connects the pharynx and trachea. The larynx can be subdivided into three regions:

1. **The supraglottis**, which includes the epiglottis, false vocal cords, and laryngeal ventricles.
2. **The glottis**, consisting of true vocal cords and the anterior and posterior commissures.
3. **The subglottis**, the region below the true vocal cords, extending down to the lower border of cricoid cartilage.

The epiglottis is a flap valve that prevents entry of food into the trachea. It is passively bent to cover the larynx during swallowing and, since it is subjected to considerable wear it is mostly covered with stratified squamous nonkeratinizing

epithelium. It has seromucous glands in the lamina propria and a core of elastic cartilage to give it the flexibility to withstand repeated bending.

The true vocal folds are 2 folds of mucosa that project into the lumen of the larynx in an anteroposterior direction. The vocal folds of the larynx control airflow and allow the production of sound. These folds are lined by stratified squamous epithelium and contain the muscle (striated, skeletal) and ligaments needed to control the tension of the vocal folds. Intrinsic skeletal muscles join cartilage plates and generate tension in the vocal folds and open and close the glottis. This is important for pitch. Above the vocal folds is the laryngeal ventricle, an elongated recess. Above this are the false vocal cords, or the vestibular folds. The false vocal cords are covered with pseudostratified, ciliated columnar epithelium with goblet cells. These cords are important for resonance.

Extrinsic laryngeal muscles move the larynx during swallowing.

The larynx is supported by a set of complexly shaped cartilages. It has hyaline cartilage to keep its passage open and is mostly covered with respiratory epithelium.

Laryngeal seromucous glands are found throughout the lamina propria, except at the level of the true cords. The lamina propria consists of loose connective tissue, usually rich in mast cells. Mast cells participate in hypersensitivity reactions leading to edema and laryngeal obstruction, a potential medical emergency. **Croup** designates a laryngotracheobronchitis in children, in which an inflammatory process narrows the airway and produces **inspiratory stridor.**

TRACHEA is the major segment of the conducting region of the respiratory system; is the continuation of the larynx. **The trachea** is a thin walled tube extending from the larynx to where it bifurcates as the two primary bronchi.

The wall of the trachea has 4 layers:

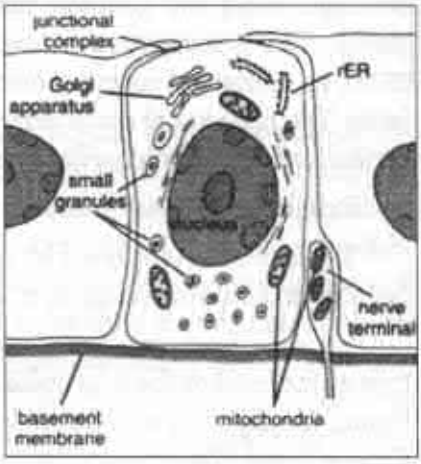
I. **Tunica mucosa** contains:
   1. **simple pseudostratified columnar ciliated epithelium**
   2. **lamina propria of mucosa** has typical loose connective tissue. It is very cellular and contains many lymphocytes and elastic fibers at its base. Furthermore, there are also plasma cells, mast cells, eosinophils and fibroblasts.

Simple pseudostratified columnar ciliated epithelium contains 4 types of cells:

a) **Columnar ciliated cells** – are the most numerous. On the apical surface have cilia that move mucus back toward oral area. **Function**: removes small inhaled particles from the lungs (cleaning the air).

b) **Goblet cells (mucus secreting)** are spread among the more numerous columnar ciliated cells. **Goblet cells** produce mucus that partially floats on top of watery secretions generated by submucosal serous glands. Turbulence of entering air results in small particles of dust sticking in the mucus. Secretions are greatly increased in response to an increase in particulate matter inhaled, during the allergic response, and when there is an infection of mucous membrane (e.g. flu).

c) **Endocrine cells (granule cells)** – are of several types. Some look like basal cells while others are taller and reach the surface. They contain secretory granules and are a part of the diffuse neuroendocrine system, in which endocrine secretory cells are distributed within tissue (most often epithelia) rather than being grouped together into endocrine glands. They produce neurotransmitters and hormones, and in the respiratory system granule cells are thought to regulate the activity of smooth muscle, cilia, goblet cells, and submucosal glands with their secretions. Release of their products is triggered by unknown stimulation from the airway or from efferent nerve endings that synapse on some of them.



A endocrine cell between two Clara cells

d) **Basal cells** – rest on the basal lamina but do not extend to the lumen, undergo mitosis – they are stem cells that will divide to provide replacements for dead or lost cells of the epithelium.

A thick basement membrane is characteristic of tracheal epithelium.

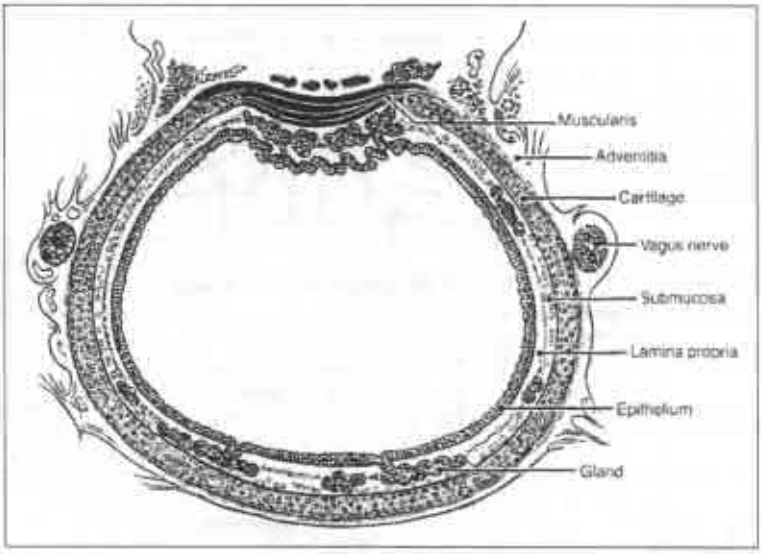
II. Tunica submucosa

- is composed of loose connective tissue. The submucosa also has mucous-secreting acini with serous demilunes. These have ducts (lined by a simple cuboidal epithelium) that empty into the tracheal lumen.
- The submucosa ends when the connective tissue blends with the perichondrium of the cartilage.

III. Tunica fibro – cartilaginous

- Consists of **C-shaped rings of hyaline cartilage** (17-20 hyaline cartilage) – each surrounded by a perichondrium with 2 layers: outer – fibrous, inner – cellular.
  - **Trachealis muscle** (smooth muscle) connects the ends of the "C" formed by the cartilage rings. The trachealis muscle helps mediate the cough response when it contracts by narrowing the trachea and increasing the velocity of escaping air.
  - The cartilage and muscle make the trachea a very rigid structure.

IV. Tunica adventitia is formed by loose connective tissue; contains large blood vessels, nerves supply and also the lymphatics.

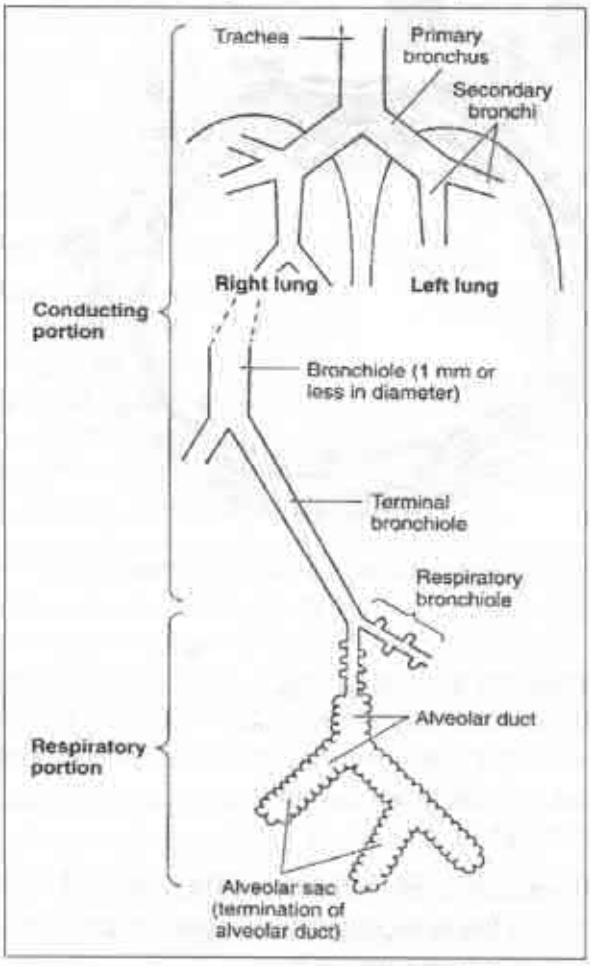


Scheme of structure of the trachea

The trachea branches to form the right and left primary bronchi entering the hilum of each lung. The hilum is the region where the primary bronchus, pulmonary artery, pulmonary vein, nerves, and lymphatics enter and leave the lung. Secondary divisions of the bronchi and accompanying connective tissue septa divide each lung into lobes.

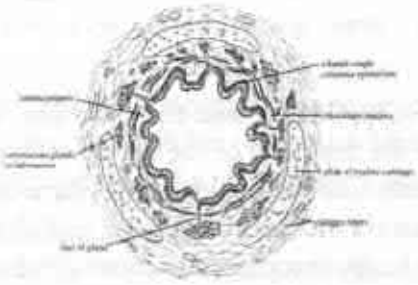
Subsequent bronchial divisions further subdivide each lobe into bronchopulmonary segments. The bronchopulmonary segment is the gross anatomic unit of the lung that can be removed surgically.

The bronchial tree has 2 primary (main) bronchi, one for each lung, which branch into lobar bronchi – secondary bronchi – (3 right lung lobes, 2 left lung lobes), which in turn branch repeatedly as they become preterminal bronchioles – segmental bronchi (tertiary) – to supply pulmonary lobules. Preterminal bronchioles branch until they become terminal bronchioles. The most distal portion of the terminal bronchioles gradually becomes lung tissue, and these transition regions are called respiratory bronchioles. The luminal diameter of bronchioles is regulated by smooth muscle at the base of the lamina propria, called muscularis mucosa. This smooth muscle is controlled by the autonomic nervous system, endocrine cells in the epithelium, and free mast cells in the lamina propria. Abnormal activity of mast cells causes constriction of bronchioles, resulting in asthma. Parasympathetic NS activity reduces the lumen and sympathetic NS activity increases the lumen.



Scheme of the extra and intrapulmonary bronchial tree

Distinguishing features between different regions of the bronchial tree is illustrated in the table.

Large (primary) bronchi Ø 10-15 mm	**Medium (secondary) bronchi Ø 5-2 mm**	**Small bronchi (bronchiole) Ø < 2 mm**
	I. Tunica mucosa: 1. Epithelium	
Simple pseudostratified columnar ciliated epithelium is tall. Goblet cells are numerous.	Simple pseudostratified columnar ciliated epithelium becomes lower than in the large bronchi. Number of Goblet cells decreases.	Simple is simple columnar to simple cuboidal Goblet cells are absent. nonciliated Clara cells apper.
	2. Muscularis interna	
is absent	appears	is well developed – circularly arranged smooth muscle cells.
	II. Tunica submucosa	
Mixed glands are numerous.	Number of glands decreases.	Glands **are absent.**
	III. Tunica fibro-cartilage	
Lamelae of hyaline cartilage.	**Islets** of cartilage. **Hyaline cartilage** is changed to **elastic.**	Is absent.
	 *Scheme of a secondary bronchi*	 *Scheme of a bronchiole*

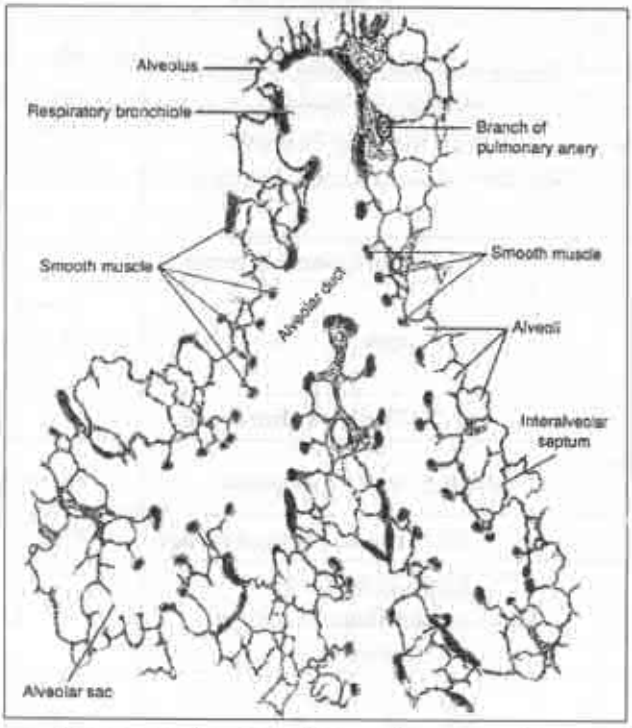
The intrapulmonary segmentation results in the organization of a pulmonary lobule and a pulmonary acinus.

II. Respiratory (gas exchange) portion:

The respiratory unit of the lung is represented by the pulmonary acinus.

PULMONARY ACINUS – is a morpho-functional unit of pulmonary tissue that includes a respiratory bronchiole (3-4 order – are covered by cuboidal epithelium), alveolar ducts, sacs, and alveoli, also called respiratory lobule.

- **12-18 acini make a lobule.**



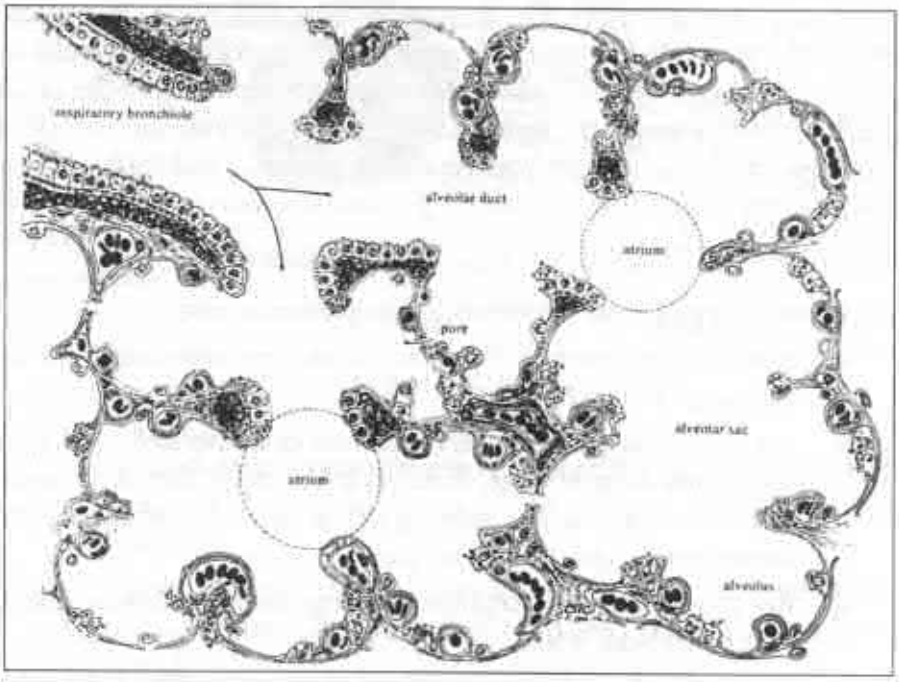
Schematic drawing of the pulmonary acinus

RESPIRATORY BRONCHIOLES make the transition into the lung tissue. Respiratory bronchioles are the first part of the bronchial tree that allow gas exchange. They have ciliated cuboidal epithelium **with Clara cells; no goblet cells, glands or cartilage;** has a lamina muscularis. Alveoli and alveolar ducts open directly onto them so they are easily recognized because there are mixed regions of cuboidal and squamous epithelia that overlie smooth muscle mixed with regions of alveoli. A few alveoli bud from their walls.

ALVEOLAR DUCTS. They are branching channels within the lung tissue that open up into an alveolar sac. Epithelia is simple squamous but some slips of smooth muscle may remain (smooth muscle sphincter).

Near the termination of the alveolar duct, clusters of alveoli share a common opening to the alveolar duct. Such a cluster of alveoli is referred to as an **alveolar sac** (sometimes a small alveolar sac is found along the length of the alveolar duct).

ALVEOLAR SACKS – are composed of 2 to 4 or more alveoli. Alveolar sacks open only into the alveolar ducts. The space into which alveolar sacks open in the alveolar duct is called the atrium.



Scheme showing alveolar duct, alveolar sac, and alveoli

ALVEOLI

They are 200μ air spaces lined by a squamous simple epithelium consisting of pneumocytes. The epithelial lining of the alveolus is of endodermal origin. Epithelium constitutes a thin cellular covering (clearly visible only with the electron microscope); separated from the endothelium of capillaries by a continuous basal lamina.

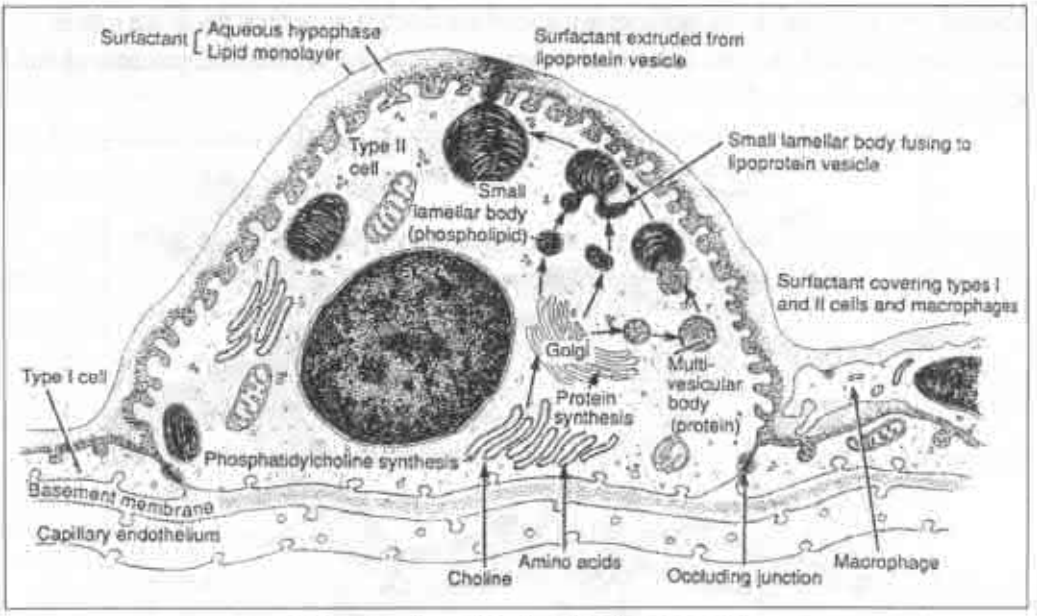
It is estimated that there are 200-600 million alveoli which present a surface area between 70 and 140 m^2 (the surface of a medium to large swimming pool). In the alveolar wall are pores (*pores of Kohn*) that provide communication between alveoli. In case of blockage of a small bronchiole, adjacent unobstructed bronchioles and associated alveoli continue to provide alveolar ventilation through the pores of Kohn.

Alveoli are separated from one another by a thin connective tissue layer (alveolar septum) with many capillaries. **Interalveolar septa** are narrow regions of loose connective tissue confined between the simple squamous epithelial lining of adjacent alveoli.

This space contains: 1) a very elaborate **capillary network** for gas exchange, 2) **elastic fibers** for stretch and rebound during respiration, 3) **reticular fibers** for structural support, and, 4) with the exception of mast cells, the usual collection of **loose CT cells**. Mast cells are absent because the edema they cause would be very damaging to the lungs. Tissue fluid that does enter this space from the capillaries must diffuse slowly through the septa until it reaches the lymphatic vessels in the interlobular septa. Lung collagen proliferation is common and there are over 100 diseases associated with lung fibrosis.

CELLS OF THE ALVEOLAR WALL *(see fig. 135, plate II)*

1. **Alveolar type I cells** (or membranous pneumocytes):
   - form the structure of the alveolus and are responsible for the gas exchange in the alveolus.
   - are squamous epithelial cells which are characterized by a superficial layer consisting of large, thin, scale-like cells; they also cover 95% of the alveolar surface, although they are only half (about 40%) as numerous as type 2 pneumocytes.
   - this type of cell is susceptible to a large number of toxic insults and cannot replicate itself.
   - Type I alveolar cells are joined together, and to type II alveolar cells by junctional complexes.
2. **Alveolar type II cells** (also called "big" cells):
   - are cuboidal cells that represent about two thirds of the cells of alveolar epithelia but cover only 3% of the area.
   - The alveolar type 2 cell is a smaller cell that can replicate in the alveoli and replace damaged alveolar type 1 cells.
   - These cells are secretory and exocytose the contents of lamellar bodies (named for their layered appearance in EM) onto the alveolar surface. Lamellar bodies contain a mixture of phospholipids, primarily phosphatidylcholine, and glycosaminoglycans (GAGS), which are constituents similar to that of mucus.
   - The cell responsible for the production and secretion of surfactant.

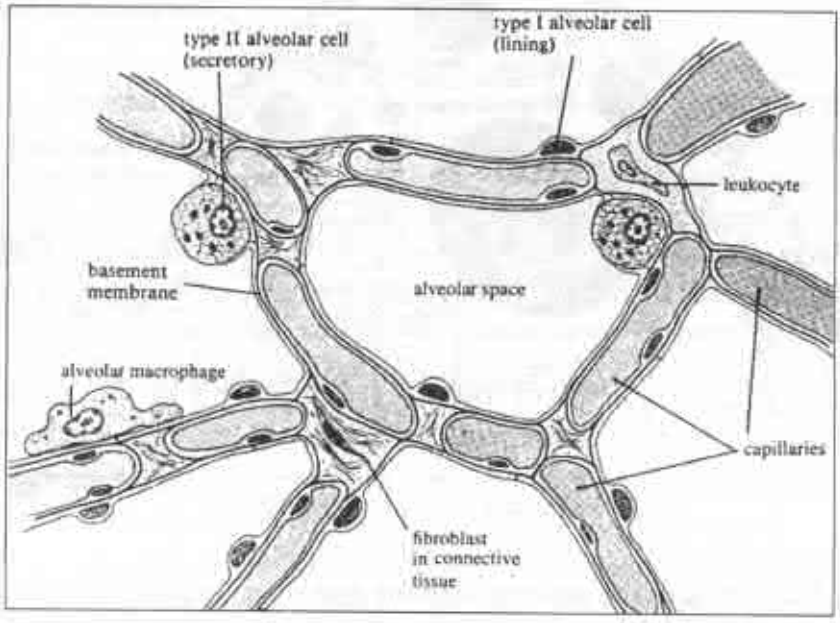


The structure of a type II alveolar cell

3. Also find **alveolar macrophages or dust cells** – are derived from monocytes, usually located free in the alveolar lumen, but also in tissues between alveoli.
   - Activity of the dust cells is relatively high, because they are located at one of the major boundaries between the body and the outside world.
   - Dust cells are frequently seen to contain granules of exogenous material such as particulate carbon that they have picked up from respiratory surfaces. Such black granules may be especially common in smoker's lungs or long-term city dwellers.
   - These cells act **to clean fine inhaled debris from the alveolar** free surface of cells by endocytoses particles.
   - Alveolar macrophages make and release a variety of substances, such as lysozyme, that fight bacterial infection.
   - **Remove degraded surfactant.**

Once they pass into an alveolus they do not return to the septa, but eventually migrate to bronchioles where they are carried out from the lung with mucus. In this manner, particulates are removed from the lungs and it is estimated that as many as 2 million macrophages may be cleared from the lungs in an hour. We

should be proud of these selfless janitorial staffs that sacrifice their tiny lives by throwing themselves into the great mucus river of the airways (a profound microanatomical drama).



The structure of the alveolus

SURFACTANT

- is a surface-active lipoprotein complex formed by type II alveolar cells. The proteins (10% – half of this is plasma proteins) and lipids (over 90%) that comprise surfactant have both a hydrophilic region and a hydrophobic region.

Functions:

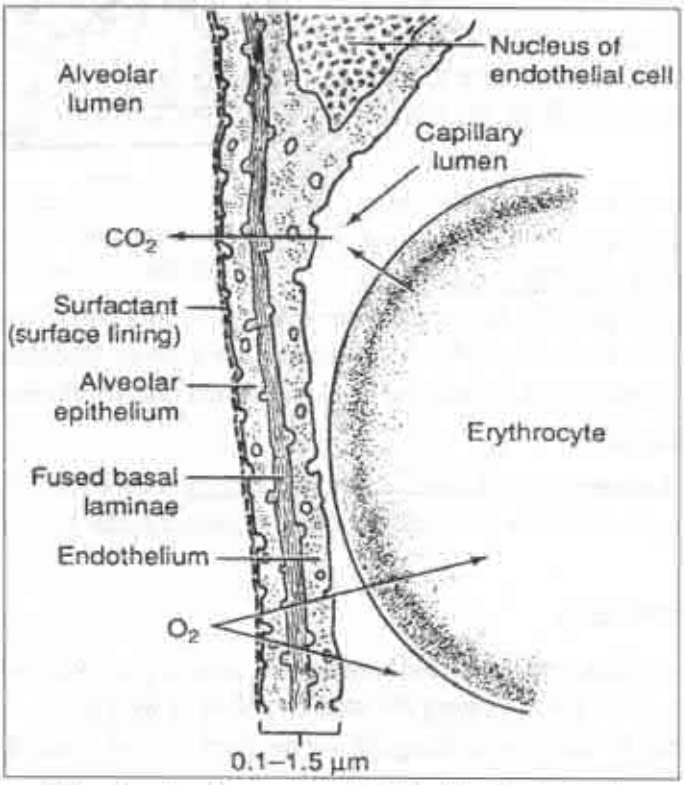
- is a component of the **blood-air barrier.**
- **prevent the lung from collapsing** at the end of expiration.
- **increase pulmonary compliance** by decreasing the surface tension of water which tends to provide a force to collapse alveolar volume (compliance is the ability of the lungs to stretch in a change in volume relative to an applied change in pressure).
- stands in the way of **excretion of the blood plasma and interstitial fluid** into the lumen of alveoli.
- **bactericidal action.**

Premature babies sometimes get respiratory distress syndrome or hyaline membrane disease when surfactant production is not activated at birth. Surfactant release can be induced in the newborn. Surfactant is constantly recycled back into interalveolar space by the pinocytic activity of type I cells.

THE BLOOD-AIR BARRIER

This refers to the substances/structures that lie between the air in the alveolar sacs and the blood in the capillaries. Starting in the alveolar lumen this barrier consists of:

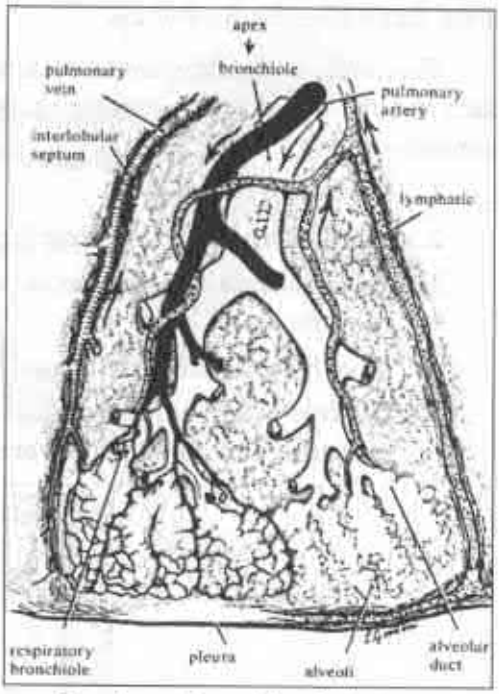
1. **Surfactant.**
2. **Type I alveolar cells** lining the alveolus.
3. **Basal lamina** of both alveolar cells and capillary endothelial cells (fused in some areas).
4. Non-fenestrated (continuous) **endothelium of capillary.**
5. In areas where basal laminas of the two endothelial layers are not fused, **reticular and elastic fibers are present.**



The structural components of the blood-air barrier

Vasculature of the lung. The lungs have a dual blood supply. The functional supply carries blood to be oxygenated in alveoli. The nutrient supply provides oxygenated blood to supporting structures in the lung. Lymphatics remove excess fluids.

A. **The Functional supply.** Blood to be oxygenated comes from the right ventricle in the pulmonary artery. The artery follows and branches with the bronchi and bronchioles to supply the capillaries of the alveoli. After being oxygenated the blood returns to the left heart in the pulmonary vein by way of interlobular and interlobar septa. Thus, in relation to the state of oxygenation of blood in the rest of the body, the pulmonary artery carries the equivalent of venous blood, and the pulmonary vein carries the equivalent of arterial blood.



Blood supply and lymph drainage of the pulmonary lobule

B. **The Nutrient blood supply,** from the bronchial arteries, also follows and branches with the bronchi and bronchioles to provide them, the pleura, and CT septa with oxygenated blood and nutrients. Bronchial arterioles anastomose with small caliber pulmonary arteries and veins just before the level of the alveolar capillaries, so there are no nutrient veins.

Lymphatic vessels collect around, but not within, lobules in CT septa. Lymph that collects in the alveolar walls diffuses out to interlobular CT before entering lymphatics.

Nerves of the lung

The lungs are innervated by both sympathetic and parasympathetic systems. Receptors of various sorts serving the stretch reflex as well as those that monitor the quality of the air exist in the lung. However, they are not evident with the light microscope.

PLEURA

The pleura consists of two layers: **a visceral layer**, and **a parietal layer**.

The surface of the lung is covered by a serous membrane that is referred to as visceral pleura. This consists of connective tissue rich in elastic fibers (continuous with the interlobular and interlobar septa of the lung) and a surface of mesothelium. Cells of mesothelium have apical microvilli. The visceral layer prevents leakage of air into thoracic cavity. The parietal layer has the same structure but is thicker.

Blood vessels to the visceral pleura derive from pulmonary and bronchial blood vessels. The vascular supply to the parietal pleura derives from the systemic blood vessels. Branches of the phrenic and intercostals nerves are found in the parietal pleura; the visceral pleura receives branches of the vagus and sympathetic nerves supplying the bronchi.

CHAPTER IX

THE INTEGUMENT

Components of the integumentary system are:

- **SKIN** (epidermis, dermis, hypodermis).
- skin's **DERIVATIVES** (sweat glands, sebaceous glands, mammary glands, hair, nails).

Skin's characteristics

- The free surface of the skin is not smooth, but has a series of fine grooves. In particular the grooves are most pronounced on the thick skin of the hands (**fingerprints**) or the feet.
- The skin in adults covers a total surface area of about **2.2m²**. The surface area increases approximately sevenfold during the lifetime from an infant to adult and it is important to estimate the area of skin surface in order **to calculate the dosage of drugs to be administered**.
- The surface: volume ratio in young children is much greater than that of adults and consequently their control of body temperature is more problematic. A child loses heat more rapidly than an adult.
- The thickness of the epidermis varies from 0.12-0.17 mm over most surfaces of the body ("thin skin"), but may reach a thickness of 0.8 mm on the palms of the hands and up to 1.4mm on the soles of the feet ("thick skin").
- Skin is the largest organ in the body. It weighs about 4 kg.

Skin and its derivatives constitute a complex organ composed of many different cell types. The diversity of these cells and their ability to work together provide a number of functions that allow the individual to cope with the external environment. Major functions of the skin are:

- regulation of body temperature.
- regulation of body fluid volume (participates in homeostasis).

- protection from U.V. radiation(acts as a ultraviolet barrier).
- protection from minor traumas (acts as a mechanical barrier).
- protection against disease organisms (provides immunologic information).
- excretion of water, fats.

Additional functions are:

- sensory reception from specialized touch receptors.
- vitamin D synthesis with the aid of sunlight (performs endocrine function).

STRUCTURE OF THE SKIN

Three distinct layers can be seen in the skin:

- **Epidermis** (ectodermal origin) – consists of keratinizing stratified squamous epithelium.
- **Dermis** (mesodermal origin) – consists of loose connective tissue (areolar) and dense irregular connective tissue.
- **Hypodermis** (mesenchyme origin) – consists mostly of white adipose tissue (sometimes referred to as the subcutis) and loose connective tissue.

EPIDERMIS

The epidermis represents the outermost layer of skin. That is:

- avascular.
- made of 4 (*thin skin*) or 5 (*thick skin*) layers of cells.
- variable thickness depending on location.
- responsible for barrier properties of skin.
- in order from outermost (surface) to innermost (deepest) are:
  - **Stratum corneum**
  - **Stratum lucidum**
  - **Stratum granulosum**
  - **Stratum spinosum**
  - **Stratum basale**

The epidermis is constantly growing from the deepest layer and sloughing off on the surface, and the layers represent stages of development of cells as they migrate upward, rather than permanent structures.

STRATUM BASALE (the basal layer) is the deepest zone and consists of cuboidal cells on a thick basement membrane. The primary cell types are epidermal stem cells, keratinocytes (primary epidermal cell type), and melanocytes (produce melanin). Some other less common cell types will be discussed below. Keratinocytes are the most numerous, and they link to basal lamina through hemidesmosomes.

STRATUM SPINOSUM (the spiny layer)

- rests on top of the basal layer.
- contains cuboidal to squamous cells with central nuclei.
- cells contain aggregates of keratin called tonofilaments.
- tonofilaments linked cell to cell by desmosomes which give a spiny appearance to the cells.
- tonofilaments are responsible for mechanical strength of epidermis.

In this layer keratinocytes produce and accumulate keratin, and take in melanin granules from melanocytes. The stratum basale and the stratum spinosum together are known as the **GERMINATIVE LAYER** (as this is the area of mitosis and generation of new keratinocytes).

STRATUM GRANULOSUM (the granular layer)

- contains mostly squamous keratinocytes.
- contains usually 3 – 5 cell layers.
- keratinocytes contain keratohyalin granules in this layer which appear as basophilic granules giving granular appearance in light microscope.

STRATUM LUCIDUM (the clear layer) is a thin and sometimes difficult layer to see because this stage is very brief. It is not obvious in thin skin.

The cells are dead, devoid of organelles (thin layer of very flat, eosinophilic, anucleate cells). The cytoplasm of the cells contains a substance called **ELEIDIN**, apparently derived from keratohyalin, and lamellar bodies. Melanin is destroyed by autolysis but in dark skinned individuals there may be enough that some remains to reach the stratum corneum.

STRATUM CORNEUM (the keratinized or cornified layer) is the outermost layer and provides a wearing surface for skin.

The keratinocytes have degenerated into flakes or squames of keratohyalin still attached to one another with desmosomes and cytoskeletal tonofilaments. This type of keratin is called soft keratin (keratin in hair and nails is hard keratin).

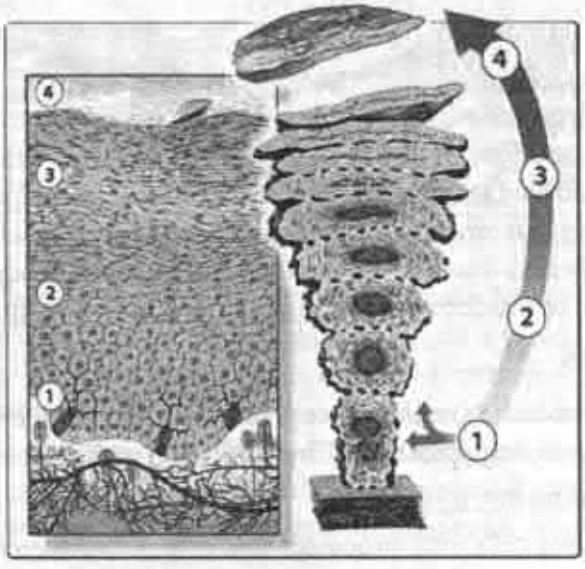
Lipid, derived from lamellar bodies, is released when the cell membrane degenerates, together with the sebum from sebaceous glands, seal the spaces between the dead cells.

The stratified squamous keratinized epithelium (epidermis) contains four distinct cell types:

- Keratinocytes (keratin production)
- Melanocytes (pigment production)
- Langerhans cells (immune system)
- Merkel cells (diffuse neuroendocrine system)

KERATINOCYTES

- are the **dominant cell type** of the epidermis, and are part of the keratinizing system.
- produce keratin, lamellar bodies (membrane-coating granules), and receive melanin from melanocytes while in the stratum spinosum. Lamellar bodies contribute to the formation of the intercellular epidermal water barrier.
- involved **in the cornification or keratinization** of the skin.
- the most **superficial cells are essentially dead cells**, or **scales**, primarily composed of keratin. These superficial keratinized cells are continuously being lost (desquamation or exfoliation) and need to be replaced.



The epidermal proliferative unit

The epidermal proliferative unit (EPU). Keratinocytes approximate a shape (tetrakaidecahedrons) that allow them to interlock together side to side, and they are stacked neatly one above another in columns. Stem cells are scattered between ordinary keratinocytes (and a few other cell types) in the basal layer, and about once a day they divide to produce a new cell that is shifted to the bottom of a nearby stack of keratinocytes, sort of like dealing cards to different players, thus moving the columns upward. A single stem cell supplies new cells to around 10-12 columns of keratinocytes, but each column can receive new cells from several different stem cells. One stem cell and the columns it supplies are called an EPU, even though the EPUs overlap. Cells spend an average of 2 weeks as keratinocytes in the spiny, granular, and clear layers, and 2 weeks as squames of keratin in the stratum corneum.

Clinical Correlation:

Psoriasis (the heartbreak of) is a manifestation of accelerated and disorganized keratinocyte production. The transit time of a keratinocyte to the surface is reduced to 1 week because stem cells proliferate rapidly in both the basal and spiny layers. Keratinocytes are not as tightly bound together as normal and there is not enough time for autolysis of melanin which results in patches of pigmented and flaky skin.

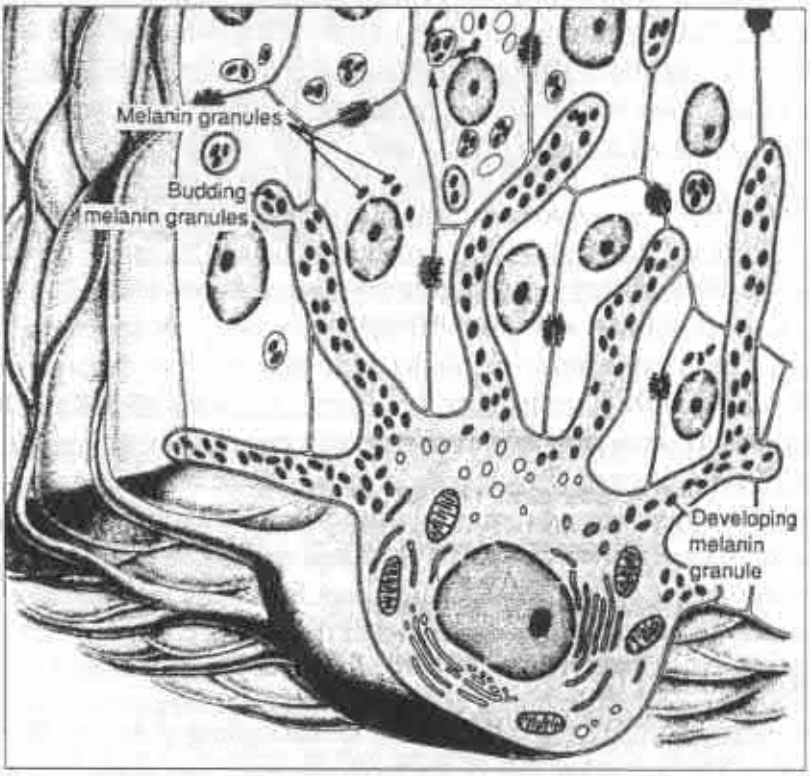
Keratin is a family of polypeptides which keratinocytes form into non-membrane bound keratohyalin granules in increasing amounts as cells progress upward. Desmosomal junctions are well developed between the cells, and this gives the skin great resistance to tearing. Connecting with the desmosomes are intracellular tonofilaments, a keratin type intermediate filament. The intermediate filament cytoskeleton of adjacent epithelial cells are bound together by desmosomes. The filaments form into bundles, called tonofibrils, which aggregate with keratohyalin granules. Lamellar bodies are membrane bound packages of GAGS and phospholipids that are stored in keratinocytes as they develop. When the cellular membrane breaks down in the stratum corneum the product of the lamellar bodies is released to produce a water resistant seal between squames.

MELANOCYTES

During embryonic life, melanocyte precursor cells migrate from neural creast and enter the developing epidermis. The epidermal melanoctes:

- are found mostly in thin skin.
- are rounded cells with processes that extend between the adjacent keratinocytes.

- are not connected to the keratinocytes.
- synthesize **melanin** (a brown pigment that protects against damage from UV light).
- melanocyte density varies with location in body.
- melanocyte number does not vary between people of different color, rather, the keratinocyte processing of melanosomes differs or the activity of the melanocytes differ.



The structure of the melanocyte

Melanin production starts with tyrosinase made by the rER. The Golgi sends tyrosinase vesicles to fuse with melanosomes in a process similar to creation of lysosomes. Receptor mediated endocytosis retrieves tyrosine through the basement membrane, and it is translocated into the melanosomes where it is transformed into melanin by tyrosinase. Completed vesicles, full of melanin, are called melanin granules. The granules are transported into the melanocyte cytoplasmic processes and are injected into keratinocytes when the tips of the processes are phagocytosed by keratinocytes. This process is known as cytocrine

secretion. Tanning, in response to ultraviolet radiation exposure, is due to both a rapid release of existing melanin granules and a subsequent increase in granule production. The pituitary gland stimulates melanin production via MSH (melanin stimulating hormone).

Skin color is due to a combination of carotene (yellow), blood (red), and two types of melanin: pheomelanin (red-beige) and eumelanin (almost black). The numbers of melanocytes vary in different regions of the body, but they exist in approximately the same numbers in the same regions in different individuals regardless of genetic background. Except for freckles, genetic variation in melanin is due to the number of melanin granules produced, not the number of melanocytes. Freckles are the result of a patchy distribution of melanocytes, not an uneven distribution of melanin production.

