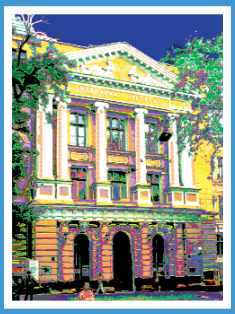

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HISTOLOGY

ОДЕСЬКИЙ МЕДУНІВЕРСИТЕТ

ОДЕСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ

THE ODESSA NATIONAL MEDICAL UNIVERSITY

Бібліотека студента-медика

Medical Student's Library

Серія заснована в 1999 р. на честь 100-річчя Одеського державного медичного університету (1900–2000 рр.)

The series is initiated in 1999 to mark the Centenary of the Odessa State Medical University (1900–2000)

L. V. Arnautova
O. A. Ulyantseva

HISTOLOGY

A course of lectures

A manual

Odessa
The Odessa National Medical University
2011

UDC 616-018: 378.16
BBC 28.8я73

Series “Medical Student’s Library”
Initiated in 1999

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It is published according to the decision of the Central Coordinational Methodical Committee of the Odessa National Medical University Proceedings N1 from 22.09.2010

Навчальний посібник містить лекції з гістології, цитології та ембріології у відповідності до програми. Викладено матеріали теоретичного курсу по всіх темах загальної та спеціальної гістології та ембріології. Посібник призначений для підготовки студентів до практичних занять та ліцензійного екзамену “Крок-1”.

Arnautova L. V.

Histology. A course of lectures : a manual / L. V. Arnautova, O. A. Ulyantseva. — Odessa : The Odessa National Medical University, 2010. — 336 p. — (Series “Medical Student’s Library”).

ISBN 978-966-443-034-7

The manual contains the lecture course on histology, cytology and embryology in correspondence with the program. The theoretical course on all the topics of general and special histology is given. The manual can be used by students during their preparation to the practical trainings and to the license test examination “Krok-1”.

The manual is recommended for the students of medical and dental faculties of higher medical universities.

UDC 616-018:378.16
BBC 28.8я73

ISBN 978-966-7733-47-6 (series)
ISBN 978-966-443-034-7

PREFACE

A necessity in the lectures course publication for English-speaking students has appeared because there is no textbook corresponding to the regulations of education issued by the Ministry of Public Health of Ukraine. As a result of conversion to the credit-modular system of education the hour-time of the lectures and practical trainings was changed, that resulted in redistribution of the schedule of the lectures course. The current textbooks on histology published abroad do not meet the requirements to our syllabus. Thus, for example biochemical processes of the cell are overviewed out in detail excessively while the morphology of vital organs (the hypothalamus, the spinal cord and so on) is not described sufficiently. Moreover, there are differences in classifications of the organs.

The histology lectures are designed to prepare the student for practical trainings. They are dedicated to the overview of basic and secondary characteristics of tissue structure and training student's skills of histological sample analysis.

Some topics of the lectures course contain too much information, so that the students really have no time to scrap-note the data along with the lecturer; this fact and the lack of a textbook makes the preparation to practical classes even more complicated for them.

Therefore, we present the lectures course in English that answers the requirements of the Ministry of Public Health of Ukraine and can be used for education of students of medical and dental faculties.

Lecture 1

INTRODUCTION TO THE COURSE OF HISTOLOGY, CYTOLOGY AND EMBRYOLOGY. CYTOLOGY

The Department of Histology and Embryology of the Novorossiysk University was organized in May 1900 and it was one of the first fundamental departments of the Medical university.

The founder of the histologists a Kiev school professor V. V. Podvysotsky was directed to Odessa for organization of the medical faculty of histologists and microbiologists in 1989. In May 1900 he was nominated as a chief of the Histology Department.

Scientific activity of professor V. V. Podvysotsky was directed to cancer research and development of some questions of immunology.

Professor A. F. Mankovsky had headed the Department of Histology, Cytology and Embryology from 1905 to 1917. The range of his scientific interests covered the questions on embryology and endocrinology.

From 1917 to 1920 the Histology Department of the Odessa Medical University had been consecutively managed by professor V. V. Voronin, academician D. K. Tretiyakov and assistant professor V. E. Yanishevsky.

Professor F. N. Zhmaylovich had headed the department from 1920 to 1923. The main direction of his scientific studies were the questions of cytology.

Professor M. N. Zaevloshin had headed the Histology Department for a long time (1923–1944). He is an author of more than 40 scientific works on histology and patomorphology.

Assistant professor B. I. Kardashevich had been at the head of the department twice, from 1944 to 1946, and from 1950 to 1954. During that period the main scientific trend of the department were the questions to age morphology.

Professor S. D. Shakhov has managed the Histology Department from 1954 to 1958. He is an author of more than 60 scientific works. The range of his scientific interests was very wide.

Professor N. D. Zaytsev had headed the Histology Department from 1958 to 1976. He is an author of 67 scientific works devoted to the questions of embryogenesis and problem of neurohistology.

Professor N. D. Zaytsev has changed the professor V. F. Pchelyakov who had managed the department from 1976 to1993. His scientific works were devoted to study of the cornea of the eye in amimals and humans.

Professor V. K. Napkhanyuk was elected the head of the department in May 1993 and prepared more than 20 research assistances. A young and perspective leader professor V. A. Ulyanov has headed the department since 2006.

CYTOLOGY

The cell is a functional unit of all living tissues, having a capacity to perform all the essential life functions. Within the different tissues and organs of the body, the constituent cells exhibit a wide range of functional specialization. Despite this extraordinary range of morphological forms and cells conform to a basic structural model which is the subject of this lecture. The process by which cells assume specialized structure and function is known as differentiation.

Even with a primitive light microscopy, it was evident that cells are divided into at least two components, the nucleus and the cytoplasm, and as microscopic techniques advanced it became increasingly obvious that both the cytoplasm and nucleus contain a number of subcellular elements called organelles.

The resolving power of the light microscope is limited to about 0.2 mm (200 nm) and the study of the ultrastructure of the cell had to await the advent of electron microscopy. The capacity of electron microscopes in current use permits the resolution of structures as small as 1.0 nm, this however, falls far short of many cellular processes which occur at the molecular level. Light and electron microscopy have been successfully combined with biochemical and immunological techniques to define the location

of many biological processes; these techniques are known as histochemical and immunohistochemical respectively.

All cells are bounded by an external limiting membrane called the plasma membrane or plasmolemma, which serves as a dynamic interphase between the internal environment of the cell and various external environments. The nucleus is the largest organelle and its substance, often referred to as the nucleoplasm, is bounded by a membrane system called the nuclear envelope. The cytoplasm contains the variety of organelles, most of which are also bounded by membranes. An extensive system of membrane-bound tubules, saccules and flattened cisternae, known as the endoplasmic reticulum, is widely distributed throughout the cytoplasm. A more distended system of membrane-bound saccules, the Golgi apparatus, is typically located close to the nucleus. Scattered free in the cytoplasm are a number of relatively large elongated organelles called mitochondria, which have a smooth outer membrane and convoluted inner membrane system.

The cytoplasmic organelles are suspended in a fluid medium called the cytosol in which much of the intermediary metabolism of the cell takes place. Within the cytosol, there is a network of minute tubules and filaments collectively known as the cytoskeleton, which provides structural support for the cell and its organelles as well as providing a mechanism for cellular and intracellular movement; elements of the cytoskeleton are only visible with very high magnification.

The current concepts of membrane structure derive from the work of Singer and Nicholson in the early 1970 year. In this model, cell membranes consist basically of phospholipid molecules arranged as a bilayer. Phospholipid molecules consist of a polar, hydrophilic (water-loving) head and non-polar, hydrophobic (water-hating) tail. The polar heads are mainly derived from glycerol conjugated to a nitrogenous compound such as choline, ethanolamine or serine via a phosphate bridge. The phosphate group is negatively charged whereas the nitrogenous group is positively charged. The non-polar tail of the phospholipid molecule consists of two long-chain fatty acids each covalently linked to the glycerol component of the polar head. The weak intermolecular forces which hold the bilayer together allow individual phospholipid molecules to move relatively freely within each layer and sometimes to ‘flip” between layers. Cholesterol molecules regulate the fluidity and stabilize the phospholipid bilayer. Associated with the bilayer are a variety of protein molecules, which make up almost half of the total

mass of the membrane. Some proteins are incorporated within the membrane (intrinsic or integral proteins) whereas others are held to the inner or outer surface by weaker electrostatic forces (extrinsic or integral proteins). Some intrinsic proteins span the entire thickness of the membrane (transmembrane proteins) to be exposed to each surface, some functioning as "pores" through which hydrophilic molecules are actively or passively transported across the membrane. Many proteins are not fixed but rather "float" within the membrane so that they are freely mobile within the plane of the phospolipid bilayer. This has led to the use of the term fluid mosaik model of membrane structure. The lipid component of the membrane principally determines its mechanical properties, the dynamic functions of the biological compartments is a function of the membrane proteins. Other integral proteins may be fixed by attachment to elements of the cytoskeleton. On the external surface of the plasma membranes of animal cells, many of the membrane proteins and some of the membrane lipids are conjugated with short chains of polysaccharide. These glycoproteins and glycolipids respectively project from the surface of the bilayer forming an outer coating, which may be analogous to the cell walls of p1ants, bacteria and fungi. This polysaccharide layer has been termed the glycocalyx and appears to vary in thickness in different cell types; a similar layer is often also present on membrane surfaces within the cell which are not exposed to the cytosol (e.g., luminal aspects of membrane systems). In some situations the glycocalyx also provides mechanical and chemical protection for the plasma membrane.