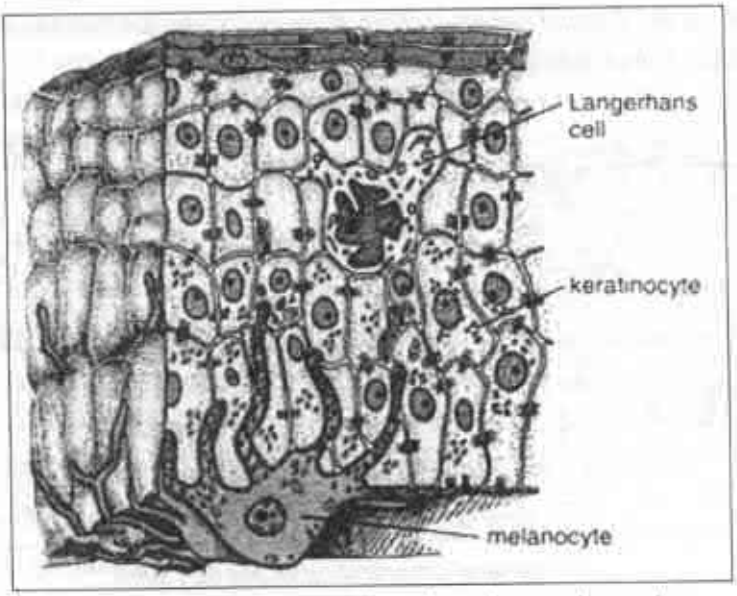
Clinical Correlation:

Albinism is an autosomal (non sex chromosome) recessive genetic defect that results in the inability to produce tyrosinase. Albinos lack all skin and eye melanin, so their skin is very pale and their eyes are pink (from capillaries in the retina). They have serious problems with skin and eye damage from sunlight. Individuals with Waardenburg syndrome (piebaldism) have a dominant heritable defect that variably affects the development of the neural crest where melanocytes are generated. This results in melanocytes being absent in confined regions of the body, or absent completely. Individuals with this syndrome usually have patches of unpigmented hair and skin, and may be deaf and have blue eyes on one or both sides. These individuals do not have pink eyes because pigment epithelial cells in the eye are not generated in the neural crest.

LANGERHANS' CELLS

- are antigen presenting cells (similar to macrophages) derived from monocytes. They constitue part of the mononuclear phagocytotic system.
- found within the stratum basale and spinosum of the epidermis of thick and thin skin.
- are star-shaped cells (dendritic) cells.
- like melanocytes they do not form desmosomes with neighbouring keratinocytes.

They phagocytose antigens and present them to T lymphocytes, which may cluster around them during an allergic or immune response. Langerhans' cells contribute to the cutaneous contact hypersensitivity reaction (contact dermatitis).



The relationship between keratinocytes, melanocytes and Langerhans cells

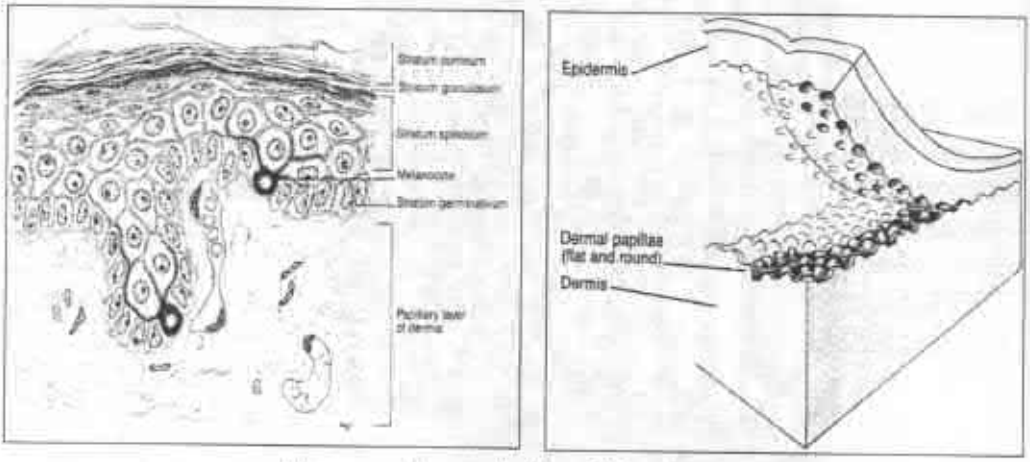
MERKEL' S CELLS

- found in the basal layer of the epidermis and form junctions with keratinocytes.
- located over the entire surface but more prominent on palms and soles of feet (thick skin).
- contain small dense granules similar to neuroendocrine cells.
- the function is still not fully established: they may function as sensitive mechanoreceptors and produce local neuroendocrine secretions.

Thick and thin skin refers to two general types. Thick skin is found on the **palmar surface of the hands and fingers, and the plantar surface of the feet and toes**. Thin skin is found on the rest of the body. It is only the epidermis that is thicker in thick skin, the dermis is often thinner than that of thin skin. The overall thickness (epidermis plus dermis) of thin skin varies considerably, and on the back is the thickest skin in the body, while on the eyelids it is the thinnest.

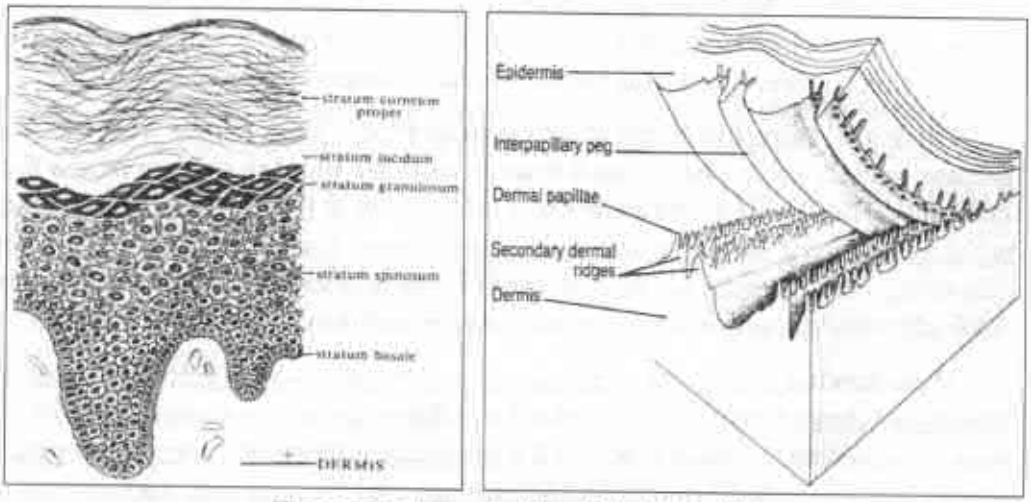
Thin skin has hair, fewer sweat glands, more melanocytes, greatly reduced or missing epidermal layers, and simpler dermal elevations. The stratum germinativum is the same as in thick skin, but the spinosum is thinner, the granulosum is a discontinuous single layer of cells, the lucidum can't be seen, and the corneum is

thin. There are no dermal ridges in thin skin, but there are broad, low, rounded, dermal papilla that are not carried to the epidermal surface.



The general organization of the thin skin

Thick skin differs from the thin in the following: it has more sweat glands, no hair follicles, fewer melanocytes, Merkel cells, Meissner's and Pacinian corpuscles in the dermis, more prominent epidermal layers, and more elaborate dermal ridges. Primary dermal ridges are large enough that the pattern is carried to the surface of the epidermis as finger or toe prints. The primary ridges are divided into secondary dermal ridges by an epidermal down growth called the interpapillary peg. This elaborate pattern provides increased area for a strong bond between the epidermal basement membrane and underlying dermis.



The general organization of the thick skin

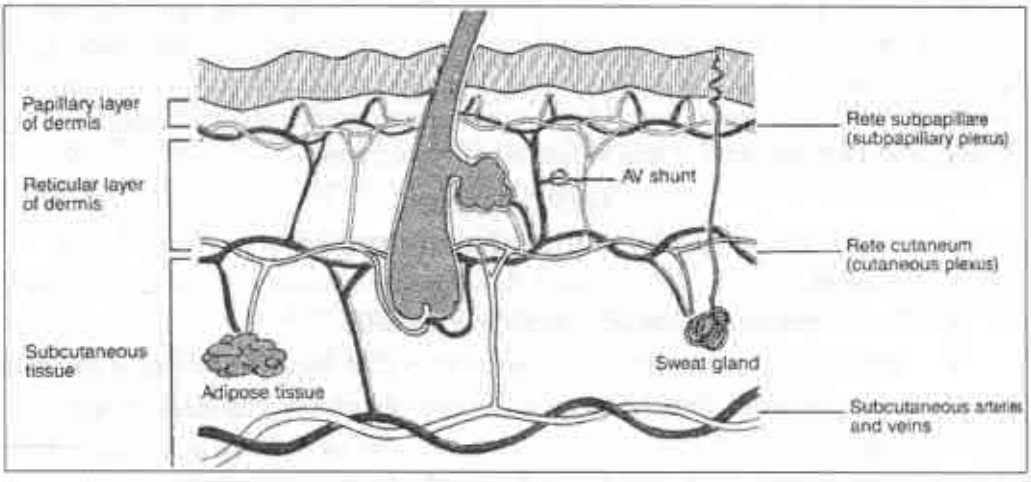
Free nerve endings are somatic afferents found in both the epidermis and dermis and are receptive to touch, pain, and temperature. The ending is an unencapsulated and non myelinated nerve process of a dorsal root ganglion cell (pseudounipolar neuron).

DERMIS

- is an inner layer of skin.
- links epidermis to hypodermis.
- sweat glands, sebaceous glands and hair follicles reside mostly in this layer.
- variable thickness over different regions of the body (the dermis of the dorsal side of the body is in general thicker than that of the ventral side).
- usually the dermis is thinner in females than in males.

Two distinct regions are present in the dermis:

- **papillary layer**
- **reticular layer**



Layers of the dermis and blood supply of the skin

The **papillary layer** consists of loose irregular CT that occupies all the dermal elevations of thick skin, the papillae of thin skin, and a narrow region just below. Like most loose irregular CT the papillary dermis has many capillaries and a watery amorphous ground that allows exchange, by diffusion, of nutrients and waste with the epidermis. This is the region where immune cells hang out. Meissner's corpuscles are found in the papillary layer within dermal papillae of thick skin.

The **reticular layer** is thicker than the papillary layer and consists of dense irregular CT with large collagen fibers that resists tearing, and large bundles of elastic fibers that make the skin stretchy and supple. The term reticular refers to the netlike arrangement of these bundles. Pacinian corpuscles are found in the deep reticular dermis and just below in the hypodermis.

Both the papillary and the reticular layers have abundant elastic fibers, responsible for the elasticity and flexibility of the skin.

The blood supply of the dermis is provided by vertical branches from larger arteries and veins of the subcutaneous tissue below. The reticular plexus (rete cutaneum) is a horizontal bed of arteries and veins in the general region of the base of the reticular layer. This plexus provides vertical branches to the subpapillary plexus (rete subpapillare) near the base of the papillary layer. Capillary loops extend from the subpapillary plexus into the papillary layer to nourish the epidermis. The vertical branches between the reticular and papillary plexuses have A-V shunts between arteries and veins. The shunts regulate blood flow in surface capillaries for controlling body temperature, especially in the hands and feet. When it is cold the shunts open and blood flows directly from artery to vein so that the papillary plexus and its capillary loops are bypassed. In this mode, body heat is conserved because warm blood does not reach the surface. When it is warm the shunts close to force blood into surface capillaries, and heat from the blood is lost by convection and radiation at the surface.

HYPODERMIS (links skin to body proper)

Consists of:

- **loose connective tissue containing fat cells**
- **arteries, veins, lymphatics, and nerves that supply and drain the skin**
- **cells:** fat cells, fibroblasts, lymphocytes, macrophages, mast cells
- **topology:**
  - variable thickness depending on location and nutritional status
  - site of deposition of the majority of body fat

SKIN GLANDS

The glands of the skin are:

- **Sebaceous glands**
- **Sweat glands**
  - **Merocrine** sweat glands
  - **Apocrine** sweat glands

- **Modified Sweat Glands**
  - **Ceruminous glands**
  - **Mammary glands**

They are all exocrine glands that form as a down growth of the epidermis early in development.

SEBACEOUS GLANDS

Sebaceous glands are dermal exocrine glands associated with hairs and which secrete an oily substance – **SEBUM** which keeps skin soft and waterproof. They are **simple alveolar branched** glands. Their mechanism of secretion is **holocrine**. The glands are composed of **alveoli (secretory portions)** and a short **secretory duct (short duct,** lined by a **stratified squamous epithelium**). In the region of infundibulum of a hair follicle, short duct of the sebaceous gland forms the pilosebaceous canal.

Secretory portion consists of two types of cells:

1. **BASAL CELLS** – divide by mitosis and regenerate sebum-producing cells lost during the holocrine secretion. The cells do not adhere to one another, like keratinocytes, and as they are forced out of the gland by generation of more cells.
2. **SEBUM-SECRETING CELLS** on top of the basal cells begin to store the oil secretion within cytoplasmic droplets, they die and the sebum is released.

The ducts open into the upper 1/3 of hair follicles all over the body, and directly onto the surface in areas that must remain soft, or are subjected to a lot of wetting. Examples of the latter are the eyelids, nipples, labia minora, and on the face, especially around the lips.

Increased androgens at puberty of both males and females stimulate sebaceous glands on the face to produce excess sebum, which can lead to acne. A sebaceous gland between a hair follicle and an arrector pili (smooth) muscle make up a unit in the skin, referred to as a pilosebaceous apparatus. Hairs lean to one side and when the arrector pili muscle contracts, the hair is straightened (erected), sebum is expressed into the follicle, and a small lump (goose bump) on the surface is formed. Activity of the arrector pili muscle is controlled by sympathetic nerve endings which release acetylcholine.

SWEAT GLANDS

- also called **sudoriferous glands.**
- are simple coiled tubular exocrine glands that secrete sweat.

- contain the **secretory units** that are simple convoluted tubular epithelial structures, which is located in deep dermis or hypodermis and a straight **secretory duct**. The secretory ducts are surrounded by myoepithelial cells, which on contraction cause the expulsion of the sweat.

Sweat glands are classified on the bases of their structure and the nature of their secretion. Two types of sweat glands are found:

- Merocrine (eccrine) sweat glands
- Apocrine sweat glands

MEROCRINE SWEAT GLANDS (ECCRINE SWEAT GLANDS)

- are simple coiled tubular glands found all over the body except the lips and portions of the genitalia. They are particularly numerous in thick skin, these are found all over the body including the thick skin. In thick skin sweat pores are conspicuous in the thick outer layer of keratin. Fingerprints are formed by secretions derived from the sweat glands. Over the whole body they can produce 10 liters/day of sweat for temperature regulation. Sweat is a filtrate of the blood that contains water, sodium, potassium, and nitrogenous waste. Secretion by the secretory cells is by the merocrine method which is just exocytosis.
- secretory portion includes **dark** and **clear** secretory cells and **myoepithelial** cells. Myoepithelial cells are contained within the basal lamina of the secretory cells, and they can contract to expel sweat. Sympathetic nerve endings, which release acetylcholine, regulate the activity of the gland.
- **duct** lined by **stratified cuboidal epithelium**

The coiled secretory unit is in the deep dermis and the straight duct travels upward through the dermis to penetrate the epidermis through the middle of an interpapillary peg, and opens on the surface as a sweat pore.

APOCRINE SWEAT GLANDS

- are found only in the **axilla** (armpit), **areolae of the breast,** and the **pubic, perineal regions,** and **anal regions**.

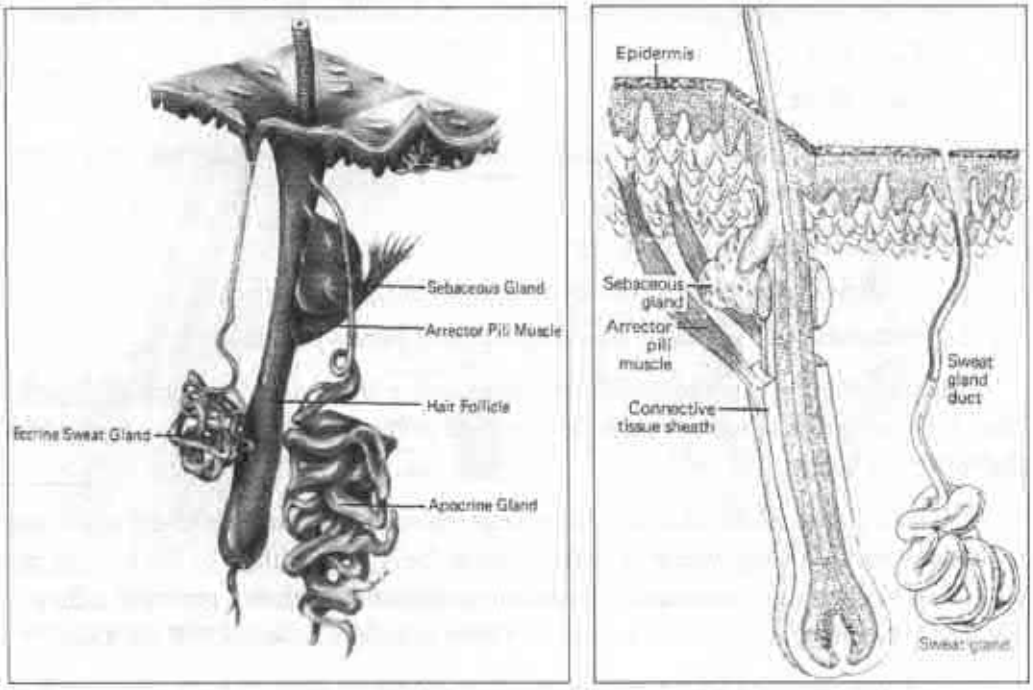
Apocrine gland structure is similar to eccrine glands, but they have a very wide lumen and they secrete into hair follicles. They have very large secretory units and myoepithelial cells, in which the apical part of the secretory cells including their contents, are secreted into the lumen (apocrine secretion). The breakdown of this secretion by bacteria is the cause of the **typical smell** of sweat from the armpits.

The apocrine sweat glands only become functional at puberty and are influenced by sex steroids (young children do not have a noticeable sweat smell). It is possible that the secretions of apocrine sweat glands contain **pheromones** (as sex chemoattractants).

Their activity is controlled by the sympathetic NS with the release of noradrenalin (note that this is a different neurotransmitter than for the arrector pili and eccrine sweat glands).

CERUMINOUS GLANDS are a special apocrine sweat gland, found in the outer auditory meatus that secrete directly onto the skin or into hair follicles.

Their secretory product combines with sebum of adjacent sebaceous glands to form cerumen, or ear wax. Ear wax protects the very thin skin in the external auditory meatus, but it is not clear why a much smaller amount of cerumen would not be adequate. Recent studies have found that cerumen repels insects, and it contains lysozyme which breaks down bacterial cell walls.



The diagram showing the position of the sweat and sebaceous glands in the dermis

HAIR

Hairs are composed of keratinized cells that develop from hair follicles.

Types of hair:

- **lanugo**: neonatal, form in utero, fine, soft, hair without a medulla (non pigmented).
- **vellus**: post-natal, short, fine shaft, no medulla (non-pigmented).
- **intermediate**: post-natal to 2 years of age, intermediate between vellus and terminal hair.
- **terminal**: post-natal and on, long, course shaft, medullated (pigmented), most common visible hair.
- **bristly hair** – eyebrows, eyelashes, external auditory tube, vestibule of the nasal cavity.

HAIR STRUCTURE

Hairs are elongated filamentous structures. Each hair consists of two parts:

1. **Hair follicle**
2. **Hair shaft**

The **HAIR FOLLICLE** is a tubular invagination of the epidermis and is responsible for the growth of hair.

The **hair follicle** consists of:

1. **external root sheath** – a down growth of the epidermis.
2. **internal root sheath** – made up of three layers of soft keratin.

The hair follicle is surrounded by a connective tissue layer – **dermal sheath**. The ***Arrector pili*** smooth muscle (also called *piloerector* muscle) is attached to the follicular bulge.

The hair follicle is divided into three segments: **infundibulum** (used as a route for discharge of sebum); **isthmus** (extend from the infundibulum to the level of insertion of the arrector pili muscle); **inferior segment** (which is a growing follicle). The end portion of the inferior segment of hair follicle is called **HAIR BULB.**

- A vascularized loose connective tissue core projects into the hair bulb – **DERMAL PAPILLA** for nourishing stem cells of the basal layer.

Like the epidermis, stem cells in the basal layer of the hair bulb continually generate keratinocytes. The keratinocytes are different from skin cells; they generate hard keratin, and when they die the squames are bound together to form

the hair shaft and internal root sheath. Melanocytes in the basal layer produce melanin and hair color is determined by a combination of three colors of melanin. Graying of hair with age is due to the decreasing ability of head hair melanocytes to produce tyrosinase.

The **HAIR SHAFT** of thick hair reveals three concentric zones containing keratinized cells:

1. **cuticle** consists of a single layer of hard, keratinized squamous cells.
2. **cortex** (located peripheral to the medulla) – contains hard keratin; keratinized, pigmented cells.
3. **medulla** (forms the central part of the shaft) – contains soft keratin; large vacuolated cells. It is **absent** in **thin** hair.

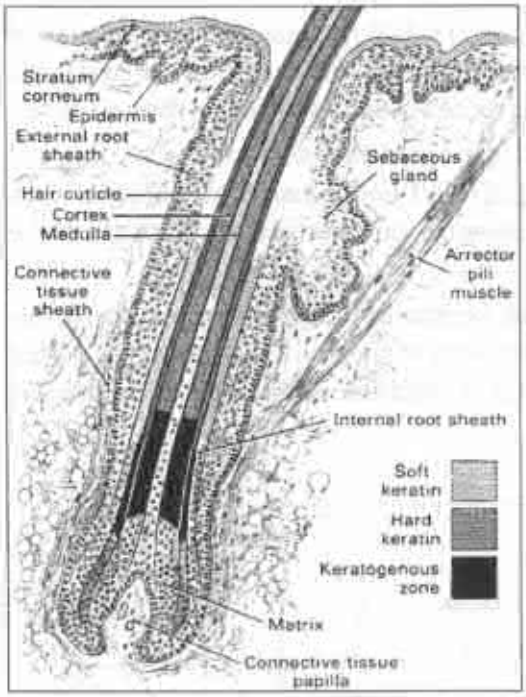
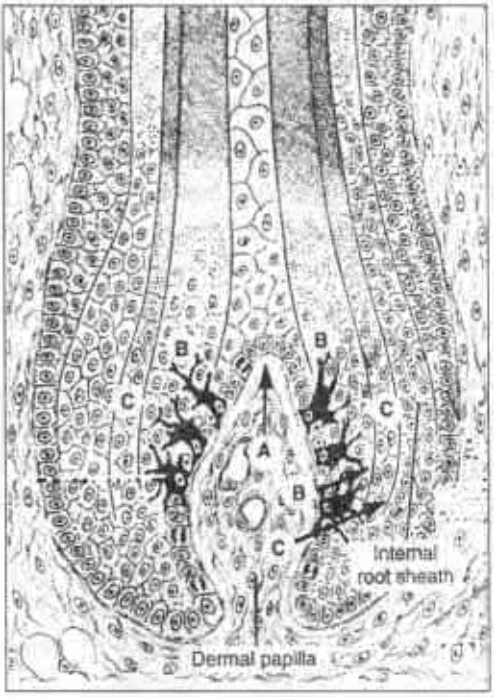


Diagram of a hair in a hair follicle, showing the distribution of soft and keratin and the keratonogenous zone



Structure of the hair bulb

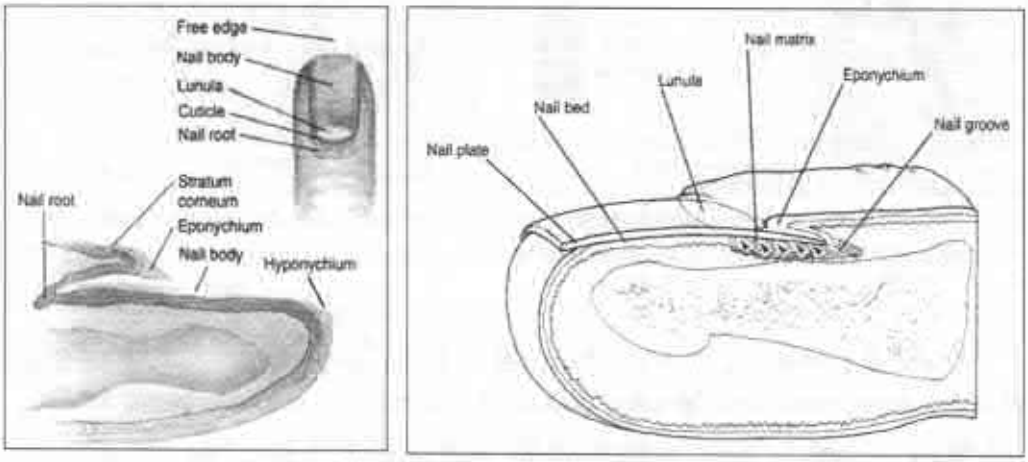
The scales interlock with similar ones on the inner root sheath (soft keratin), pointed downward, to help hold the hair in place.

Hair growth is cyclic with growing and resting phases. For example, eyebrows grow for 1-2 months and rest for 3-4 months and head hair grows for 2-3

years and rests for 3-4 months. When a hair begins growing again, a new one is started and the old one falls out. The duration of growth periods is the limiting factor for how long hair grows in different regions of the body and on different individuals. The hair cycles of a region may be synchronized to some extent, and larger patches completing the rest period explains why the amount of hair you find in your comb varies from time to time. Hair grows at 0.4 to 0.5 mm/day, but this, and whether terminal or vellus hairs are produced, is influenced by hormones, especially androgen. An autosomal dominant genetic trait that interacts with androgen concentrations triggers the growth of vellus instead of terminal hair on the scalp to produce baldness.

NAILS – are horny plates of keratin on the dorsal surface of the terminal phalanges of the hand and foot. The parts of the nail are:

- **Nail root** (proximal part) is buried in a fold of epidermis and covers the cells of the germinative zone. Stratum basale and spinosum of epidermis are present here forming nail matrix cells which synthesize the nail plate.
- The nail lies in a depression in the epidermis surrounded by the nail groove, and rests on the nail bed. **Nail bed** consists of epithelial cells that are continuous with the stratum basale and stratum spinosum of the epidermis.
- **Nail plate** is a closely compacted, keratin enriched with hard interfibrillar material; it is the stratum corneum of the nail. Nail keratin is a hard keratin, like that of the hair cortex. Unlike the soft keratin of the epidermis, it does not desquamate.



The structure of the fingernail

- **Eponychium (cuticle)** is a junction between skin stratum corneum and base of nail plate. It is also composed of hard keratin and for this reason it does not desquamate.
- **Hyponychium** represents a junction between the skin stratum corneum and the tip of the nail plate. It secures the free edge of the nail plate at the fingertip.
- **Lunula** represents the light or white region at the base (eponychium) of nail plate. The crescent shaped white lunula is the nail matrix seen through the nail.

Nails grow at about 0.5 mm/wk and they grow faster in the summer than in the winter.

CHAPTER X

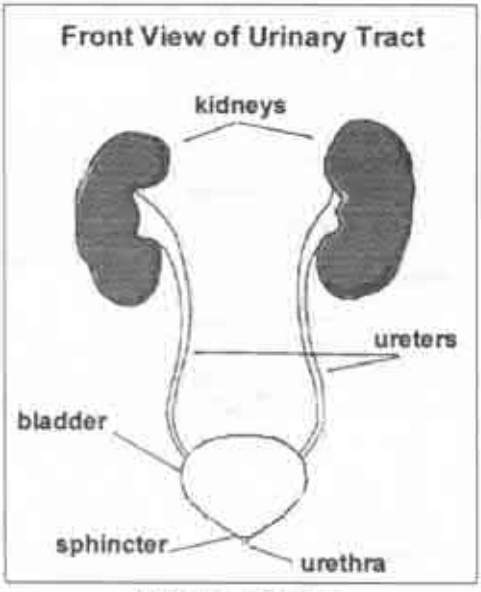
URINARY SYSTEM

Functions of the urinary system:

- Clears the blood of nitrogenous and other waste metabolic products (urea, uric acid, toxic stuff, drugs) by filtration and excretion.
- Regulation of:
  - blood volume
  - concentration of blood solutes
  - pH of extracellular fluid
- Endocrine function: synthesis of **erythropoietin, renin, prostaglandins.**
- Makes calcitrol (from Vit D3: stim Ca2+ absorption by intestinal epithelium).
- Recovers by reabsorbtion small molecules (amino acids, glucose, and peptides), ions (Na, Cl, Ca, PO), and water, in order to maintain blood homeostasis.
- Assists liver in detoxification of poisons.

The urinary system contains the following organs:

- **Kidneys**
- **Ureters**
- **Urinary bladder (storage)**
- **Urethra**



Scheme of organs of the urinary system

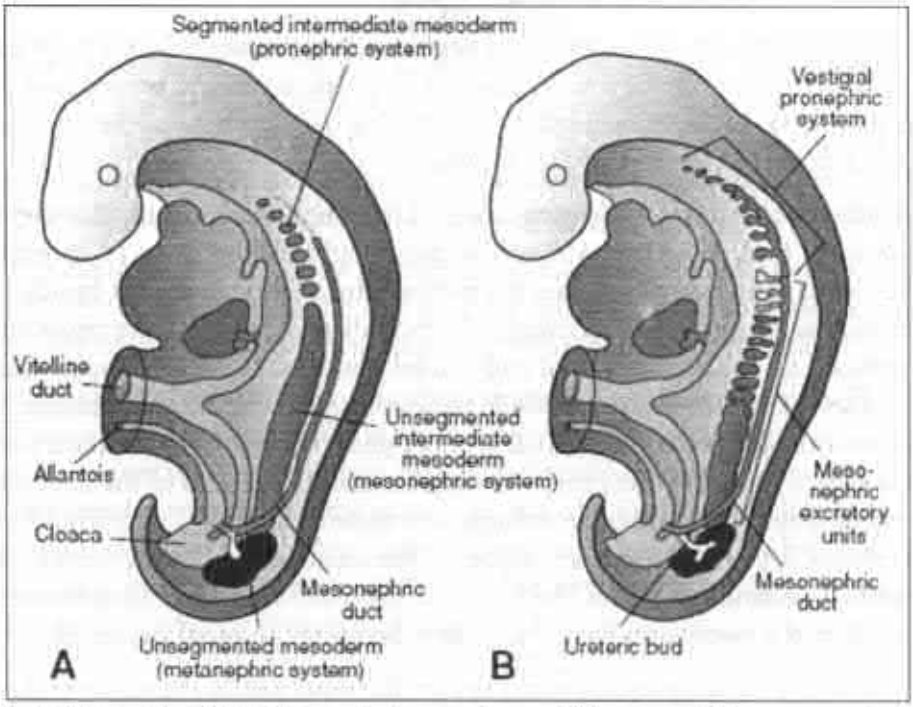
DEVELOPMENT

The urinary and genital systems are closely tied through development which explains their use of common ducts. Both systems originate from intermediate mesoderm which gives rise to the urogenital ridge from which the two organ systems emerge.

Three slightly overlapping kidney systems are formed in a cranial to caudal sequence during intrauterine life in humans: the **pronephros, mesonephros,** and **metanephros.** The first of these systems is rudimentary and nonfunctional; the second may function for a short time during the early fetal period; the third forms the permanent kidney.

PRONEPHROS

At the beginning of the fourth week, the pronephros is represented by 7 to 10 solid cell groups in the cervical region. These groups form vestigial excretory units, nephrotomes, that regress before more caudal ones are formed. By the end of the fourth week, all indications of the pronephric system have disappeared.



A. *Relationship of the intermediate mesoderm of the pronephric, mesonephric, and metanephric systems.* **B.** *Excretory tubules of the pronephric and mesonephric systems in a 5-week-old embryo.*

MESONEPHROS

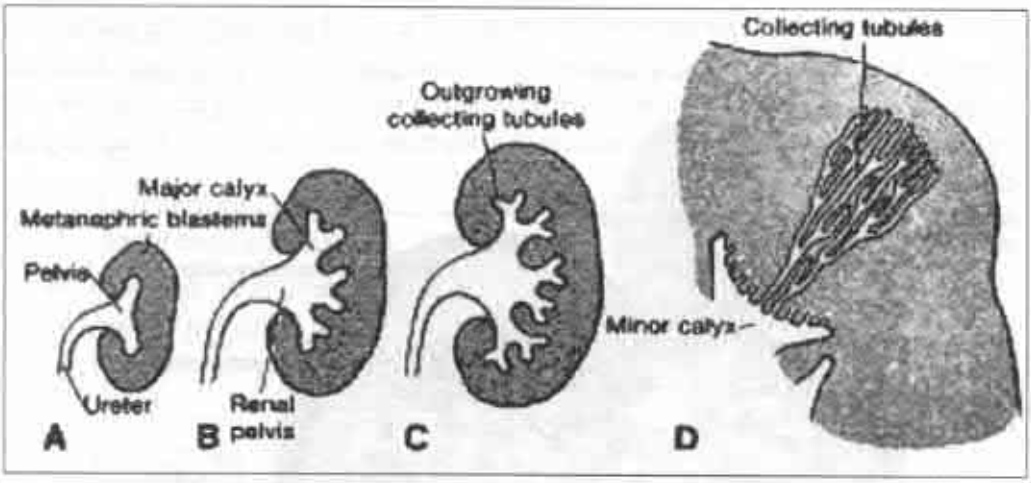
The mesonephros and mesonephric ducts are derived from intermediate mesoderm from upper thoracic to upper lumbar (L3) segments. Early in the fourth week of development, during regression of the pronephric system, the first excretory tubules of the mesonephros appear. They lengthen rapidly, form an S-shaped loop, and acquire a tuft of capillaries that will form a glomerulus at their medial extremity. Around the glomerulus the tubules form **Bowman's capsule,** and together these structures constitute a **renal corpuscle.** Laterally the tubule enters the longitudinal collecting duct known as the **mesonephric** or **wolffian duct.** In the middle of the second month the mesonephros forms a large ovoid organ on each side of the. Since the developing gonad is on its medial side, the ridge formed by both organs is known as the **urogenital ridge.** While caudal tubules are still differentiating, cranial tubules and glomeruli show degenerative changes, and by the end of the second month the majority have disappeared. In the male a few of the caudal tubules and the mesonephric duct persist and participate in formation of the genital system, but they disappear in the female.

METANEPHROS: The Definitive Kidney

The third urinary organ, the **metanephros,** or **permanent kidney,** appears in the fifth week. Its excretory units develop from **metanephric mesoderm** in the same manner as in the mesonephric system. The development of the duct system differs from that of the other kidney systems.

Collecting System. Collecting ducts of the permanent kidney develop from the **ureteric bud,** an outgrowth of the mesonephric duct close to its entrance to the cloaca. The bud penetrates the metanephric tissue, which is molded over its distal end as a cap. Subsequently the bud dilates, forming the primitive **renal pelvis,** and splits into cranial and caudal portions, the future **major calyces.** Each calyx forms two new buds while penetrating the metanephric tissue. These buds continue to subdivide until 12 or more generations of tubules have formed. Meanwhile, at the periphery more tubules form until the end of the fifth month. The tubules of the second order enlarge and absorb those of the third and fourth generations, forming the **minor calyces** of the renal pelvis. During further development, collecting tubules of the fifth and successive generations elongate considerably and converge on the minor calyx, forming the **renal pyramid.**

The ureteric bud gives rise to the ureter, the renal pelvis, the major and minor calyces, and approximately 1 million to 3 million collecting tubules.



Development of the renal pelvis, calyces, and collecting tubules of the metanephros. **A.** *6 weeks.* **B.** *At the end of the sixth week.* **C.** *7 weeks,* **D.** *Newborn. Note the pyramid form of the collecting tubules entering the minor calyx.*

KIDNEYS

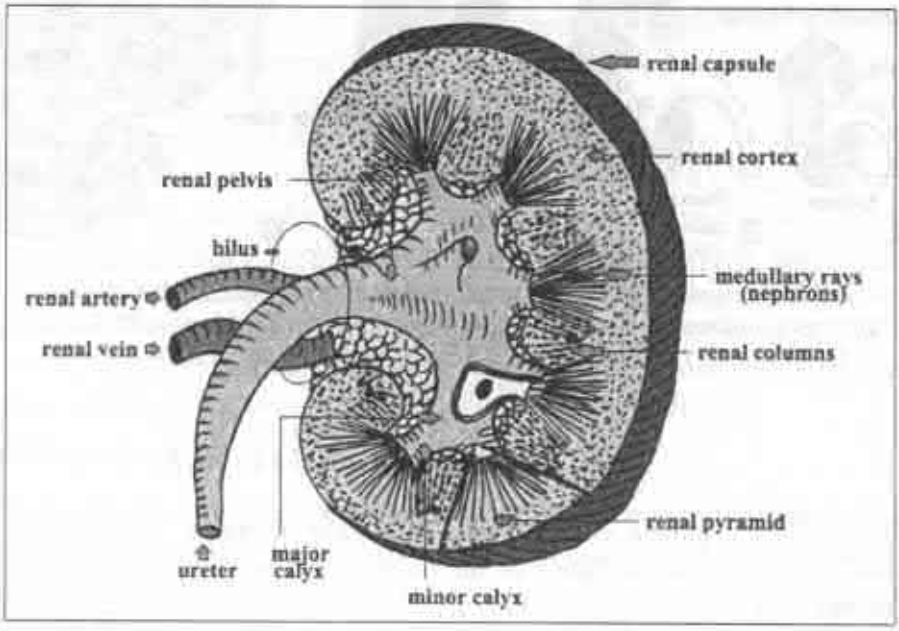
The kidney is paired, encapsulated bean-shaped organ. The hilum is a depression on the medial surface that serves as portal for the renal vessels, nerves and ureter. The renal sinus is the cavity deep to hilum that is occupied by the renal pelvis and vessels. The renal pelvis (L, basin) is simply an expansion of the ureter which receives urine from the major calyces.

The capsule of the kidney in humans is bi-laminar, consisting of an outer layer of dense connective tissue and an inner layer composed of loose connective tissue with many fibroblasts. The function of the inner layer is debated.

Each kidney is divided into an **outer cortex** and an **inner medulla.** The cortex is the outer region; the medulla is the deeper one. The cortex has large numbers of renal corpuscles, discernible as round structures containing small blood vessels. The renal corpuscles are the actual site of blood filtration and the initial stage of urine production. The cortex (outer region) is grossly divisible into alternating medullary rays (which appear striated to the eye) and cortical labyrinths. The medullary rays are rich in straight tubules and collecting ducts; the cortical labyrinths are rich in renal corpuscles, convoluted tubules and collecting tubules.

The medulla, deep to the cortex, has no renal corpuscles, only tubules. The medulla (inner region) is divisible into alternating pyramids and renal columns. Renal columns are simply extensions of cortex into the medullary region and

their composition is the same as the cortex. The renal pyramids are variable in number and contain straight tubules (thick and thin), collecting ducts and vasa recta. The apex of each pyramid terminates in a papilla The papilla is perforated by numerous openings of the terminal collecting ducts and its surface is described as the *area cribosa*



Medullary pyramid – conical masses with their bases located at the cortico-medullary border. Each kidney has over 10-18 renal pyramids. The tubular composition of a medullary pyramid varies between its inner and outer portions. In the outer portion (adjacent to the cortex) thick tubules predominate; conversely, in the inner portion thin tubules are more commonly found. Each pyramid drains at its papillae into a minor calyx; several minor calyxes unite to form a major calyx. In turn, major calyxes unite to form the renal pelvis which is drained by ureter.

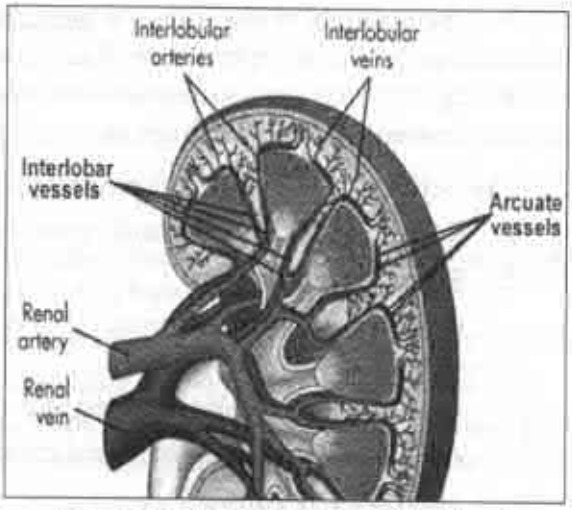
- A medullary pyramid, together with the associated covering cortical region, constitutes a renal lobe.

BLOOD CIRCULATION OF THE KIDNEY

Arterial system:

- Renal artery (musculo-elastic artery from aorta, enters at hilum).
- Interlobar arteries (between the lobes in the columns of Bertin).
- Arcuate arteries (arched vessels at cortico-medullary junction).

- Interlobular arteries (in cortex, parallel to medullary rays).
- Afferent arterioles.
- Glomerular tuft (and filter in renal corpuscle).
- Efferent arterioles (surround proximal and distal tubules for reabsorption).



Schematic representation of the blood circulation of the kidney

Venous system:

The venous vessels accompany and parallel the arterial vessels.

- Interlobular veins
- Arcuate veins
- Interlobar veins
- Renal vein (at hilum)

The pyramids of the medulla are composed mainly of straight collecting tubules. Each tubule is accompanied by straight arterioles and venules (known as vasa recta) that originate in the arcuate arteries and return to the arcuate veins.

The main function of the kidney is **to filter the blood.**

- The kidneys receive 20-25% of the total cardiac output per minute and filter about 1.25 L of blood per minute. All the blood of the body passes through the kidneys every 5 minutes.
- About 90% of the cardiac output goes to the renal cortex; 10% of the blood goes to the medulla.
- Approximately 125 ml of filtrate are produced per minute, but 124 ml of this amount are reabsorbed.
- About 180 L of fluid ultrafiltrate are produced in 24 hours and transported through the uriniferous tubules. Of this amount, 178.5 L are recovered by the tubular cells and returned to the blood circulation, whereas only 1.5 L are excreted as **URINE.**

The **NEPHRON** is the kidney's **basic functional unit**. There are a lot of them: about 2 million nephrons per kidney, so there's a great deal of capacity for processing urine. Enough so that the loss of a kidney to disease or to injury can be compensated by the surviving one.

The nephron consists of 2 components:

1. **Renal corpuscle** (Bowman's capsule and glomerulus).
2. **Renal tubule:**
   - Proximal thick segment (proximal convoluted tubule and proximal straight tubule).
   - Thin segment (descending and ascending limbs of ***loop of Henle***).
   - Distal thick segment (distal convoluted tubule and distal straight tubule).

TYPES OF NEPHRONS

Depending on the distribution nephrons can be:

- **Cortical (85%)**
  - are located in the outer region of the cortex.
  - their loop of Henle is short and does not enter the medulla.
  - Site of the most of reabsorption and secretion of the urine.
- **Juxtamedullary (15%)**
  - are located in the cortex region adjacent to the medulla.
  - their loop of Henle is longer and extends deep into the medulla.
  - are responsible for producing the urine-concentrating mechanism of the kidney.

1. RENAL CORPUSCLE

The renal corpuscle is the site where blood enters the nephron and undergoes **the process of glomerular filtration**. In order to understand the ultrastructure of the renal corpuscle, it is important to know that it is made up of two component parts *(see fig. 143, plate II)*:

1) **Glomerulus**

2) **Bowman's capsule**

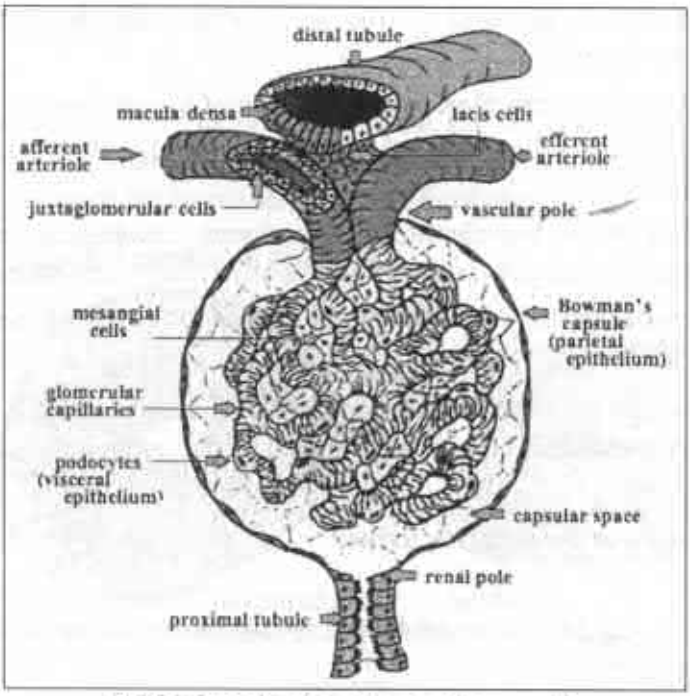
GLOMERULUS – consists of tufts of fenestrated capillaries; located between an afferent arteriole and efferent arteriole.

BOWMAN'S CAPSULE – is a double-walled epithelial capsule. The internal layer (the **visceral layer**) of the capsule envelops the capillaries of the glomerulus. The external layer forms the outer limit of the renal corpuscle and is called the **parietal layer** of Bowman's capsule. Between the two layers of Bowman's capsule is the **urinary space,** which receives the fluid filtered through the capillary wall and the visceral layer.

The parietal layer of Bowman's capsule consists of a simple squamous epithelium supported by a basal lamina and a thin layer of reticular fibers.

The visceral layer of the capsule is attached to the capillary glomerulus. It is formed by a modified simple squamous cells which are called PODOCYTES.

– **Podocytes** – have a cell body from which arise several long **primary processes.** Each primary process gives rise to numerous **secondary processes,** called **pedicels,** that embrace the capillaries of the glomerulus at a periodic distance, the secondary processes are in direct contact with the basal lamina. The pedicels, from the same podocyte or adjacent podocytes, interdigitate to cover the basal lamina and are separated by gaps, the **filtration slits** (are bridged by a membranous material, the **filtration slit diaphragm**) *(see fig. 146, plate II)*. However, the cell bodies of podocytes and their primary processes do not touch the basement membrane.



Schematic drawing of a renal corpuscle

Each renal corpuscle has a **vascular pole,** where the **afferent arteriole** enters and the efferent arteriole leaves, and a **urinary pole,** where the proximal convoluted tubule begins.

The glomerulus forms the **renal filtration barrier** *(see fig. 145, plate II)* of the nephron and is comprised of 3 components:

1. **Capillary fenestrated ENDOTHELIUM.**
2. **BASEMENT MEMBRANE.**

– Much thicker than typical basement membrane. The basement membrane is derived from the fusion of capillary and podocyte – produced basal laminae. With the aid of the electron microscope can be distinguished three layers:

- **Lamina rara externa** – an electron-lucent zone.
- **Lamina densa** – an electron dense intermediate zone.
- **Lamina rara interna** – an electron-lucent zone.

3. **Bowman's Capsule VISCERAL EPITHELIUM (podocytes).**

In addition to endothelial cells and podocytes, the glomerular capillaries have **mesangial** cells adhering to their walls. Mesangial cells have several functions:

- they give structural support to the glomerulus, synthesize extracellular matrix.
- provide **contraction and regulation of blood flow.**
- serve a phagocytic function and keep the basement membrane clear of debris.
- endocytose and dispose of normal and pathological (immune complex) molecules trapped by the glomerular basement membrane.
- probably produce chemical mediators such as cytokines and prostaglandins.

In the vascular pole but outside the glomerulus, there are the so-called **extraglomerular mesangial cells** that form part of the juxtaglomerular apparatus (described below).

Composition of the primary urine

- Water
- Ions (K, Ca, Mg, bicarbonate, phosphate, sulfate ions)
- Glucose

- Small-weight proteins (less than 69,000 Daltons).
- Amino acids
- Urea

2. RENAL TUBULE – site of selective re-absorption / secretion of solutes.

PROXIMAL CONVOLUTED TUBULE

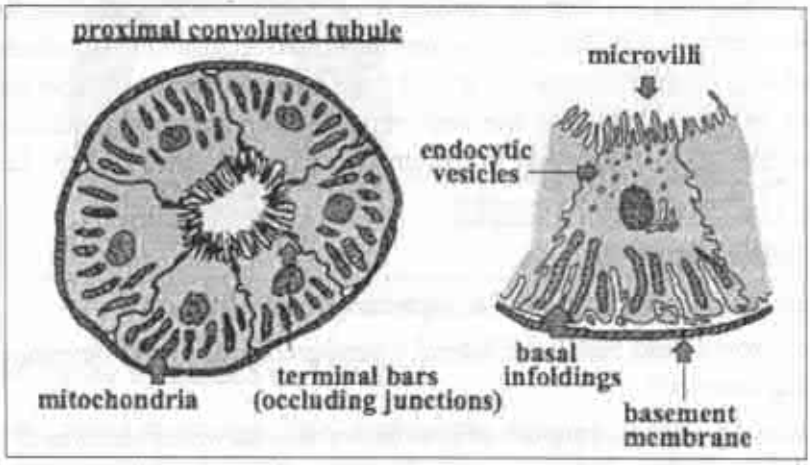
Most of the cortical tissue around the renal corpuscles is proximal tubules, among which the corpuscles are interspersed.

Functions:

- receives filtrate from urinary space
- site of selective re-absorption of most solutes:
  - all glucose and amino acids
  - 60 – 80% of NaCl (active) and water (passive)
- proteins absorbed by pinocytosis followed by lysosomal degradation and release of amino acids.
- re-absorbed materials released to peritubular capillary network.
- site of pH balancing.
- site of creatinine secretion.

Structure:

At the urinary pole of the renal corpuscle, the squamous epithelium of the parietal layer of Bowman's capsule is continuous with the cuboidal, or low columnar, epithelium of the proximal convoluted tubule.



Schematic drawing of proximal convoluted tubule cells

- The apical surface is covered with microvilli creating a light microscopic brush border that increases the surface area for ion absorption.
- The cells are tightly bound to one another to seal off the intercellular spaces from the lumen using junctional processes apically and interdigitating plicae (folds) laterally.
- Basally, interdigitating processes contain numerous mitochondria which create light microscopic basal striations that are associated with ion transport.

Histological appearance

- most abundant tubule in cortex.
- eosinophilic cytoplasm with basal nucleus (polarized):
  - brush border rarely preserved producing occluded lumen.
  - indistinct cell margins due to basal and lateral border interdigitations.

PROXIMAL STRAIGHT TUBULE

- They are located within or near medulla, depending upon type of nephron.
- They are formed by lower cuboidal epithelium and their microvilli and basal and lateral interdigitations are less well developed.
- Histologically they are similar to proximal convoluted tubules.

LOOP OF HENLE

Henle's loop is a U-shaped structure consisting of a **thick descending limb,** a **thin descending limb,** a **thin ascending limb,** and a **thick ascending limb.** The thick limbs are very similar in structure to the distal convoluted tubule. In the outer medulla, the thick descending limb continues as the thin descending limb. The lumen of this segment of the nephron is wide because the wall consists of squamous epithelial cells whose nuclei protrude only slightly into the lumen.

DESCENDING THIN TUBULE

- located within medulla.
- are lined by low cuboidal to squamous epithelium.
- microvilli and basal and lateral interdigitations poorly developed creating leaky cell.
- **site of passive transport of ions (inward) and water (outward) between lumen and interstitium.**

ASCENDING THIN TUBULES

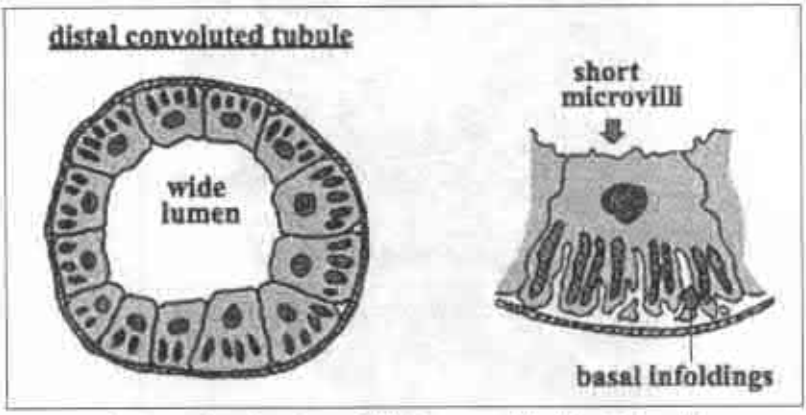
- located within medulla.
- similar in appearance to descending thin tubules.
- **water impermeable; passive transport of NaCl into interstitium.**

DISTAL STRAIGHT TUBULE

- Located within medulla and cortex.
- Is lined by simple cuboidal epithelium with sparse microvilli and lacking lateral interdigitations.
- The nucleus is apical and basal interdigitations with abundant mitochondria are present.
- Function: **water impermeable**; site of ion transport from lumen to interstitum which establishes ion gradient of medulla.

DISTAL CONVOLUTED TUBULE

- are located within cortex.
- They are approximately 1/3 as long as their proximal counterparts.
- They contact the renal corpuscle forming a macula densa which is part of the juxtaglomerular apparatus.
- Histologically they are similar to the distal straight tubules.
- Function: ion exchange.



Schematic drawing of distal convoluted tubule cells

COLLECTING TUBULES and DUCTS

Urine passes from the distal convoluted tubules to collecting tubules that join each other to form larger, straight collecting ducts, which widen gradually

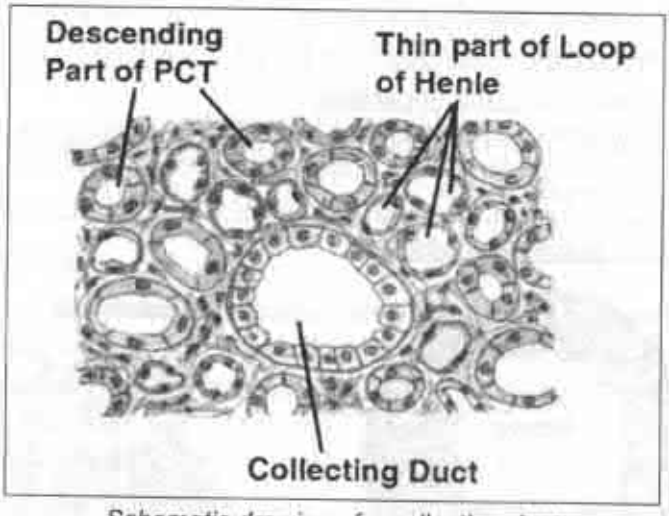
as they approach the tips of the medullary pyramids. They terminate at the tip of the renal pyramid as the papillary ducts.

Collecting tubules start in cortex and descend through medulla.

The smaller collecting tubules are lined with cuboidal epithelium. As they penetrate deeper into the medulla, their cells increase in height until they become columnar. The epithelium is composed of two cell types:

- **Principal cells** (light-staining) – resorb Na^+ and water and secrete K^+ in a Na^+, K^+ – ATPase pump-depending manner.
- **Intercalated cells** (dark-staining) – have abundant mitochondria and secrete both H^+ and HCO_3^-. They are important regulators of acid-base balance.

The collecting duct is the site of osmotic concentration; modulated by ADH secreted by the hypophysis: ADH increases the water permeability of collecting duct cells allowing water into the interstitium and back into blood via vasa recta resulting in a more concentrate urine.



Schematic drawing of a collecting duct

URINE FORMATION:

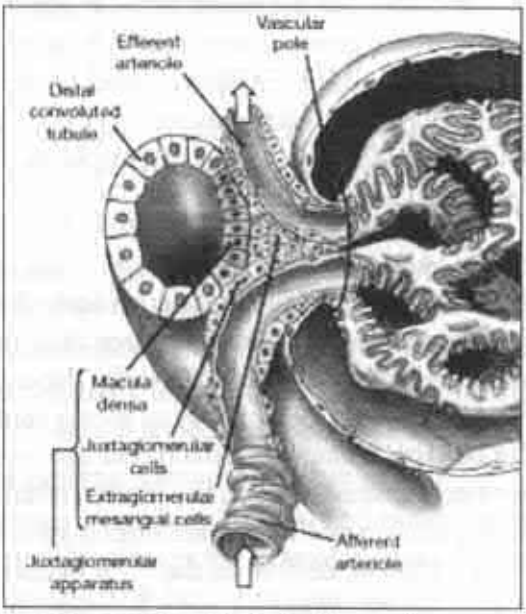
Process of urine formation consists of three main phases:

1. **Blood filtration** and formation of the primary urine.
   - Body fluid is collected via filtration thru selective permeable membrane via Hydrostatic pressure forcing water and small solutes thru it, large molecules are retained = primary urine (filtrate).

2. **Reabsorption** and formation of the secondary urine.
   - Selective reabsorption and secretion of solutes. Active transport reabsorbs solutes as glucose, salts and amino acids. Large molecules are actively secreted into the filtrate.
   - Active and passive concentration of filtrate expels an ultrafiltrate fluid = secondary urine.
3. **Secretion** and formation of the final urine.

JUXTAGLOMERULAR APPARATUS – site of blood pressure regulation via renin-angiotensin-aldosterone system. It is located at the vascular pole of Bowman's capsule and is formed by the conjunction of cells of:

- **Macula densa**: specialized cells in *distal convoluted tubule adjacent to renal corpuscle.*
  - These cells have receptors for Na. If it is necessary they stimulate production of aldosterone.
- **Juxtaglomerular cells**: modified smooth muscle cells of *afferent and efferent arterioles*
  - They produce renin. Renin provides the transformation of angiotensinogen into angiotensin I, which transforms into angiotensin II (in lungs) that elevates the blood pressure.



Schematic drawing of a juxtaglomerular apparatus

- **Juxtavascular cells:** extraglomerular mesangial cells.
  - Their function is not well known. Probably they are involved in the renin and erythropoietin secretion and blood pressure regulation.

Mechanism of the juxtaglomerular apparatus's action:

Steps:

- macula densa cells monitor NaCl levels in afferent arteriole.
- renin secretion of the juxtaglomerular cells is stimulated by paracrine activity from the macula densa.
- renin is a protease that cleaves plasma angiotensinogen into angiotensin I.
- angiotensin I converted to angiotensin II in the lung (by enzyme in capillaries).
- angiotensin II promotes vascular smooth muscle contraction and release of aldosterone from the adrenal cortex.
- aldosterone stimulates absorption of NaCl and water in the distal convoluted tubule thus increasing blood volume.
- net result is to increase blood pressure.

Renal Interstitium

The interstitium is the connective tissue matrix of the kidney. It occupies a very small volume in the cortex but increases in the medulla. The renal interstitium contains a small amount of connective tissue with fibroblasts, some collagen fibers, and, mainly in the medulla, a highly hydrated ground substance rich in proteoglycan. In the medulla are found the secreting cells called **interstitial cells**. They contain cytoplasmic lipid droplets and are implicated in the synthesis of prostaglandins and prostacyclin.

Aging and Kidneys

With advancing age the kidneys shrink due to a loss of nephrons within the cortical region of the kidney. There is also evidence of collapsing of glomeruli and sclerotic changes in the larger renal blood vessels. The end result is a decrease in renal blood flow from a level of 1200 ml/min in young adults to 600 ml/min in people over the age of 80. This change in renal blood flow compromises our ability to eliminate unwanted substances from the blood stream. There is considerable variability in the population and many individuals have much loss of kidney function. But on average a linear decline in the GFR occurs after the age of 40. Other functions like glucose resorption are also decreased in the aged.

This decrease in GFR means that, for drugs excreted by the kidney, the doses of drugs need to be adjusted to compensate for the age-related decrease in renal function. If adjustments are not made, there is an age-related risk for drug overdose. The risk for age-related drug complications is compounded further by age-related decreases in the liver metabolism of drugs.

The loss of nephrons also results in a decrease in the production of renin and secondarily, aldosterone. The decrease in aldosterone decreases potassium secretion by the nephron and can produce a condition known as hyperkalemia (potassium deficiency). Hyperkalemia is just one of many causes of hypertension in the elderly. This age-related problem can be further exacerbated by certain drugs (i.e. ibuprofen) which block the ability of aldosterone to help rid our blood stream of excess potassium.

The URINARY TRACT consists of:

1. renal calyces (minor and major) into which the large collecting ducts in the medullary papillae discharge their urine.
2. the renal pelvis in the hilum of the kidney.
3. the ureter, a muscular tube which conveys the urine toward the urinary bladder.
4. the urinary bladder which acts as a reservoir for urine and a pump that expels the urine during micturition.
5. the urethra, through which urine is voided from the body.

URETER

The ureter is muscular tubes connecting the renal pelvis to the urinary bladder. It drains urine from kidney to urinary bladder.

Structure – the wall of ureter has three layers:

- **Tunica mucosa** – lined by a transitional epithelium over connective tissue – lamina propria.
  - **transitional epithelium** – impermeable to water and salts; distensible.
  - **lamina propria –** loose connective tissue, is thick and elastic (as it is important that it is impermeable).
- **Muscularis externa** – smooth muscle layer
  - **bi-laminar**: inner longitudinal and outer circular (this is the opposite to the situation in the gastrointestinal tract); produce peristalsis. The distal third of the ureter contains another layer of outer longitudinal muscle.

- **Adventitia,** like elsewhere is composed of loose fibrous connective tissue, that binds it to adjacent tissues/ **Serosa** – connective tissue coat with mesothelial covering.

URINARY BLADDER

Urinary bladder represents hollow muscular organ; works as a distensible reservoir for urine (full: ~1 liter). It contains three openings: two for the ureters and one for the urethra. The triangular region defined by these three openings is called the **trigone**. **Trigone** of the urinary bladder has no folds (no submucosa; the tunica mucosa contacts directly with the muscularis externa); the lamina propria of mucosa in this area contains glands. These differences reflect the embryologic origins of the trigone and the rest of the bladder wall: the trigone is derived from the embryonic mesonephric ducts, and the major portion of the wall originates from the cloaca.

The gross regions of the urinary bladder are:

- **Fundus**
- **Body**
- **Neck**

The histology of the urinary bladder is as follows:

- **Tunica mucosa** – is lined by a transitional epithelium. Beneath is located the lamina propria (loose connective tissue).
- **Tunica submucosa** – represented by a connective tissue with blood supply.

The wall of the urinary bladder contains folds (rugae), which are formed by a tunica mucosa and submucosa. As the bladder fills with urine, the rugae flatten and the volume of the bladder increases with minimal increase in intravesical pressure.

- **Muscularis externa** – the muscularis contains numerous bundles of smooth muscle cells arranged in 3 layers: outer and inner longitudinal layer and a middle circular layer. Smooth muscle cells forms the **the detrusor muscle** specialized distally as internal urethral sphincter.
- **Serosa/Adventitia** – have typical structure.

URETHRA

The urethra is a fibro-muscular tube connecting the bladder to the external urethral orifice. It is sexually dimorphic. In the males it is the terminal duct for both the urinary and genital systems. In the female it empties only the bladder.

Female urethra:

- is about 3-5 cm long and opens between the clitoris and the vaginal opening.
- its direction is obliquely downward and forward; it is slightly curved with the concavity directed forward.

Histology: the wall of the urethra consists of 4 tunics:

Mucosa. The epithelium of the urethra starts off as transitional cells as it exits the bladder. Further along the urethra there are pseudostratified columnar cells, then stratified squamous cells near the external meatus (exit hole).

The lamina propria lacks papillae, but is folded longitudinally. This gives the lumen an irregular, crescentic shape. There are small mucus-secreting ***urethral glands*** that help protect the epithelium from the corrosive urine.

Submucosa. It is a deeper stratum, rich in elastic fibers and veins. The veins constitute a plexus of prominent; they represent a sort of spongy, semi-erectile tissue.

Muscularis externa. There is a rather thick coat of smooth muscle. It contains two layers: the inner layer is arranged longitudinally; the outer layer is arranged circularly. At the neck of the bladder it condenses, forming an **involuntary sphincter**.

Bundles of circular skeletal fibers occur outside the smooth fibers. This voluntary muscle is the ***constrictor urethra***. At the lower end of the urethra it forms a **voluntary sphincter**.

Adventitia. This coat is indefinite because of fusions with surrounding structures. Dorsally merge with the fibrous coat of the vagina.

Male urethra:

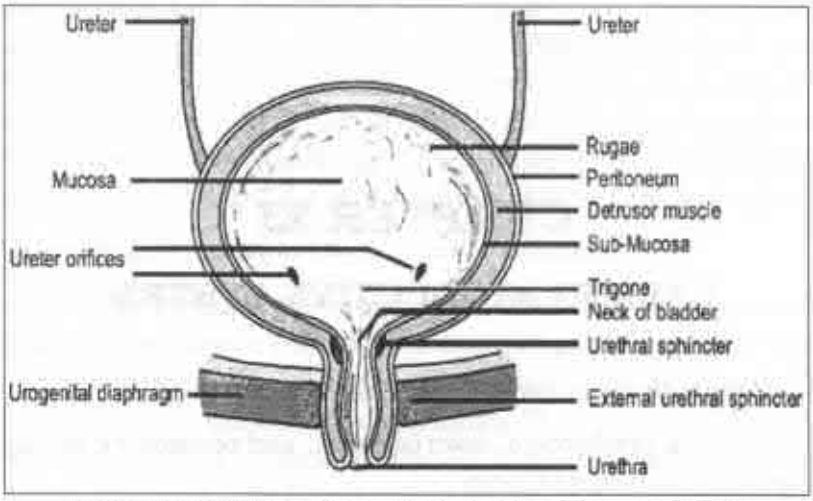
- The urethra is about 17.5 cm long and opens at the end of the penis. The inside of the urethra has a spiral groove (like rifling in a gun barrel), which makes the urine flow in a wide stream.
- The urethra is divided into four parts in men, named after the location:

Region	Description	Epithelium
pre-prostatic urethra	This is the intramural part of the urethra and varies between 0.5 and 1.5 cm in length depending of the fullness of the bladder.	Transitional
prostatic urethra	Crosses through the prostate gland. There are several openings: (1) a small opening where sperm from the vas deferens and ejaculatory duct enters, (2) the prostatic ducts where fluid from the prostate enters, (3) an opening for the prostatic utricle, but nothing is added from it. These openings are collectively called the verumontanum.	Transitional
membranous urethra	A small (1 or 2 cm) portion passing through the external urethral sphincter. This is the narrowest part of the urethra. It is located in the deep perineal pouch. The ducts from the bulbourethral glands enter here.	Pseudostratified columnar
spongy urethra (or penile urethra, or cavernous urethra)	Runs along the length of the penis on its ventral (underneath) surface. It is about 15-16 cm in length, and travels through the corpus spongiosum. The ducts from the urethral gland enter here. Some textbooks will subdivide the spongy urethra into two parts, the bulbous and pendulous urethra.	Pseudostratified columnar – proximally, Stratified squamous – distally

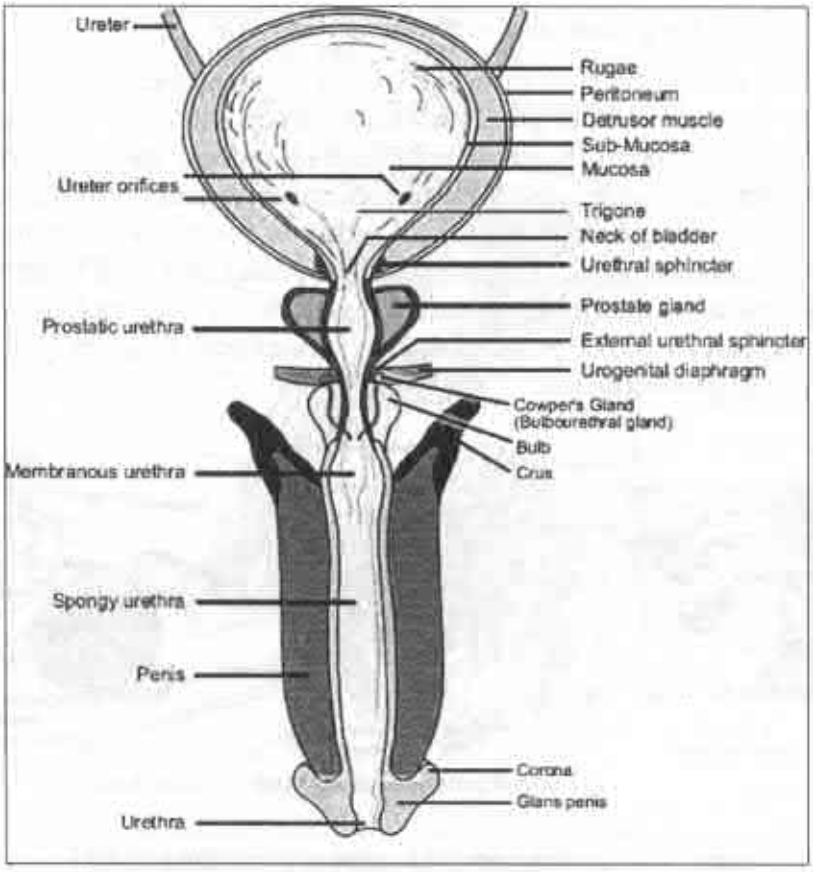
The length of a male' s urethra, and the fact it contains a number of bends, makes catheterization more difficult.

Muscularis externa contains two layers: the inner layer is arranged longitudinally; the outer layer is arranged circularly (it is best developed at the neck of the bladder, forming a *sphincter* there). The muscular tunic occurs chiefly in the prostatic and membranous segments. The cavernous segment lacks typical smooth-muscle layers.

Adventitia. There is no typical adventitial tunic. The prostatic urethra is surrounded by the tissue of the prostate gland. The membranous urethra is encircled by a sphincter of skeletal muscle belonging to the deep transverse perineal muscle. The cavernous urethra is surrounded by erectile tissue and a dense outer sheath.



Schematic drawing of urinary bladder and female urethra



Schematic drawing of urinary bladder and male urethra

CHAPTER XI

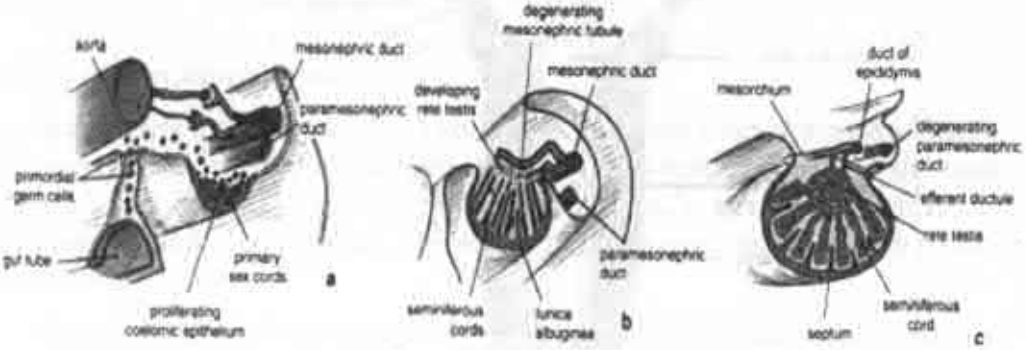
MALE REPRODUCTIVE SYSTEM

The male reproductive system is responsible for:

1. Continuous **production, nourishment**, and **temporary storage** of the haploid male gamete (sperm).
2. The **synthesis** and **secretion** of male sex hormones (androgens).

Development of the gonads and extra-testicular ducts

As noted in the urinary system, the urinary and reproductive systems share a common developmental origin. In males, the testes and extra-testicular ducts arise from the 3 different tissues: intermediate mesoderm, mesodermal epithelium and primordial germ calls. The intermediate mesoderm forms the urogenital ridge on the posterior abdominal wall and gives rise to the (1) stroma of the testes and (2) mesonephric (Wolffian) duct (the duct of a fetal renal system). The mesodermal (coelomic) epithelium gives rise to the (1) Sertoli cells and (2) paramesonephric duct. The primordial germ cells migrate from the yolk sac and give rise to the spermatagonia.



Development of the gonad

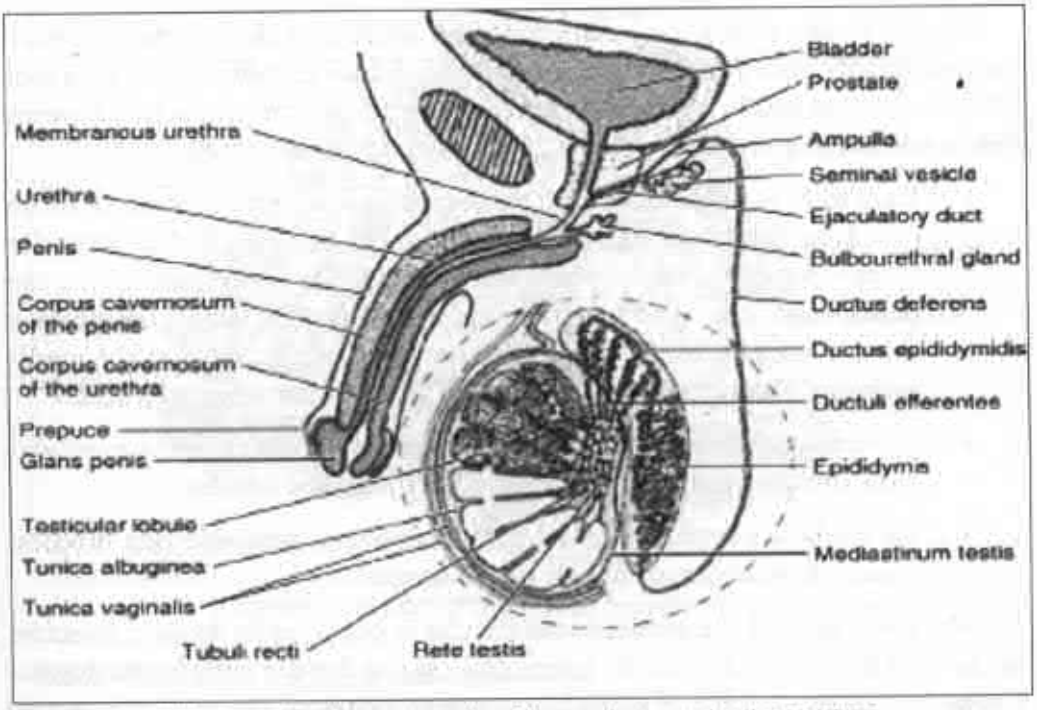
During embryonic development the mesonephric duct (Wolffian) develops tubules that connect to the developing seminal vesicles. These tubules form the

(1) efferent ductules and (2) proximal ductus epididymis. The duct proper forms the remainder of the ductus epididymis, ducts deferens, seminal vesicles, and ejaculatory ducts. The paramesonephric (Mullerian) duct degenerates but small portions persist as the prostatic utricle (male homolog of the uterus) and appendix testis. The urethra develops from the primitive cloaca, the urogenital sinus, the common terminus of the digestive, reproductive and urinary systems.

Sex determination

Genetic sex in humans is determined at fertilization by the presence or absence of the Y-chromosome. Gonadal sex determination begins at 7 weeks gestation. At this time the transcription of the SYR gene on the Y-chromosome begins synthesis of a protein called testis-determining factor (TDF). Secretion of this protein stimulates (1) the nascent Leydig cells to produce testosterone leading to an elaboration of the mesonephric duct (see above) and (2) Sertoli cells to secrete Mullerian-inhibiting factor (MIF) leading to the regression of the paramesonephric duct. Conversion of testosterone to DHT (dihydrotestosterone) induces the urogenital sinus to form the male external genitalia, prostate and urethra.

In the absence of SYR, the default program is female.



The structural components of the male reproductive system

Male reproductive system consists of:

1. **The testis**, which produce sperm and synthesis and secrete androgens.
2. **The epididymis, vas deferens, ejaculatory duct, and a segment of the male urethra**, which form the excurrent duct system responsible for the transport of spermatozoa to the exterior.
3. **Accessory glands – the seminal vesicle, the prostate gland, and the bulbourethral glands**, whose secretions form the bulk of the semen and provide nutrients to ejaculated spermatozoa.
4. **Penis**, the copulatory organ, formed of erectile tissue; its duct, the urethra, serves as the terminal passage for both the urinary and reproductive systems.

TESTIS

Functions of the testis:

- **spermatogenesis** (produce sex cells).
- **endocrine** (act as endocrine gland, produce testosterone).

The testes are paired oval shaped organs, located in the scrotum, outside the abdominal cavity. **Scrotum is** a sac-like pouch located behind the penis that holds each testis and helps regulate temperature for sperm production (**sperm development** need temperature with 2°F below body temperature).

- Each testis is surrounded by a thick capsule of fibrous connective tissue called the **tunica albuginea.** It is composed of dense fibrous irregular connective tissue, and appears white in life. The innermost layer is the tunica vasculosa, which is looser and more vascular. Tunica albuginea is thin on the anterior side and thick on the posterior, to form the **mediastinum**, where rete testis, nerves and testicular vessels are located.
- Numerous fibrous septa extend from tunica albuginea to the mediastinum, dividing the testis into 250 to 300 pyramidal lobules.
- Each lobule contains 1 to 4 highly convoluted **seminiferous tubules,** where *spermatogenesis* occurs (make sperm).

The space around the seminiferous tubules is occupied by loose connective tissue with blood vessels and the interstitial cells or **Leydig cells** (make **testosterone** in response to LH and **stimulate spermatogenesis).** Electron microscopy of these cells shows a cytoplasm rich in sER and mitochondria and the presence

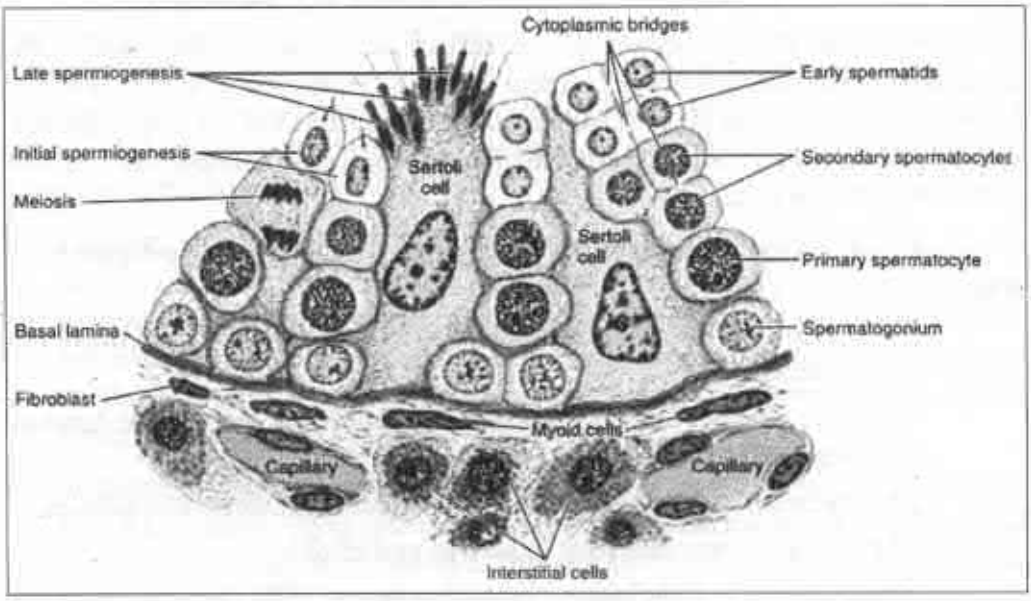
of Reinke crystals, a crystallized protein of unknown composition. Testosterone maintains spermatogenesis, male libido, and the function of the male accessory glands (prostate and seminal vesicle). Macrophages are also present, but other connective tissue cells including typical fibroblasts are normally absent.

The seminiferous tubules usually form an anastomic loop. Near the apex of the lobule the seminiferous tubules open into the tubuli recti, which form the first segment of genital duct system. These ducts are continuous with the rete testis, a system of epithelial-lined spaces in the mediastinum.

The seminiferous tubules are surrounded by a lamina propria. It is constituted of basement membrane, **contractile myoid cells** (responsible for the rhythmic contractile activity that propels the nomotile sperm to the rete testis) and an outer layer of adventitial cells.

The seminiferous tubules consist of a stratified epithelium resting upon a thickened basal lamina. The epithelium consists of 2 distinct cell populations:

1. the **Sertoli cells.**
2. the **spermatogenic cells.**



Schematic diagram of the wall of the seminiferous tubule

SERTOLI CELLS (sustenacular cells)

- Are columnar, non-replicating (post-mitotic) support cells extending from the basal lamina to the lumen of the seminiferous tubule.

- They have extensive apical and lateral projections that envelop the adjacent germ cells and make contact with adjacent Sertoli cells.
- At their basolateral domain, Sertoli cells form occluding junctions. Occluding junctions subdivide the seminiferous epithelium into a **basal compartment** (below the junctions) and an **adluminal compartment** (above the junctions).
- The basal compartment houses the spermatagonia (stem cells). The adlumenal compartment houses the spermatocytes (primary and secondary), spermatids and spermatozoa.
- The occluding junctions of the Sertoli cells also establish the blood-testis barrier. **Blood testis barrier contains:** testicular capillaries which are fenestrated, but tight junction between Sertoli cells prevents passage of many larger macromolecules from reaching the developing lineage. This barrier is both physiological and immunological in function; the barrier permits the adlumenal fluid to differ from that of the interstitium creating a more nutritive environment for the gametes and prevents an immunological response to the antigenic haploid spermatids.

Ultrastructurally, the Sertoli cells exhibit an active synthesizing profile. The nucleus is euchromatic with prominent nucleoli. Extensive sER and rER and abundant mitochondria fill the cytoplasm. Like the interstitial (Leydig) cells, Sertoli cells contain diagnostic crystalloids of unknown function known as Charcot-Bottcher inclusion bodies.

Functions of the Sertoli cells: – serve as nurse cells to the spermatogenic cells.

- These cells provide nutrients to them and remove wastes from the developing gametes.
- They also phagocytose and digest cytoplasm that is shed by the developing spermatids, thus recycling nutrients.
- They secrete fluid to carry mature sperm out of seminiferous tubules.
- They secrete hormones such as activin and inhibin.
- They respond to follicle-stimulating hormone (FSH) stimulation, which regulates the synthesis and secretion of androgen-binding protein (ABP) – secretory protein with high binding affinity for the testosterone.
- They act to compartmentalize the developing gametes, separating them from the body's immune system and the effects of certain hormones.

SPERMATOGENIC CELLS – are comprised of spermatogonia, spermatocytes, spermatids and spermatozoa *(see fig. 149, plate II).*

The spermatogonia divide by mitosis to create stem cells and primary spermatocytes; the latter undergo further differentiation as they move apically through the seminiferous epithelium to form secondary spermatocytes, then spermatids and finally spermatozoa.

- **Spermatogonia** (46 chromosomes; 2n, 1d (DNA)) are the stem cells found in the basal compartment of the seminiferous tubule adjacent to the basal lamina. There are 3 types (Ad, Ap, and B) that differ in their nuclear morphology and cell fate. Spermatogonia type A are stem cells whose daughter cells (mitosis) remain either (1) as a pair of stem cells (Type Ad (dark) or (2) into a pair that initiate a spermatogenic lineage (Type Ap (pale)). Not surprisingly, type Ad has an oval, dark staining nucleus whereas type Ap has an oval, light staining nucleus. Spermatogonia type B are simply differentiated descendents of type AP with spherical nuclei and obvious clumps of chromatin.

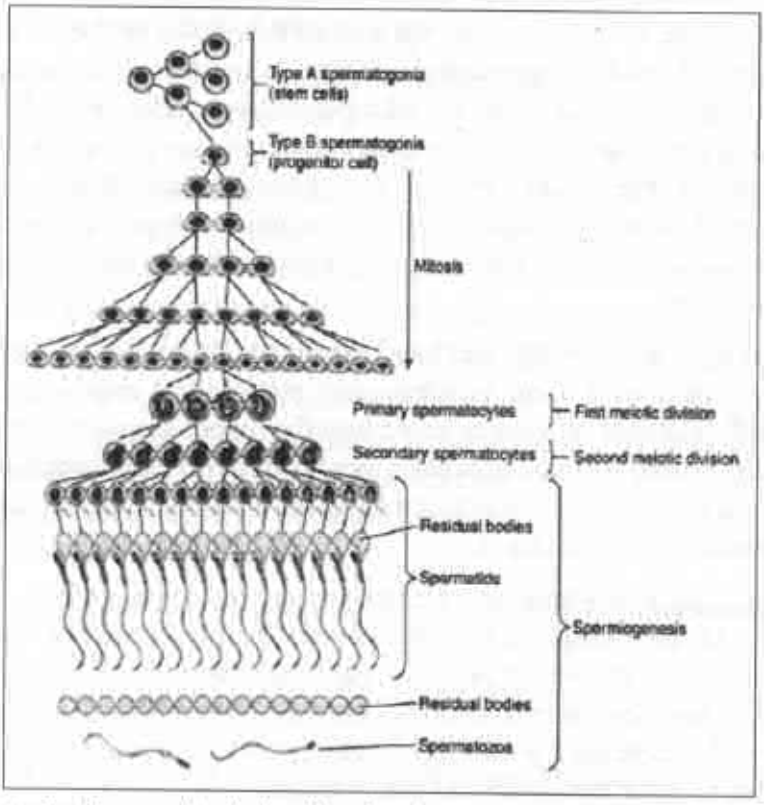
Interestingly, the type Ap daughter cells do not undergo cytokinesis following mitosis, nor does it occur in subsequent mitotic and meiotic cell divisions until the differentiated cells are released into the lumen as spermatozoa. The resulting multi-nucleated mass is called a **syncytium** and its intercellular connection accounts for the synchronous development of all the daughter cells derived from the initial type Ap division.