Transport Across Plasma Membranes

Plasma membranes mediate the exchange of molecules between the internal and external environments of the cell in four principal ways enab1ing the cell to control the quality of its internal environment.

Passive diffusion. This type of transport is entirely dependent on the presence of the concentration gradient across the plasma membrane. Lipids and lipid-solub1e metabolite such as ethanol pass freely through plasma membrane, which also offer little barrier to the diffusion of gases such as oxygen and carbon dioxide. The plasma membrane is, in general, impermeable to hydrophilic molecules. Nevertheless some small molecules including water and urea, and inorganic ions such as bicarbonate, are able to pass

down osmotic and electrochemical gradients through the membrane via hydrophilic regions, the nature of which remains obscure.

Facilitated diffusion. This type of transport is also concentration-dependent and involves the transport of larger hydrophilic metabolites such as glucose and amino acids. This process is strictly passive but requires the presence of so-called "carriers" to which the metabolites bind specifically a manner analogous to the binding of substrate with enzyme.

Active transport. This mode of transport is not only independent of concentration gradients but also often operates against extreme concentration gradients. The classical example of this form of transport is the continuous transport of sodium out of the cell by the "sodium pump". This process requires the expenditure of energy provided in the form of ATP. Active transport is mediated by "dynamic pores" consisting of transmembrane protein systems. Both active and passive transport processes are enhanced if the area of the plasma membrane is increased by folds of projections of the cell surface as exemplified by the absorptive cells lining the small intestine.

Bulk transport. This involves large molecules or small particles being engulfed by the plasma membrane, thus forming membrane-bound vacuoles (vesicles) within the cytoplasm. When the process involves the creation of small vacuoles, it is known as pinocytosis, and when large vacuoles are formed, it is called phagocytosis. The term endocytosis, encompassing both processes, is probably a more appropriate term for bulk transport. Endocytotic vesicles either discharge their contents directly into the cytoplasm or fuse with membrane-bound organelles called lysosomes which contain a variety of enzymes which are capable of degrading carbohydrates, lipids, proteins, nucleic acids and other organic molecules. Liposomal enzymes digest engulfed material which is then made available for metabolic processes. In many secretory processes, bulk transport occurs in the opposite direction when it is termed exocytosis. In some tissues where cells form a barrier between two extracellular environments, bulk transport involving pinocytotic vesicles is used to transport large molecules from one side of the cell to the other without the involvement of lysosomes; this is described as transcytosis.

Histologically, the passive and active processes of transport can only be observed indirectly; for example, cells suspended in hypotonic solutions swell due to passive uptake of water whereas cells placed in hypertonic solutions tend to shrink due to outflow of water. Radioisotope labeling tech-

niques can be used to follow active transport processes. Bulk transport, however, is readily observable by microscopy.

Endocytosis. The first stage of phagocytosis involves recognition of matter to be ingested. This then becomes enveloped by plasma membrane which may involve the formation of cytoplasmic extensions called pseudopodia. When the particle is completely surrounded, the encompassing plasma membranes fuse and the membrane surrounding the engulfed particle forms a vesicle, known as a phagosome or endocytotic vesicle, which detaches from the plasma membrane to float freely within the cytoplasm. The phagosome is then in some way recognition by one or more *lysosomes (primary lysosomes)* which fuse with the phagosome to form a *secondary lysosome*. This exposes the engulfed material to a battery of lysosomal enzyme. When digestion is complete, the lysosomal membrane may rupture, discharging its contents into the cytoplasm. Undigested material may remain within membrane-bound vesicles called *residual bodies*, the contents of which may be later discharged at the cell surface by exocytosis; alternatively residual bodies may accumulate in the cytoplasm. Membrane-bound organelles containing multiple small vesicles called multivesicular bodies, are also found in some cells and are through to represent a form of secondary lysosome or residual body containing multiple pinocytotic vesicles or their remnants. The typical lysosomal enzyme is *acid phosphatase*.

Peroxisomes. Peroxisomes are small, spherical, membrane-bound organelles (also known as micro bodies) which closely resemble lysosomes in size and electron microscopic appearance. They are, however, distinguished from lysosomes by their content of an entirely different set of enzymes which can be demonstrated by hystochemical techniques. Peroxisomes contain oxidases, involved in certain catabolic pathways (beta oxidation of long chain fatty acids) utilizing molecular oxygen and resulting in the formation of hydrogen peroxide. Peroxisomes also contain *catalase*, which regulates hydrogen peroxidase concentration, utilizing in the oxidation of a variety of potentially toxic metabolites and ingested substances including phenols and alcohol.

The peroxisomes of many species have a central crystalloid structure which contains the enzyme *urate oxidase*. This is not present in human which thus lack the ability to metabolize urates. The peroxisomes of the liver and in kidney are particularly large and abundant reflecting the func-

tions of these organs in lipid metabolism and management of metabolic waste products.

Endoplasmic reticulum. The endoplasmic reticulum consists of an interconnecting network of membranous tubules and flattened sacs (cisternae) which ramifies throughout the cytoplasm. Much of its surface is studded with ribosomes giving a rough or granular appearance leading to the name rough or granular endoplasmic reticulum (rER or gER). Proteins are synthesized by the ribosomes on the external surface of the rER and are then passed into reticular lumen. Protein synthesis by rER is destined either for secretion or incorporation in lysosomes; integral membrane proteins are also synthesized on rER. In contrast, proteins which are synthesized on free ribosomes are utilized within the cytosol.

Lipid biosyntheses. Lipids are synthesised by all cells in order to repair and replace damaged or worn membranes. Cell may also synthesis lipid as a means of strong excess energy (as cytoplasmic droplets), for lipid transport (e.g., chylomicron production by cells of the small intestine) and in the form of steroid hormones. The precursor molecules (fatty acids, cholesterol) are available to the cell from dietary source of lipid stored in other cells or can be synthesis by most cells using simple sources of carbon such as acetyl-Co A and other intermediates of glucose catabolism. Fatty acids and triglycerides are mostly synthesis within the cytosol, whereas cholesterol and phospholipids are synthesis in areas of endoplasmic reticulum devoid of ribosomes called smooth endoplasmic reticulum.

Smooth endoplasmic reticulum. Smooth endoplasmic reticulum (sER) consists of an irregular network of membranous tubules and vesicles devoid of ribosomes. It forms part of the intracellular membrane system being continuing with the rough endoplasmic reticulum and Golgi apparatus. The principal functions of smooth endoplasmic reticulum are lipid biosynthesis and intracellular transport in liver cells, endoplasmic reticulum also plays a major role in the metabolism of glucogen and detoxification of various noxious metabolic products and alcohol. In highly contractile cells (muscle), sER is involved in storage and release of calcium ions which activates the contractile mechanism. In general, most cells do not have a prominent system of smooth endoplasmic reticulum but, rather, scattered elements can be seen among the other organelles. The notable exceptions are the liver and those cells specialized for lipid biosynthesis such as the steroid hormone-secreting cells of the adrenal glands and the gonads.

Golgi apparatus. Excretion or secretion of small molecular weight compounds or lipid-soluble materials rarely involves bulk transport, whereas secretion of proteins and protein complexes almost always involves this model of transfer. Prior to release from the cell proteins and other secretory products are packed within membrane-bound vesicles. These then fuse with the surface plasma membrane thus releasing their contents by the process of exocytosis. The Golgi apparatus (also called Golgi body or Golgi complex) is the organelle primarily responsible for the packaging process. By a similar process, the Golgi is also responsible for lysosomes formation.

The Golgi apparatus is also involved in modification of certain proteins and contains the enzymes required for the synthesis of glycoproteins. These include plasma membrane glycoproteins forming the glycocalyx. Likewise, the Golgi is also responsible for the elaboration of membrane glycolipids. During the secretory process large amounts of intracellular membrane become incorporated into the plasma membrane and the Golgi system recycles excess plasma membrane returning it to an internal "pool" of membrane. The Golgi apparatus also elaborates new membrane necessary for cell growth and formation of membrane-bound organelles such as lysosomes, as well as replasing membrane lost or damaged during normal metabolic activities.

The Golgi apparatus consists of a system of cisternae with the concave facing the nucleus. Proteins, synthesized on ribosomes of the rough endoplasmic reticulum, are transported within the endoplasmic reticulum to the vicinity of the Golgi apparatus. Small membrane-bound vesicles containing protein, known as transfer vesicles, bud off from the endoplasmic reticulum and then coalesce with the convex surface of the Golgi apparatus known as the forming face. By a mechanism still unresolved, secretory product is passed towards the concave surface, the maturing face new vesicles containing secretory product are formed. These secretory vesicles are of much greater dimensions than the transfer seen at the Golgi forming face. After release from the maturing face, the contents of secretory vesicles become increasingly condensed to form mature secretory vesicle, often termed secretory granules, which are then liberated at the cell surface by exocytosis.

The Golgi apparatus is a dynamically changing structure, the appearance of which varies according to the functional state of the cell. For this reason the "classic" appearance of the Golgi apparatus is in practice, rarely seen.

Moreover, a cell may contain as many as 100 Golgi stacks or even, all being linked by an anastomotic membrane network.