- **Primary spermatocytes** (46 chromosomes; 4n, 2d) result from the mitotic division of spermatogonia type B. They are the site of the first meiotic division and have a prolonged prophase (up to 22 days), thus are commonly observed in histological preparations. They are large cells with coarse chromatin threads; these are the cells that migrate through the Sertoli cell junctional complex to enter the adluminal compartment.

- **Secondary spermatocytes** (23 chromosomes; 2n, 1d) result from the first meiotic division. These cells rapidly move to the second meiotic division and thus are not commonly observed. They are small cells with indistinguishable chromatin found in the adluminal compartment.

- **Spermatids** (23 chromosomes; 1N, 0.5d) are haploid cells resulting from the second meiotic division. Found within the adlumenal compartment, they are small cells with condensed chromatin. Early spermatids are spherical in shape whereas late spermatids develop flagella and acrosomal caps.

- **Spermatozoa** represent the final stage in differentiation and separate from their intercellular bridges to enter the lumen. **The mature spermatozoa** have lost the majority of its cytoplasm and have become specialized for **locomotion, penetration of the ovum, and transmission of genetic material to the next generation.** However, they do not acquire motility until after transport to the epididymis.
- In humans, the entire process from the first mitotic division of spermatogonial cell to fully formed spermatozoa takes about 64 days.



Schematic diagram illustrating the clonal nature of the various generations of spermatogenic cells

Spermatogenesis can be divided into three stages:

1. ***spermatogonial phase***: Spermatogonia divide by mitosis to create stem cells and primary spermatocytes.
2. ***spermatocyte phase***: primary spermatocytes undergo two meiotic divisions (reducing both chromosome number and amount of DNA) to produce haploid spermatids.

3. ***spermatid phase***: Spermatids differentiate into mature sperm cells (spermatozoa).

During the spermatogonial phase the type Ap spermatogonia (daughter cells of type Ad) undergo repeated cell divisions (mitotic) to produce multiple clones; cytokinesis during these divisions is incomplete and the daughter cells are all linked by a cytoplasmic bridge forming a **syncytium**. As noted above, this cytoplasmic continuity is responsible for the synchronous development of the linked "cells". At the end of this phase the linked type Ap spermatogonia differentiate into type B spermatogonia.

During the spermatocyte phase mitotic division of each type B spermatogonia produces 2 daughter primary spermatocytes that migrate into the adlumenal compartment of the seminiferous tubule. Prior to undergoing meiosis I, these cells replicate their DNA so that the chromatids are doubled (4N) as is the amount of DNA (2d). As meiosis I begins crossing over can occur (one source of genetic variation) and that during metaphase the maternal and paternal chromosome are randomly segregated (2nd source of genetic variation). At the end of meiosis I, two secondary spermatocytes (2N, 1d) are formed. The secondary spermatocytes move rapidly into meiosis II without DNA synthesis (S phase) resulting in the formation of spermatids (1N, 0.5d).

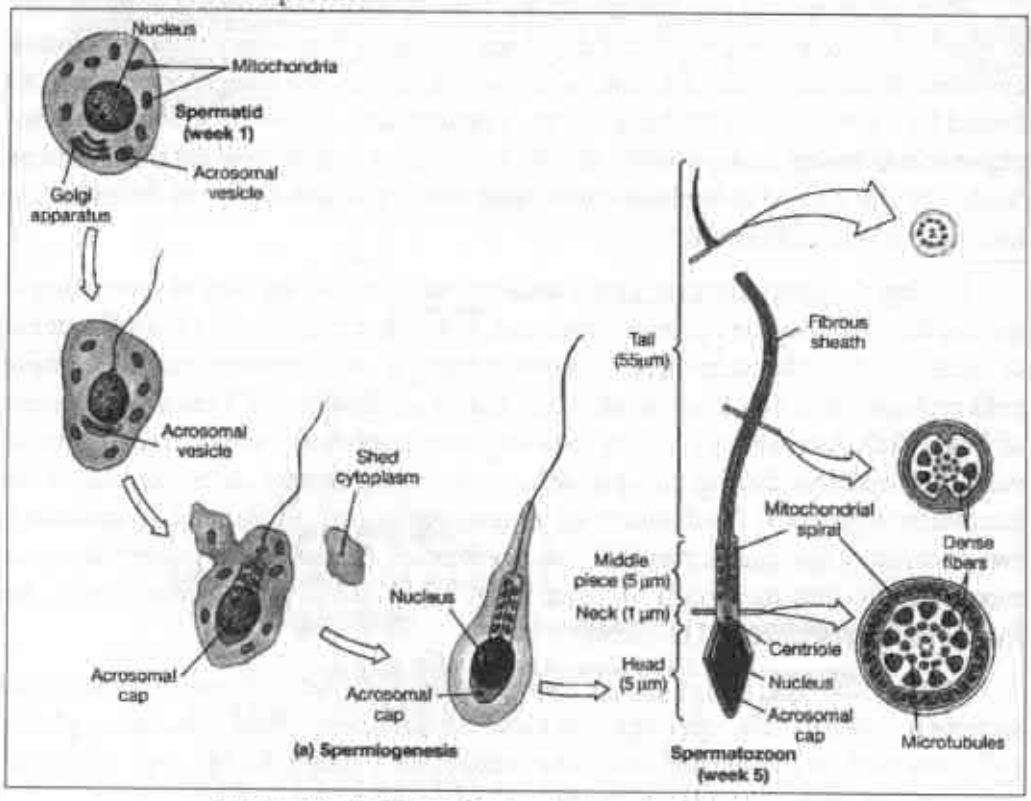
During the spermatid phase (***spermiogenesis***) the spermatids (immature gametes) develop into spermatozoa (mature gametes) while remaining physically attached to the Sertoli cells. This occurs in 4 stages: Golgi, cap, acrosome and maturational.

a. During the Golgi stage the polarity of the spermatids is established. The acrosomal vesicle (rich in glycoproteins) develops from the Golgi complex and marks the anterior (head) pole of the sperm. Following this, the centrioles migrate to the opposite end to establish the posterior pole and initiate flagellum formation.

b. During the cap stage the acrosomal vesicle spreads over the nucleus as the acrosomal cap. The nucleus begins to condense and the flagellum starts to form.

c. During the acrosome stage the spermatid re-orients so the flagellum projects into the lumen and the acrosome points toward the basal lamina. The nucleus flattens and elongates and the cytoplasm moves posterioraly to concentrate the mitochondria around the flagellum. The centrioles migrate back to the nucleus and form the connecting piece (neck).

d. During the maturational phase the connecting residual bodies of cytoplasm are shed and the spermatids are released into the lumen as spermatozoa or sperm.



Schematic diagram of spermiogenesis in the human

In addition, in a given histological section (in humans), not all portions of the wall of the seminiferous tubules are in the same part of a given cycle.

a. Thus different parts of a given tubule will contain different associations of the various gamete stages.

b. Some regions may appear to contain mostly mature spermatozoa, while other regions will contain a mixture of primary and secondary spermatocytes and early spermatid stages.

Structure of mature sperm

A mature spermatozoon is approximately 60 micrometers long and 1 micrometer wide. The head consists of the nucleus and acrosomal cap; the latter is rich in enzymes responsible for producing the acrosome reaction. The neck contains the centrioles. The tail contains the locomotory flagellum and is divided into the

middle, principal and end pieces. In the middle piece the flagellum is surrounded by a sheath of mitochondria that provide the energy for movement. In the principle piece the flagellum is covered by a fibrous sheet that is lacking in the end piece.

INTRATESTICULAR DUCTS and acquisition of motility

The newly released sperm are non-motile. Suspended in a fluid secreted by the Sertoli cells, they are transported to the epididymis by peristaltic contraction of the myoid cells. In doing so, they pass through the length of the seminiferous tubule into the intra-testicular duct system formed by the tubuli recti and the rete testis. The tubuli recti are the terminal straight portions of the seminiferous tubules that are lined only by Sertoli cells. The tubuli recti feed into the rete testis, a labyrinth of ducts within the highly vascularized mediastinum testis. These channels are lined by a simple low cuboidal epithelium with apical cilium and short microvilli.

The spermatozoa reach the epididymis by passing through the first part of the extra-testicular duct system, the efferent ductules. Within the epididymis the sperm become motile; however, transport through the remainder of the male reproductive system is accomplished largely by peristalsis of the smooth muscle lining the duct system, the sperm resting themselves in preparation for the external exertions to come.

ENDOCRINE REGULATION OF MALE REPRODUCTIVE FUNCTION

1. **Overview**: The process of spermatogenesis and all other aspects of male reproductive function depend on the presence of hormones produced by the hypothalamus, the anterior pituitary, and the testis forming what is known as the hypothalamic-pituitary-testis axis. Failure of any component of this system will result in infertility, loss of secondary sex characteristics, and if occurring embryonically failure to develop into a male. Furthermore, this axis is subject to feedback regulation through which the various hormones levels are controlled. We will now consider each component in turn.
2. **The Hypothalamus**: Certain neurons of the hypothalamus release gonadotropin releasing hormone (GnRH) into the hypothalamic-pituitary portal system where it then travels to the anterior pituitary to affect the secretion of pituitary hormones called gonadotropes.
3. **Anterior Pituitary**: Cells of the anterior pituitary called gonadotropes respond to GnRH by increasing secretion of two hormones called follicle stimulating hormone (FSH) and luteinizing hormone (LH). These hormones, together

known as gonadotropins are released into the blood stream where they circulate to end in the testis to interact with their respective target cells.

4. **Testis**: The target cells for gonadotropins are:
   - **Leydig cells**: are the target cells for LH and in response increase their secretion of testosterone. Some of this testosterone remains locally to affect Sertoli cells, whereas much of it is released into the blood stream where it circulates to affect other organs of the male reproductive system as well as other tissues and organs.
   - **Sertoli cells** are the target cell for FSH and in response increase functions which support spermatogenesis. Actually testosterone from Leydig cells and FSH together result in a number of changes such as an increase in the amount of androgen binding protein (which chaperones testosterone to developing spermatocytes and throughout the male reproductive tract) and increases in metabolic support to spermatocytes.
5. **Feedback Regulation**: Testosterone inhibits release of GnRH from the hypothalamus and LH release from the pituitary. Interestingly, it does not affect FSH secretion at the level of the pituitary, and only does so indirectly by its effect on GnRH. Instead FSH secretion is inhibited by a regulatory molecule released from Sertoli cells called inhibin.
6. **Effect of Androgens**: Testosterone and other androgens interact with a large variety of target cells, but all function by combining with an intracellular receptor called the androgen receptor. In some target cells, the testosterone is converted to another potent androgen called dihydrotestosterone (DHT) in a process catalyzed by an enzyme called 5α reductase. DHT interacts with the same androgen receptor, which functions by combing to the target cell DNA and affects gene transcription. Among the effects of androgens in the body are:
   - Development of the male internal and external genitalia in embryonic and fetal development.
   - Development of male secondary sexual characteristics (beard, deep voice, etc).
   - Anabolism of various tissues such as an increase in muscle and bone mass, etc.
   - Development of sexual function: including stimulating spermatogenesis and supporting male sex drive (libido).

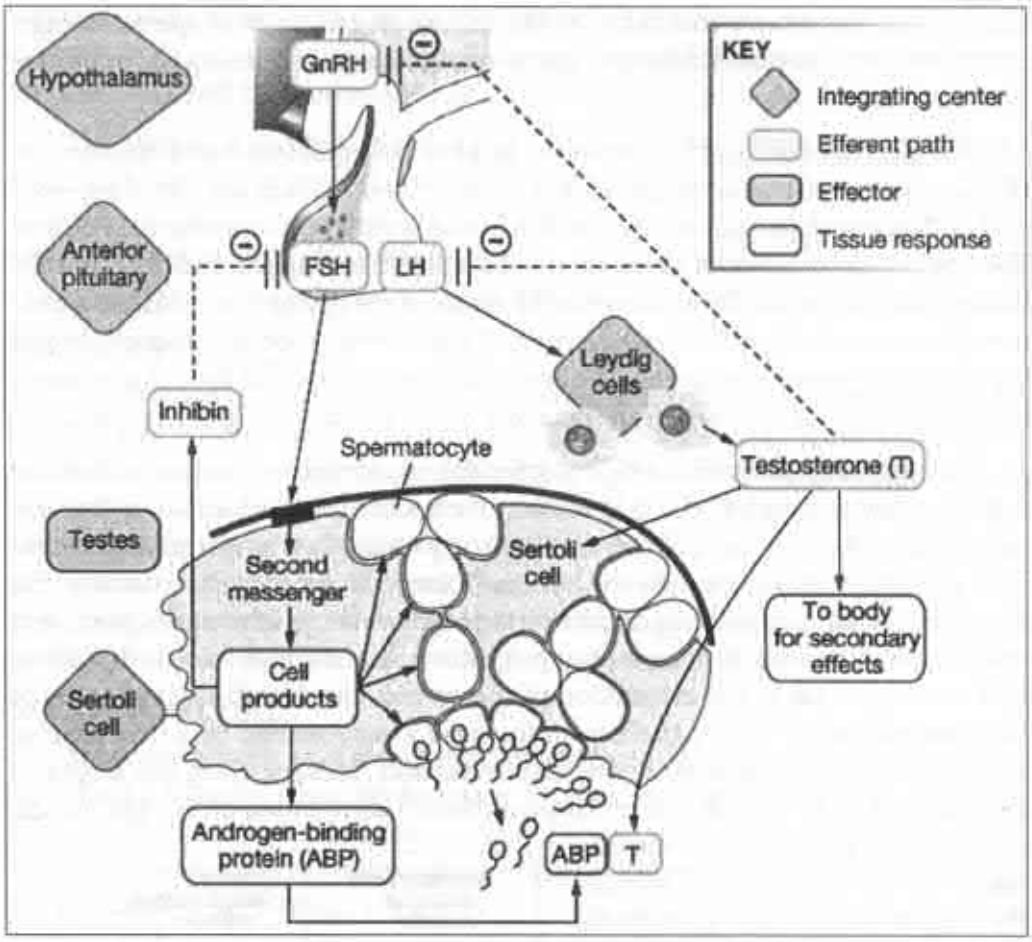


Diagram depicting the hormonal regulation of male reproductive function

EXTRA-TESTICULAR (EXCURRENT) DUCT SYSTEM

The extra-testicular duct system transports sperm from the rete testis (terminus of the intratesticular duct system) to the urethra within the prostate gland. It has a different developmental origin than the testes (specifically the mesonephric or Wolffian duct) and is divided into 4 parts: efferent ductules, duct of the epididymis, ductus deferens, and ejaculatory ducts.

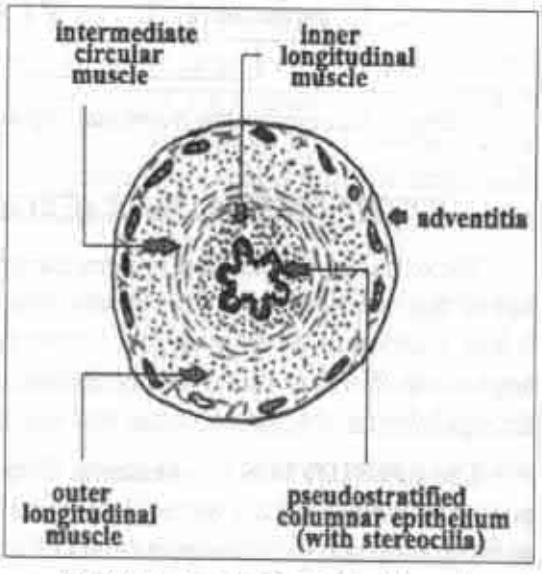
The **EPIDIDYMIS** is a crescent shaped organ lying along the superior and posterior surfaces of the testis. It contains both the efferent ductules and the duct of the epididymis (ductus epididymis). Surrounding the ducts are vessels, smooth muscle and a connective covering. The epididymis is divided into a head (adja-

cent to the testis), body and tail, with the tail serving as a site of sperm storage. Functions: **phagocytoses** damaged spermatozoa; **stores** spermatozoa; **maturate** spermatozoa.

The **efferent ductules** lie within the head of the epididymis and connect the channels of the rete testis to the duct of the epididymis. They consist of approximately 20 convoluted ducts lined with a pseudostratified columnar epithelium. The epithelium consists of basal stem cells, taller ciliated cells and shorter cells with apical microvilli. The different cell heights of the epithelium result in a characteristic saw-tooth outline to the lumen. The microvilli absorb seminal fluid and the cilia help propel the sperm. The epithelium is surrounded by a thin smooth muscle layer which also assists in transport of the sperm *(see fig. 151, plate II)*.

The **ductus epididymis** is a highly coiled tube that receives the efferent ductules in the head of the epididymis. It is lined by pseudostratified columnar epithelium consisting of basal (stem cells) and columnar principal cells. The principal cells have stereocilia projecting into the lumen. [Stereocilia are long, non-motile cilia which are better termed long microvilli as they lack the microtubules that form true cilia and consist of cytoplasmic projections like normal microvilli]. These cells' secretions aid in the maturation of sperm and they resorb any remnants of the residual bodies. The epithelium in the head is surrounded by a thin layer of circumferential smooth muscle that elaborates and thickens along the length of the duct so that in the tail it is tri-laminar. Neural stimulation of this tail muscle during ejaculation moves the sperms out of the tail storage site. Although the sperm within the epididymis is motile, transport through the epididymis is largely by peristalsis *(see fig. 150, plate II)*.

The **DUCTUS DEFERENS** is the longest portion of the extra-testicular duct system and runs from the tail of the epididymis to the seminal vesicles. It is lined with the same epithelium as the ductus epididymis but with a highly irregular lumen. Beneath the lamina propria is a thick muscularis consisting of three layers (inner and outer – longitudinal; middle – circular) of smooth muscle



Drawing of a cross-section through a vas deferens

surrounded by a connective tissue adventitia. The distal termination of the ductus deferens is dilated to form the ampulla. In the ampulla the lumen is expanded and the muscular wall thinned to serve as a (yet another) sperm storage site.

The terminal portion of the extra-testicular duct, **THE EJACULATORY DUCT**, is formed by the union of the ductus deferens (ampulla) and duct of the seminal vesicle. The ejaculatory ducts run within the prostate to the prostatic portion of the urethra. Its epithelium is similar to that of the ducts deferens but the muscular wall is replaced by the fibromuscular tissue of the prostate gland.

URETHRA

The urethra in males serves as the common final duct for both the urinary (urine) and reproductive (semen) tracts. It is divisible into three (toponymic) portions: prostatic, membranous and penile. The prostatic urethra lies within the prostate gland and is lined by transitional epithelium. The membranous urethra lies within the urogenital diaphragm (UG membrane) and is lined by a stratified or pseudostratified columnar epithelium. The penile urethra lies within the corpus spongiosum of the penis and transitions from a pseudostratified columnar at its beginning to a stratified squamous at the urethral orifice.

SPERMATIC CORD

Spermatogenesis is temperature sensitive requiring a cooler environment than the body cavity. Thus, the testes are located peripherally in an out-pocketing of the abdominal wall called the scrotum. During their descent into the scrotum the testes carry along portions of the peritoneal cavity and abdominal wall (muscle and connective tissue). The cavity becomes the cavity of the tunica vaginalis and the abdominal wall becomes the coats (tunics) of the spermatic cord.

The spermatic cord contains the proximal half of the ductus deferens and the vascular supply to the testes (testicular artery and venous pampiniform plexus). The cremaster muscle lies within the tunics.

Together these structures assist in the cooling of the seminiferous tubules. Cooling is promoted by the following mechanisms:

1. peripheral location within the scrotum.
2. **dartos muscle** – this is a layer of smooth muscle within the dermis of the scrotum. Contraction decreases the surface area (SA) of the scrotum conserving heat; relaxation increase SA to release heat.
3. vascular counter-current heat exchange: The testicular artery is encased within the venous pampiniform plexus; heat of the arterial blood is transferred to the cooler returning venous blood.

4. **cremaster muscle:** This muscle elevates the testes closer to the body to conserve heat; it has a limited range of motion in human.

ACCESSORY GLANDS

There are:

1. Seminal vesicles
2. Prostate gland
3. Bulbourethral glands

Functions of accessory glands:

- **Activate** spermatozoa.
- Carry nutrients for **motility.**
- **Serve as buffers** for vaginal acidity.

SEMINAL VESICLES

The seminal vesicles occur bilaterally on the posterior aspect of the urinary bladder. They develop as an evagination of the ductus deferens. They consist of a single, highly convoluted tube lined by a pseudostratified columnar/cuboidal epithelium. The columnar cells are secretory and the basal cells are stem cells. The wall is comprised of a mucosa (epithelium and lamina propria), muscularis (smooth muscle) and adventitia. The epithelium produces a viscous secretion containing: fructose that serves as the principal metabolite for sperm; **prostaglandins** that help **sperm transport**, and clotting proteins. The seminal vesicles provide 60% volume of the seminal fluid Secretory activity is stimulated by testosterone.

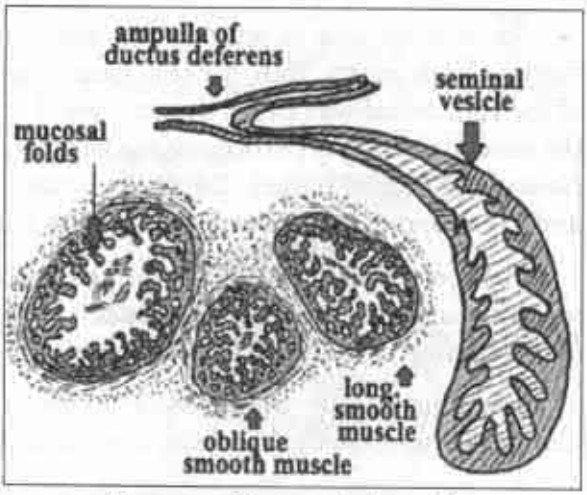


Diagram of the seminal vesicles

PROSTATE GLAND

The prostate gland is a large, midline (unpaired) gland underlying the urinary bladder and perforated by the prostatic urethra and ejaculatory ducts. Covered by

a fibroelastic capsule, it consists of 30-50 tubuloalveolar glands which open to the urethra. The epithelium is extremely variable (columnar or pseudostratified) and secretes semen components including: citric acid (nutrient), fibrinolysin (keeps semen liquefied), minerals, other enzymes and serine protease (PSA). All these substances **neutralize vaginal acids** and give adequate physical environment for sperm *survival* in female reproductive tract.

Like the seminal vesicle, secretory activity of the prostate is stimulated by testosterone. The glands are surrounded by a fibromuscular stroma which contracts during ejaculation to empty the glands' lumens. In older individuals the lumen may contain prostatic concretions (corpora amylacea), a precipitate of the secretions, rich in glycoproteins and, sometimes, a site of **calcium deposition**.

Glands can be separate in 3 groups which are arranged concentrically around urethra.

1. **main prostatic glands** (bulk of organ).
2. **submucosal** (outer periurethral) **glands**.
3. **mucosal** (inner periurethral) **glands**.

BULBOURETHRAL (COWPER'S) GLANDS

The bulbourethral (Cowper's) glands are small, bilateral glands within the urogenital diaphragm. These tubuloalveolar glands secrete a mucous that lubricates the urethra prior to ejaculation. The glands are formed by a simple columnar epithelium and their ducts open to the penile urethra. Secretory activity is stimulated by testosterone.

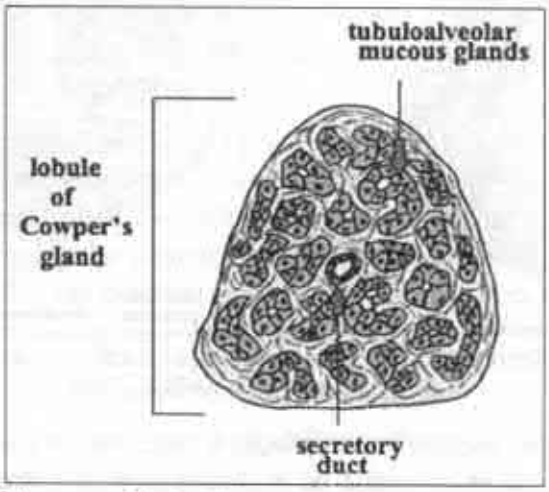


Diagram of the bulbourethral (Cowper's) glands

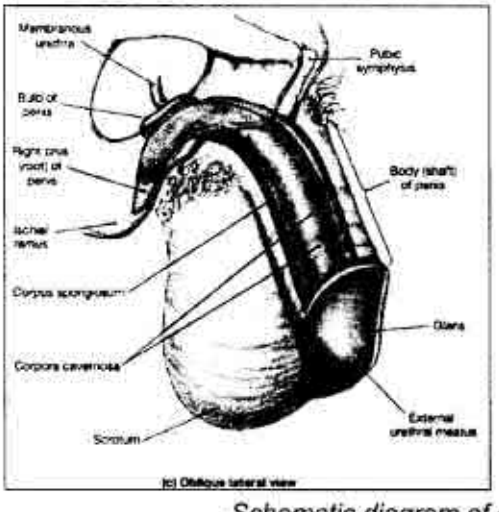
PENIS

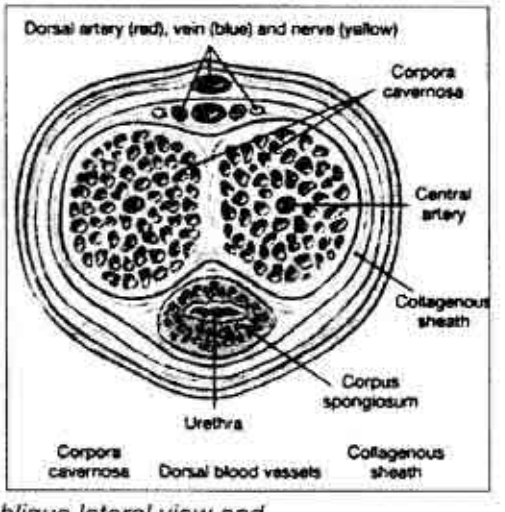
The penis is the male copulatory organ. It is formed by three **spongy** erectile bodies surrounded by a dense fibroelastic capsule (tunica albuginea, *which it's not as well defined in the corpus spongiosum*). The three erectile bodies are the (1) left and (2) right **corpus cavernosus** which are positioned dorsally and the unpaired ventral **corpus spongiosum**. The corpus spongiosum contains the spongy urethra and its erectile tissues expand distally as the glans.

The erectile tissue consists of numerous wide spaces lined with vascular endothelium and surrounded by smooth muscle. Erection is mediated by vasodilation of the incoming arteries and constriction of the venous outflow; this results in the vascular space becoming engorged with blood.

The penis consists of root, body, and glans. The skin of the penis is thin and loose except over the glans. It is highly innervated by both somatic sensory and autonomic nerves which are important in erection and ejaculation. These nerves cross the prostate to reach the penis and damage to them during prostate surgery can result in impotence.

The skin covering the forms a cuff of skin over the glans penis called the foreskin. Often this tissue is surgically removed shortly after birth in a procedure called circumcision.





Schematic diagram of a oblique lateral view and a cross-section through the penis

Surprisingly, the penis in humans lacks a baculum (os penis) positioned between the erectile bodies, as would be expected given the phylogenetic distribution of these bones in mammals.

MALE SEXUAL RESPONSE:

1. **Erection**: Normally the penis is flaccid because the venous spaces in erectile tissues are empty as a result of constriction of the arteries supplying these spaces. As a result blood is shunted via arterio-venous channels thereby by-passing these regions. Upon sexual excitement, mediated by the parasympathetic nervous system, these arteries dilate allowing these venous spaces to fill with blood giving rise to an increase in pressure and an enlargement of the penis – called erection. This vasodilation is mediated by release of nitric oxide, a potent vasodilator. The parasympathetic nerves also stimulate the secretions of the bulbourethral glands which produce mucus which not only neutralizes the acidic urine which may be present, but also results in release of this mucus from the penis aiding in lubrication. A variety of inputs including sensory and mental can lead to stimulation of vasodilatory parasympathetic nerves leading to erection. Failure to achieve an erection is called impotence which can result from a variety of factors ranging from vascular, nerve, psychological, or even temporary factors such as alcohol or certain drugs.

2. **Ejaculation**: actually consists of two distinct phases called emission and ejaculation, both under the control of the sympathetic nervous system. When sexual excitement reaches a maximum, there is a massive discharge of the sympathetic system which results in:

   - **Emission**: involves contraction of smooth muscle of the vas deferens which propels the spermatozoa forward through the ejaculatory duct into the prostatic urethra. Shortly after, the seminal vesicles contract forcing their contents forward followed by the prostate. These materials all mix in the prostatic urethra.

   - **Ejaculation Proper**: Due to stretch of the prostatic urethra, a second wave of nerve impulses pass to skeletal muscles associated with the bulb of the penis called the bulbospongiosus muscles. Contraction of these muscles forces the contents (semen) out the end of the penis. Another aspect is that the external urethral sphincter contracts preventing the semen from passing into the bladder.

OTHER RELATED CONCERNS

Circumcision: a process that surgically removes the flap of skin that covers the glans of the penis. This is usually done a few hours or days after birth. It is commonly done for religious reasons or to make it easier to keep clean. It is not

done to all males. Uncircumcised and circumcised penises look a little different, but function the same way.

Ejaculation: the passage of sperm from the penis, a result of a series of muscular contractions.

Semen: a combination of fluid that is produced in the seminal vesicles, prostate gland, and Cowper's gland. This fluid nourishes and helps sperm move through the urethra.

Nocturnal Emission (wet dream): a normal, involuntary ejaculation of semen while a male is asleep.

Impotence: the failure to get or maintain an erection. The reasons for impotence may be emotional or physical.

Vasectomy: surgical procedure for sterilization of the male. The vas deferens is severed or a portion is cut out to prevent sperm from entering the semen.

CHAPTER XII

FEMALE REPRODUCTIVE SYSTEM

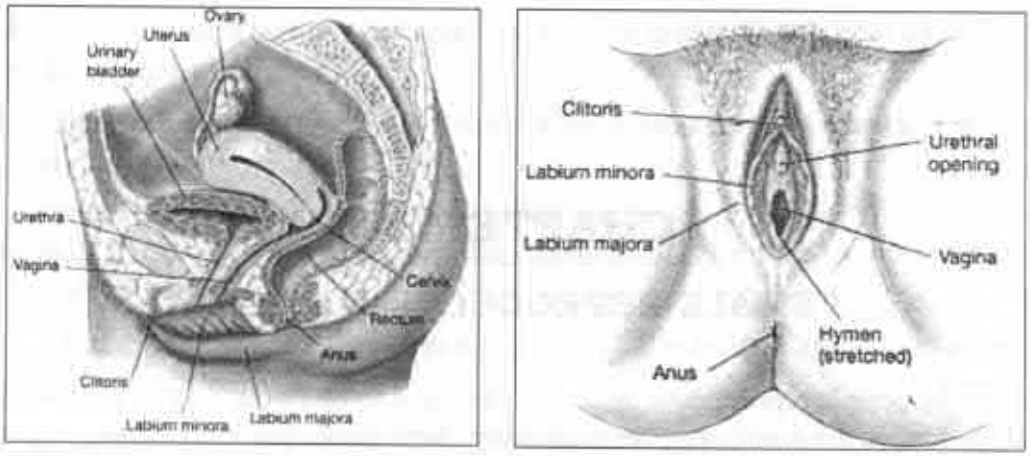
The female reproductive system is designed to carry out several functions. It produces the female egg cells necessary for reproduction, called the ova or oocytes. The system is designed to transport the ova to the site of fertilization. Conception, the fertilization of an egg by a sperm, normally occurs in the fallopian tubes. After conception, the uterus offers a safe and favourable environment for a baby to develop before it is time for it to make its way into the outside world. If fertilization does not take place, the system is designed to menstruate (the monthly shedding of the uterine lining). In addition, the female reproductive system produces female sex hormones (estrogen and progesterone) that maintain the reproductive cycle.

During menopause the female reproductive system gradually stops making the female hormones necessary for the reproductive cycle to work. When the body no longer produces these hormones a woman is considered to be menopausal.

The reproductive system consists of **2 ovaries**, **2 uterine tubes**, the **uterus**, **vagina**, and **external genitalia**. After **menarche**, when menses begin, the ovaries and uterine lining undergo a regularly repeated cycle of histological changes, usually 28 days in length. This cycle is under hormonal control and continues until **menopause**, the cessation of menses.

Organs of the female reproductive system are divided into:

1. Internal organs:	2. External genitalia:	3. Mammary glands
• **Ovaries**	• **Pubis**	
• **Oviducts**	• **Labia majora & minora**	
• **Uterus**	• **Clitoris**	
• **Vagina**	• **Vestibule**	
	• **Opening of vagina**	



Schematic drawing of the components of the female reproductive system

OVARIES

Functions:

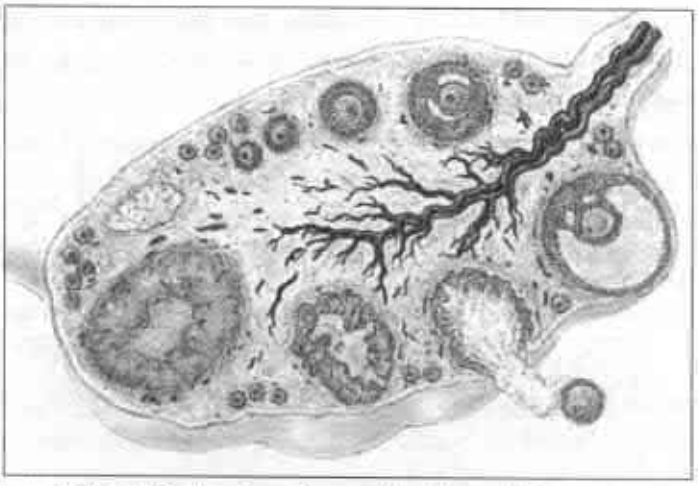
- The production of gametes (gametogenesis – oogenesis) – at birth, it is estimated that 400,000 – 800,000 primary oocytes are present, but only 400-500 are ovulated during a woman's reproductive life; the remaining 99% undergo degeneration **(atresia)**.
- Endocrine gland: secrete the steroid hormones (estrogens and progesterone). Estrogens promote growth and maturation of internal and external sex organs and are responsible for the typical female characteristics that develop at the time of puberty. They also act on mammary glands to promote breast development. Progesterone prepares sex organs, mainly the uterus, for pregnancy by promoting secretory changes in the endometrium. It prepares the mammary glands for lactation.

STRUCTURE OF THE OVARY

Grossly, the ovaries are flattened, almond-shaped structures, 2 – 5 cm long, attached by the **mesovarium** to the **broad ligament** of the uterus.

- The surface of the ovary is covered with **surface epithelium** (is known as the **germinal epithelium**), a simple epithelium which changes from squamous to cuboidal with age. Immediately beneath this surface epithelium there is a dense connective tissue capsule, which is called the **tunica albuginea**.

- The substance of the ovaries is distinctly divided into an outer **cortex** and an inner **medulla**. The cortex appears more dense and granular due to the presence of numerous ovarian follicles in various stages of development (least mature to most mature), corpus albicans, luteal body, atretic follicles, interstitial gland cells and stromal elements. The medulla is loose connective tissue with abundant blood vessels, lymphatic vessels, and nerve fibers.



Schematic drawing of a section through the ovary

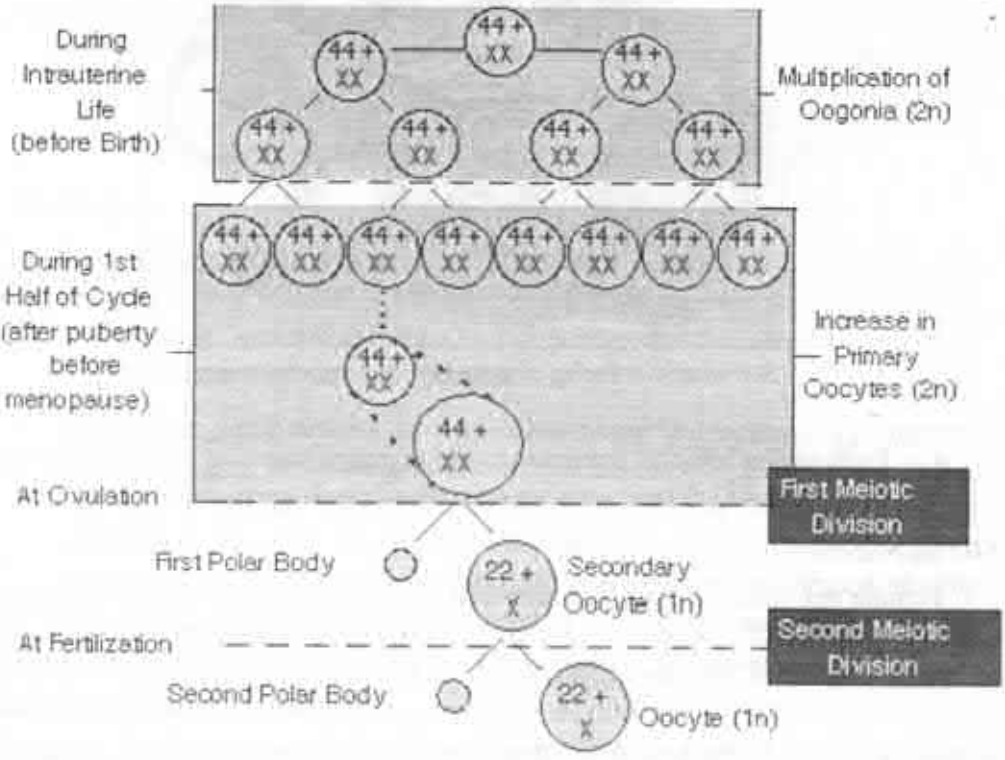
- Each of the follicles consists of one or more layers of follicular (granulosa) cells which surround an oocyte, a female germ cell.

OOGENESIS

Female sex cells, or gametes, develop in the ovaries by a form of meiosis called oogenesis. The sequence of events in oogenesis is similar to the sequence in spermatogenesis, but the timing and final result is different. Early in fetal development, primitive germ cells in the ovaries differentiate into oogonia. These divide rapidly to form thousands of cells, still called oogonia, which have a full complement of 46 (23 pairs) chromosomes. Oogonia then enter a growth phase, enlarge, and become primary oocytes. The diploid (46 chromosomes) primary oocytes replicate their DNA and begin the first meiotic division, but the process stops in prophase and the cells remain in this suspended state until puberty. Many of the primary oocytes degenerate before birth, but even with this decline, the two ovaries together contain approximately 700,000 oocytes at birth. This is the lifetime supply, and no more will develop. This is quite different than the male

in which spermatogonia and primary spermatocytes continue to be produced throughout the reproductive lifetime. By puberty the number of primary oocytes has further declined to about 400,000.

Beginning at puberty, under the influence of follicle-stimulating hormone, several primary oocytes start to grow again each month. One of the primary oocytes seems to outgrow the others and it resumes meiosis I. The other cells degenerate. The large cell undergoes an unequal division so that nearly all the cytoplasm, organelles, and half the chromosomes go to one cell, which becomes a secondary oocyte. The remaining half of the chromosomes go to a smaller cell called the first polar body.



Meiosis in the female

The secondary oocyte begins the second meiotic division, but the process stops in metaphase. At this point ovulation occurs. If fertilization occurs, meiosis II continues. Again this is an unequal division with all of the cytoplasm going to the ovum, which has 23 single-stranded chromosomes. The smaller cell from this division is a second polar body. The first polar body also usually divides in meio-

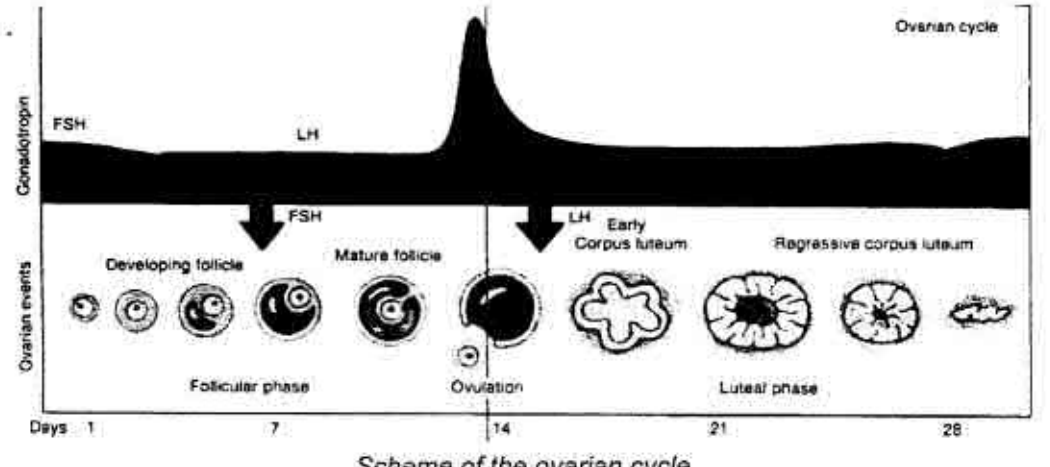
sis I to produce two even smaller polar bodies. If fertilization does not occur, the second meiotic division is never completed and the secondary oocyte degenerates. Here again there are obvious differences between the male and female. In spermatogenesis, four functional sperm develop from each primary spermatocyte. In oogenesis, only one functional fertilizable cell develops from a primary oocyte. The other three cells are polar bodies and they degenerate.

OVARIAN FOLLICLE DEVELOPMENT

Maturation of several follicles occurs each month and depends on **Follicle Stimulating Hormone (FSH)** secreted by the pituitary; although many follicles in various stages of maturity can be found in the cortex, generally **only one oocyte, within the dominant follicle, matures each month and is ovulated**; the dominant follicle develops in only one ovary. This is a random process – the ovaries do not always alternate with each other as to which one will ovulate during a given monthly cycle.