Mitochondria. Mitochondria vary considerably in size and shape but are most often elongated "cigar-shaped" organelles. They are motile and tend to localize at intracellular sites of maximum energy requirement. The number of mitochondria in cells is highly variable: the liver cells contain as many as 2,000 mitochondria whereas inactive cells contain very few. Each mitochondrion consists of two layers of membrane. The outer membrane is relatively permeable and contains enzymes that convert lipid substrates into forms that can be metabolized within the mitochondrion. The inner membrane is thrown into folds called cristae projecting into the inner cavity which is filled with an amorphous substance called matrix. The matrix contains a number of dense matrix granules, the nature and function of which are unclear. The inner mitochondrial membrane is closely applied to the outer membrane leaving a narrow intermembranous space, which extends into each crista. Aerobic respiration takes place within the matrix and inner mitochondrial membranes and this process is enhanced by the large surface area what provided by the cristae. The matrix contains most of the enzymes involved in oxidation of fatty acids and the Krebs cycle. The inner membrane contains the cytochromes, the carrier molecules of the electron transport chain, and the enzymes involved in ATP production.

As organelles mitochondria have several most unusual features. The mitochondrial matrix contains a strand of DNA arranged as a circle in a manner analogous to the chromosomes of bacteria. The matrix also contains ribosomes, which have a similar structure to bacterial ribosomes. Mitochondria synthesis at least some of their own constituent proteins, others being synthesis by the cell in which they reside. In addition, mitochondria undergo self-replication in a manner similar to bacterial cell division. It has thus been proposed that mitochondria are semi-autonomous organelles, which arose during evolution as bacterial intracellular parasites of larger more advanced cells.

The cytoskeleton and cell movement. In order to maintain structural stability, there is within every cell a supporting framework of minute filaments and tubules known as the cytosceleton. Nevertheless, the cell membrane and intracellular organelles are not rigid or static structures but are in constant state of movement to accommodate process such as endocytosis, phagocytosis and secretion. Some cells have actively motile membrane spe-

cialized such as cilia and flagella, whilst other cells (e.g., muscle cells) are highly specialized for contractility. In addition, cell division is a process which involves extensive reorganization of cellular constituent. The cytoskeleton thus incorporates features which accommodate all these dynamic functions. The cytoskeleton of each cell contains structural elements of three main types, microfilaments, microtubules and intermediate filaments as well as many accessory proteins responsible for linking these structures to one another, to the plasma membrane and to the membranes of the intracellular organelles.

Microfilaments. Microfilaments are extremely fine strands (5 nm in diameter) of a protein known as actin. Each actin filament consists of two strings of bead-like subunits twisted together like a rope. The globular subunits are stabilized by calcium ions and associated with ATP molecules which provide energy for contractile processes.

Actin filaments are best demonstrated histologically in skeletal muscle cells where they are arranged in bundles with another type of filamentous protein called myosin. Contraction occurs when the actin and myosin filaments side relative to one another due to the rearrangement of intermolecular bonds fuelled by the release of energy from associated ATP molecules. Cells not considered to be overtly contractile also contain the globular subunit of various subtypes of actin which appear to assemble readily into microfilaments and then dissociate, thereby providing a dynamically changing structural framework for the cell.

Beneath the plasma membrane, actin in association with various transmembrane and linking proteins (predominantly filamin) forms a robust supporting meshwork called the cell cortex which protects against deformation yet can be rearranged to accommodate change in cell morphology.

Microtubules. Microtubules (25 nm in diameter) are much larger than microfilaments, but like them, are made up of globular protein subunits which can readily be assembled and disassembled to provide for alterations in cell shape and position of organelles. The microtubule subunits are two types, alpha and beta tubulin, which polymerize to form a hollow tubule; 13 tubulin molecules make up a circle. Microtubule-associated protein stabilize the tubular structure and including capping proteins which stabilize the growing ends of the tubules. Two attachment proteins, dynein and kinesin (which can move along the tubule towards and away from the cell center respec-

tively), may become attached to membranous organelles (mitochondria, vesicles) providing a means by which they can be moved about within the cytoplasm. In cilia, nine pairs of microtubules are disposed in a cylindrical structure, and movement occurs by rearranged of chemical bonds between adjacent microtubule pairs.

The organizing center for the cytoskeleton appears to be located near the nucleus in an area called the centrosome (cell centre) which contains a pair of centrioles. Each centriole consists of nine triplets of microtubules arranged in a cylindrical manner, the pair of centrioles being disposed at right angles to one another. The centrosome appears to act as a nucleation center for microtubules which radiate from here towards the cell periphery. Centrioles appear to be necessary for microtubular function. For example, prior to cell division the pair of centrioles is duplicated, the pairs migrate towards opposite ends of the cell. Here they act as organizing centers for the microtubules of the spindle, which controls distribution of chromosomes to the daughter cells. Likewise a pair of centrioles, known as a basal body, is found at the base of the microtubules of cilia. The destruction of microfilaments and intermediate filaments tends to be complementary to that of the microtubules and there is experimental evidence that, at least in some situations, microtubules may form a temporary framework around which more permanent cytoskeleton structures can be built up. The elements of the cytoskeleton are attached to one another and to the plasma membrane and the membranes of cytoplasmic organelles by a variety of linking proteins. In addition, some of the metabolic enzyme systems the cytosol appears to be bound to various elements of the cytoskeleton. Moreover, the cytoskeleton appears to provide a mechanism where can be transported within the cell by some molecules. Such molecules are bound to microfilaments and microtubules and these elongate and shorten; the molecules are moved from one site to another. The cytoskeleton elements may then disassemble leaving the transported molecules in new positions.

Nucleus. Protein synthesis. Proteins are not only a major structural component of cell but, in the form of enzymes mediate every metabolic process within the cell. Thus the nature and quantity of proteins present within any individual cell determines the activity of that cell. Both the structural proteins and enzymes of the cell are subject to wear and tear and are replaced continuously. Many cells also synthesize proteins for export; such proteins include granular secretions and extracellular structural components

of tissues. Protein synthesis is, therefore, as essential and continuous activity of all cells and the major function of some cells.

The principal organelles involved in protein synthesis are the nucleus and ribosomes. The nucleus of every cell contains within its complement of DNA a template for each protein that can be made by that individual as a whole. The process of protein synthesis involves transpiration of the DNA code for a particular protein by synthesis of the specific, complementary messenger RNA molecule. The mRNA molecule then enters the cytoplasm to associate with ribosomes upon which protein synthesis occurs; the amino sequence of the resulting protein is determined by translation of the mRNA code.

Ribosomes are minute cytoplasmic organelles, each composed of two subunits of unequal size. Each subunit consists of a strand of RNA (ribosomal RNA) with associated ribosomal proteins; the ribosomal RNA strand and associated proteins are folded to form a condensed, globular structure. Ribosomes are highly active structures with specific receptor proteins which align mRNA strands so that transfer RNA molecules carrying the appropriate amino acids may be brought into position prior to the addition of their amino acids to the growing polypeptide chain. Other ribosomal proteins are involved in catalysing peptide bond formation between amino acid. Individual ribosomes are too small to be clearly resolved by electron microscopy although they are visible as small electron-dense structures at high magnification: nevertheless, the detail of ribosome structure and function are well established at the molecular level. Ribosomes may be present in the cytoplasm either alone of attached to messenger RNA molecules in spiral-shaped aggregations called *polyribosomes* or *polysomes*. Ribosomes and polyribosomes may also be attached of the extensive intracytoplasmic membrane system known as the endoplasmic reticulum.

The nucleus not only contains DNA, which comprises less than 20% of its mass, but also contains a large quantity of protein called nucleoproteins and some RNA. Most of the nucleoproteins are closely associated with DNA, these DNA-binding proteins being of two major types, *histones* and *non-histones*. Histones, which comprise the bulk of DNA-associated protein, are relatively low molecular weight proteins with a high content of positively charged amino acids, which bind them readily to the negatively charged DNA strands. Histones may be involved in regulation of gene activity. The remaining nucleoproteins include enzymes responsibly for DNA

and RNA synthesis. All nucleoproteins are synthesized in the cytoplasm and imported into the nucleus. The nuclear RNA represents newly synthesized messenger transfer and ribosomal RNA which has not yet passed into the cytoplasm.

Except during cell division, the chromosomes, each comprising a discrete length of the DNA complement, exist as tangled strands, which extend throughout the nucleus and cannot be visualized individually by direct electron microscopy. Nuclei appear as heterogeneous structures with electron-dense and electron-lucent areas. The dense areas, called *heterochromatin*, represent that portion of the DNA complement and its associated nucleoprotein, which is not active in RNA synthesis. Heterochromatin tends to be clumped around the periphery of the nucleus but also forms irregular clumps throughout the nucleus. In females, X-chromosome (equivalent to the Y-chromosome of the male) forms a small discrete mass known as a Barr body. Barr bodies are seen at the edge of the nucleus in a small proportion of female cells. The electron-lucent nuclear material, called *euchromatin,* represents that part of the DNA, which is active in RNA synthesis. Collectively, heterochromatin and euchromatin are known as chromatin, a name from the strongly coloured appearance of nuclei when stained for light microscopy.