Morphological changes of the ovary during the menstrual cycle is called ovarian cycle. **Ovarian cycle has 3 phases:**

1. FOLLICULAR PHASE (the first 14 days of the cycle) – **in which the primordial follicle develops into a mature or Graafian follicle.** Follicular phase is regulated by FSH (follicular-stimulating hormone) of the pituitary gland.
2. LUTEAL PHASE (the second 14 days) – **consists in the formation of the corpus luteum, a major-secreting gland.** Luteal phase is regulated by LH of the pituitary gland.
3. **At the middle of the ovarian cycle the OVULATION takes place.**

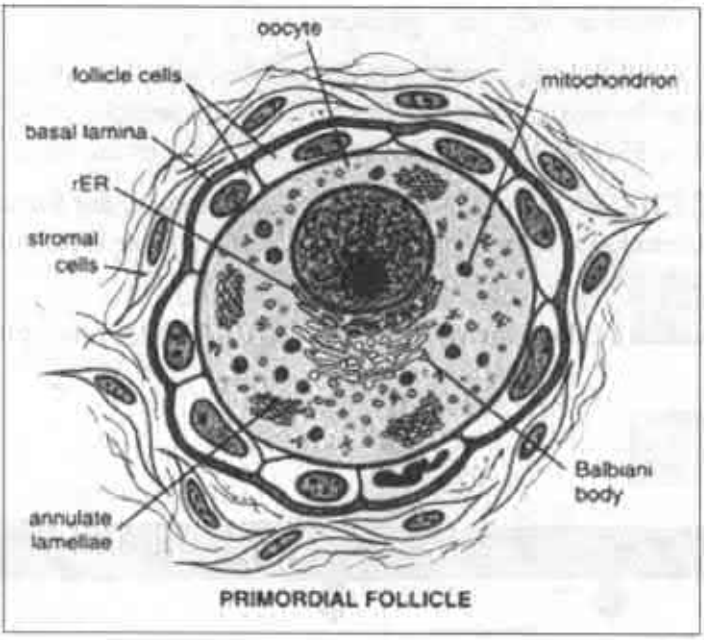


Scheme of the ovarian cycle

FOLLICULAR DEVELOPMENT

PRIMORDIAL FOLLICLES *(resting follicles)*

- are the most numerous type of follicle in the ovary.
- appear in the ovaries during the third month of fetal development.
- are located at the periphery of the cortex just beneath the tunica albuginea.
- each primordial follicle consists of a primary oocyte that were arrested in prophase of the first meiotic division before birth, and a single layer of ***squamous*** (flattened) follicular cells, the outer surface of which is bounded by a basal lamina. The oocytes are large, about 25 – 30μm in diameter. The nucleus of the oocyte is positioned eccentric in the cell. It appears very light and contains a prominent (large) nucleolus. Most organelles of the oocyte aggregate in the centre of the cell, where they form the vitelline body. Also, the cytoplasm contains many mitochondria, RER and several Golgi complexes *(see fig. 152, plate II)*.



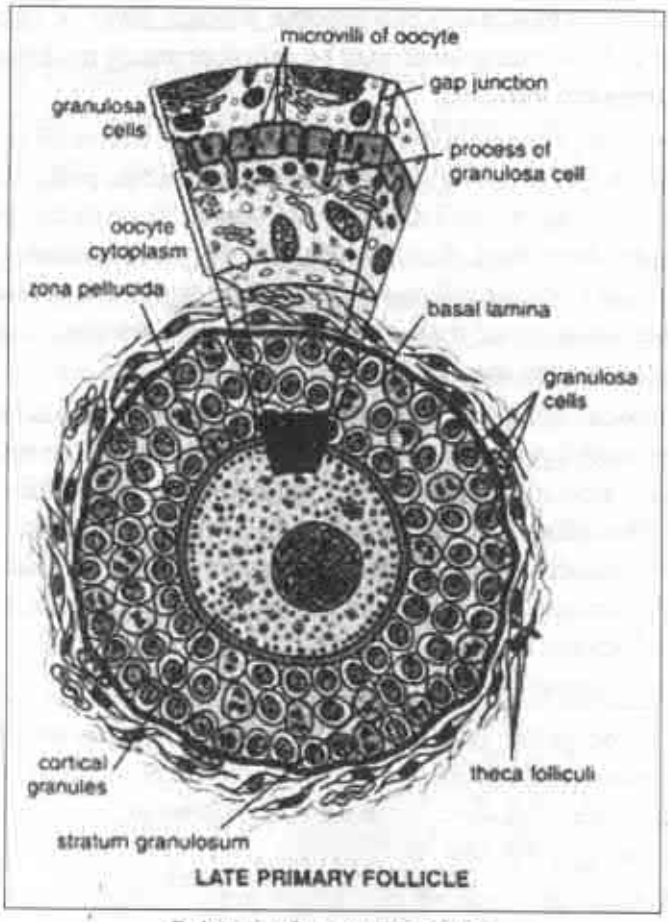
Scheme of a primordial follicle

PRIMARY (preantral) FOLLICLES *(growing follicles)*

Primary follicles represent the first step in the maturation of an oocyte:

- the oocytes begin to increase in size (to about 80μm).

- the squamous follicular cells become a single layer of **cuboidal** or **columnar** cells, forming what may be called **primary unilaminar follicles** ***(early primary follicles).***
- Between the primary oocyte and the adjacent follicular cells appear an amorphous, protective glycoprotein coat, the **zona pellucida** (manufactured by the oocyte and the follicular cells). It protects the oocyte and separates it from the follicular cells. *[During **fertilization**, sperm binds to receptors on the zona pellucida which then triggers the **acrosome reaction** (enzymes are released from the sperm to allow penetration of zona pellucida so that sperm can reach the oocyte)].*
- The cuboidal follicular cells undergo mitosis as the oocyte grows, forming several layers of follicular cells that surround the oocyte, forming what may be called **primary multilaminar follicles** ***(late primary follicles).*** The follicular cells are now called **granulosa cells.** The granulosa cells communicate with each other by gap junctions, and develop FSH, LH and estrogen receptors which allow them to become more receptive to gonadotropic stimulation.
- The granulosa cells also communicate with the oocyte by sending processes through the zona pellucida which form gap junctions with the oocyte plasmalemma. This allows for exchange of nutrients and regulatory molecules that are crucial for oocyte gene expression. The **zona pellucida** becomes thicker.
- The stromal cells around the follicle are arranged circumferentially to form the **theca folliculi**, which is divided into an inner **theca interna** and outer **theca externa**. The theca interna consists of **cuboidal cells** (thecal cells) that are separated from the granulosa by a basement membrane; a capillary network grows into the developing theca interna whose cells are aquiring **LH receptors**. The theca externa contains collagen, and smooth muscle fibers *(see fig. 153, plate II).*
- The growing follicles migrate centrally toward the ovarian medulla.
- During each cycle, a few primary follicles will continue to develop into secondary follicles.

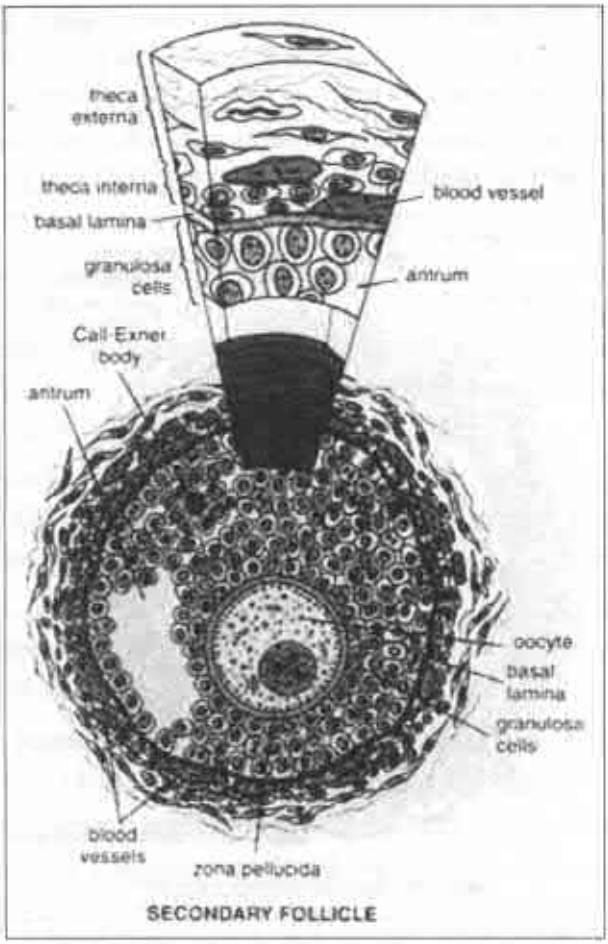


Scheme of a growing follicle

SECONDARY (antral) FOLLICLES are oval-shaped and contain a crescentic, fluid-filled cavity (the **antrum**); the oocyte is eccentrically positioned (off to one side).

- *OOCYTE:* The primary oocyte has reached its full size (~120μm); further growth is prevented by **oocyte maturation inhibitor** (secreted into antral fluid by granulosa cells). The oocyte lies within a local thickening of the granulosa cells called the **cumulus oophorus** which projects into the antrum.
- *GRANULOSA CELLS:* The granulose cells begins to secrete **follicular fluid**. Small pockets of fluid (**liquor folliculi**) between granulosa cells begin to appear. The spaces among the granulosa cells will coalesce to form the **antrum**, and the entire follicle continues to enlarge.

- The granulosa cells begin to express large numbers of **FSH receptors**. LH receptors are expressed, but in much lower quantity.
- Granulosa cells also secrete **inhibin,** which directly inhibits FSH secretion at the pituitary level.
- ***THECA CELLS:*** Cells in the theca interna (now expressing **LH receptors**) are large, rounded and epithelial-like; in response to LH stimulation they synthesize and secrete the androgens that are precursors of estrogens. **Estradiol** subsequently diffuses back across the basement membrane where it enters the blood vessels of the theca interna and raises the level of the hormone in the general circulation. (Estradiol also has a **proliferative effect** on granulosa cells, which develop estradiol receptors in response to increased levels of the hormone) *(see fig. 154, plate II).*

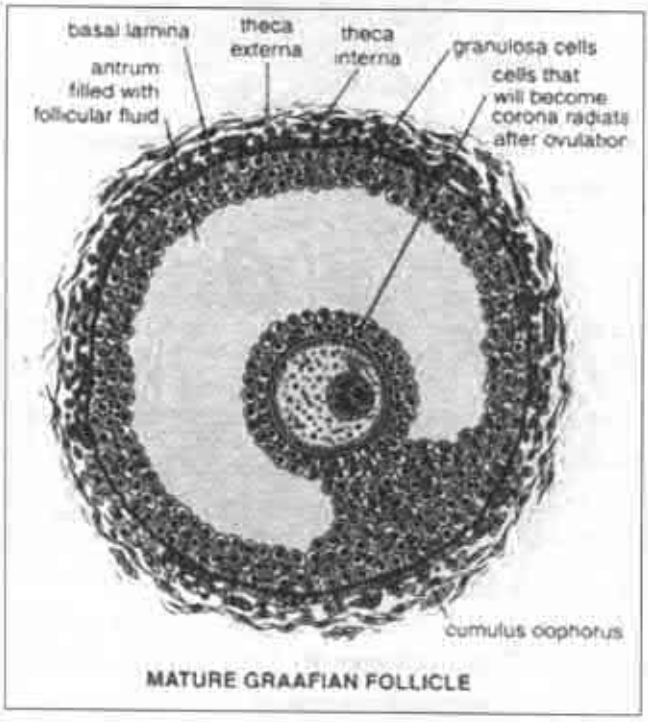


Scheme of a secondary follicle

PREOVULATORY (GRAAFIAN) FOLLICLE:

Early in the follicular phase of each cycle, one antral follicle maintains a greater rate of granulosa cell proliferation and produces more estradiol than the others in its cohort; this becomes the **dominant follicle,** which will be ovulated. The other follicles in its cohort degenerate (become ***atretic***) after they have grown to various sizes (between 2 – 10 mm). The dominant follicle grows to become the mature **preovulatory follicle.** It has reached its full size (10 – 20 mm) and bulges from the free surface of the ovary.

- The most rapid enlargement occurs 5 – 6 days prior to ovulation.
- The **single layer** of granulosa cells closest to the zona pellucida is firmly attached to it and forms the **corona radiata;** they communicate with oocyte via gap junctions.
- The oocyte, with its zona pellucida and corona radiata, is loosened from the rest of the granulosa cells by the development of new, liquid-filled intercellular spaces within the cumulus oophorus *(see fig. 156, plate II).*
- The corona radiata and many cumulus cells will remain with the ovum as it travels down the oviduct.



Scheme of a mature graafian follicle

OVULATION occurs when the dominant follicle ruptures and the ovum is released.

- It is a hormone-mediated process resulting in the release of the secondary oocyte.
- Ovulation takes place at the middle of the menstrual cycle.
- It is stimulated by LH.
- A clear, cone-shaped area forms on one side of the follicle. This **stigma** is produced by lack of blood flow in this area and by the action of enzymes such as collagenase (activated by LH surge).
- The stigma ruptures, gently releasing follicular fluid and blood with the ovum, which is still surrounded by the zona pellucida and the cumulus oophorus.
- The factors include:
  - increase in the volume and pressure of the follicular fluid.
  - contraction of smooth muscle fibers in the theca externa.

ENDOCRINE CONTROL OF OVULATION involves the **pituitary gland, hypothalamus** and the **ovary** itself.

1. **Gonadotropin Releasing Hormone (GnRH)** is released by neurons within the hypothalamus every 90 minutes in the human. It is carried to the anterior pituitary via the hypophyseoportal vessels, where it stimulates synthesis and release of **Follicle Stimulating Hormone (FSH)** and **Luteinizing Hormone (LH)** by the gonadotrophs.
2. **FSH** is carried to the ovary in the general circulation where it stimulates growth of follicles and their maturation (increases the number of gap junctions, induces aromatase activity in granulosa cells so estradiol production is increased).
3. **LH** causes the thecal cells to organize and to secrete androgens; also causes maturation of the oocyte in the growing follicles.
4. **Estradiol** is secreted in increasing amounts, mostly by the dominant follicle, during the first 14 days of the cycle (the follicular phase).
   - Promotes mitotic activity of granulosa cells of antral follicles.
   - Estradiol has a negative feedback effect on the hypothalamic secretion of GnRH and on the pituitary gonadotropes, causing them to slow the release of **FSH** and **LH** (which now accumulates in the gonadotropes).

The increasing level of **inhibin** (from granulosa cells of growing follicles) also directly inhibits FSH synthesis and secretion.

- However, at mid-cycle (14 days), the dominant follicle reaches maturity and secretes a maximum level of estradiol which now exerts a positive feedback effect on the secretion of GnRH, causing more to be released from the hypothalamus, and also causes the pituitary gonadotropes to be more sensitive and responsive to GnRH.
- The **LH** that has been stored in the pituitary gonadotropes is abruptly released into the circulation, triggering the process of ovulation to begin; this phenomenon is known as the **LH surge**. A smaller surge of **FSH** also occurs that helps to trigger ovulation (stimulated by **LH-induced progesterone synthesized by granulosa cells).**
- Ovulation occurs about 36 hours after the LH surge begins, and ~12 hours after LH reaches its peak concentration in the blood.
- LH also stimulates the oocyte to resume meiosis and complete the first meiotic division – now it is a ***secondary oocyte***. The second meiotic division is then begun and is arrested in metaphase. It is completed if the ovum is fertilized. If there is no fertilization the secondary oocyte will die in 48 hours.

After ovulation and in response to luteinizing hormone, the portion of the follicle that remains in the ovary enlarges and is transformed into a corpus luteum.

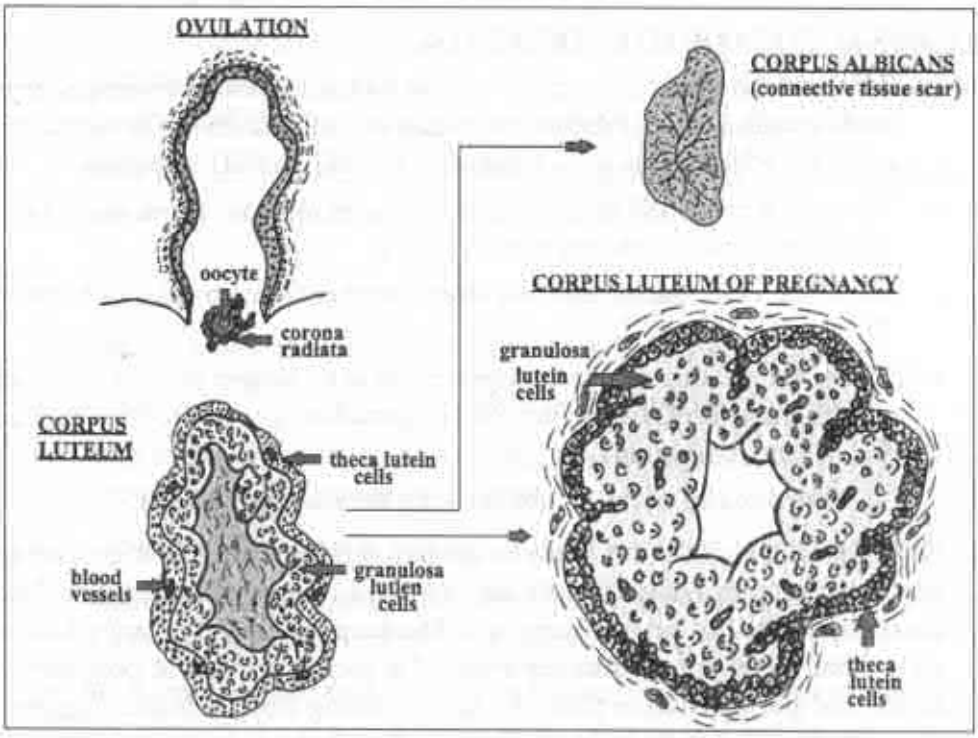
CORPUS LUTEUM (yellow body)

In the corpus luteum development are distinguished next phases:

1. ***PROLIFERATION*** – after the follicle ruptures, its wall collapses and the granulosa cell lining becomes convoluted. **LH** stimulates the cells within the ruptured follicle to start proliferation and to organize into a **corpus luteum**. The basal lamina separating the granulosa cells from the theca cells dissolves, and blood vessels from the theca invade the granulosa layer.
2. ***MORPHOLOGICAL CHANGES*** – the future corpus luteum consists of two types of luteal cells:
   - **granulosa lutein cells**, very large, centrally located cells derived from the granulosa cells. The granulosa cells undergo hyperplasia (proliferation), hypertrophy (enlargement) and are transformed into granulosa lutein cells.
   - **theca lutein cells**, smaller, peripherally located cells derived from the cells of the theca interna layer.

Both types of cells enlarge, accumulate lipid, and have all the characteristics of steroid secreting cells, including abundant smooth endoplasmic reticulum, mitochondria and lipid droplets.

3. ***FLOWERING*** – the resulting structure secretes **progesterone** and smaller amounts of **estradiol**. These hormones stimulate the growth and secretory activity of the endometrium, to prepare it for the implantation of the blastocyst.



The development of the corpus luteum

The fate of the corpus luteum depends on whether fertilization occurs. There are two types of corpus luteum: **corpus luteum of pregnancy** (if fertilization and implantation occurs) and **corpus luteum of menstruation** (formed in the absence of fertilization and implantation).

CORPUS LUTEUM OF PREGNANCY:

- The corpus luteum of pregnancy grows to a diameter of 5 cm and continues to secrete progesterone until birth occurs; it decreases in size during the last 3 months of pregnancy, and manufactures and releases the polypeptide hormone **relaxin** (promotes dilation of the cervix, softens connective tissue of symphysis pubis).

- Human **chorionic gonadotropin (hCG)** produced by the trophoblast of the chorion will stimulate the corpus luteum (CL) for about 6 months & prevents its degeneration. hCG can be detected in the serum as early as 6 days after conception and in the urine as early as 10-14 days of pregnancy. Detection of hCG in the urine is the basis of most pregnancy tests. Estradiol, IGF I and II (from ovary), LH, Prolactin (from anterior pituitary) and insulin also contribute to the formation of the CL of pregnancy.

CORPUS LUTEUM OF MENSTRUATION:

- **LH** causes the corpus luteum to secrete increasing amounts **progesterone and estradiol** which inhibits the release of GnRH from the hypothalamus – the net effect of this is the **inhibition of FSH and LH secretion.**
- Because it needs LH to survive, the CL can last for only about 14 days (the luteal phase of the menstrual cycle).
- the luteal cells shrink and lose their organization; stromal cells invade the remains of the CL.
- When the CL degenerates, progesterone is no longer present in the circulation and GnRH again acts on the gonadotropes to produce FSH and LH; a new cycle begins.
  - The remains of the CL form a scar, the **corpus albicans,**

4. *INVOLUTION* – is the last phase in the fate of corpus luteum. It degenerates and is replaced by connective tissue forming a **corpus albicans,** that slowly decrease in size but never disappears. The corpus albicans (scar) which results from the eventual disintegration of a corpus luteum of pregnancy is larger and persists longer than the scar resulting from a corpus luteum of menstruation.

CORPUS ALBICANS

The cellular components of the corpus luteum are replaced by fibrous connective tissue.

FOLLICULAR ATRESIA is the programmed death of some follicles on different stages of their development.

- At the beginning of each cycle, several secondary follicles start to grow but only one follicle will mature completely and be ovulated. The rest of the follicles will undergo atresia at various stages in their maturation.
- Atresia is the name for the degenerative process by which oocytes (and follicles) perish without having been expelled by ovulation. Only about

400 oocytes ovulate – about 99.9 % of the oocytes that where present at the time of puberty undergo atresia. Atresia may affect oocytes at all stages of their "life" – both prenatally and postnatally. Large numbers of follicle undergo atresia during fetal development. By the sixth month of gestation about 7 million oocytes and oogonia are present in the ovaries. By the time of birth this number is reduced to about 2 million. Of these only about 400.000 survive until puberty.

- Atresia is also the mode of destruction of follicles whose maturation is initiated during the cyclus (10-15) but which do not ovulate. Such degenerate follicles are called **atretic follicles**.
- They appear to contain a dark pink-staining material which is probably the remains of the zona pellucida of the follicle.

OVIDUCT (UTERINE TUBE)

The uterine tubes (also called Fallopian tubes or oviducts):

1. transport the ovum from the ovary to the site of fertilization.
2. help transport spermatozoa, the haploid male gametes, from the site of deposition to the site of fertilization.
3. provide an appropriate environment for fertilization.
4. transport the fertilized ovum (embryo) to the uterine horns where implantation and further development may occur.

In the mature female, each oviduct measures 10 – 12 cm long. The wall of the oviduct consists of a **mucosa, muscularis externa and serosa**. The serosa is continuous with the mesosalpinx (the upper free margin of the broad ligament of the uterus). The lumen communicates with the uterine cavity at one end and the peritoneal cavity at the other end. There are 4 identifiable segments of this muscular tube:

1. **Infundibulum** – wide, funnel-shaped opening into the peritoneal cavity; has complex folds with fringe-like processes (**fimbria**) on its free margins which embrace the ovary during ovulation and sweep the ovum into the oviduct.
2. **Ampulla** – intermediate region with expanded lumen where fertilization is likely to occur; mucosa forms elaborately branched folds. Comprises 2/3 of the tube.

3. **Isthmus** – medial 1/3 of the oviduct close to uterine wall; has narrow lumen with less complex mucosal folds.
4. **Intramural portion** (pars interstitialis) – the segment which penetrates the uterine wall; few mucosal folds.

The **MUCOSA** consists of a **simple columnar epithelium** resting on a **lamina propria** which forms the luminal folds. The lamina propria consists primarily of loose connective tissue, collagen fibers, and fibroblasts, although lymphocytes, monocytes and mast cells may be present. There are two types of epithelial cells which are probably different functional states of the same cell:

1. **Ciliated columnar cells** – the cilia beat toward the uterus and prevent microorganisms from passing into the peritoneal cavity. The height of the cells and number of cilia is dependent on estradiol, so in the early follicular stage, when there is little estrogen, the cells are low with few cilia. By the time ovulation occurs, they are tall and have the maximal number of cilia that facilitate the transport of the ovum toward the uterus. During the luteal phase, the cells become shorter (cuboidal) and have fewer cilia.
2. **Secretory cells** ***(peg cells)*** – are non-ciliated, but have apical microvilli; secrete substances that will nourish the ovum and promote capacitation (activation) of sperm. Progesterone causes an increase in the number of secretory cells. True glands are absent in the oviduct *(see fig. 158, plate II).*

MUSCULARIS EXTERNA – consists of an inner circular or spiral layer of smooth muscle and an outer longitudinal layer. The boundary between the two layers is indistinct. The muscularis increases in thickness toward the uterus (sparse in the infundibulum and the ampulla but thick in the isthmus and intramural portion). Peristaltic movements help to move the ovum toward the uterus.

SEROSA – the uterine tube is covered by loose connective tissue with an outer mesothelium.

During ovulation, the veins in the lamina propria which extend into the fimbriae become distended; this engorgement plus smooth muscle contractions bring the infundibulum close to the ovary. ***Peristaltic contractions of the muscularis and the beating of the mucosal cilia transport the ovum to the uterus.***

Clinical consideration: The uterine tubes are the site of tubal ectopic pregnancies. They can also be the site of bacterial infection which can lead to Pelvic Inflammatory Disease, a major cause of infertility in women.

UTERUS

Functions of the uterus:

1. serves to receive the sperm.
2. transports sperm from site of deposition to uterine tubes for fertilization.
3. provides suitable environment for:
   a. *implantation of the embryo.*
   b. *nourishment of the embryo and foetus during pregnancy.*
4. provides mechanical protection of the foetus.
5. expels the mature foetus at the end of pregnancy.

The wall of the uterus is formed of 3 tunics. From the outside inward there are:

- **Perimetrium** = tunica serosa.
- **Myometrium** = tunica muscularis.
- **Endometrium** = tunica mucosa (with basal & functional layers).

PERIMETRIUM

- is the tunica serosa of the uterus. It has the typical composition of loose connective tissue with mesothelium (visceral layer of peritoneum), but contains a large number of lymphatic vessels. The primetrium covers the entire posterior surface of the uterus but only part of the anterior surface. The remaining part of the anterior surface consists of adventitia.

MYOMETRIUM is very thick and consists of 3 ill-defined layers of smooth muscle:

- **Submucosal** layer – smooth muscle bundles are oriented parallel to the long axis of the uterus.
- **Vascular** layer – is the thickest layer. Contains numerous large blood and lymphatic vessels. Smooth muscle bundles are oriented in a circular or spiral pattern interlaced with each other.
- **Supravascular** layer – the arrangement of smooth muscle bundles is the same as in the submucosal layer.

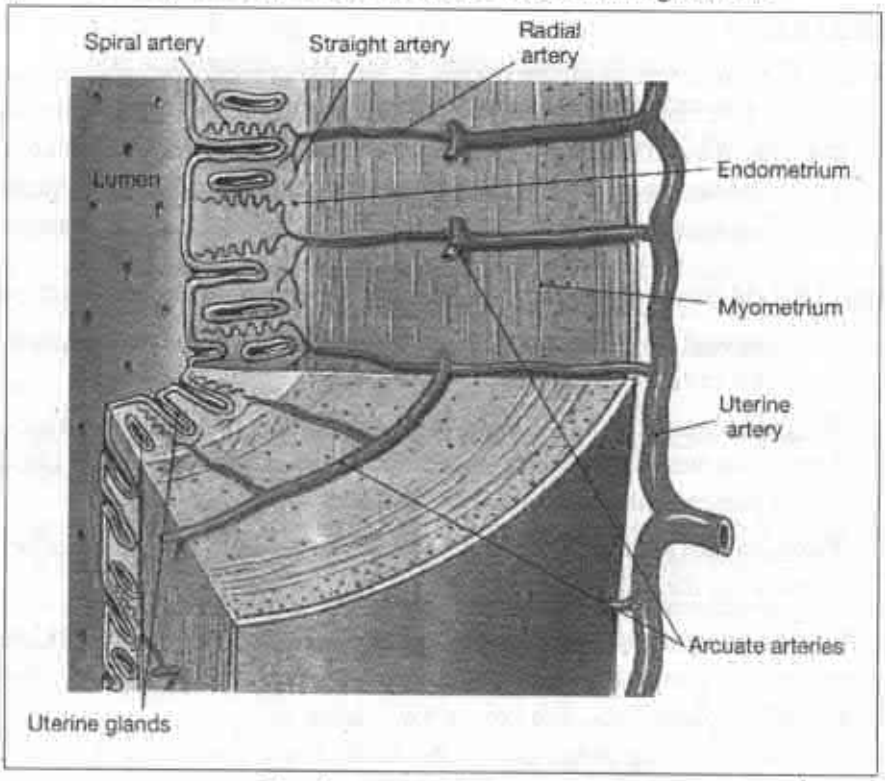
In the non-pregnant state, the smooth muscle fibers are 30-50 μm long, but during pregnancy they undergo hypertrophy and enlarge to 500μm. They also proliferate (hyperplasia) and the connective tissue increases too. Thus the myometrium thickens during pregnancy. After birth, the smooth muscle shrinks, the extra cells degenerate and are lost; the uterus returns to its ≈ original size.

ENDOMETRIUM is dependent on hormones for its appearance and maintenance. It consists of:

1. **The epithelium** which is usually **simple columnar ciliated** (with ciliated and secretory cells).
2. **The lamina propria** is composed of loose connective tissue full of neutrophils and lymphocytes.

The epithelium invaginates into the lamina propria to form **uterine glands** which are simple tubular nonbranched glands; ciliated cells are generally not present within the glands.

The lamina propria contains a rich system of blood vessels. The **blood supply of the endometrium** arises from the **uterine arteries** that run in the broad ligaments whose branches penetrate into the vascular layer of the myometrium to become **arcuate arteries**. Branches of the arcuate arteries penetrate into the stratum basalis; some are coiled and spiral as they ascend to the functionalis layer. These **spiral arteries** are dependent on ovarian hormones for their maintenance; during menses they undergo necrosis, bleed and are sloughed off.



Blood supply of the uterine wall

Throughout the reproductive life span, the endometrium undergoes cyclic changes each month that prepare it for the implantation of the embryo. Changes in the secretory activity of the endometrium during the cycle are correlated with the maturation of the ovarian follicles.

During reproductive life, the endometrium consists of 2 layers that differ in structure and function: **functional layer** and **basal layer**.

1. **Functional layer** – the thick part of the endometrium, which is sloughed off at menstruation and depends on ovarian hormones.
2. **Basal layer** – lies next to the myometrium and is not sloughed off. It serves as the source for regeneration of the functional layer.

MENSTRUAL CYCLE

The endometrium is **directly** controlled by OVARIAN hormones (estrogen, progesterone), **not** by pituitary hormones.

Menstrual cycle has 3 phases:

- **Proliferative phase** is regulated by estrogens.
- **Secretory phase** is under the control of progesterone.
- **Menstrual phase** results from a decline in the ovarian secretion of progesterone and estrogens.

PROLIFERATIVE PHASE – day 4 of cycle to day 14 (*until ovulation*)

- corresponds to the ***follicular phase*** of the ovarian cycle.
- under the control of **estrogenes** the stromal and epithelial cells in the stratum basalis begin to proliferate.
- epithelial cells in the basal portion of glands rapidly proliferate, reconstituting the glands and migrating to cover the denuded endometrial surface; the glands lengthen but remain tubular and straight.
- stroma, glands, spiral arteries grow toward lumen.

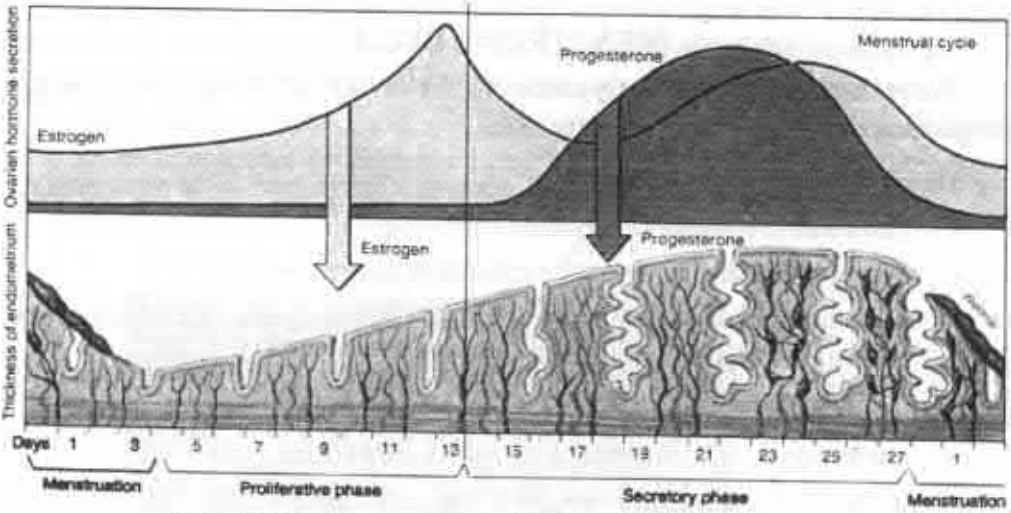
SECRETORY PHASE – day 14 to day 28 (*menses*)

- corresponds to the ***luteal phase*** of the ovarian cycle.
- under the control of **progesterone** the uterine glands become intensely coiled, with large lumin. They begin to secrete glycogen and mucin.
- arteries become more coiled, extend nearly to the surface of the endometrium.
- the stromal cells transform in decidual cells (rich in glycogen).

- The functionalis layer can now provide a nutritive environment for a fertilized ovum ***(blastocyst)***.

MENSTRUAL PHASE – day 1 – 4 of cycle

- If implantation of a blastocyst does not occur, progesterone levels decrease because the corpus luteum degenerates. The spiral arteries located between the glands in the functional layer become constricted and vascular stasis occurs.
- A reduction in the normal blood supply-causing intermittent ischemia-and the consequent hypoxia determine the necrosis of the functional layer of the endometrium, which sloughs off during the menstrual phase.



Schematic diagram illustrating the morphological changes in the endometrium during the menstrual cycle

***Clinical consideration*:**

ORAL CONTRACEPTIVES: Most consist of a combination of estrogens and progesterone, which fool the pituitary into thinking that a woman is pregnant; therefore it does not release hormones that stimulate the ovary. Contraceptive estradiol inhibits ovulation by suppressing FSH and LH; progesterone also inhibits ovulation by suppressing LH (therefore no LH surge occurs).

CERVIX of the uterus:

The internal surface of the cervix (**endocervix** or **cervical canal**) is covered by a **simple columnar epithelium** that secretes mucous and invaginates into the cervical wall to form branched tubular mucous-secreting glands which lubricate

the vagina. The consistency of the mucous depends on ovarian hormones – generally, it is quite viscous, and is able to impede sperm and bacteria from entering the uterus. However, during ovulation the amount of mucous increases 10-fold and the secretion is thin; this enhances sperm entry into the uterus. The cervical mucosa is **not** sloughed off during menstruation.

The wall of the cervix is composed of very dense connective tissue and smooth muscle. The epithelium changes at the external surface or **ectocervix** which projects into the vagina. Here, it is covered by **stratified squamous epithelium** continuous with the vaginal epithelium. This transition zone is utilized for Pap smears, as it is the primary site of cervical cancer.

VAGINA

The vagina is a fibromuscular tube. The **mucosa** consists of non-keratinized, stratified squamous epithelium with an underlying lamina propria. The lamina propria contains many leukocytes, many **elastic fibers**, a large **venous plexus** and **NO GLANDS**. Lubrication is provided by the cervical glands and by the vestibular mucous glands (present at the opening [vestibule] of the vagina; Bartholin's glands). The epithelial cells are continually desquamated and contain much **glycogen** when estrogen levels are high (ovulation). Bacteria in the vagina ferment the glycogen to form **lactic acid**, thus lowering the pH. The acidic environment inhibits the growth of some pathogenic microorganisms. Post-menopausal women do not secrete much glycogen because of their low estradiol levels; the subsequent higher vaginal pH can lead to increased vaginal infections.

The **muscular layer** consists of a thin inner circular layer and a thicker layer of longitudinally-directed smooth muscle fibers. Inferiorly, the striated, voluntary bulbospongiosus muscle forms a sphincter around the vagina.

An outer **adventitial layer** also contains numerous elastic fibers, blood and lymphatic vessels, and nerves.

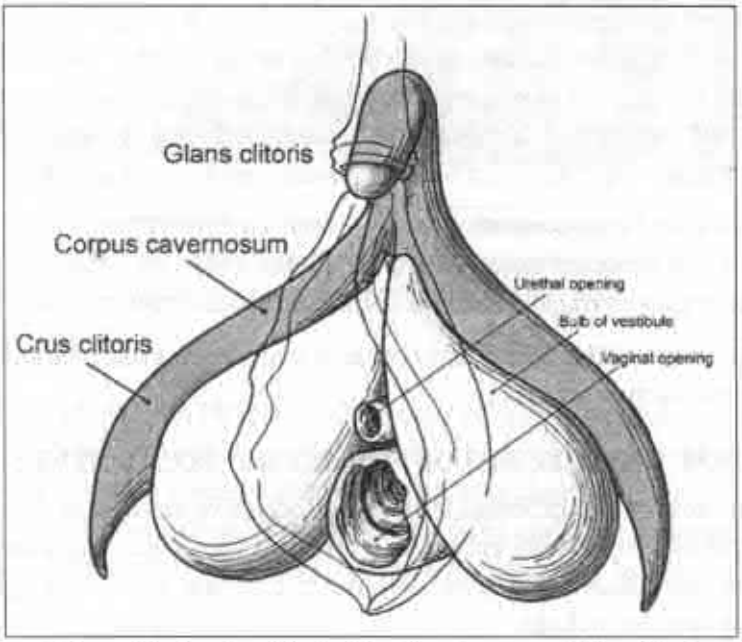
EXTERNAL STRUCTURES OF FEMALE REPRODUCTIVE SYSTEM

The function of the external female reproductive structures (the genital) is twofold: To enable sperm to enter the body and to protect the internal genital organs from infectious organisms. The main external structures of the female reproductive system include:

- **Labia majora**: The labia majora enclose and protect the other external reproductive organs. Literally translated as "large lips," the labia majora are relatively large and fleshy, and are comparable to the scrotum

in males. The labia majora contain sweat and oil-secreting glands. After puberty, the labia majora are covered with hair. The inner surface of the labia is soft, smooth and hairless.

- **Labia minora**: Literally translated as "small lips," the labia minora can be very small or up to 2 inches wide. They lie just inside the labia majora, and surround the openings to the vagina and urethra. Each is a long, high but relatively thin fold of mucous membrane. The labia minora contain sebaceous glands on both of it surfaces.
- **Bartholin's glands**: These glands are located next to the vaginal opening and produce a fluid (mucus) secretion. These glands correspond to the bulbo-urethral glands in the male.
- **Clitoris**: The two labia minora meet at the clitoris, a small, sensitive protrusion that is comparable to the penis in males. The clitoris is covered by a fold of skin, called the prepuce, which is similar to the foreskin at the end of the penis. Like the penis, the clitoris is very sensitive to stimulation and can become erect. It has two cavernous, erectile bodies and a rudimentary glans. It lacks a urethra and corpus spongiosum.