Nucleoli. Many nuclei, especially those of cells highly active in protein synthesis, contain one or more dense structures called nucleoli which are the site of ribosomal RNA synthesis and ribosome assembly. The many different ribosomal proteins imported from the cytoplasm are conjugated with ribosomal RNA to form the ribosomal subunits, which then pass into the cytoplasm before being assembled into fully active ribosomes. Nucleoli are heterogeneous structures, the paler areas being the sites of DNA coding for ribosomal RNA and the dark areas being the sites of partially assembled ribosomes. Each cell type has a characteristic nuclear morphology and, in general, the degree of activity of any cell may be judged by the intrastructural appearance of its nucleus. Relatively inactive cells have small nuclei in which the chromatin is predominantly in the condensed form (heterochromatin) and in which the nucleolus is small or absent. In highly active cells, the nuclear material is dispersed (euchromatin) and nucleoli are a prominent feature,

Nuclear envelope. The nuclear envelope consists of two layers of membrane (each layer of standard phospholipid bilayer structure) which repre-

sent a specialized part of the endoplasmic reticulum. The intermembranous (perinuclear) space is continuous with that of the endoplasmic reticulum, and the outer surface of the nuclear envelope is connected with ribosomes. On the inner aspect of the nuclear envelope there is an electron-dense fibrillar layer, the nuclear lamina of polypeptides bound to membrane proteins and linked with condensed peripheral chromatin. A network of filamentous proteins is attached to the nuclear lamina, which provide internal support for the nuclear contents and nucleoli and thus represent the nuclear cytoskeleton.

The nuclear envelope contains numerous nuclear pores at the urging of which the inner and outer membranes become continuous. Each pore contains an electron-dense structure known as a *nuclear pore complex,* which have the octagonal symmetry and consists of tubular and globular proteins. Nuclear pore complex is formed by the 3 lines of proteins consisting 8 globular molecules in the each line. Two lines occupy peripheral disposition (nuclear and cytoplasmic sides) and one is located in the central part of the pore. Fibrillar proteins connect peripheral and central globular proteins forming structure similar to diaphragm. Nuclear pores permit and regulate the exchange of metabolites, macromolecules and ribosomal subunits between nucleus and cytoplasm.

Lecture 2

EPITHELIAL TISSUE

As explained previously, the body is composed of only three basic elements, i.e., cells, intercellular substances, and body fluids. During development, the embryo consists of three cellular layers (ectoderm, mesoderm, and endoderm), each specialized in respect of function, future development. All adult tissues develop from these and, in the adult, only four primary tissues are present. A primary or basic tissue may be defined as a evolutionary formed system of cells and intercellular substance specialized in a common direction and able to perform a common function.

In turn, organs are formed from these tissues and, usually, all four types are present in a single organ. A student who has learned to recognize these four basic tissues will have taken a large step toward the understanding of histology and the identification of tissues. A section that at first appears complex and confusing when seen under the microscope is, in fact, composed only of the four basic tissues. A few characteristics usually will prove adequate for identification.

The four primary tissues are *epithelium, connective tissue, muscle,* and *nervous tissue.*

Epithelium. The cells are closely packed with very little cementing substance between, and they are arranged as sheets covering or lining surfaces or as masses of cells in glands.

Connective tissue. The cells usually are widely separated by a relatively large amount of intercellular substance. This group includes certain specialized tissues such as blood and blood-forming tissues, bone, and cartilage.

Muscle. The cells are elongated, containing cytoplasm filaments, are relatively closely associated, and separated by fine, vascular connective tissue.

Nervous tissue. This consists of cells, some of which are very large, and their long processes, which are usually grouped as relatively isolated masses or bundles.

Epithelial tissues are formed by closely posed polygonal cells with little or without intercellular material. They occur as *membranes* and as *glands*. Membranes are formed by sheets of cells and cover an external surface or line an internal surface. Glands develop from epithelial surfaces by down growths into underlying connective tissue, and usually the connection to the surface remains as the duct of the gland. Such are *exocrine* glands, the secretion passing externally to the surface. In some cases, the surface connection is lost and the gland secretes internally into the vascular system; these are *endocrine* glands.

All epithelia lie upon or are surrounded by a *basal lamina* that separates the epithelium from subjacent connective tissue, blood vessels, and nerves lying in that connective tissue. Functionally, epithelia form the coverings or linings of surfaces, provide secretions from both membranes and glands, and are involved in the process of absorption. A few specialized epithelial cells are contractile (myoepithelial cells), and a few are sensory (neuroepithelial).

Being packed closely together, most epithelial cells are polygonal in outline although they may be highly irregular. Their shape and arrangement in layers are the two factors that provide the basis for classification of membranes.

With respect to shape, the epithelial cells are squamous, cuboidal, and columnar, with intermediate forms. *Squamous* cells are very flat, with height much less than width. In profile, they show a central thickening at the site of the nucleus. *Cuboidal* cells are box like, with height and width approximately equal and *columnar* cells have a much greater height than width. Nuclei of all the cells are parallel to the main axis of the cell with a shape corresponding to that of the cell. Thus, nuclei are spherical and generally central in position in cuboidal cells, flattened in squamous cells, and ovoid in columnar cells.

Cells are arranged in membranes in one or more layers. *Simple* epithelia are those with cells in a single layer; all the cells contact the basal lamina

and reach the surface. *Stratified* epithelia are those with cells in two or more layers, only the cells of the deepest or basal layer contacting the basal lamina. *Pseudostratified* epithelia are those in which all the cells contact the basal lamina, but not all reach the surface. Basically, several cell types are present in a single layer, usually with nuclei at different levels giving the false appearance of several layers.

Classification uses these two factors with that concerning cell shape applying only to the surface layer of cells in stratified epithelia. Thus, there are, for example, simple and stratified squamous epithelia, the former formed by a single layer of squamous cells, the latter by several layers of cells of which the surface layer is formed by squamous cells. Further descriptive terms are used for subclassification, for example, pseudostratified columnar epithelia may be *ciliated* or *non-ciliated.* It is not feasible to classify epithelia on the basis of embryonic origin. Since all three germ layers give rise to epithelia of the skin and those lining the oral and anal regions being of ectodermal origin. Those lining respiratory and digestive tracts — of endodermal one. Those of the urinary and reproductive tracts, serous layers — from mesoderm, lining blood and lymph vessels — from mesenchyme.

As cells lie in epithelial membranes, for descriptive purposes the surface adjacent to the lumen is termed the *apical surface* or *pole,* that toward the basal lamina the *basal surface,* and those surfaces between adjacent cells as *lateral cell surfaces.* There are no blood or lymph vessels in the epithelium. Nutrition occurs by diffusion of tissue fluid from vessels of the underlying connective tissue. Epithelial cells thus are kept moist mainly by fluid from beneath; although in many cases an epithelial membrane lines a moist cavity, e.g., the digestive tract. Numerous small nerve fibers are located in the connective tissue beneath epithelial membranes, and fine terminal branching from them may penetrate the basal lamina to run among epithelial cells. The cell nuclei are lying at different levels in a perpendicular section, thus giving the impression that the membrane is composed of more than one layer of cells. Some of the cells may not reach the lumen, although all are adjacent to the basal lamina. Such an epithelium lines the larger excretory ducts of many glands and parts of the male urethra. This type of the epithelium may be ciliated, usually in association with goblet cells, and is found lining the larger respiratory passages and some of the excretory ducts of the male reproductive system.

CLASSIFICATION

Because of epithelia lining surfaces and cavities, they commonly have a free margin, which faces the outside environment or the lumen of a particular organ, and a surface, which faces underlying or surrounding connective tissue. The free margin is termed the apical surface or pole of the epithelium, and the connective tissue-facing pole is termed the basal surface. The surfaces of cells, which face neighboring epithelial cells within the epithelium, are sometimes referred to as the lateral cell surfaces. In nearly all epithelia (with some outstanding exceptions) the cells bordering the basal surface secrete a submicroscopically thin extracellular coat, the basal lamina along that surface. It serves to separate the epithelium from underlying connective tissue. The basal lamina is reinforced by layers of connective tissue collagen. The total structure becomes thick enough to be discerned by light microscopy and is referred to as a basement membrane. For convenience, epithelia are classified into different types on the basis of the number of cell layers and the shape of the cells at the apical surface. The epithelia of only one layer are termed simple, and they are subdivided according to the height of the cells when viewed in cross section, into *simple sqamosus, simple cuboidal*, and *simple columnar.* The epithelium composed of two or more layers is said to be stratified, and is subdivided according to the shape of the cells at the apical surface into *stratified sqamous, stratified cuboidal*, and *stratified columnar.* Stratified sqamous epithelium is the most commonly found stratified type. Only the upper layers are sqamous in it; those along the basal lamina and for a considerable distance above it are columnar and polyhedral. The cells of the basal areas are frequently mitotically active, serving to replenish the layers above. Moreover, the functions served by the cells of various layers may be quite different. This is particular truth in the epidermis of the skin. The epithelium of some parts of the body consists of a single layer of cells of variable height and arrangement, with all cells resting on the basement membrane, but with only some of the cells reaching the apical surface. The nuclei of these cells are seen at different levels above the basement membrane and, on first inspection, one might conclude that the epithelium is stratified. Only careful study discloses that this epithelium is really composed of only one layer of cells. It has been traditionally classified as *pseudostratified.*

The special modification of the stratified epithelium is found in the urinary system, where the number of cell layers and the shapes of the cells vary with distention and contraction of the organ. This type of the epithelium is classified as transitional. In addition to the above criteria for classification, the epithelia are often described in terms of products they accumulate or release, or in terms of cellular appendages that may characterize the apical surface. Hence, an epithelium, which has a high population of specialized cells which synthesize and release mucus into the apical surface (goblet cells) can be termed a mucous epithelium. Likewise, because the cells of the apical epidermis accumulate high concentrations of a tough protein material called keratin, this epithelium can be keratinized. When the apical surface of an epithelium bears cilia, particularly if they are numerous, the epithelium is said to be ciliated. If the same surface possesses large numbers of more minute projections, the microvilli, the epithelium is said to have a "*striated", "brushed*", or *microvillious border.*

Combining these various schemes, it is common to describe an epithelium in rather complex fashion. For example, the epidermis of the skin is termed stratified sqamous keratinized epithelium and the lining of the trachea is a pseudostratified ciliated columnar epithelium. The lining epithelium of the intestine is a simple columnar epithelium displaying a striated microvillous border.