Schematic drawing illustrating the structure of the clitoris

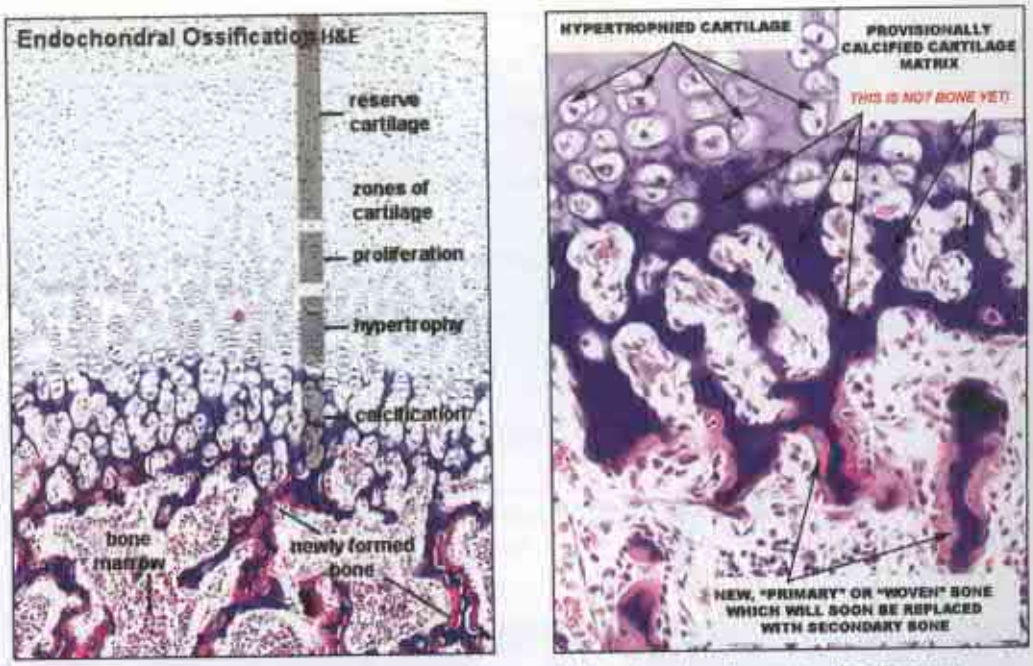


Fig.56. Photomicrographs of the epiphyseal plate, showing its zones, the changes that take place in the cartilage, and the formation of bone

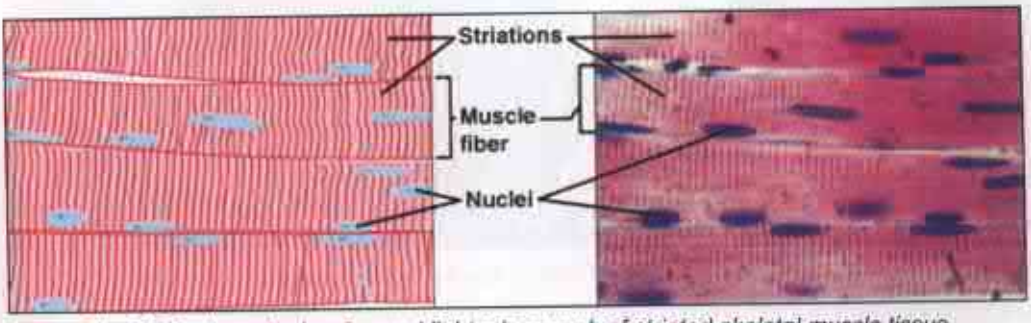


Fig. 57. Schematic drawing and light micrograph of striated skeletal muscle tissue

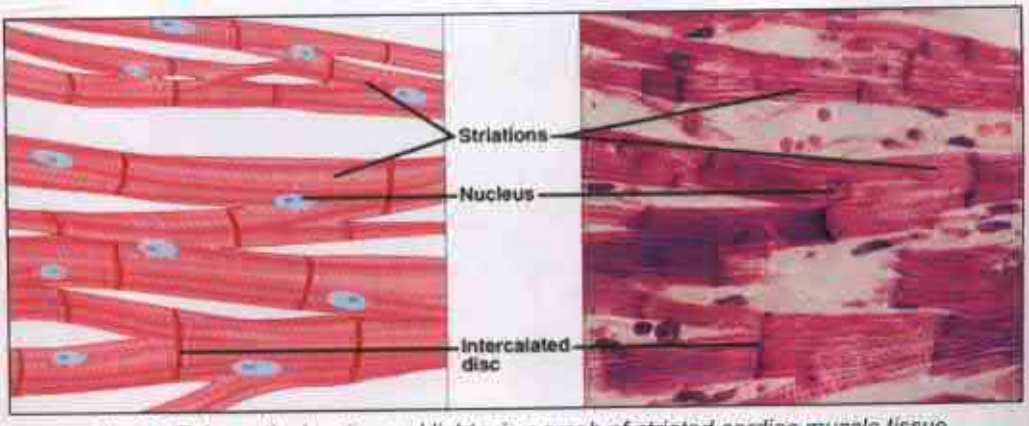


Fig. 58. Schematic drawing and light micrograph of striated cardiac muscle tissue

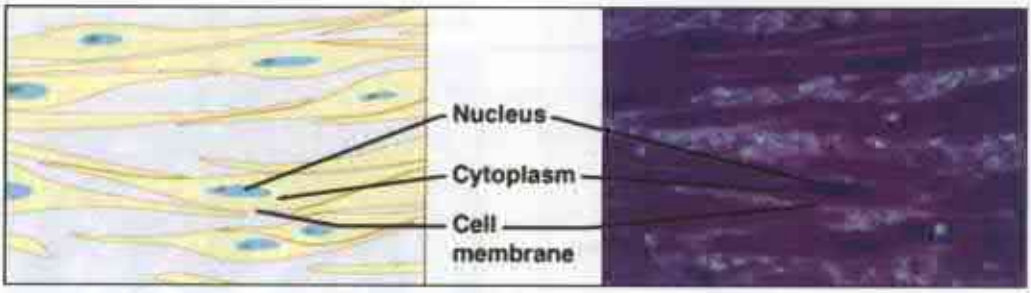


Fig. 59. Schematic drawing and light micrograph of smooth muscle tissue

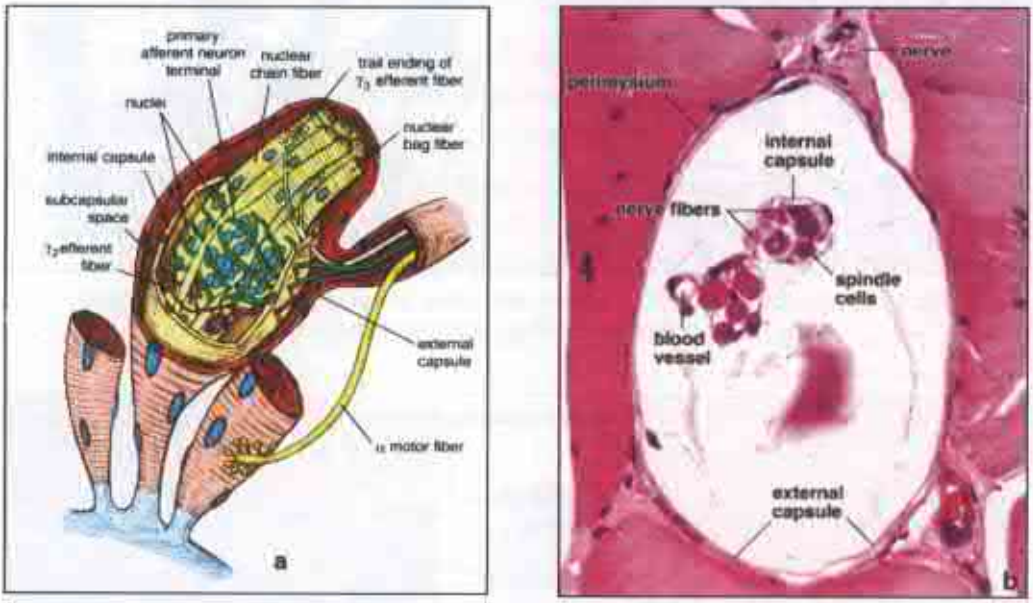


Fig. 60. Schematic drawing (a) and light micrograph (b) showing the structure of a muscle spindle

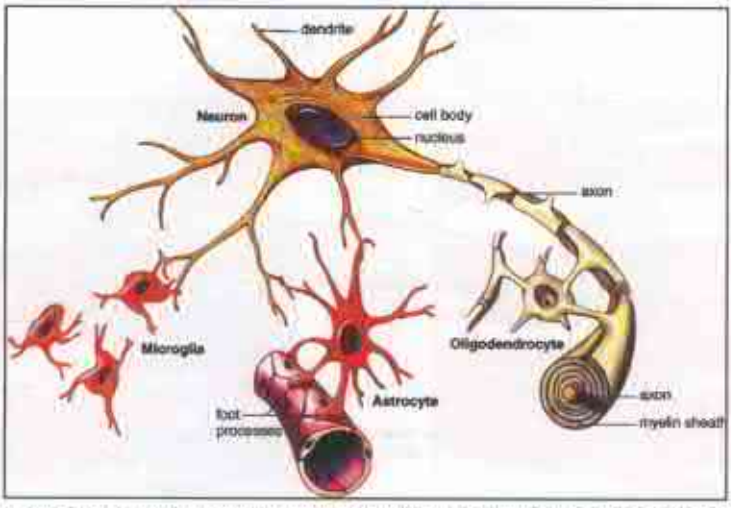


Fig. 61. Schematic diagram illustrating relationship between neuron and glial cells

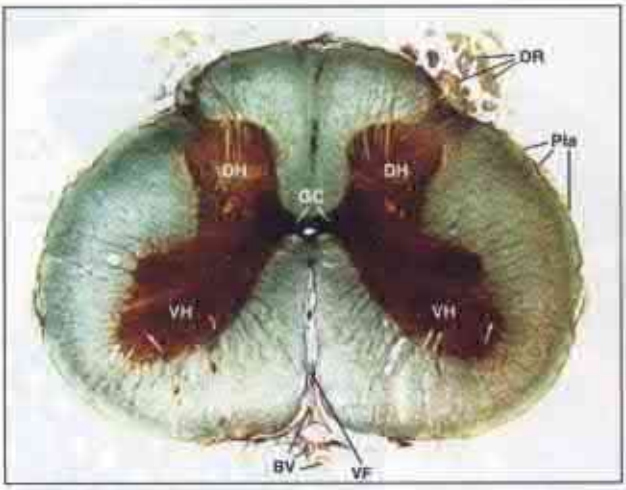


Fig. 62. Cross section through the lumbar region of the spinal cord

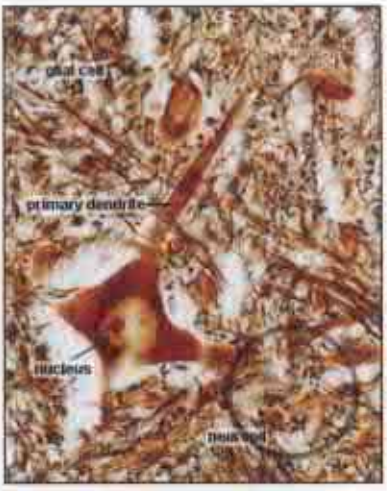


Fig. 63. This preparation shows a ventral motor neuron

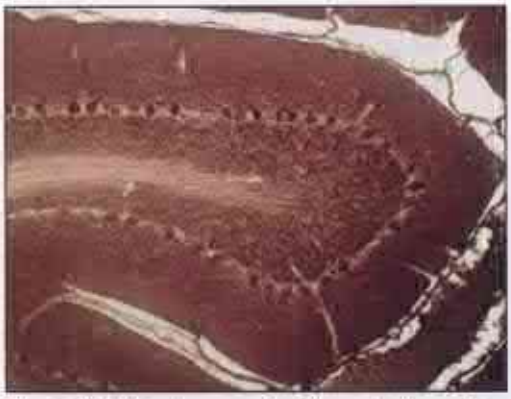


Fig. 64. Light micrograph of the cebellar cortex

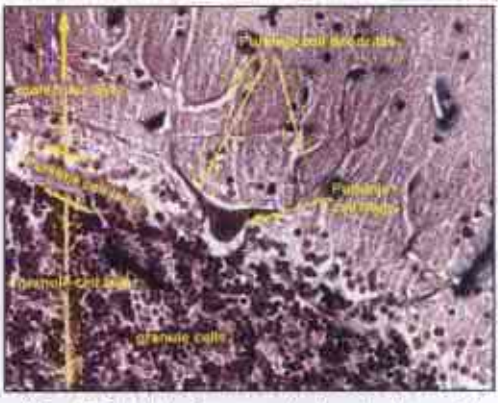


Fig. 65. Light micrograph showing the cell types of the cebellar cortex

Fig. 66. Light micrograph of the cerebral cortex

Fig. 67. The structure of the giant Betz cell

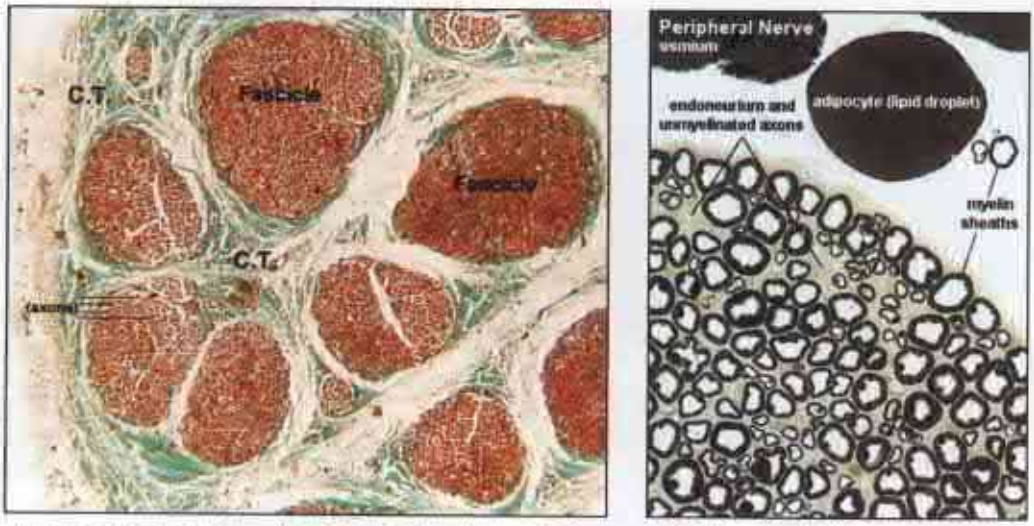


Fig. 68. Photomicrographs of a peripheral nerve in cross section (low and high magnification)

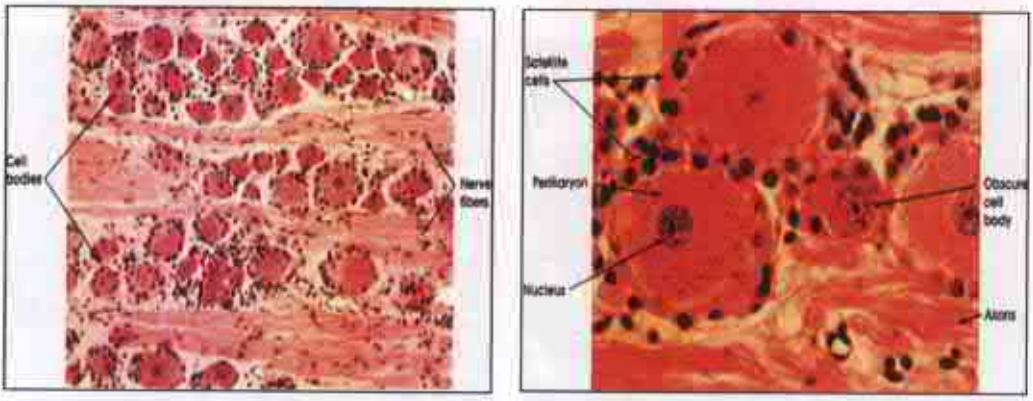


Fig. 69. Photomicrographs of a spinal ganglion (high and low magnifications)

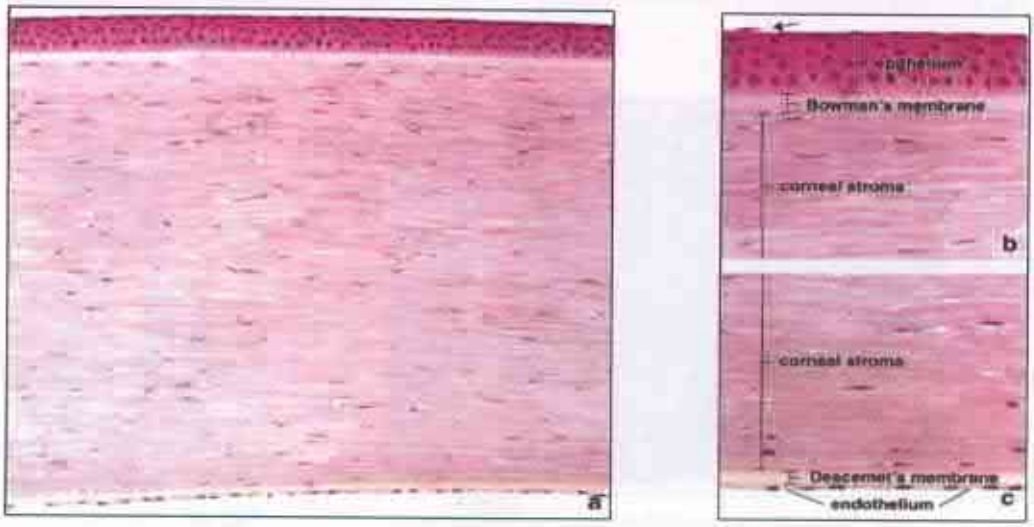


Fig. 70. Photomicrograph of a cornea (section through the full thickness of the cornea)

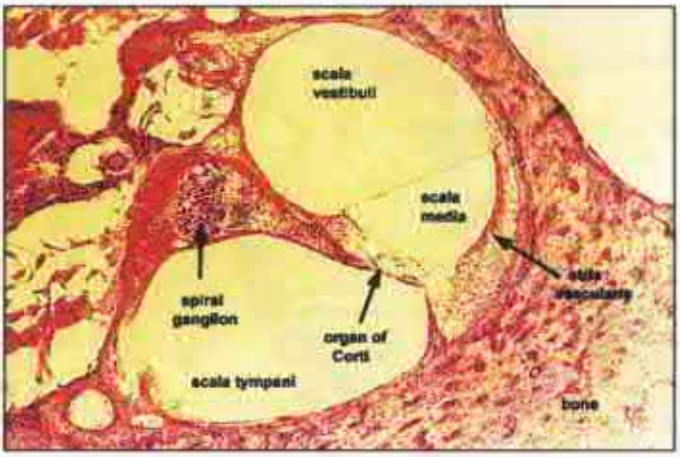


Fig. 71. Light micrograph of a cochlea

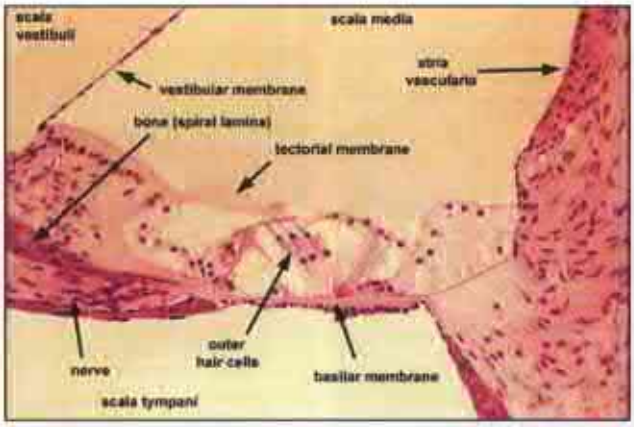


Fig. 72. Light micrograph showing the structure of an organ of Corti

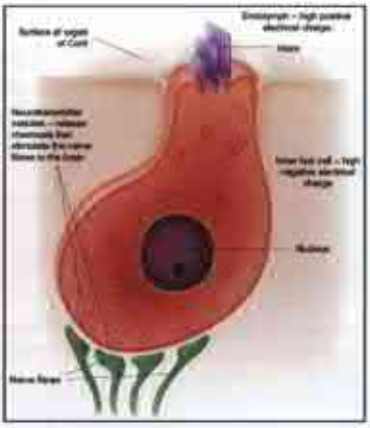


Fig. 73. Schematic view of structure of inner hair cell

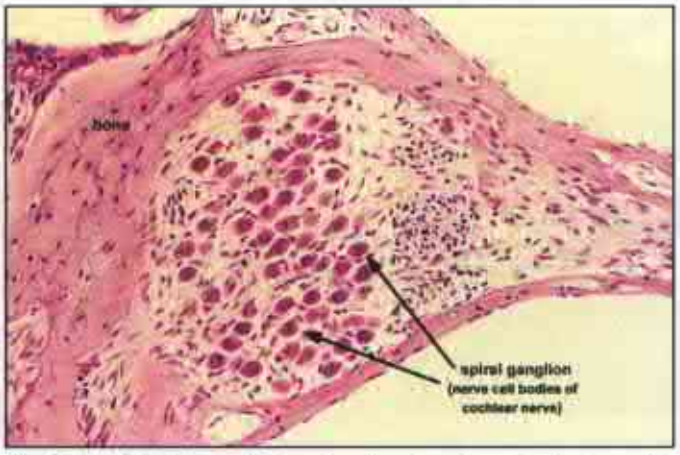


Fig. 74. Light micrograph showing the structure of spiral ganglion

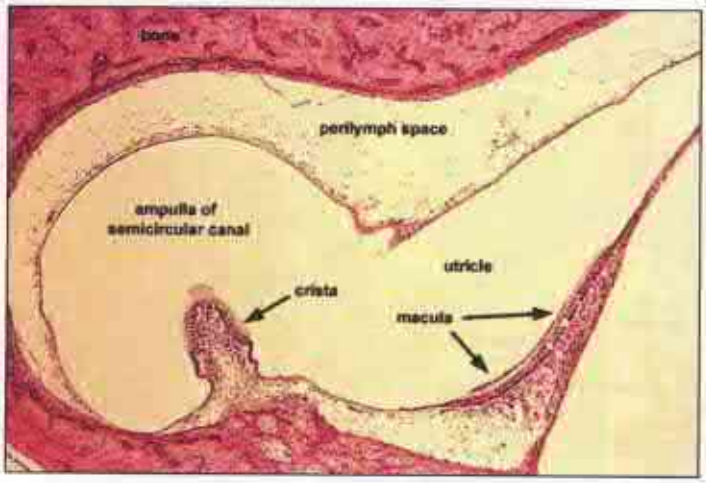


Fig. 75. Light micrograph of a portion of the macula utriculae and ampulla of semicircular canal

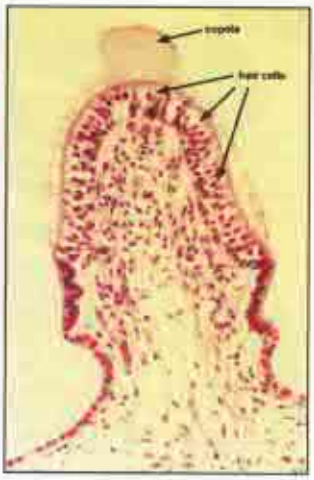


Fig. 76. Photomicrograph of a crista ampullaris

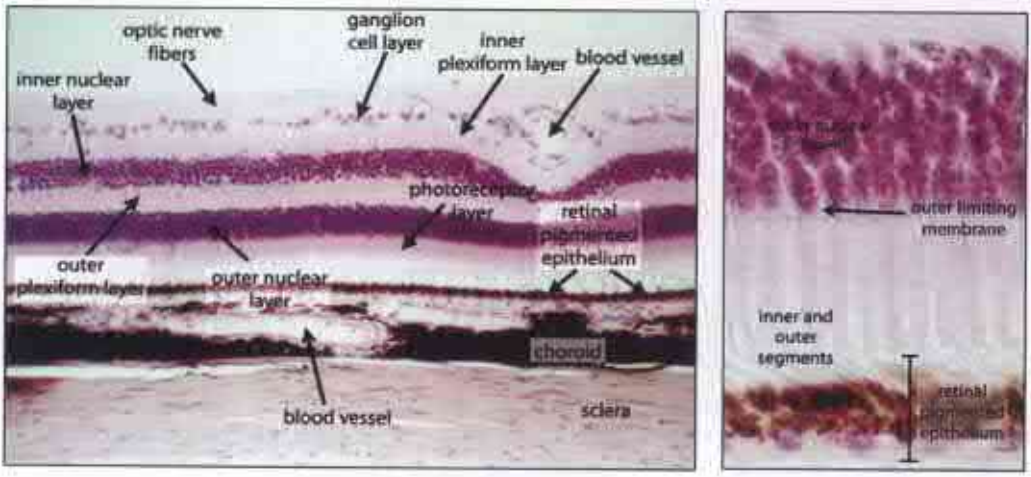


Fig. 77. Photomicrographs of the retina

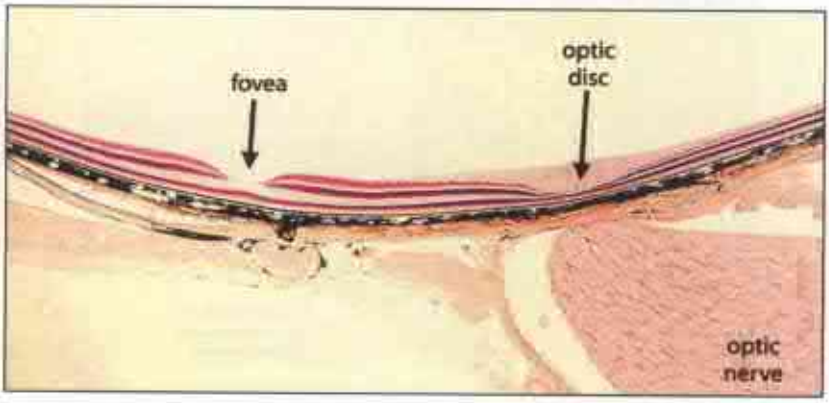


Fig. 78. Photomicrograph of the retina showing a fovea and optic disc

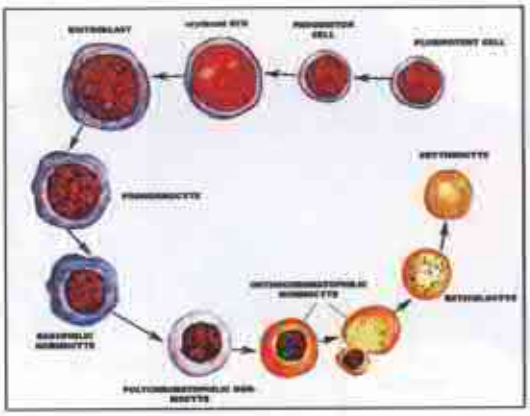


Fig. 79. Summary of erythrocyte maturation

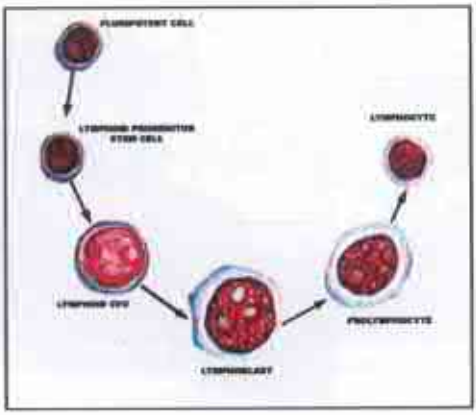


Fig. 80. Summary of lymphocyte maturation

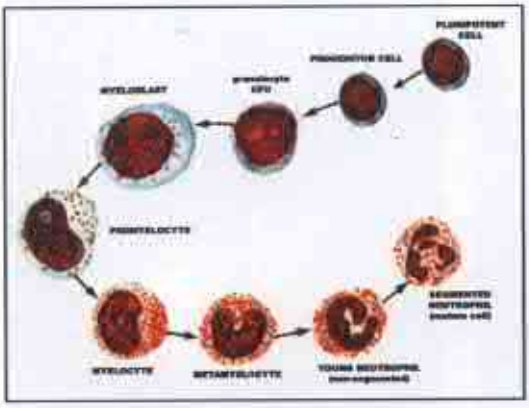


Fig. 81. Summary of granulocyte maturation

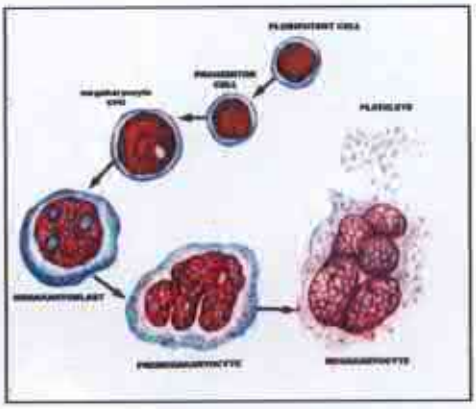


Fig. 82. Summary of development of platelets

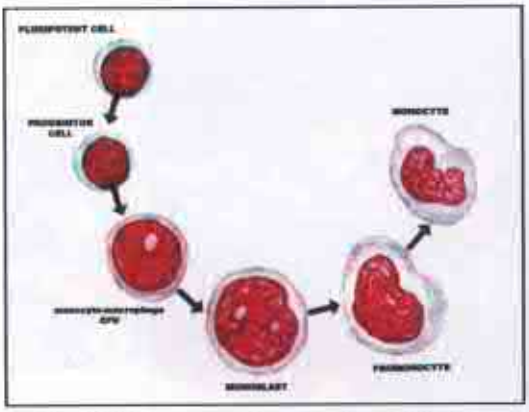


Fig. 83. Summary of monocyte maturation

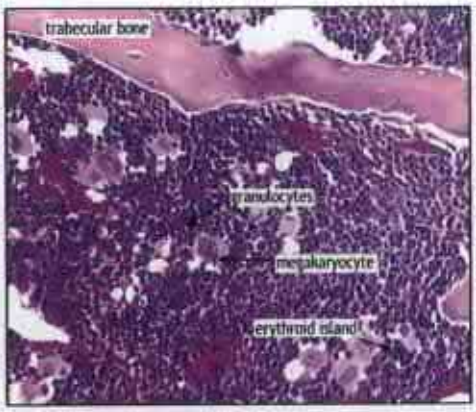


Fig. 84. Light micrograph of a red bone marrow

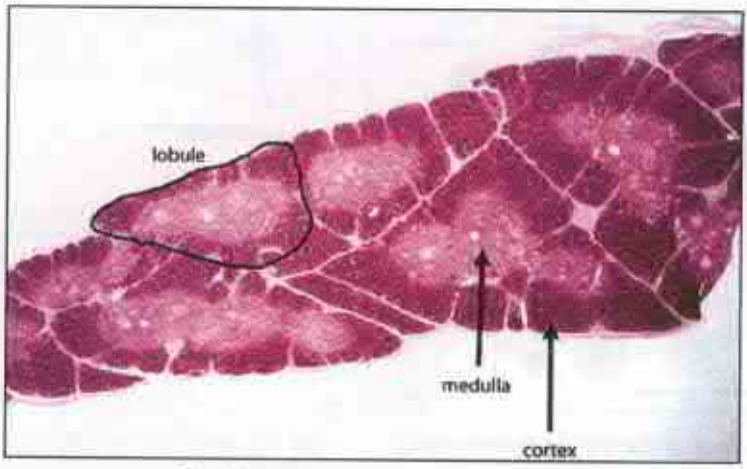


Fig. 85. Light micrograph of a thymus

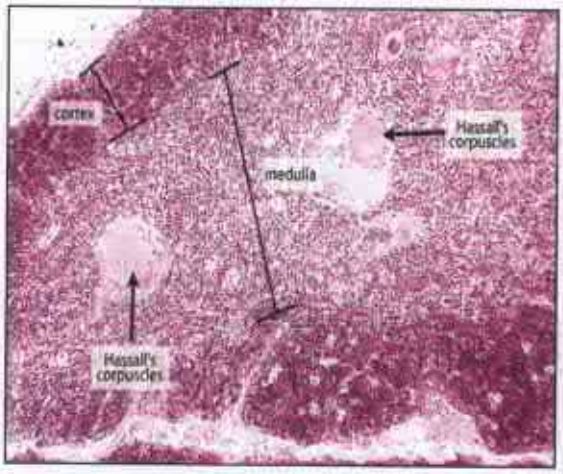


Fig. 86. Light micrograph of a thymic lobule

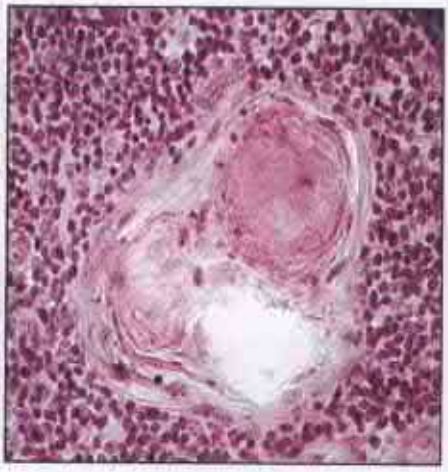


Fig. 87. Photomicrograph of a thymic corpuscle

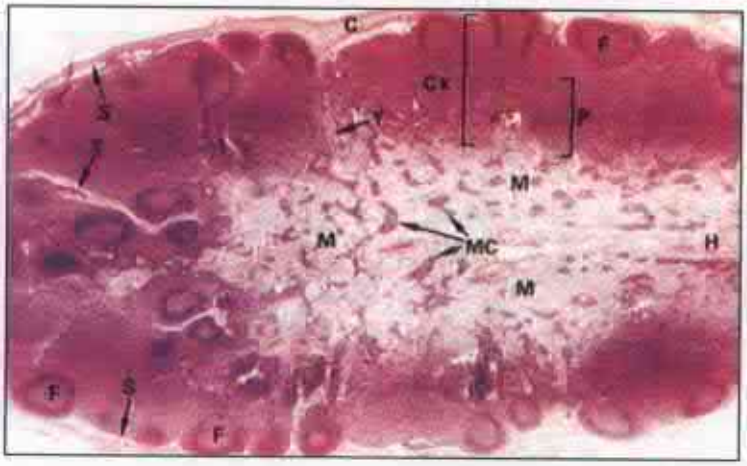


Fig. 88. Light micrograph of a lymph node

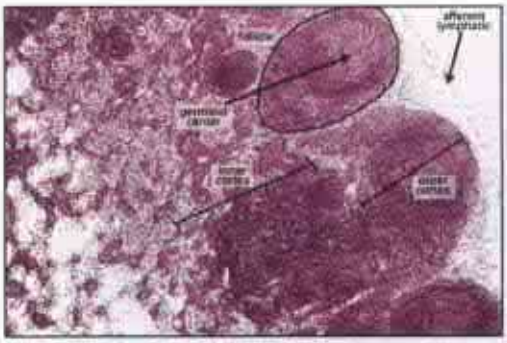


Fig. 89. Section of a portion of the cortex of a lymph node

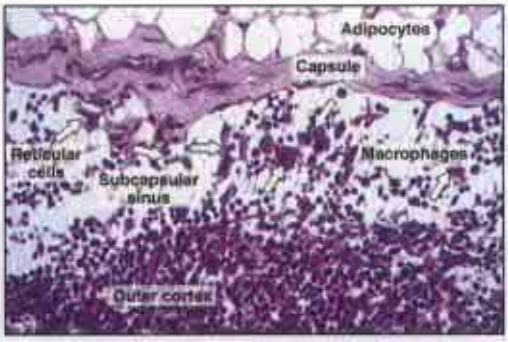
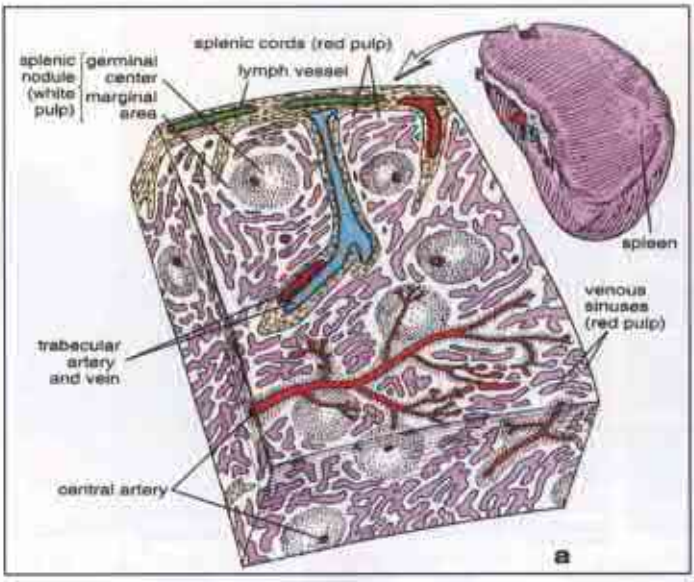


Fig. 90. Photomicrograph showing the subcapsular sinuse



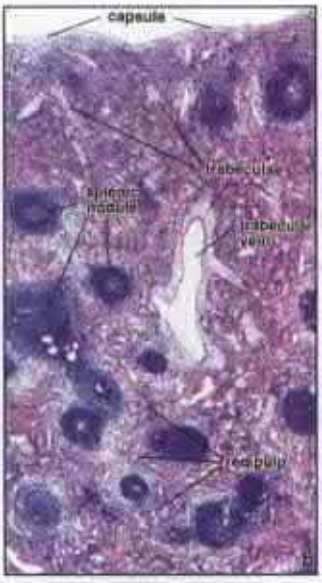


Fig. 91. Schematic drawing (a) and light micrograph (b) of spleen structure

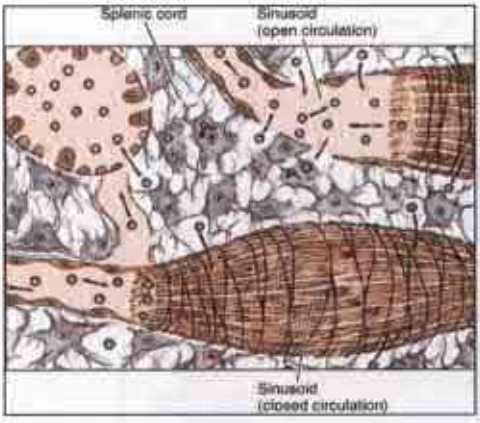


Fig. 92. Schematic view of the blood circulation in the spleen

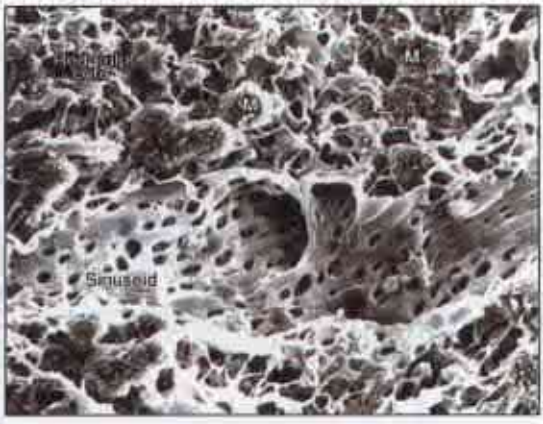


Fig. 93. Discontinuous capillaries (sinusoids)

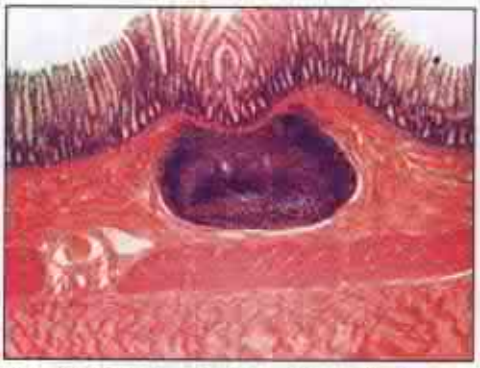


Fig. 94. The lymphoid nodule of the large intestine

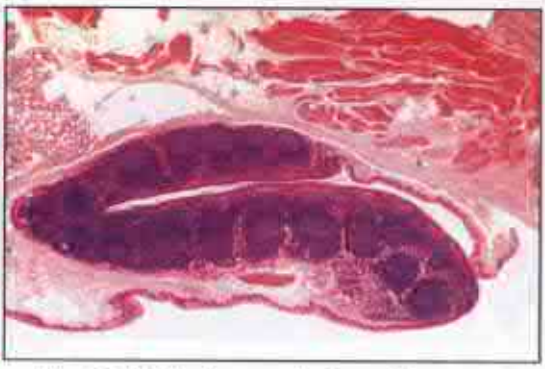


Fig. 95. Light micrograph of a palatine tonsil

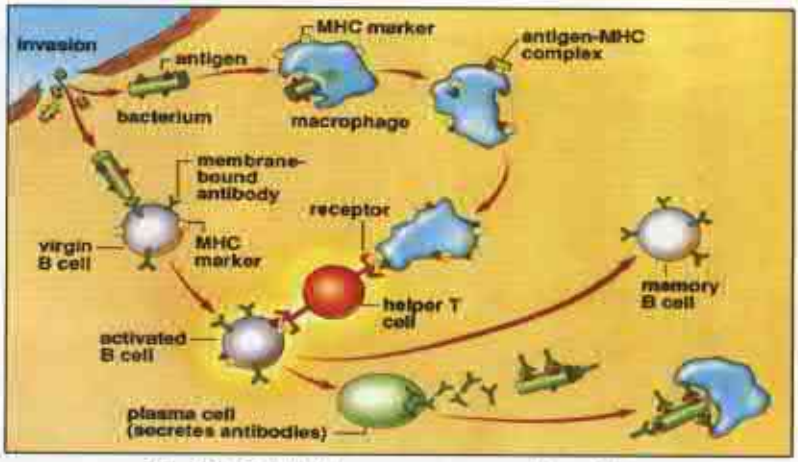


Fig. 96. Detailed immune response description

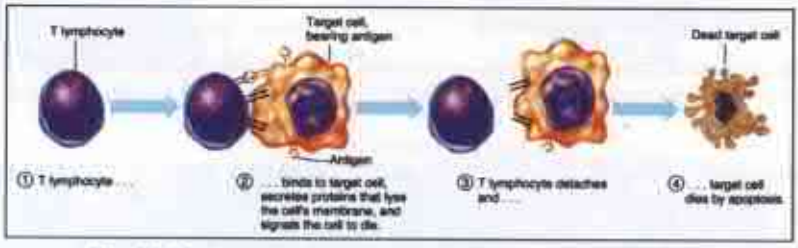


Fig. 97. Schematic diagram of activation of T lymphocytes

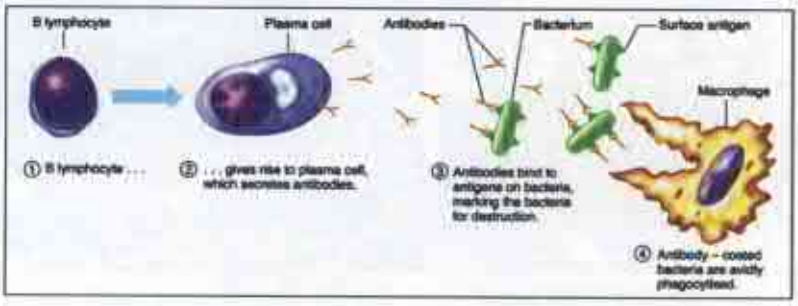


Fig. 98. Schematic diagram of activation of B lymphocytes

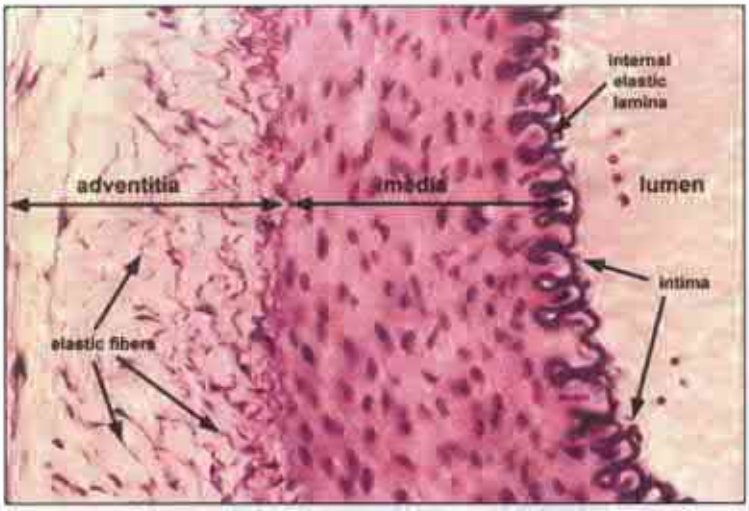


Fig. 99. Transverse section showing part of a muscular artery

Fig. 100. Internal elastic lamina

Fig. 101. Transverse section of a large elastic artery showing a well-developed tunica media

Fig. 102. Concentric fenestrated lamellae (SEM)

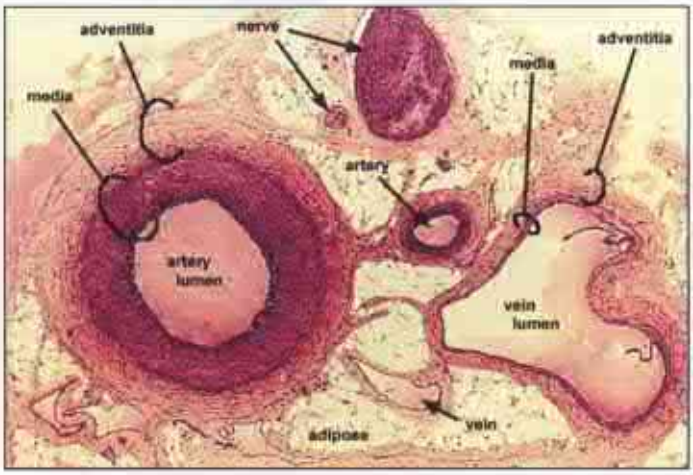


Fig. 103. Light micrograph showing muscular artery and muscular vein

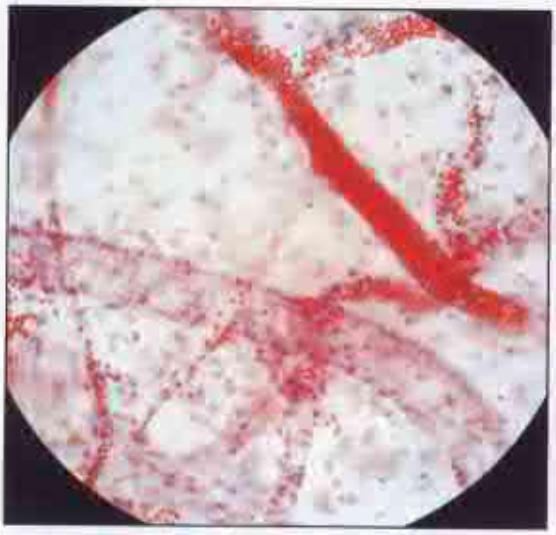


Fig. 104. Light micrograph of microcirculatory bed (arterioles, vnules, and capillaries)