Simple Epithelium

Simple squamous epithelium. The simple squamous epithelium (covering epithelium) consists of flat scale-like or plate-like cells arranged in a layer only one cell thick. On surface view the cells appear as a delicate mosaic. The edges of the cells are usually slightly interdigitated with those of their neighbors, but may be smooth. The nucleus, situated in the center of the cell, is spherical or ovoid, causing a bulge.

The simple squamous epithelium is widely distributed. It lines the peritoneal, pleural, and pericardial cavities (mesothelium), the heart and all blood and lymph vessels (endothelium), the membranous labyrinth of the internal ear, portions of the uriniferous tubule, and portions of the rete testis.

Endothelium and mesothelium are excellent examples of the simple squamous epithelium. Pinocytotic vesicles are particular numerous in the cytoplasm of endothelial cells, and they apparently play a role in the transport

of some substances (large molecules such as proteins) across the cell. Mesothelium differs in that it apparently can be regenerated from cells of the underlying connective tissue. Mesothelial cells can also change into fibroblasts. They seem to be less specialized than other types of epithelial cells and appear to retain some of the multipotency of mesenchyme. Mesenchymal epithelium is sometimes given to the simple squamous cells, which line other connective tissue-enclosed cavities: the subarachnoid and subdural cavities, the chambers of the eye, and the perilymphatic spaces of the ear. The structure of this epithelium is generally similar to that of mesothelium.

Simple columnar epithelium. Simple columnar epithelium in its various modifications represents the chief secretory and absorptive tissue of the body. It consists of a single layer of tall cells resting on a continuous basal lamina. The height varies considerably and the term cuboidal epithelium is applied when height and thickness of the cells are about equal. All transitions from low cuboidal to high columnar types of cells are encountered. The secretory units or acini of most glands are lined by cuboidal or columnar epithelial cells whose broad bases rest on the basement membrane and whose apices face the narrow lumen. This is termed pyramidal or glandular epithelium. In simple columnar epithelial cells, the nucleus is oval and usually placed basally. In the basal perinuclear portion of the cytoplasm, there are numerous mitochondria and abundant rough endoplasmic reticulum, particular in cells that are secretory. The apical portion may contain granules or vesicles of stored products of the cell (zymogen, mucin, etc.). The cytoplasmic constitution varies greatly under different conditions of cellular activity. The striated border may be absent, as in most glandular epithelium, or very prominent, as in the high columnar absorptive epithelium of the small intestine. The important cellular variation, found in columnar epithelia, is the goblet cell. This cell is characterized by the accumulation of membrane-bounded mucin droplets and a relative paucity of microvilli. The mucin droplets accumulate in the apical end of the cell and push the nucleus and most of the remaining cytoplasm toward the base. Thus, the cell assumes a goblet shape. The mucin droplets become closely packed but remain membrane-bounded and separate until they escape by exocytosis from the apical pole of the cell. Sometimes goblet cells secrete cyclically and at other times continuously, depending upon the stimuli and demands in a given location. Most mucins are not preserved and stained in the routine preparations for light microscopy.

Pseudostratified epithelium. In this epithelium type, the nuclei lie at different levels, giving it a stratified appearance. Fill of the cell reach the basement membrane, but not all of them extend to the free surface. Those cells, which do reach the surface, are columnar containing one or more thin processes which extend to the basement membrane. These processes are difficult to see in routine histological preparations. Between the slender processes, there are ovoid or spindle-shaped cells. This type of epithelium usually has either cilia or stereocilia. It occurs mainly as the lining of the passages of the respiratory and the male reproductive systems.

Stratified Epithelium

All stratified epithelia can withstand more trauma than the simple types and thus are located in sites where they are subjected to friction and shearing forces, but because of their thickness they are not membranes through which absorption can occur readily.

Stratified squamous epithelium. The stratified squamous epithelium is a thick membrane, and only the more superficial cells are flat. The deeper layers of cells vary from cuboidal to columnar, and often the basal layer, i.e., that adjacent to the basal lamina, shows considerable irregularity. That covering the cornea of the eye lies upon connective tissue with a smooth, regular surface, but in other locations the underlying connective tissue is raised into ridges and folds that appear as finger-like processes (papillae) in perpendicular section. Such arrangement is found, for example, in the vagina, the esophagus, and the skin. In the vagina and esophagus, the surface of the epithelium is moist, and here the epithelium is non-keratinized; in the skin, the surface is dry and the surface cells undergo a transformation into a tough, resistant, nonliving layer of material called keratin — hence the name stratified squamous keratinizing epithelium.

Stratified columnar epithelium. The stratified columnar epithelium is also relatively rare. Usually the basal layer or layers consist of relatively low, irregularly polyhedral cells, and only the cells of the superficial layer are of the tall columnar type: such an epithelium lines a part of the male urethra and is found also in some larger excretory ducts and in the conjunctiva.

Transitional epithelium. Transitional epithelium is so termed because originally it was believed to represent a transition between the stratified

squamous non-keratinizing and stratified columnar types. It is found lining the urinary system from the renal pelvis down to the urethra, sites where it is subject to considerable variations in internal pressure and capacity. Hence, its appearance varies with the degree of distention. The basal layer is cuboidal or even columnar in type, the intermediate levels are cuboidal and polyhedral, and the superficial layers vary from cuboidal to squamous, depending upon the degree of distention. The superficial cells lining a non-distended organ characteristically have a convex free border and are often binucleate; i.e., they exhibit polyploidy.

Stratified cuboidal epithelium. Stratified cuboidal epithelium is found only in the ducts of sweat glands in the adult and consists of two layers of cuboidal cells. As this type lines a tube, it is obvious that the cells of the superficial layer or layers are small functions not shown by ordinary simple squamous epithelium. For example, endothelial and mesothelial cells are actively phagocytic, can form fibroblasts by cell division, and are responsible for a variety of interesting tumors. They formerly were called "false epithelia" or "pseudoepithelia".

BASEMENT MEMBRANES

The epithelial, muscle and nervous tissues, of which all specialized composite tissues and organs are comprised, derive mechanical and nutritional support from supporting connective tissue, the boundary being invariably marked by a condensed layer of extracellular material traditionally known as the basement membrane. In the context of muscle and nervous tissue, the term external lamina may also be applied. Epithelia in particular are almost entirely composed of closely packed cells with minimal intercellular material between them. The basement membrane provides structural support as well as binding the epithelium to the underlying supporting tissue. Basement membrane is also involved in the control of epithelial growth and differentiation forming an impenetrable barrier to downward epithelial growth; this is only breached if epithelia undergo malignant transformation. Epithelium is devoid of blood vessels and the basement membrane must therefore permit the flow of nutrients, metabolites and other molecules to and from the epithelium. Where an epithelium acts as a selective barrier to the passage of molecules from one compartment to another (e.g., between the

lumen of blood vessels and surrounding tissues), the basement membrane assumes a critical role in regulating permeability. Critical association of basement membranes with epithelial structure and function was thus responsible for the basement membrane being traditionally discussed as if it was a unique epithelial structure. Increasing understanding demands that it be considered as one of the supporting tissues. The main constituents of basement membranes and external laminae are the glycosaminoglycan — heparan sulphate, the fibrous protein collagen type 4, and the structural glycoproteins fibronectin, laminin and entactin. Fibronectin appears to be produced by fibroblasts of the supporting tissue but the remainder is at least partly, if not exclusivity, elaborated by the tissues being supported.

CELL ADHESION IN EPITHELIAL MEMBRANES

Within an epithelial membrane and other tissues, there is cell-to-cell adhesion that can resist considerable mechanical forces tending to separate the cells, e.g., in the stratified squamous non-keratinizing epithelium lining the oral cavity and esophagus, where relatively hard food material passes over the surface. As indicated above, spaces between adjacent epithelial cells are narrow, of the order of 15 to 20 nm (150 to 200 A), and this space is occupied by the glycocalyx of adjacent cells. The binding action of the exposed carbohydrates of these glycoproteins provides some adhesion. The material also contains cations, particularly calcium, which is also important in cell adhesion. In many instances also, plasma membranes of two adjacent cells do not run in parallel fashion but show reciprocal tongues and grooves. These are termed “zipper” or “jigsaw” interlocking. In addition, there are several specializations of the cell surface or junctional specializations. These are found also in tissues different from the epithelium, although they are perhaps developed maximally in epithelium; they concern not only cell adhesion but also permit cells to interrelate functionally in several ways.

CELL JUNCTIONS

Although several terminologies have been used to describe the various types of specialized junctional regions between cells, two factors are taken into account usually:

1. The shape and extent of the contact area. This can be in the form of a spot or punctate area of limited extent called a *macula;* or it can pass around the entire cell in a belt- or corona (crown)-like manner called a *zonula* or as a sheet or strip-like area called a *fascia.*

2. The relative closeness and nature of the cell contact. Here the terms *occludens, adherens,* and *gap* are used. In the occludens type *(occluding* or *tight junction),* the intercellular space is virtually obliterated with outer surfaces of the two plasma membranes apparently in contact or even fused. In *adhering junctions* the intercellular space is apparent, usually 20 to 25 nm wide, and with dense material in the space and associated with the cytoplasmic surfaces of the opposed membranes. The third type, the *gap junction* or *nexus,* shows a very slender intercellular gap of about 2 nm and is concerned with inter cellular communication rather than cell adhesion. Terms different from these are also used, but the text will now focus on the three main types of junctional specialization.

Tight or occluding junctions with apparent fusion of the outer leaflets of the two plasma membranes. In fact, fusion occurs at a series of points only and the freeze-etch technique demonstrates a net work of linear ridges and complementary grooves, each ridge formed by a double row of 3 to 4 nm particles. These particles are interpreted as integral membrane proteins, one row arising from each membrane and making contact like the teeth in a zipper, effectively obliterating the intercellular space at the ridges. These ridges or sealing strands physically bar the passage of molecules, preventing intercellular transport from lumen to extracellular space (or vice versa) through the intercellular space. There is a considerable variation in the number and complexity of sealing strands from tissue to tissue, the more impermeable junctions usually having more strands.

Fascia occludens is similar in structure, but strip-like in form. This type occurs, for example, between endothelial cells lining some blood capillaries. Such junctions obviously do not form an uninterrupted seal between the constituent cells because of its limited extent.

Adhering junctions. Adjacent plasma membranes at adhering junctions are separated by an intercellular space containing bonding material, and these junctions function in cell adhesion. Two types are recognized on their form and associated filamentous material.

Zonula adherens. Zonula adherens, or *belt desmosome,* forms a complete band around epithelial cells adjacent to the luminal surface but just to

the basal side of zonula occludens. Apposed plasma membranes are parallel with an intercellular space of 15 to 20 nm filled with fine filamentous material. The cytoplasmic surfaces of inner leaflets show some electron-dense material associated with filaments, some of which may pass as a flat horizontal band into the terminal web (to be described). These filaments are 7 nm in diameter and appear to contain *actin,* a contractile protein. Zonulae adherents of epithelia and other tissues, e.g., cardiac muscle, function in mechanical attachment and may transmit forces generated within cells. By keeping the terminal web taut, zonulae adherents aid in contraction of microvilli (described later) in some epithelia.

Macula adherens. The second type of adhering junction is macula adherens, or *spot desmosome.* These are small, discoid structures about 410 nm by 250 nm, with their long axes perpendicular to the basal lamina in epithelial membranes and located at various levels on lateral cell interfaces. At the desmosome, there is an intercellular space of 20 to 30 nm between parallel plasma membranes. The space is filled with filamentous material and is bisected by a linear density termed the "central stratum." Cytoplasmic surfaces of inner leaflets of the plasma membranes show dense "attachment" plaques into which there pass 10 nm tonofilaments. These are non-contractile and part of the cytoskeleton. They arise within the cytoplasm, pass into the dense plaque material, and then loop back into the cytoplasm. Thinner filaments appear to extend into the intercellular space to the central stratum as "transmembrane linkers," providing a direct mechanical linkage between tonofilament networks of adjacent cells. The freeze-etch technique demonstrates small particles associated with desmosomes, and these may represent filaments that were broken in the fracturing process. Desmosomes are found in many cell types, but are numerous in cells subject to mechanical stress.

Hemi- or *half desmosomes* occur on basal surfaces adjacent to the underlying basal lamina and connective tissue: they are morphologically half a spot desmosome with tonofilament bundles anchoring in the hemidesmosome and are found in cells where mechanical stress occurs, e.g., in epidermis and cervical epithelium.

Gap Junctions. Here, adjacent plasma membranes are separated by a space of only 2 to 3 nm situated in tangential or grazing sections, within the gap. Usually, these are in the form of a belt completely encircling a cell near its terminal or apical border and termed *zonula occludens.* Often on elec-

tron microscopy a laminar structure is seen, i.e., three dense lines separated by two electron lucent lines, are seen a hexagonal array of 7 to 9 nm particles or connexons, each particle is cylindrical and with a central channel. In freeze-etch preparations, gap junctions vary from discoid, macula-like structures to extensive belt-like regions and show densely packed particles or reciprocal pits. The particles appear to be composed of six subunits arranged around a central coated within the two plasma membranes in register so that their central channels are confluent, permitting direct cell-to-cell interchange. Gap junctions are widely distributed in the body, being absent only in skeletal muscle and blood cells. It is believed that these channels permit transfer of small molecules such as ions, sugars, amino acids, and some hormones. In some tissues (e.g., smooth muscle of the intestine and cardiac muscle, where they usually are called "nexuses"), gap junctions transmit electrical impulses; this permits synchronization of activity between cells that thus are electronically coupled.

Terminal bars are found on lateral epithelial cell interfaces near the free (luminal) surface and are seen particularly well on light microscopy after staining with iron hematoxylin. In grazing sections near the cell surface or in whole mounts, terminal bars outline cells in a hexagonal pattern and are seen as dense dots near the luminal surface in perpendicular sections. By electron microscopy, the terminal bar shows two of the cell junction specializations. The whole complex usually is referred to as the *junctional complex.* Immediately beneath the free surface, on the lateral inter face, there is a tight junction or zonula occludens, and, more basally, is a zonula adherens, both extending around the entire cell perimeter like a crown. The whole complex covers a depth of up to 0.5 micron (nm). Deep to the zonula adherens, i.e., lying on the lateral cell interfaces closer to the base of the cell, are scattered desmosomes or maculae adherents, but these are limited in extent and are not part of the junctional complex.

SPECIALIZATIONS OF THE CELL SURFACE IN EPITHELIA

Such specializations are developed to different degrees in different sites, and these will be indicated later during description of the organ systems.

Microvilli. Microvilli are small, slender, finger-like projections of the apical cell surface consist channel 1.5 nm in diameter, and these cylindrical units appear to be loving of tube-like invaginations of the plasma membrane of the apical surface containing a core of cytoplasm. Individually, they are too small to be seen with the light microscope. In many epithelia, particularly high cuboidal and columnar types, they are numerous and of regular dimensions and form a brush or striated border, visible by light microscopy.

Microvilli of the brush border contain a bundle of axial or core microfilaments that contain actin and pass deeply into apical cytoplasm, where they mesh into a network of other filaments called the "terminal web." This web also contains actin filaments, but these filaments pass peripherally to the zonula adherens. More deeply in the web are intermediate 10 nm tonofilaments passing into spot desmosomes at the cell periphery. The terminal web also contains myosin, and it is believed that actin-myosin interaction results in shortening of the microvillous core bundles with resultant contraction of microvilli. At the same time, the terminal web provides rigidity to permit this actin-myosin interaction. The terminal web of, for example, columnar absorptive cells of the intestine (enterocytes) can be stained with the tannic acid and thus seen by light microscopy just beneath the brush border.

In epithelial cells lining part of the male generative tract, the so-called *stereocilia* are present on the apical surface. By light microscopy they appear as long, slender, sometimes branching, processes that are non-motile. By electron microscopy, it can be demonstrated that stereocilia bear no resemblance to true cilia; rather, they are composed of groups of extremely long, slender, often branching microvilli.

Basal infoldings. At the basal surface of epithelial cells, the plasma membrane may show numerous infoldings, thus forming "pockets" of basal cytoplasm. These infoldings are one method of increasing the surface area at the base of a cell, functioning in this respect similarly to microvilli, which increase the apical surface area. Indeed, in many epithelial cells, both of these specializations are present, as, for example, in the convoluted tubules of the kidney. Such epithelia show rapid absorption and/or secretion of fluid.

Cilia. They project from the free apical surfaces of some epithelial cells and may be very numerous. For example, there are some 270 cilia on each

ciliated cell lining the trachea. In movement, each cilium undergoes a rapid forward beat with a slower recovery stroke, the beat appearing as a wave of movement in a ciliated epithelial membrane, transporting material (e.g., mucus) in one direction along the surface of the membrane. The mechanism of cilia beat is not clear, but its direction is associated with the orientation of cilia filaments.

Cilia occur also in the maculae and cristae of the inner ear and in a modified form as retinal rods of the eye; in such sites they appear to be nerve receptors. In other sites single cilia have been considered chemoreceptors. The single flagellum of spermatozoa is of similar structure and, of course, is motile.

GLAND EPITHELIUM

As has been noted previously, cells of epithelial membranes in many instances secrete materials in addition to their other functions such as protection and absorption. However, this function of secretion is often of secondary importance becouse a cell which is highly specialized for protection or absorption cannot also be highly specialized for secretion. In addition, the epithelial surfaces of the body are of inadequate surface area to accommodate the numbers of secretory cells required. Thus, a system of multicellular glands is present, each composed of masses of epithelial cells highly specialized for secretion. The secretory product of these cells is passed into a system of tubes or ducts which then transport it to a surface. The glandular secretion consists of an aqueous fluid containing the secretory product, e.g., an enzyme, or mucin. This process of synthesis involves interaction of cell organelles and the expenditure of energy.