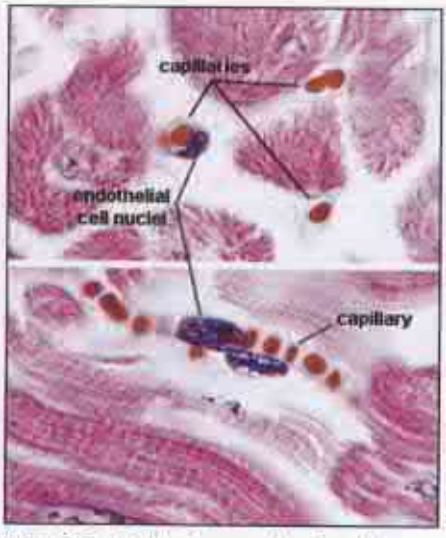


Fig. 105. Light micrograph of continuous capillaries

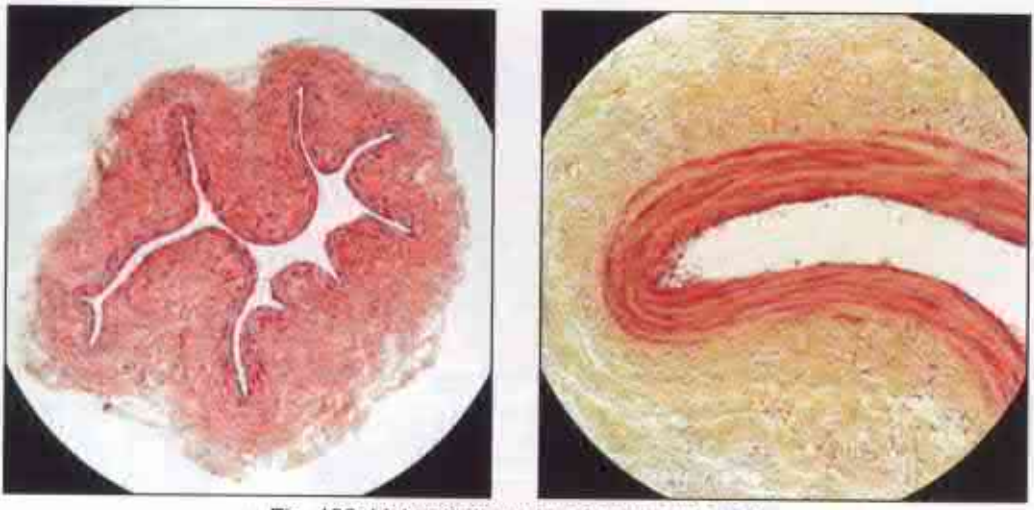


Fig. 106. Light micrographs of a muscular vein

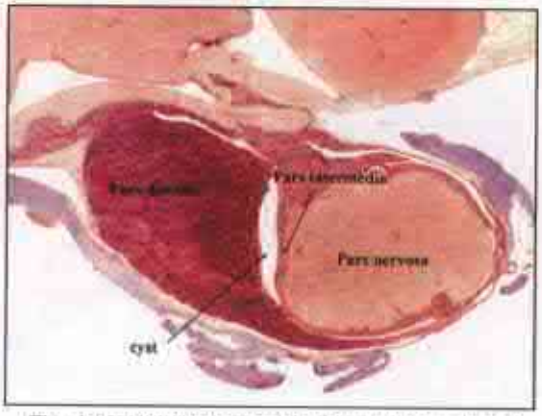


Fig. 107. Photomicrograph of a pituitary gland

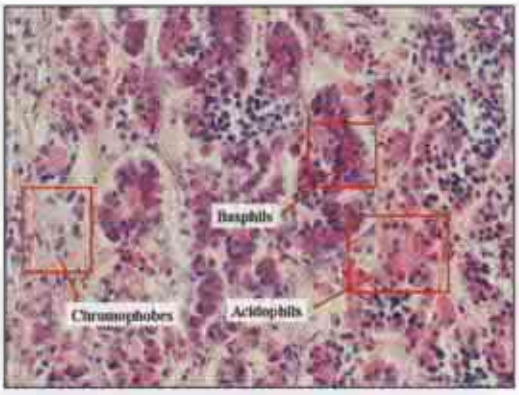


Fig. 108. Light micrograph and schematic drawing which show cells of pars distalis of adenohypophysis

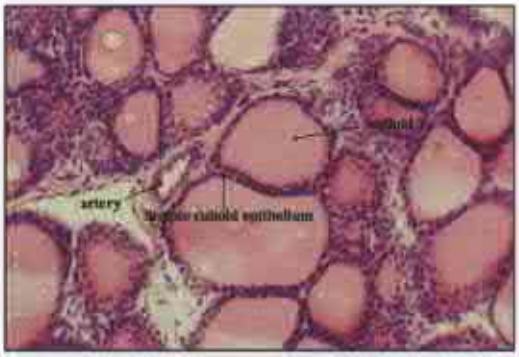


Fig. 109. Photomicrograph of a thyroid gland

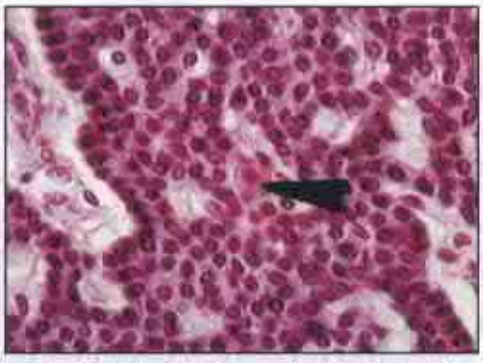


Fig. 110. Photomicrograph of parathyroid gland showing oxyphil cell

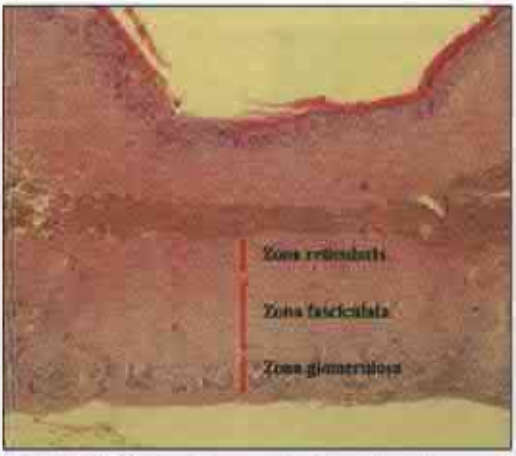


Fig. 111. Photomicrograph of a adrenal gland

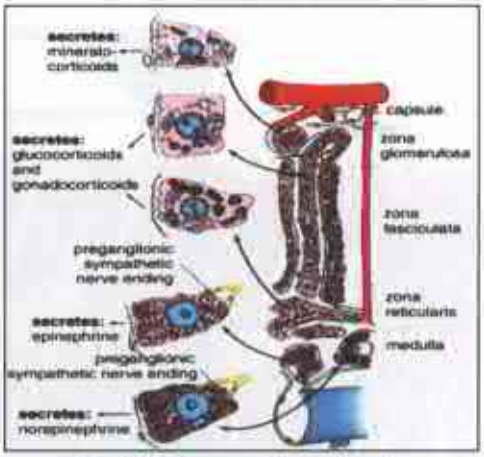


Fig. 112. Diagram illustrating the organization of the cells within the adrenal gland and their relationship to the blood vessels

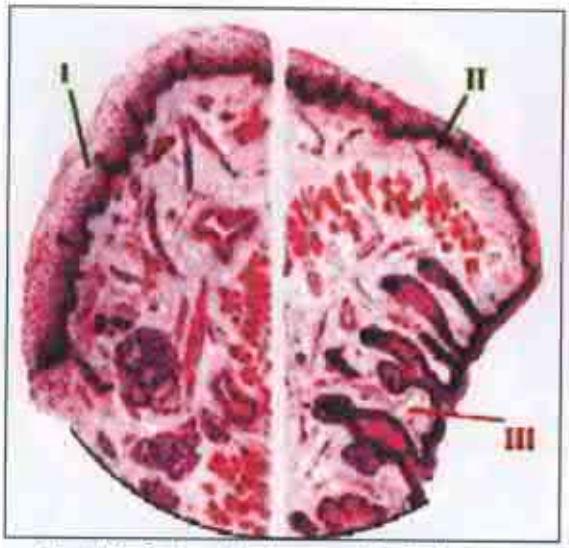


Fig. 113. Schematic diagram of a lip (I – inner surface; II -"red" area; III - cutaneous area)

Fig. 114. Light micrograph of the taste bud

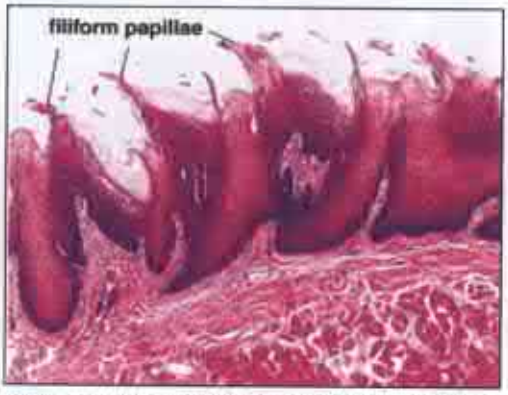


Fig. 115. Light micrograph of filiform papillae

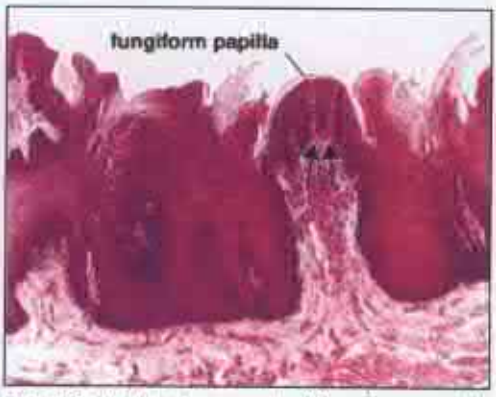


Fig. 116. Light micrograph of fungiform papilla

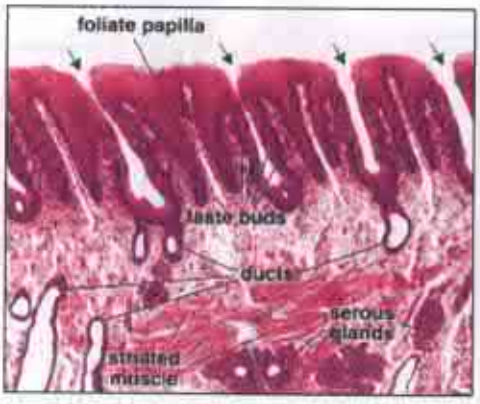


Fig. 117. Light micrograph of foliate papillae

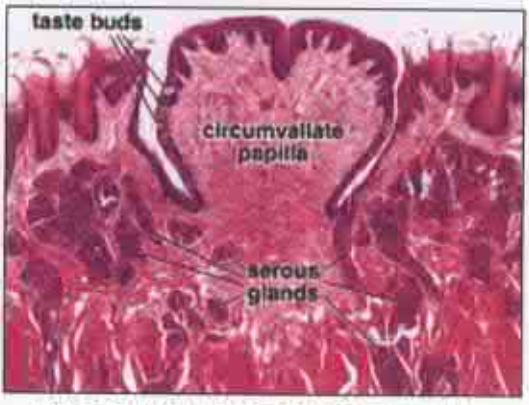


Fig. 118. Light micrograph of circumvallate papilla

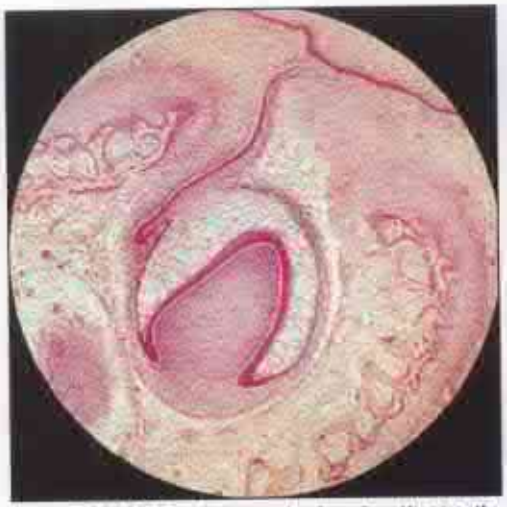


Fig. 119. Light micrograph showing the tooth development (cap stage; proliferation stage)

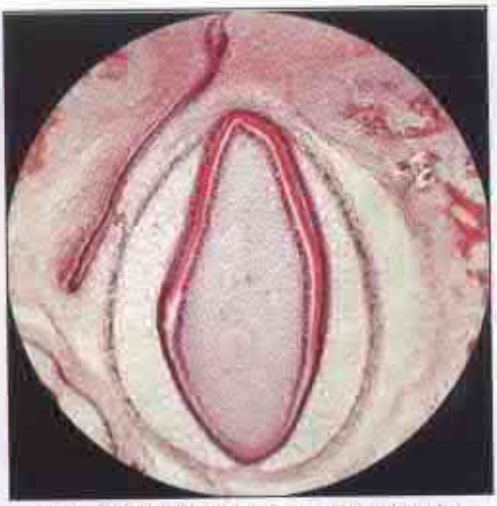


Fig. 120. Light micrograph showing the tooth development (bell stage; histo and morphodifferentiation stage)

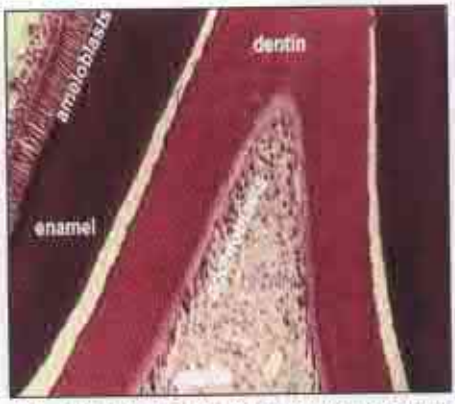


Fig. 121. Dental germ in the crown stage

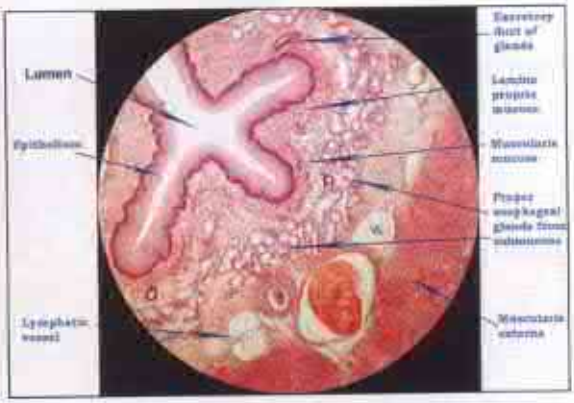


Fig. 122. Light micrograph of esophagus

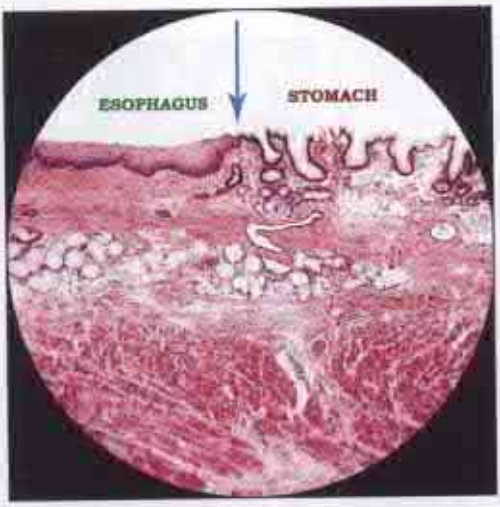


Fig. 123. Light micrograph of a gastro-esophageal junction

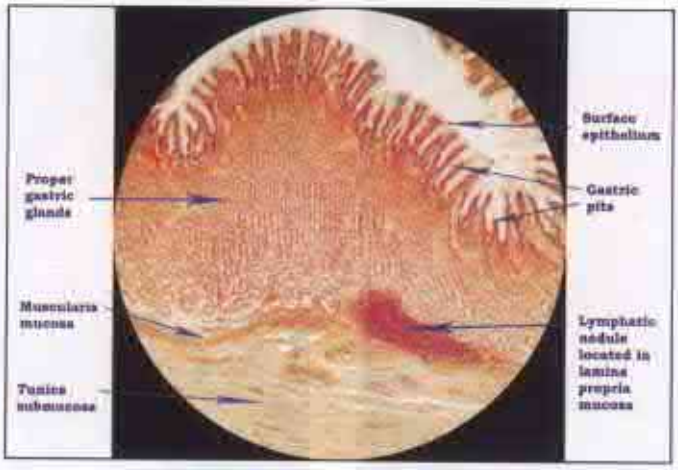


Fig. 124. Light micrograph of a stomach (fundic region)

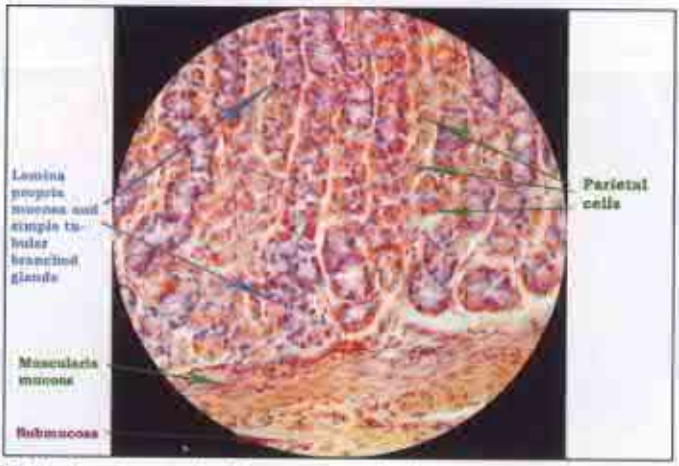


Fig. 125. Light micrograph of proper gastric glands showing the parietal cells

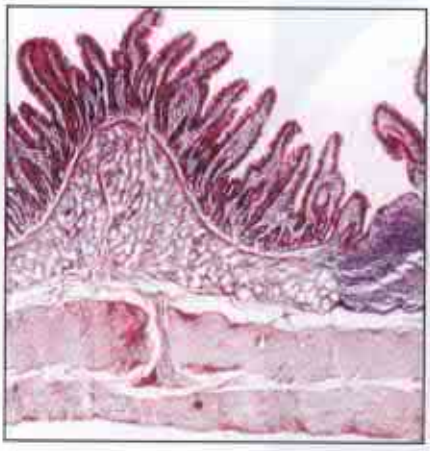


Fig. 126. Light microphotograph of a duodenum

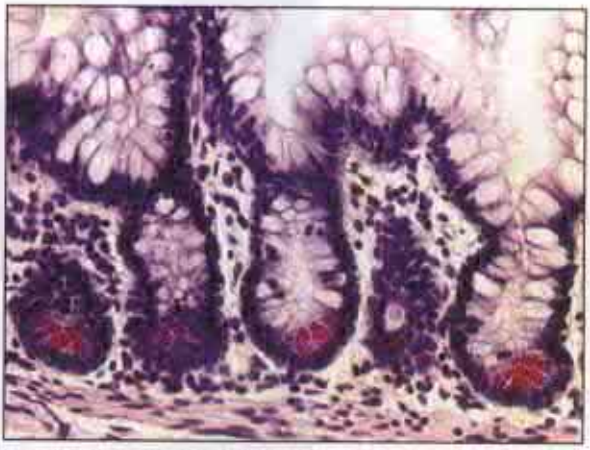


Fig. 127. Light micrograph of intestinal crypts showing Paneth cells

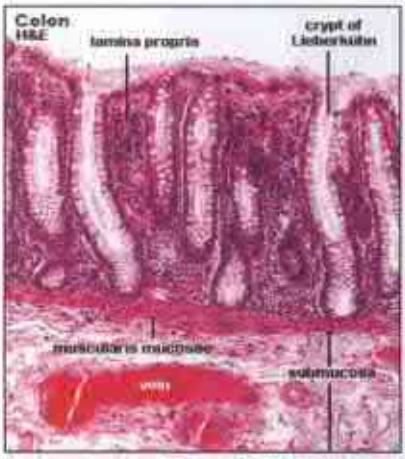


Fig. 128. Light microphotograph of a large intestine

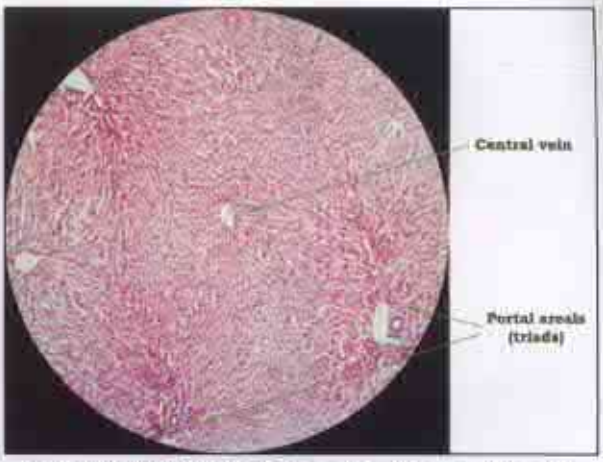


Fig. 129. Light microphotograph of classical hepatic lobule

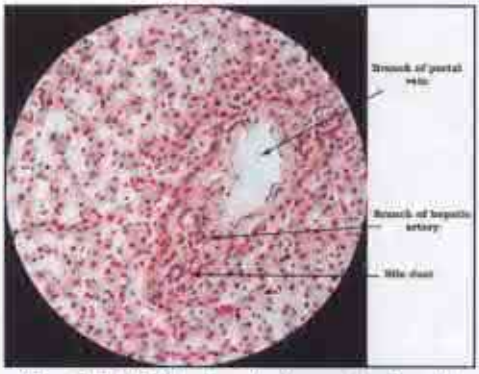


Fig. 130. Light microphotograph of portal area (hepatic triad)

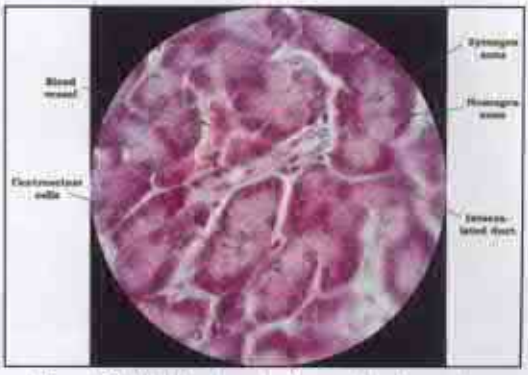
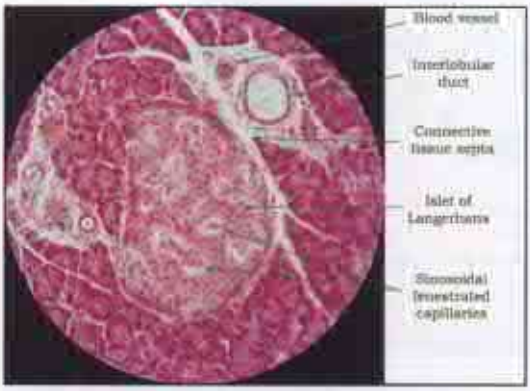


Fig. 131. Light microphotograph of exocrine pancreas



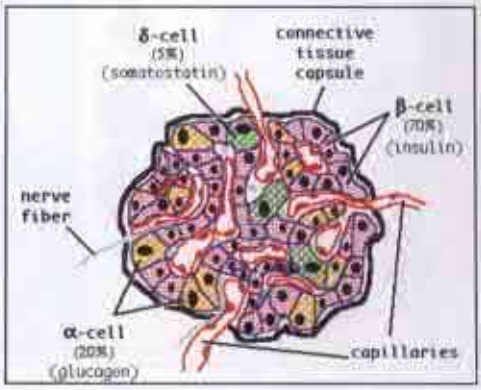


Fig. 132. Photomicrograph and schematic drawing of a pancreatic endocrine islet

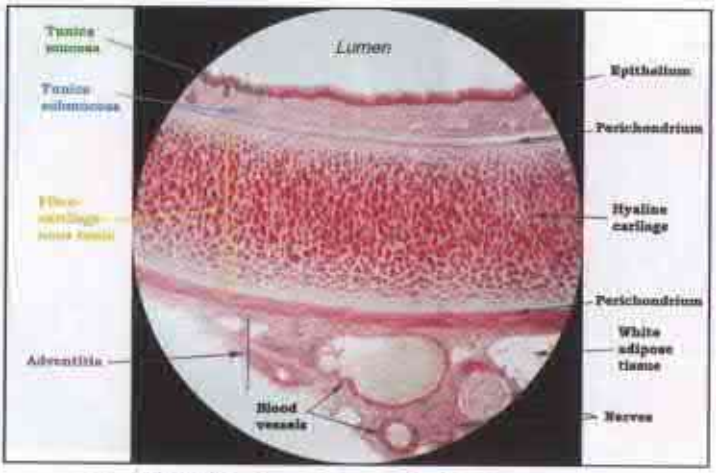


Fig. 133. Light microphotograph of trachea structure

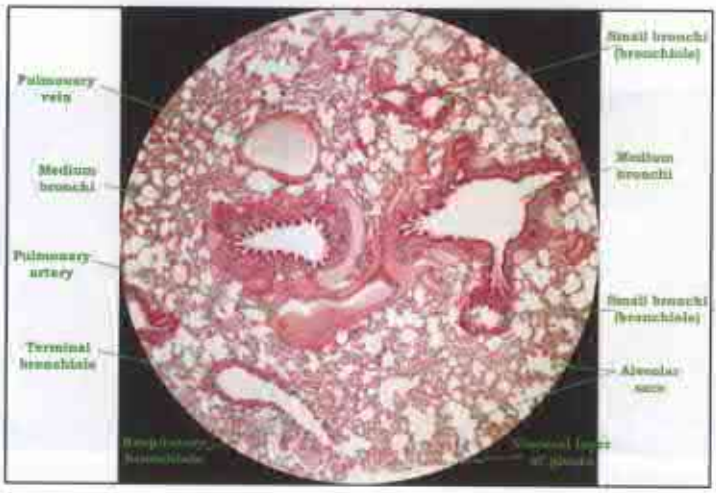


Fig. 134. Light photomicrograph of a lung

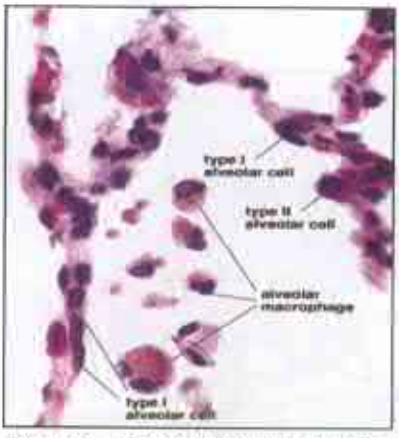


Fig. 135. Photomicrograph showing cells of the alveolar wall

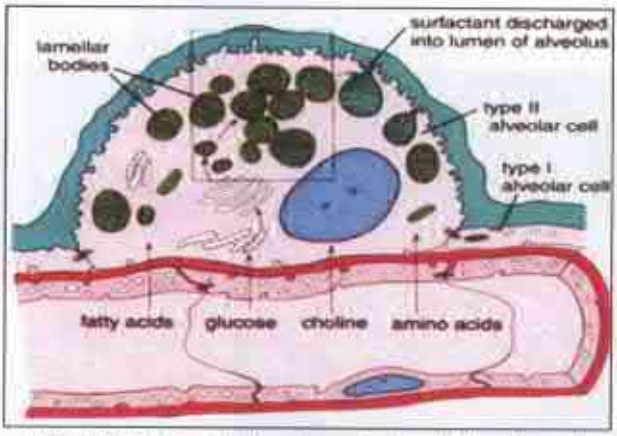


Fig. 136. Schematic drawing of a type II alveolar cell

Fig. 137. Light micrograph of a thick skin

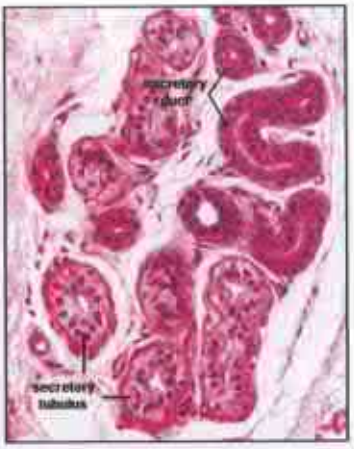


Fig. 138. Light micrograph of a sweat gland

Fig. 139. Light micrograph of a hair follicle and other skin appendages

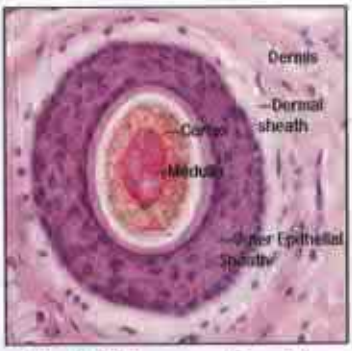


Fig.140. Cross section of the hair

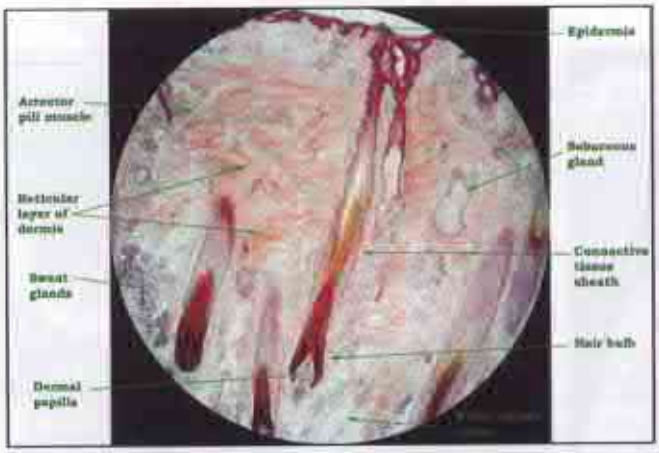


Fig. 141. Light micrograph of thin skin with hairs

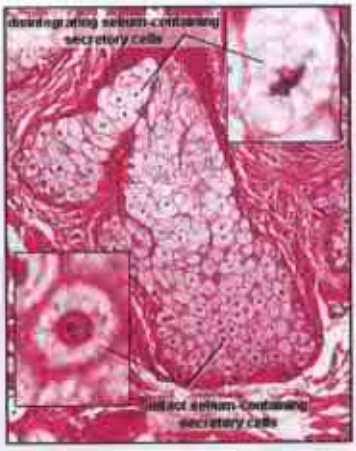


Fig. 142. Light micrograph of a sebaceous gland

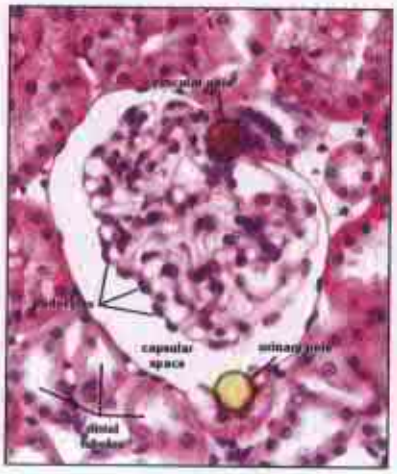


Fig. 143. Light micrograph of a renal corpuscle

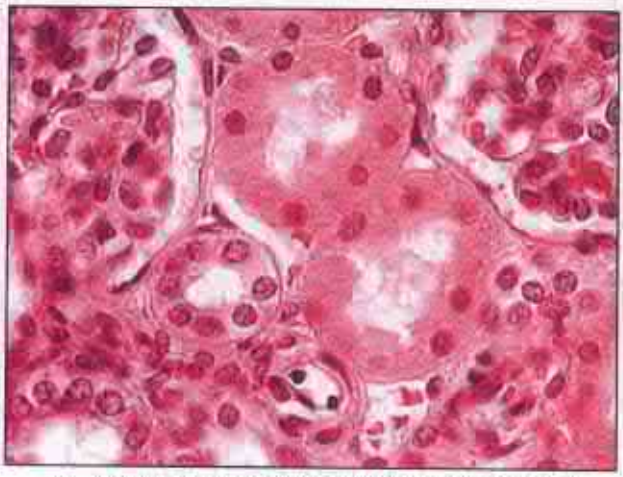


Fig. 144. Light micrograph of kidney showing renal tubules

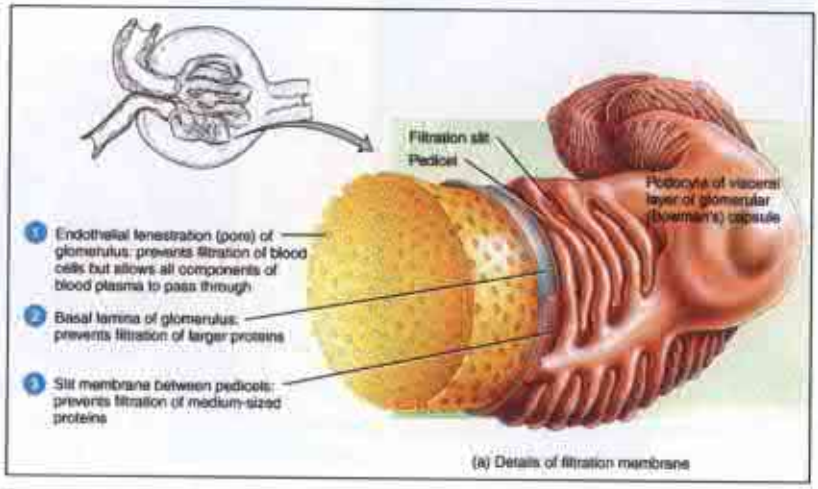


Fig. 145. Schematic representation of the filtration barrier in a renal corpuscle

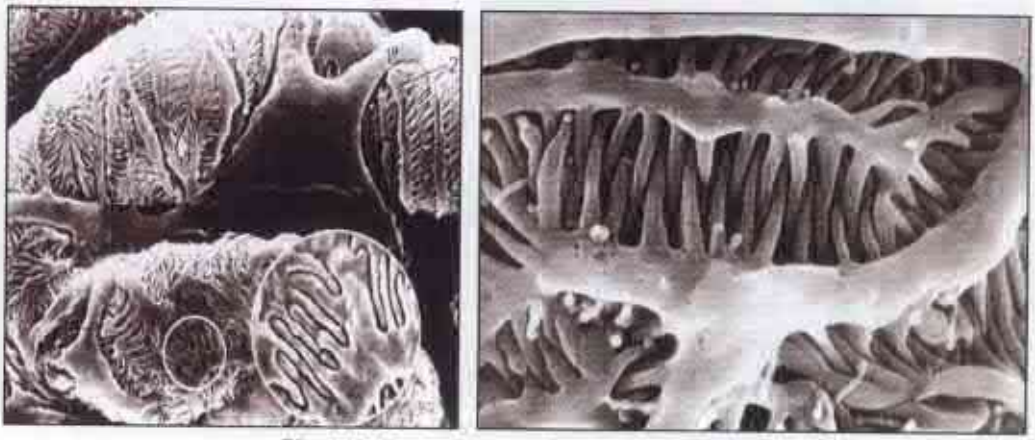


Fig. 146. Podocytes and filtration slits (SEM)

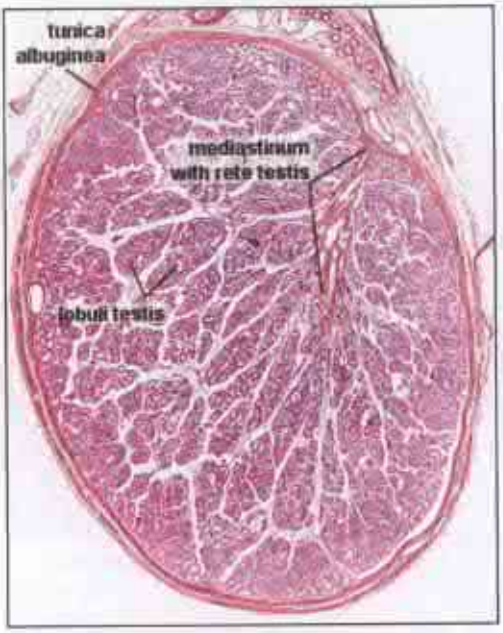
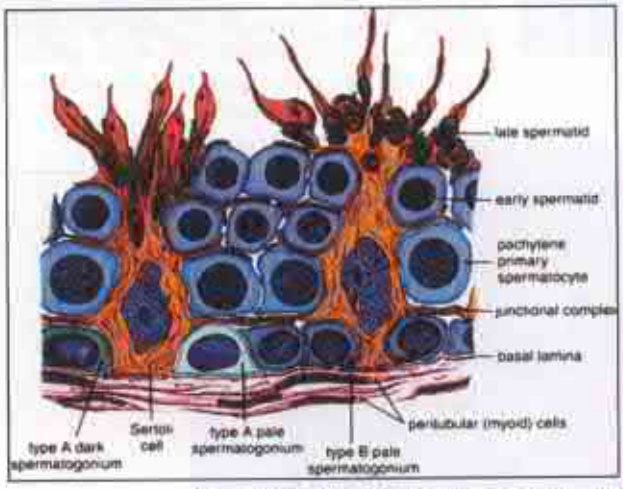


Fig. 147. Sagital section of a testis

Fig. 148. Higher magnification of photomicrograph of a section of a testis



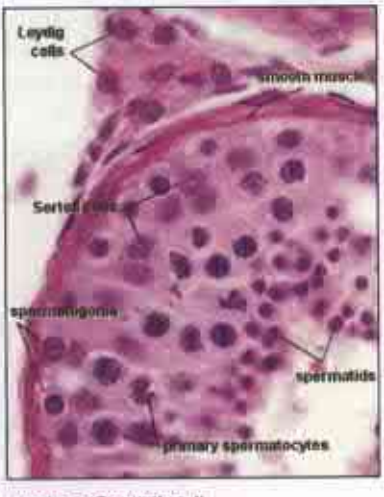


Fig. 149.Schematic drawing and light micrograph of Sertoli cell and adjacent spermatogenic cells

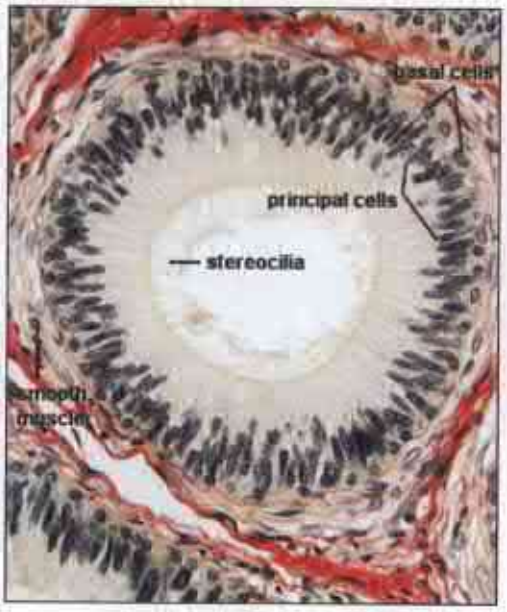


Fig. 150. Photomicrograph of ductus epididymis

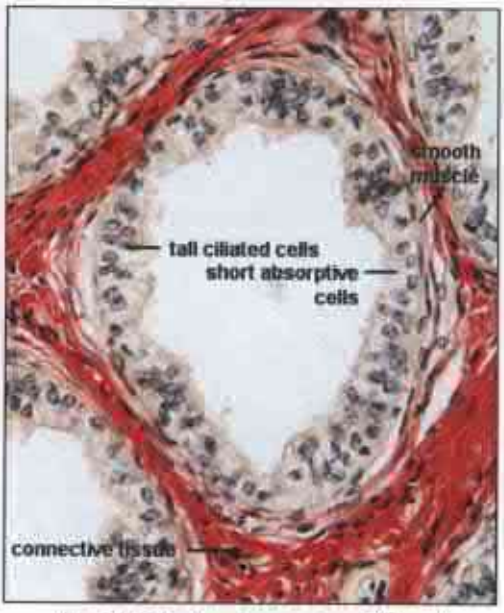


Fig. 151. Photomicrograph of efferent ductules

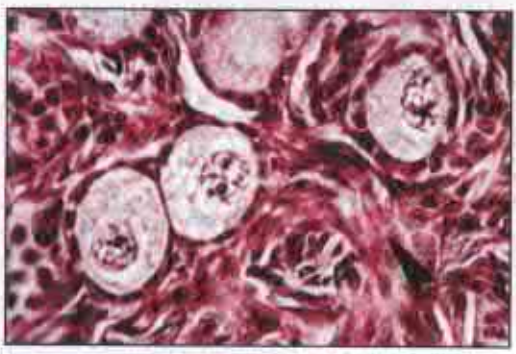


Fig. 152. Photomicrograph of primordial follicles

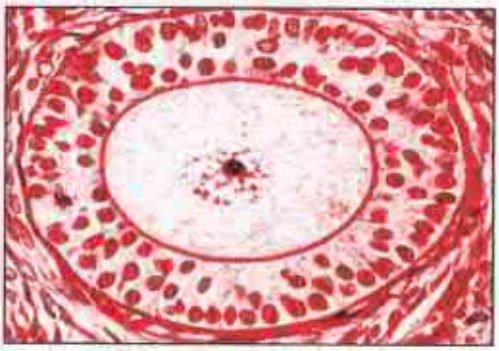


Fig. 153. Photomicrograph of late primary follicle

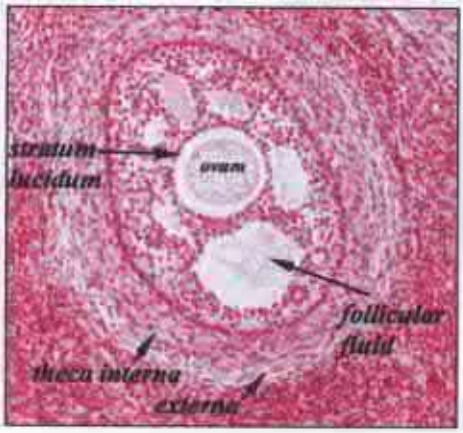


Fig. 154. Photomicrograph of secondary follicle

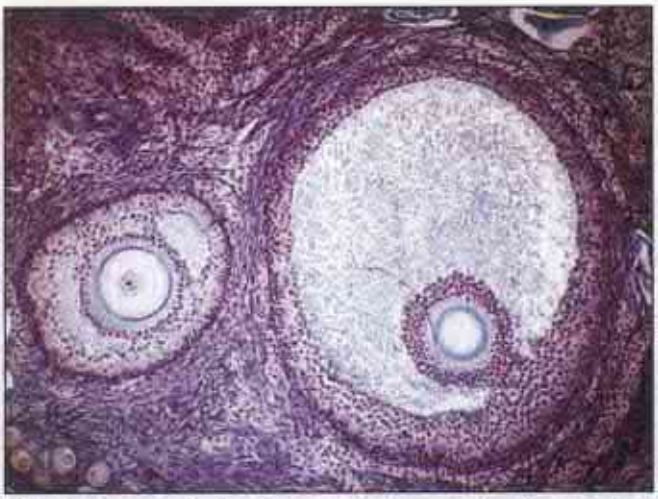


Fig. 155. Photomicrograph showing a secondary and mature graafian follicles

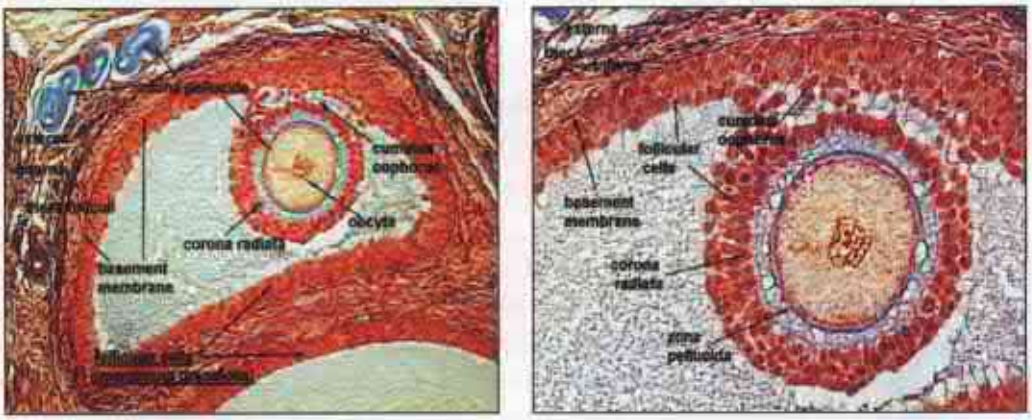


Fig. 156. Photomicrographs showing details of structure of the mature graafian follicle