CLASSIFICATION OF GLANDS

The glands usually are divided into two main groups: *exocrine* and *endocrine.* The gland of the exocrine type passes its secretion to a duct system and thus to a body surface; i.e., there is an external secretion. The endocrine gland passes its secretion or hormone directly into the blood or into the lymph; i.e., it is an internal secretion, hormone is transported through out the body and thus to the target organ or organs where its action is

performed. Both types of glands develop in the embryo in a similar fashion by an invagination of epithelial cells into the connective tissue underlying an epithelial membrane. In exocrine glands the site of the original invagination persists as the duct system, whereas connection with the epithelial membrane is lost in endocrine glands, the secretion then passing into the vascular system.

In some glands the secretion characteristically contains intact, living cells, e.g., the sex glands, which secrete living germ cells. Three other types of secretory cells are described according to the manner in which their secretory product is elaborated.

1. *Holocrine.* In some glands the entire secretory cell, having formed and accumulated secretory products within its cytoplasm, dies, disintegrates, and is discharged from the gland as the secretion. Such a gland, where the entire cell is secreted, is called holocrine; obviously, cell division in such a gland must be rapid to replace cells lost in secretion. Examples of this type are sebaceous and tarsal glands.

2. *Apocrine.* In apocrine glands the secretory product accumulates in the apical portion of the cell, which is then pinched off; the cell thus loses some of its apical cytoplasm together with the specific secretory product. The cell then passes through another secretory cycle after a short recovery period. Examples of apocrine glands are the mammary gland and certain sweat glands. However, in general, electron microscopy studies have failed to demonstrate loss of apical cytoplasm in apocrine glands. Nevertheless, the term is retained.

3. *Merocrine.* The great majority of glands are of the merocrine type whose secretory product is formed in and discharged from the cell without the loss of any cytoplasm. Examples of this type are the sweat glands, the salivary glands and pancreas.

Unicellular glands. The unicellular glands, in which a single cell forms a gland, are represented by mucous or goblet cells, mentioned previously in relation to epithelial membranes. These cells in the epithelia lining the trachea and the large and small intestines characteristically resemble a goblet or wine glass in shape. The distended apical portion is filled with a mass of mucigen droplets that are pale staining in an H and E preparation but that can be stained specifically by other methods. The mucin that is secreted by these cells is a protein-polysaccharide complex that forms *mucus* in water; mucus is a slimy, lubricating fluid. It should be emphasized that not all

mucin-secreting cells are goblet cells. The stomach epithelium, for example, contains three different types of columnar, mucin secreting cells, none of which is goblet shaped, and each of which is a little different from the others both in structure and in chemical composition of the mucin secreted.

Multicellular glands. The multicellular glands are represented most simply by an epithelial sheet composed of secretory cells, but the majority arises as an invagination of an epithelial sheet into underlying connective tissue. Thus, a gland consists of epithelial elements lining its duct system, epithelial elements of the secretory units, and the supporting fibrous connective tissue. The latter contains an extensive network of blood vessels and nerve terminals of the autonomic nervous system. In exocrine glands the epithelial cells of the secretory elements are supported by a basal lamina which separates them from the blood capillaries, but the basal lamina usually is not demonstrable in the endocrine glands (e.g., the thyroid).

Exocrine glands. The various types of multicellular exocrine glands are classified according to whether the duct branches and according to the shape of the secretory unit (tubular, alveolar, or mixed). The duct may be unbranched or branched, and this distinction provides two large groups of *simple* and *compound* glands. In simple glands the duct may be straight or coiled. The secretory unit situated at the termination of a duct, or a small branch of a duct in a compound gland, may be *tubular* or flask-shaped, the latter being called *alveolar* or *acinar* (like a hollow vessel or like a berry). In many glands the secretory units are mixed and the gland is then termed *tubulo-alveolar.* The nature of the secretion may be either *mucous* or *serous,* the latter being a clear, watery fluid usually containing enzymes. The cells responsible for the production of these two types of secretion differ greatly in their appearance. Often either serous and mucous alveoli or acini are found in the same gland, which then is called a *mixed* gland. An acinus, which contains cells of both types, is called a mixed acinus. It is by these three characteristics that exocrine glands are classified; thus we describe, for example, simple tubular serous glands and compound alveolar mucous glands.

Connective tissue elements. During development of both exocrine and endocrine glands, invagination of cells from an epithelial membrane extends into the connective tissue (mesenchyme) underlying that membrane. This connective tissue forms the fibrous connective tissue capsule and supporting framework of the gland. The amount of such tissue varies, being rela-

tively profuse and dense in salivary glands but much thinner and finer in the pancreas. From the capsule, septa of connective tissue extend into the center of the gland but are never complete, in that they are pierced by blood vessels. The major septa subdivide the glands into lobes, and each lobe is subdivided by finer connective tissue into lobules. The supporting tissue of the lobule is very thin connective tissue, containing within its mesh the secretory units and ducts, and connected at the periphery of the lobule to the more substantial fibrous connective tissue surrounding the lobule.

Blood vessels, lymphatic vessels, and nerves are carried in the connective tissue of the gland, entering the gland through its capsule and then being distributed along interlobar and interlobular septa. The small arteries are passing through interlobular septa into the lobule where they break up into a capillary network in the intralobular reticular tissue between secretory units.

Epithelial duct elements. Usually one major duct supported and surrounded by connective tissue leaves a gland, and, to use an analogy, this is like the trunk of a tree whose branches and twigs are the smaller ducts and whose leaves are the secretory units. The smallest ducts are the *intercalary* or *intercalated ducts,* the term indicating that they are inserted between (and therefore connect) secretory units and intralobular ducts. The *intralobular* ducts are supported by very fine connective tissue and are lined by small cuboidal cells, the nuclei of which resemble a necklace of beads when the duct is cut in cross section. Several of these intralobular ducts join to form a larger *lobular duct,* this also laying between acini, and in turn, several lobular ducts unite to form an *interlobular duct,* which is situated in the fibrous connective tissue of an interlobular septum. At the apex of the lobe, the interlobular ducts of that lobe unite to form a lobar duct, this being surrounded by relatively dense fibrous connective tissue. Finally, the lobar ducts unite to form (usually) a single duct that drains the entire gland and terminates by opening onto a surface.

The epithelium lining the duct system varies from squamous or low cuboidal of the intercalated duct through cuboidal and columnar to stratified columnar or stratified squamous in the main duct. Although the duct epithelium functions mainly as a passive lining of the drainage system of the gland, there is evidence that in many glands it can modify the nature and concentration of the secretion. In passing from smaller to larger ducts (i.e., from twigs to branches of the tree) not only does the lining epithelium

become more robust, and the supporting elements of the duct change from fine reticular to fibrous connective tissue.

Glandular units. As indicated previously, exocrine glands generally are classified into serous, mucous, or mixed glands, each being identified on a histological slide by the appearance of its secretory units. In all instances, the units of a lobule will be cut in different planes, some showing a lumen and occasionally continuity with an intercalated duct, and others appearing as a solid clump of cells, the plane of section having missed the lumen. The following features should serve to distinguish the types of exocrine glands.

Serous glands. Usually the cytoplasm is darkly staining, being pink or pinkish-purple with H and E stain, and somewhat basophilic toward the cell base. Cell membranes often are not easily defined. Nuclei are regularly spherical or ovoid and situated near, but not at, the base of the cell. In the apical cytoplasm, zymogen (secretory) granules or droplets are present and may be stained specifically. The lumen of the acinus is usually definite and smaller in diameter than that of a mucous acinus. By electron microscopy, an extensive granular endoplasmic reticulum is present in the basal cytoplasm with quite of the features of smooth muscle cells. They can be clearly demonstrated by the alkaline phosphatase technique. They are thought to be contractile and thus to aid in expelling secretion from the gland. Myoepithelial cells can be demonstrated also in relation to the smaller ducts of mucous, serous, and mixed glands — for example, around the secretory units of sweat glands.

Endocrine glands. The endocrine glands are much simpler histologically than exocrine glands. Usually they are surrounded by a thin connective tissue capsule, from which incomplete septa extend into the glands to divide them into lobes. The main supporting tissue, however, is composed of very fine reticular (connective tissue) fibers associated with a very rich blood capillary or sinusoidal meshwork. Between the fine blood channels are the epithelial cells that elaborate the specific hormone or hormones of the gland. Each cell is adjacent to a fine blood vessel into which it passes its secretion.

Two types of endocrine glands are classified, based on cell grouping and hormone storage. Storage of hormones is intracellular in most cases with cells arranged in anastomosing cords and clumps between dilated blood capillaries. However, in some glands, e.g., the thyroid, a group of cells may

pass their secretion centrally to form a vesicle or follicle surrounded by the secretory cells, the hormone when required passing back through the cells and into blood capillaries located between follicles.

Mixed endocrine and exocrine glands. Many glands are mixed, having both exocrine and endocrine functions. Liver cells not only form bile as an exocrine secretion, passing it into a duct system, but also secrete internal secretions directly into the blood system. In other mixed glands, i.e., the pancreas, testis, and ovary, one group of cells secretes into a duct system and another group secretes directly into the blood system.