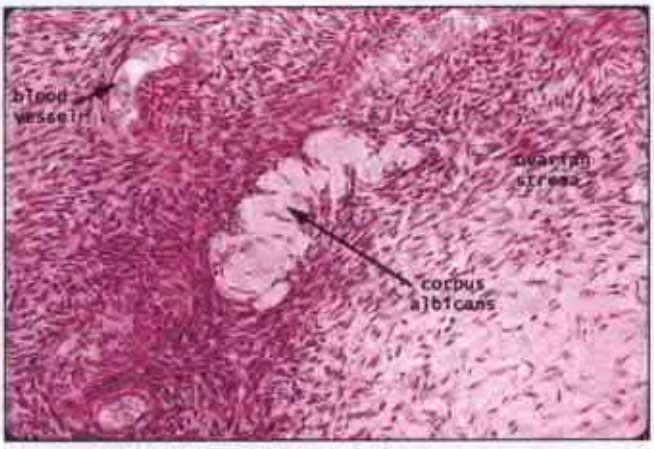


Fig. 157. Photomicrograph of the corpus albicans

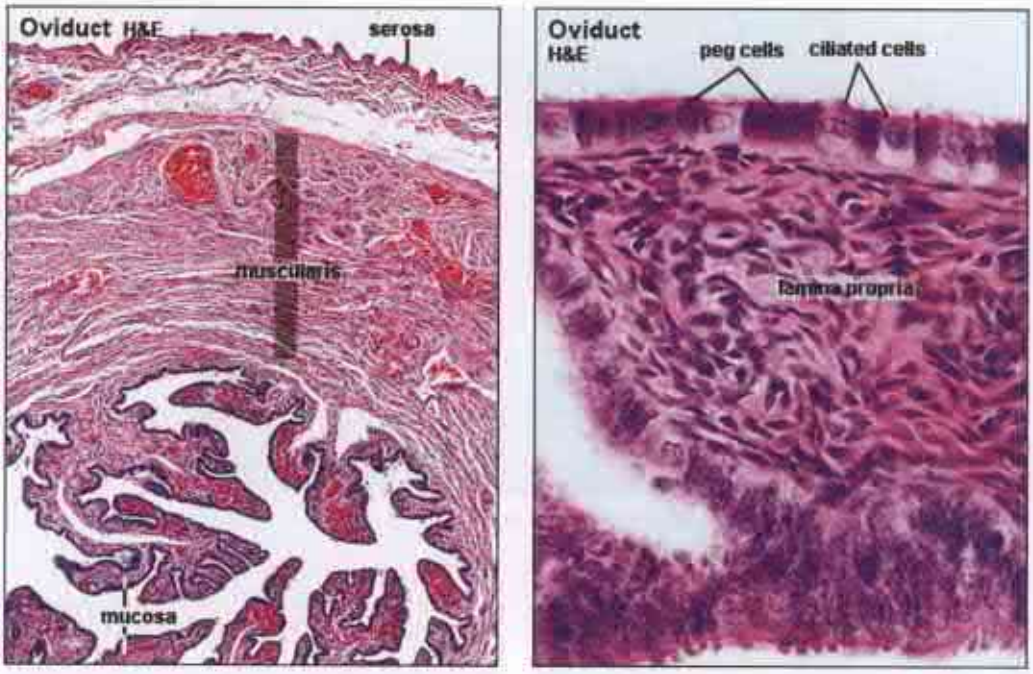


Fig. 158. Light micrographs of a uterine tube structure

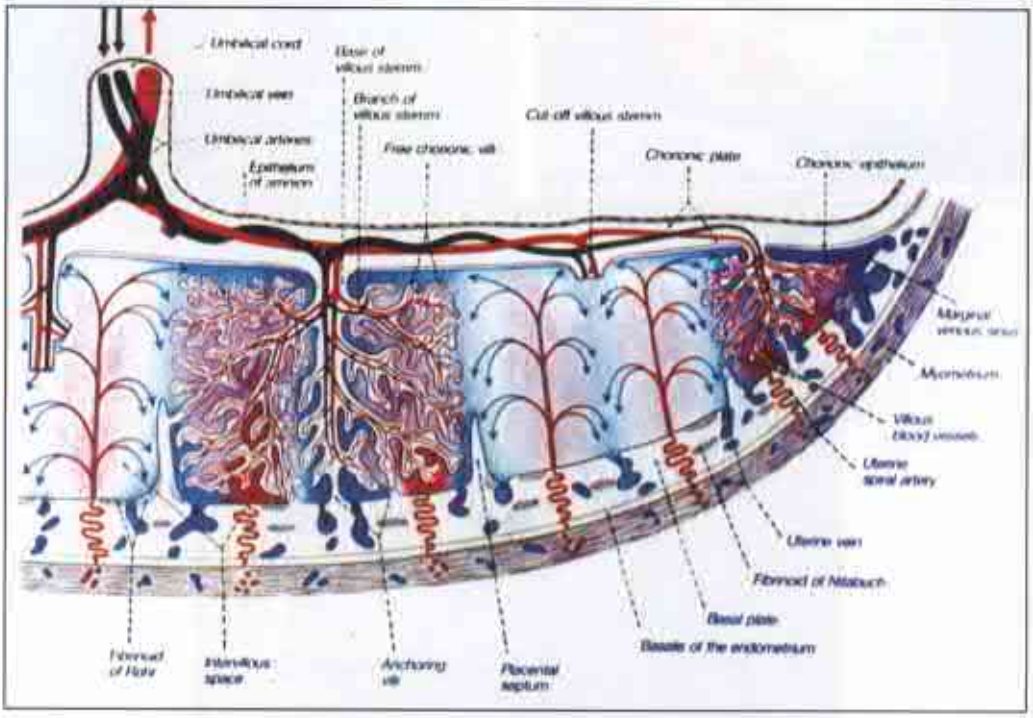


Fig. 159. Schematic diagram of the placenta structure

CHAPTER XIII

MAMMARY GLANDS

Mammary glands are specialized accessory glands that have evolved in mammals to provide for the nourishment of their offspring. They are really **modified apocrine sweat glands** that are **ectodermally derived**. The histological appearance of the breast varies with age and functional status.

DEVELOPMENT:

A. **Embryo** – during the fourth week of development, a pair of epidermal thickenings called **mammary ridges** develop along either side of the body from the axilla to the inguinal region. In humans, the ridges normally disappear except in the thoracic area.
   1. At the site of breast development (and the future nipple), the epidermis proliferates into the underlying dermis to form the **primary bud**. By the twelfth week, several **secondary buds** have formed, which begin to lengthen and branch. 15 – 25 secondary buds become canalized to form **lactiferous ducts,** which open into the mammary pit, which will become the nipple.
   2. Occasionally, portions of the mammary ridge persist, usually in the axillary region and **supernumary nipples** result **(polythelia)**.

B. **Infantile** – the breast consists of rudimentary (lactiferous) ducts surrounded by abundant connective tissue.

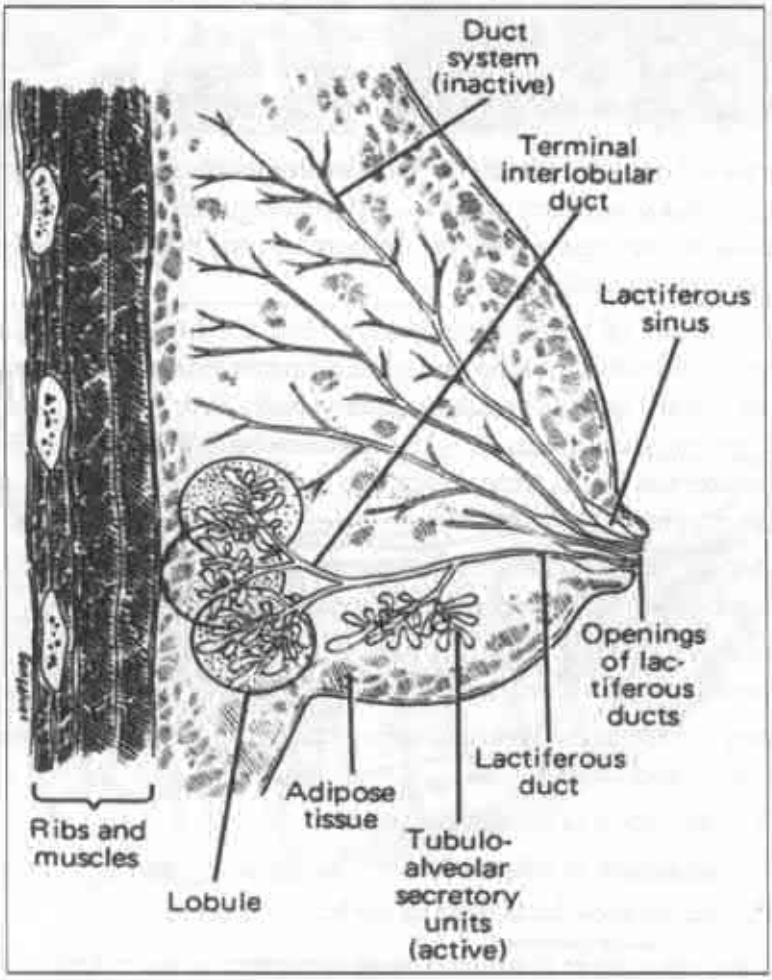
C. **Puberty** – in females, breasts develop under the influence of **ovarian estrogens** via 3 mechanisms:
   1. Accumulation of connective tissue.
   2. Accumulation of adipose tissue.
   3. Proliferation of ducts deep in the breast to form lobules.

In males, since there is minimal estrogen present, the mammary glands retain the infantile structure.

STRUCTURAL ORGANIZATION

A. The breast consists of **15 – 20 compound tubuloalveolar glands** that reside in **15 – 20 lobes.** The lobes are separated by dense connective tissue. Lobes contain **lobules** that contain clusters of **ducts**, which, in the active breast, become **secretory units (alveoli)**. The intralobular connective tissue is slightly less dense and is more cellular than the interlobular connective tissue.

1. **Secretory alveoli** are present with the lobules of each lobe. They are lined by a single layer of epithelial cells that may be cuboidal or columnar depending upon the secretory activity of the alveolus. **Myoepithelial cells** lie between the base of the epithelium and the basement membrane; these are an important part of the milk ejection reflex.



Schematic drawing of the breast

2. **Intralobular ducts** reside within the lobules and drain the secretory alveoli. The epithelium is usually simple cuboidal; myoepithelial cells are present beneath the epithelium – they also secrete and are difficult to identify.
3. The intralobular ducts of each lobule drain into **interlobular ducts** (located in the CT between lobules) that are lined with simple cuboidal epithelium; myoepithelial cells are also present.
4. Each lobe is drained by a **lactiferous duct** which receives the contents of the interlobular ducts within the lobe. The epithelium is stratified cuboidal or stratified columnar.
5. Each lactiferous duct widens within the nipple to become a **lactiferous sinus**. The epithelium becomes stratified squamous at the opening on the nipple surface.

B. The **nipple** has a conical shape and is usually pigmented; it is covered by keratinized stratified squamous epithelium (skin) that is continuous with the pigmented **areola** and the surrounding skin. The connective tissue around the lactiferous ducts (sinuses) in the nipple is rich in smooth muscle and sensory nerve endings, which are essential for the **milk ejection reflex** to occur during nursing.

FUNCTIONAL CHANGES IN THE ADULT MAMMARY GLANDS

A. **Resting (inactive) breast** is not secreting or preparing to secrete milk, so there are few secretory alveoli. The lobules contain small clusters of blind-ended ducts embedded in loose connective tissue.

B. **Active breast** – if pregnancy occurs, the hormones **estrogen, progesterone** (from placenta and corpus luteum), **prolactin** (from pituitary), **human chorionic somatomammotropin (hCS; a.k.a. placental lactogen)** are released. **Estrogen** and **progesterone** act on breast tissue and cause intense proliferation of ducts and secretory alveoli. Estrogen and progesterone inhibit the effects of prolactin and hCS prior to birth; after birth the effects of hCS allow lactation to begin. The simple columnar epithelium of the alveoli becomes low cuboidal as milk production increases.

1. **Lactation – milk** contains **lipids** (triglycerides), **proteins** (e.g. caseins, lactalbumin), immunoglobulins (secretory **IgA**) and **sugars** (e.g. lactose). Proteins are formed in the RER, packaged into vesicles by the Golgi and are secreted by exocytosis **(merocrine secretion)** into the lu-

men of the alveolus. The lipid arises as free-floating droplets in the cytoplasm, which fuse into larger droplets as they move toward the apical cytoplasm. They project into the lumen, covered by the plasmalemma and break free, enveloped by a small amount of cytoplasm and a portion of the cell membrane. This is **apocrine secretion**.

2. **Colostrum** is a very low fat, high protein secretion that is produced right after birth. It contains **secretory IgA** derived from plasma cells present in the connective tissue; these immunoglobulins confer **passive immunity** (particularly within the gut lumen) to the suckling infant.
3. After birth, the placental hormones are no longer secreted and the breast activity is maintained by **prolactin**. The adenohypophysis of the pituitary gland is stimulated to secrete prolactin by the infant suckling; the production of hypothalamic **prolactin inhibitory factor (PIF)** is inhibited by suckling. PIF inhibits the production & release of prolactin.
4. The **milk ejection reflex** occurs when the tactile receptors in the nipple are stimulated by suckling. In this reflex, the hormone **oxytocin** is released from the **neurohypophysis** (neural lobe of pituitary gland) into the bloodstream. Oxytocin rapidly stimulates contraction of the myoepithelial cells surrounding the secretory alveoli and ducts, resulting in the release of milk from the nipple. Suckling also stimulates the pituitary to release prolactin, which allows the breast to continue lactation until weaning occurs.

C. **Regression of the mammary gland** occurs when suckling by the infant ceases (weaning). During the first few days, the alveolar epithelium is flattened due to the distension of the alveoli with milk. The alveoli gradually collapse and there is an increase in connective tissue and adipose tissue. The alveolar epithelium contains numerous autophagic vacuoles, and the number of intraepithelial macrophages increases. Most of the alveoli are replaced by the connective tissue and the remaining alveoli appear as scattered cords of epithelial cells.

D. **Involution of the mammary gland** occurs after menopause because of the reduction in circulating estrogen. The epithelium of the alveoli and ducts atrophies, there is a reduction in adipose tissue and the gland returns to a prepubertal condition (mostly connective tissue with a few ducts).

Clinical significance:

Chronic cystic disease is common in women between the ages of 30 and 50. The intralobular ducts may lose their continuity with the remainder of the duct system and fluid filled cysts of varying sizes are formed.

Gynecomastia is the most common **male breast disorder**. It is not a tumor but rather just an increase in the amount of a man's breast tissue. Gynecomastia is common among teenage boys due to changes in hormone balance during adolescence. The same condition is not unusual in older men and is also due to changes in their hormone balance. Rarely, gynecomastia can occur because tumors or diseases of certain endocrine glands cause a man's body to produce more estrogen.

The breast is the most common site of **cancer** in women. **Carcinomas** can arise from the glandular and ductal structures of the breast; most arise from the lactiferous ducts.

1. **Ductal carcinoma *in situ* (DCIS):** This is breast cancer at its earliest stage (stage 0). The cancer is confined to the ducts and has not spread through the walls of the ducts into the fatty tissue of the breast. Nearly all women with cancer at this stage can be cured. The best way to find DCIS is with a mammogram.
2. **Infiltrating (invasive) ductal carcinoma (IDC):** This cancer starts in a duct, breaks through the wall of the duct, and invades the fatty tissue of the breast. From there it can spread to other parts of the body. IDC is the most common type of breast cancer; it accounts for nearly 80% of cases.
3. **Infiltrating (invasive) lobular carcinoma (ILC):** This cancer starts in the milk glands (lobules). It can spread to other parts of the body. Between 10% and 15% of invasive breast cancers are of this type.
4. There are also several less common types of breast cancer. Men can develop breast cancer; the most common type is IDC.

CHAPTER XIV

PLACENTA

The placenta has five basic functions:

1. It serves as the barrier separating the maternal and fetal circulations.
2. It transports nutrients from mother to fetus and waste products in the other direction.
3. It is the major endocrine organ, producing both steroid and polypeptide hormones (progesterone, estrogens, human chorionic gonatotropin, melanin spreading factor, other hormones also manufactured by the hypothalamus & pituitary).
4. It transfers maternal antibodies to the fetus, providing immunological protection both in uterus and in postnatal life.
5. It initiates parturition (birth).

Also the placenta acts as respiratory organ and excretory organ (*exchange of gases, nutrients waste products without any mixing of maternal & fetal blood*).

The initial formation of the placenta and the trophoblast-mediated invasion of the endometrial decidua (functional layer of the endometrium) begin approximately 6 days after fertilization as the newly formed embryo undergoes implantation. Essential to this process is the formation of the trophoblast layer of cells.

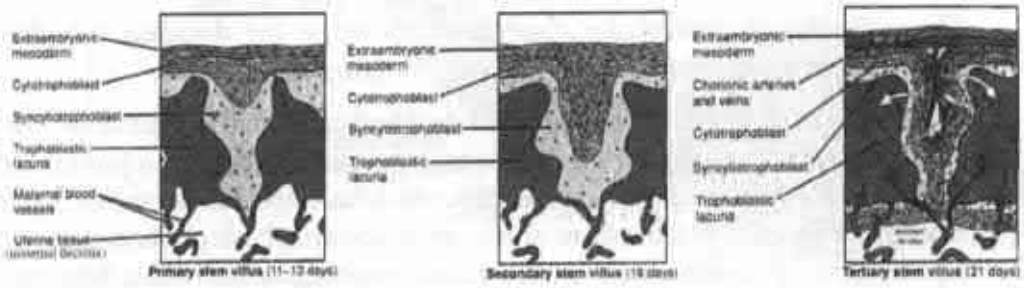
Status of the uterus at the time of fertilization:

1. The endometrium is in the secretory stage and provides a nutritive environment for implantation of a blastocyst.
2. The glands contain glycogen, mucin and some lipid (**uterine milk**).
3. The ***implantation window*** is 6 – 10 days after LH surge. This is when actions of estrogen and progesterone render the endometrium receptive for implantation. Anti-progesterone drugs compete for receptors on

endometrium to block the action of progesterone, thus causing the endometrium to become non-supportive for embryonic growth; implantation does not occur.

Development of placental villi

1. By day 11, the blastocyst is completely embedded in the endometrium. By day 13, the **cytotrophoblast** cells have grown into the syncytiotrophoblast to form ***primary villi.***
2. By day 16, the **extraembryonic mesoderm** derived from the inner cell mass invades the center of the primary villi, producing ***secondary villi.***
3. By day 21, **fetal vessels** (part of the umbilical circulation) grow into the secondary villi, forming ***tertiary villi.***
4. The lacunae coalesce to form large **intervillous spaces.** Some of the tertiary villi extend into the maternal decidua and firmly attach, forming ***anchoring*** or ***stem villi.***



primary chorionic villi *secondary chorionic villi* *tertiary chorionic villi*

5. As development continues, small tertiary villi sprout from the larger teriary villi, similar to a tree sprouting new branches. This increases the surface area for maternal/fetal exchange.
6. The outer surfaces of the villi are covered by **syncytiotrophoblast** with cytotrophoblast lying just beneath it. However, during the second half of pregnancy, the **cytotrophoblast** disappears, leaving only the syncytiotrophoblast. This thinner layer covering the villi allows for more efficient maternal/fetal exchange.

Structure of placenta:

The placenta consists of tissues derived from both the mother and the embryo. The fetal part being derived from the embryonic membranes, and the maternal part from the uterine wall. It is this boundary between the maternal and fe-

tal tissues that necessitates the immunologic role of the placenta as the embryo is normally semi-allogeneic to the mother. This initially small area of fetomaternal apposition is enormously increased during pregnancy as the placenta develops by folding and refolding while it proliferates in parallel with the fetus in order to provide the fetus with nutrients it needs to develop normally and to remove the waste products the fetus produces.

The ***fetal portion*** of the placenta described above is known as the **chorion**, which consists of the trophoblast and extrambryonic mesoderm. During the first eight weeks, villi cover the entire chorionic surface. After that, it can be divided into:

1. The **chorionic villi (chorion frondosum).**
2. The **chorionic plate** (closest to the fetus) from which the **chorionic villi** arise.
3. The smooth chorion **(chorion laeve)** extends from the chorionic plate and envelops the developing embryo, forming the chorionic sac. The chorionic villi that were located here have degenerated.

The ***maternal portion*** of the placenta is known as the **decidua,** a highly modified uterine endometrium.

1. The endometrial cells are converted into **decidual cells** after implantation. Decidual cells are large, slightly basophilic and the nucleus contains a prominent nucleolus. They secrete **decidual prolactin,** which has tropic effects on the corpus luteum and **relaxin,** which softens up the cervix and symphysis pubis in preparation for birth **(parturition)**. They also secrete **prostaglandins**, which help prevent immunologic rejection of the embryo. Early in pregnancy, their content of **lipid and glycogen** help to nourish the implanting blastocyst. Decidual cells are usually located in the upper portion of the decidua (closest to fetus).
2. **Giant cells** are also present in the decidua. They are trophoblast derivatives that migrate into the deciduas from the cytotrophoblastic shell. They are **multi-nucleated**, may be involved in some secretory activity and aid in establishing a **cleavage plane** for placental separation after birth.
3. After 4 – 5 months, placental septae divide the surface of the fetal placenta into 15 – 25 areas ***(cotyledons)***.

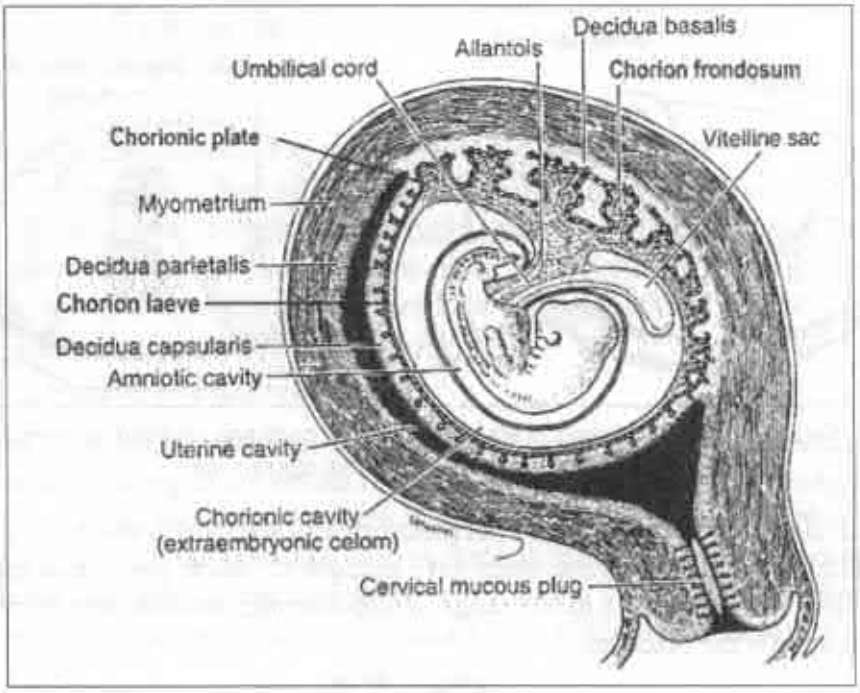
   The decidua has 3 parts until the beginning of the 4th month:

   a. **Decidua basalis** – the portion at the base of the placenta to which the anchoring villi attach. It attaches to a more compact layer called the **basal plate.**

b. **Decidua capsularis** – encapsulates the superficial portion of the chorionic sac.

c. **Decidua parietalis** – includes the remainder of the uterine lining.

By the 5th month the capsularis and parietalis fuse, obliterating the uterine lumen.



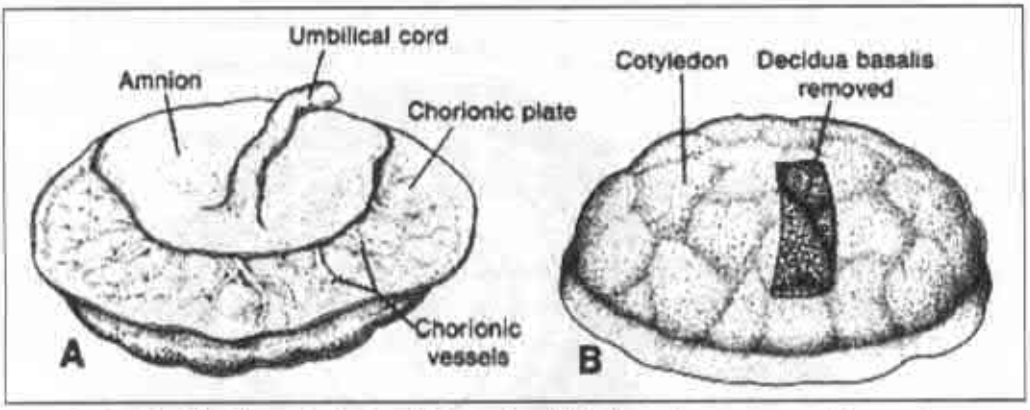
Schematic drawing of human pregnancy depicting the development of the placenta

The intervillous space between the maternal and fetal components contains circulating maternal blood. Arterial blood, derived from the open ends of the spiral arteries, flows into the intervillous space and moves blood into the uterine vein.

The placenta continues to grow and develop throughout pregnancy and at term is an organ of about 20-25 cm in diameter, with a thickness of 3 cm and a weight of about 500-600 g. At birth, it is torn from the uterine wall and, approximately 30 minutes after birth of the child, is expelled from the uterine cavity.

After birth, when the placenta is viewed from the **maternal side,** 15 to 20 slightly bulging areas, the **cotyledons,** covered by a thin layer of decidua basalis, are clearly recognizable. Grooves between the cotyledons are formed by decidual septa.

The **fetal surface** of the placenta is covered entirely by the chorionic plate. A number of large arteries and veins, the **chorionic vessels,** converge toward the umbilical cord. The chorion, in turn, is covered by the amnion. Attachment of the umbilical cord is usually eccentric and occasionally even marginal. Rarely, however, does it insert into the chorionic membranes outside the placenta (**velamentous insertion**).



A. *Fetal side. The chorionic plate and umbilical cord are covered by amnion.*
B. *Maternal side showing the cotyledons.*

The chorionic villi have an enormous area of exchange, reaching 14m² at term and the capillaries within them have a length of 50km. The rate of maternal blood flow to the uterus is in the range of 500-700ml/min with 80% of that destined to supply the placenta.

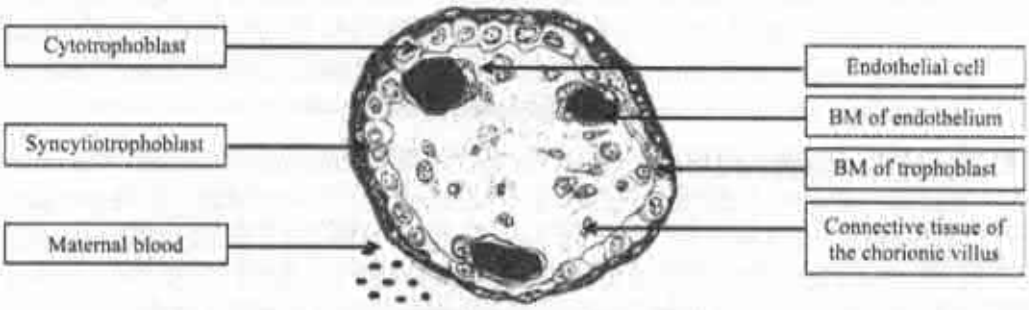
CIRCULATION OF THE PLACENTA

Cotyledons receive their blood through 80 to 100 spiral arteries that pierce the decidual plate and enter the intervillous spaces at more or less regular intervals. The lumen of the spiral artery is narrow, so blood pressure in the intervillous space is high. This pressure forces the blood deep into the intervillous spaces and bathes the numerous small villi of the villous tree in oxygenated blood. As the pressure decreases, blood flows back from the chorionic plate toward the decidua, where it enters the endometrial veins. Hence, blood from the intervillous lakes drains back into the maternal circulation through the endometrial veins. Collectively, the intervillous spaces of a mature placenta contain approximately 150 ml of blood, which is replenished about 3 or 4 times per minute. This blood moves along the chorionic villi *(see fig. 159, plate II).*

Placental exchange does not take place in all villi, only in those whose fetal vessels are in intimate contact with the covering syncytial membrane. In these villi, the syncytium often has a brush border consisting of numerous microvilli, which greatly increases the surface area and consequently the exchange rate between maternal and fetal circulations. The separation of the fetal and maternal blood, referred to as the placental barrier, is maintained primarily by the layers of fetal tissue. The **Placental barrier** consists of:

1. Syncytiotrophoblast.
2. Cytotrophoblast.
3. Basement membrane of trophoblast.
4. Connective tissue of the chorionic villus – two types of cells are recognized in the stroma of the villi: the fibroblast and the Hofbauer cells. The role of the Hofbauer cells, which are more common in early placenta, is not known, but morphologically they have characteristics of macrophages.
5. Basement membrane of endothelium.
6. Cytoplasm of endothelial cell.

From the fourth month on, however, the placental membrane thins, since the endothelial lining of the vessels comes in intimate contact with the syncytial membrane, greatly increasing the rate of exchange. The **placental barrier** is not a true barrier, since many substances pass through it freely. Because the maternal blood in the intervillous spaces is separated from the fetal blood by a chorionic derivative, the human placenta is considered to be of the **hemochorial** type.



Structural components of a placental barrier (cross section of a villus)

Modes of placental transfer include:

1. **Simple diffusion** (e.g. gases, H_2O, steroids, lipid soluble vitamins, thyroxine). Molecules that have high lipid solubility can cross the barrier by direct

diffusion across the lipid bilayers of the SCT. Steroid hormones and their precursors are of particular significance. This is a bidirectional process, with CO_2, urea and other waste products diffusing from fetus to mother. In addition, electrolytes and some small molecules like glucose can diffuse through the tight junctions between cells. Diffusional processes in many cases are inadequate. Specific cellular channels (for water, urea, etc.) are present in the cell membranes which significantly enhance the rate of diffusion across the SCT.

2. **Facilitated diffusion** (e.g., glucose, amino acids, lactate, I-, Fe, Zn, and water soluble vitamins). These molecules are transported across the two membranes of the SCT by specialized membrane proteins. Some of these transporters do not have directionality but equilibrate maternal and fetal pools. Others use the energy of ATP to concentrate molecules or make use of the electrochemical membrane potential to transport ions across the two lipid bilayers of the SCT. The surface facing the maternal blood has plasma membrane specializations (microvilli) that increase surface area.

3. **Surface receptor binding and endocytosis** (e.g., lipids and cholesterol from maternal LDL, folate, Fe^{++}, Cu^{++}). Molecules that are transported in the bloodstream complexed to proteins are generally taken up by receptor-mediated endocytosis into the trophoblast. The LDL receptor, for example, mediates the uptake of LDL, which contains cholesterol. In endosomes and lysosomes, the nutrient molecules like cholesterol are liberated from the complexes. Some of the molecules are used by the SCT cells themselves, while others are released through the basal surface of the cell where they associate with fetal carrier proteins and enter the fetal circulation. The maternal carrier proteins themselves are not transported across the SCT.

4. **Receptor-mediated transcytosis** for IgG type of immunoglobulins. The fetus and the newborn are incapable of mounting their own immune responses. To protect the newborn until its own systems take over, maternal immunoglobulins (Ig) in the blood are equilibrated with the fetal circulation across the placenta in the third trimester. A specific protein receptor at the microvillar plasma membrane of the SCT binds IgG, and the receptor/ligand complex undergoes endocytosis and re-insertion at the basal membrane, where the IgG molecules are released on the fetal side. The receptors are recycled back to the microvillar surface for reuse. The receptor resembles that involved in IgG uptake by neonatal intestine from maternal colostrum (milk), which is another source of IgG.

Placental Hormones

1. **Human chorionic gonadotropin (hCG)**
   - a glycoprotein which binds to the LH receptor of the ovarian corpus luteum to stimulate massive maternal ovarian progesterone production until 8-10 weeks gestation, at which time placental progesterone production is adequate to maintain the pregnancy. hCG is produced by the syncytiotrophoblast and its synthesis is positively modulated by CT-derived GnRH (gonadotropin releasing hormone) and negatively modulated by CT-derived inhibin and activin. hCG also stimulates the Leydig cells in the fetal testes to produce testosterone, promoting sexual differentiation in male fetuses.
   - hCG is first detectable in the maternal circulation around day 6-10 after ovulation and peaks at 10 weeks. It is also present in the urine and one of the principle molecules used to detect pregnancy. Because Down syndrome pregnancies produce lower amounts of hCG, the assessment of hCG levels in the maternal circulation at 16 weeks gestation is recommended as the primary biochemical screening test for fetal Down syndrome.
2. **Placental lactogen (hPL),** also known as **human chorionic somatomammotropin** – a glycopeptide with 94% homology to growth hormone and 67% homology to prolactin. It is produced by the syncytiotrophoblast. hPL acts as a potent anti-insulin and lipolytic agent in the maternal circulation to increase the availability of glucose, amino acids, free fatty acids and ketones for the fetus. It has the side effect of making the mother mildly 'diabetic' that is, having glucose levels above normal after meals (insulin normally lowers blood glucose). Placental lactogen stimulates the growth of a mammary gland.
3. **Corticotropin releasing hormone (CRH)**
   - a glycopeptide originally identified in the hypothalamus, where its release into the portal circulation increases pituitary adrenocorticotropin (ACTH) release which, in turn, enhances adrenal cortisol production. CRH has been found to be synthesized in the placenta, fetal membranes (amnion and chorion) and the uterine decidua in amounts that increase dramatically near term.
   - While hypothalamic release of CRH is inhibited by glucocorticoids establishing a negative feed-back loop, placental, fetal membrane and decidual synthesis is stimulated by glucocorticoids, creating a potential positive feed-back loop. Newly synthesized placental CRH will stimulate the fetal pituitary and the placenta itself to produce corticotropin

(hcACTH, see below), which results in stimulation of adrenal production of the steroid cortisol (a corticosteroid).

- CRH can enhance prostaglandin production by the fetal membranes and decidua to potentially initiate parturition (labor and delivery). Prostaglandins as well as oxytocin stimulate uterine muscle contraction. Rapid increase in circulating CRH, potentially driven by rising fetal and maternal cortisol production, occurs during the final few weeks of pregnancy. This suggests that CRH is part of the biological clock mechanism regulating the onset of parturition. It is not known how cortisol itself inhibits progesterone function, thereby disrupting decidual function. The best hypothesis is that it does so by binding to the same receptors and antagonizing the progesterone response.

4. The placental trophoblast cells synthesize the steroid hormones **progesterone** and **estrogen** and are the only major source after the first trimester, when the corpus luteum degenerates.
   - Both hormones are required for the maintenance of the maternal decidua. In their absence, the decidua will degenerate and the fetus cannot remain attached to the uterine wall.
   - In general, the principle source of progesterone is through de novo synthesis by trophoblast using cholesterol obtained through the uptake of maternal LDL. The fetal adrenal synthesizes pregnenolone during gestation, which crosses into the placenta for conversion to progesterone, providing an alternative precursor. Estrogen is produced from dehydroepiandrosterone sulfate (DHAS) synthesized by the fetal adrenal gland. Fetal steroids, including cortisol, are produced in response to progressive increases in fetal adrenocorticotropin (ACTH) production [ACTH stimulates adrenal steroid synthesis] across gestation. This axis is also important for initiating parturition.
5. **Relaxin** is synthesized by decidual cells and softens cervix and pubic symphysis
6. Other growth factors: fibroblast growth factor, colony-stimilating factor, prostaglandins (help to prevent immunologic rejection of fetus), **IGF I and II**; various other **growth factors** that stimulate trophoblast growth.

Transplacental Antibody Passage

The placenta is an immunologically privileged organ. This means that the mother does not ordinarily make antibodies against the trophoblast itself even

though it may express foreign, paternally derived antigens on its surface. Part of the explanation for this is that the placenta produces substances which locally suppress the immune response (the nature of these is unknown). In addition, effective immune responses against cells require the presence of so-called histocompatibility antigens produced by all cells. Trophoblast cells are an exception, producing instead their own unique histocompatibility antigen. This antigen not only cannot participate in the immune response, it is thought to suppress the maternal immune response.

Antibodies are not synthesized by the fetus prenatally. Maternal IgG (which is the major type of antibodies produced) is specifically transported across the placenta, particularly late in gestation. As mentioned above, specific receptors for IgG exist on the microvillar surface of the trophoblast which transport it across the SCT cells. Since the fetus is a separate immunological entity, if the mother is exposed to immunologically unique, paternal-derived fetal antigens, she will generate anti-fetal antigen antibodies. In the case of erythrocyte (red blood cell) antigens such as Rh, maternal antibodies can cross the placenta to cause alloimmune hemolytic anemia (killing of erythrocytes), also known as Rh disease, in the fetus. Rh disease can cause cardiac failure due to fluid accumulation (hydrops fetalis), intrauterine death, or even severe jaundice leading to mental retardation. In most cases, Rh disease does not affect the first pregnancy. Exposure to fetal cells does occur naturally late in gestation when pieces of placental villi break off and enter the maternal circulation, often lodging in the lungs. The fetal red blood cells therein can trigger a maternal immune response, but significant antibody accumulation would take place only very late in gestation and therefore the fetus is not affected significantly. However, in subsequent pregnancies the problem potentially may become much more severe as the maternal immune response is more vigorous. Rh immunoglobulins (a sort of blocking antibody) given to the mother can prevent damage to the fetus.

A very similar type of antibody response can affect platelets. A mother who does not express the PLA-1 platelet antigen, but whose fetus does, can produce anti-PLA-1 antibodies that can cross the placenta to cause profound alloimmune thrombocytopenia (deficiency of platelets) with fetal intracerebral hemorrhage. Again, this is more common after the first pregnancy.

Maternal auto-antibodies that cause autoimmune diseases in the mother can also cross the placenta: a) anti-acetylcholine receptor antibodies, leading to neonatal Myasthenia Gravis (impaired muscular function); b) anti-TSH receptor antibodies, leading to fetal and neonatal Graves disease (impaired thyroid function);

c) anti-ribonucleoprotein antibodies, leading to congenital heart block; and d) anti-phospholipid antibodies causing fetal or neonatal thrombosis (blood clots).

Transport of Infectious Agents

A number of viruses, including cytomegalovirus, rubella (German measles), varicella-zoster (chicken pox), measles, and poliovirus, can cross the placenta and infect fetal tissues. Rubella in particular was a significant pathological agent before the advent of vaccines. It was a major cause of craniofacial abnormalities including congenital deafness. Today, the most important virus that can infect the fetus is HIV, which is transmitted to about 1/4 of infants of HIV-infected mothers who are not being treated with anti-viral drugs. AZT and other HIV virus-suppressing drug therapies administered to mothers with HIV have reduced this percentage dramatically in the last few years. Fetal infection is believed to occur around the time of parturition, either through microscopically damaged areas of the placenta that allow maternal blood to enter the fetus or during delivery. Cesarean delivery before the onset of labor is therefore recommended in these cases. Because maternal antibodies are present for the first 2-3 months after birth, conventional AIDS testing, which detects circulating antibodies to HIV in the bloodstream, cannot be used to detect HIV infection in newborns.

Bacteria and other protozoa do not ordinarily cross the barrier. Exceptions include treponema palladium (syphilis) and toxoplasma gondii that can cause abnormalities of the brain and eyes. Toxoplasma is primarily transmitted by maternal ingestion of uncooked meat.