Mucous glands. The cytoplasm stains much lighter in an H and E preparation and may have a foamy, "moth-eaten" appearance. Specific stains for mucoprotein, e.g., alcian blue, mucicarmine, and periodic acid-Schiff (PAS), demonstrate mucous acini well. The nuclei are usually small, dark, and thin and are flattened against the basal plasma membrane of the cells. Normally the lumen is small and irregular.

Mixed glands. As explained previously, a mixed gland is one in which both mucous and serous acini are present or one in which component acini contain both mucous and serous cells. A mixed acinus is basically a mucous acinus with a small group of serous cells at its termination; the serous cells are ranged in a crescent or half-moon fashion. The cells of these serous demilunes pass their secretion into tiny intercellular canals between adjacent mucous cells and thus into the lumen of the acinus.

Polarization in epithelial cells. Polarization of the epithelial cells is based on their bordering position between two mediums — supporting, supplying, so called "home", situated under the basement membrane. And opposite "foreign", turned to outer sometimes aggresive environment.

Polatization is characteristic of both simple and stratified epithelial layers. But for stratified epithelial polarization emplies structural changes from basal to apical (superficial layer).

Because the majority of materials entering or leaving the body pass through epithelia, there often is within epithelial cells a specific arrangement or polarization of cytoplasmic organelles. Nuclei tend to be centrally located, often nearer the basal than the apical surface, with the Golgi apparatus and associated secretory material supranuclear or apical in position. The apical surface itself in many simple epithelia shows microvilli and cilia. Basally, as already indicated, basal infoldings with many mitochondria lying in the cytoplasmic "pockets" between the infoldings are

seen commonly in epithelia, e.g., of kidney tubules, those actively pump ions across the epithelium. Granular endoplasmic reticulum is situated primarily in basal cytoplasm and is present as large masses in those cells concerned with protein secretion. In many types of epithelial cells, there exists a complex network of tonofilaments (10 nm filaments) forming a cytoskeleton that, as already explained, is connected to certain types of surface specializations. Apically, fine filaments lie in the cytoplasm and extend into the cores of microvilli; many of these are of actin, which suggest that they are concerned with subtle changes in cell shape and, perhaps, with movement of the microvilli. Numerous mitochondria scattered throughout the cell. The Golgi apparatus is well developed and situated on the apical side of the nucleus. Zymogen granules of varying density, each surrounded by a single membrane, are present in the apical cytoplasm.

Lecture 3

BLOOD AND LYMPH

Among all the derivatives of the mesenchyme the tissues and organs of the blood system possess a very important place, where the blood system includes not only the two most important extracellular fluids i.e. blood and lymph but also the organs of hemopoiesis (the bone marrow, the thymus, the spleen, the lymph nodes etc.). Within the blood system all the components are interrelated histogenetically and functionally, and are regulated and coordinated by the neurohormonal system.

Blood and Lymph as the Internal Components (Environment) of the Body

All the cells line within the body in a fluid environment upon the stability of which, in terms of physicochemical characteristics each cell in dependence of its normal functioning. This fluid environment whilst being maintained at a steady state within certain limits, also allows for the diffusion of metabolites between cells and between the external and internal environments. In unicellular organisms unaided, diffusion is necessary for getting nutrients and for escaping gases and waste products to pass to and from the individual cells to satisfy its metabolic demands.

The biology of the circulating cellular components of the blood and lymphatic system and their development in the bone marrow and lymphoid tissue collectively constitute the hemo-lymphoid system. Within the system the various components function in the body. They are also interrelated by the circulation of lymphocytes from the lymph to blood.

BLOOD

Blood is an opaque turbid fluid with a viscosity somewhat greater than that of water. When oxygenated it is scarlet and when deoxygenated it is dark red to purple in color. It is a heterogeneous fluid containing plasma and formed elements. The total volume of plasma by weight is 55–60% and that of formed elements 40–45%. The total weight of blood comprises 5 to 9% of total body weight and 35% of total extracellular fluid. The total blood in the body is between 5 to 5.9 L.

Functions of blood:

1. ***Trophic*** and ***transportation function*** helps in transportation of nutrients, waste products, hormones, dissolved gases, biologically active components etc.

2. ***Respiratory function*** consists in transportation of oxygen from lungs to blood and from blood to lungs, transportation of CO_2.

3. ***Immune function*** participates in the humoral and cellular immunity.

4. ***Homeostatic function*** maintains the constancy of inner invironment within the body.

5. ***Transport*** of humoral agents and cells of the immune system that protect the body from pathogenic agents, foreign proteins, and transformed cells, i.e., cancer cells.

Plasma

Plasma is a clear slightly yellowish fluid which contains 90–93% of water with 7–10% of colloids dispersed in it, out of which 6.80–8.52% comprises of protein and 1.50–3.52% of other organic and mineral components. It gives a weak alkaline reaction due to its pH of 7.35. The plasma is rich in minerals Na^+, Cl^--ions and contains small amount of bicarbonate, phosphate, calcium and magnesium ions with other ions. It contains glucose, amino acids etc. The colloid includes high molecular weight plasma proteins.

Blood as a tissue. Blood has many affinities with connective tissue, as e.g., in mesenchymal origin of its cells, the free exchange of leukocytes with the connective tissues and the relatively low cell matrix ratio. Many of the plasma substances and some of the cells, however, arise from the variety of sources (e.g., any of the proteins associated with clotting are formed in the liver) and so blood is really a composite tissue pool.

Blood contains three groups of formed elements: red and white blood corpuscles and platelets.

Red Blood Corpuscles

The erythrocytes form the greater proportion of the blood cells (99% of the total number), with a count of $4.1–6.0\times10^6$/ml in adult males and 3.9–5.5 $\times10^6$/ml in females. Each cells is a biconcave disc with a diameter of 6.3–7.9 mm (mean 7.1 mm) and a rim thickness of 1.9 mm, in wet preparation the mean diameter is 8.6 mm. Erythrocytes lack nuclei and are pale red by transmitted light with paler centers because of their biconcave shape. They show a tendency to adhere to one another by their rims to form loose piles of cells called "Rouleaux", a character determined by the properties of their cell coat. Different types of erythrocytes are classified depending upon their shapes and sizes. Shapes:

— *discocytes* —disc shaped and constitute about 80% of all the erythrocyte number;

— *planocytes* — flat plate shaped;

— *stomatocytes* — dome shaped;

— *spherocytes* — spherical shaped;

— *echinocytes* — of spinous structure.

Spherocytes and echinocytes are older forms of erythrocytes (discocytes). These age changes are caused by the decrease in the metabolic activity of the cell on the maturation resulting in the decrease of ATP content, which causes the decrease in possibility of active transport thus changing the ionic concentration across the membrane and causing change of the shape based on envalid intracellular skeleton.

Size. 0n average the size of an erythrocyte is between 7.1 to 7.9 μm and thickness of 1.9 to 2.5 μm towards the periphery and 1 μm towards the center. 75% of all erythrocytes of normal size *are* called ***normocytes***, while 12.5% are large in size and called ***macrocytes***. Also there exist smaller erythrocytes ***microcytes***. The total surface area of an erythrocyte is 125 μm and volume of 30 μm. As a result all the erythrocytes in the body give a total area of 3500–3700 m. Plasmolemma of erythrocytes is about 20 nm thick. Defining its chemical composition it contains 60% lipid and glycolipid and 40% protein and glycoproteins. More than 15 classes of proteins present including two major types.

Firstly, the glycoproteins *glycophorins A* and *glycophorins B* span the membrane, and its negatively charged carbohydrates chains project from the outer surface of the cell.

Secondly, some of the *Band 2* proteins which may bear certain antigenic groups; *Band 3* proteins are also a transmembrane group of macromolecule, composed of four subunits, which fit together and lean a hole through which ions can pass, thus constituting the chloride channels in the membrane. Other proteins include several enzymes, some of them included in the process of ionic regulation and others, with addition of lipids, to the cell membrane from serum lipids due to lack of the cells' own synthetic apparatus.

The shape of the erythrocyte is largely determined by the protein ***Spectrin***, which conjugates to a ***Band 3*** protein on the inner surface of the membrane via short lengths of actin filaments. The other special protein called ***Ankyrin*** forms a stabilizing network giving stiffness to the membrane, which is aided by cholesterol in the membrane.

This unique cytoskeletal mobile proteins arrangement contibutes to the shape of the erythrocyte and imparts elastic properties and stability to the membrane.

Hemoglobin

Hemoglobin is a globular protein with a molecular weight of 67,000, consisting of globulin molecules bound to hem, an iron containing porphyrin group. Each molecule is made up of four subunits each in turn consisting of a coiled polypeptide chain with a cleft holding a single hem group. In normal blood, four types of polypeptide chains can occur, named α, β, χ, where each hemoglobin contains two — α and two — any of the others. So that several types of combinations and hence different types of hemoglobin molecules are possible: *hemoglobin A (HbA)*, which contains 2α and 2β chains; *hemoglobin F (HbF)*, in fetal and early postnatal life, contains 2α and 2χ chains. In fetus HbF is 80% and HbA is 20% while in adults there are 98% of HbA and 2% of HbF. Sometimes Fe^{2+} may change into Fe^{3+} state then this hemoglobin is called *methemoglobin*, which is unable to carry oxygen (this state is present due to presence of toxic substances). Another iron-containing compound called *ferritin* is often present in newly formed erythrocytes from the differentiation of erythrocytes in the bone marrow along with the persisting remnants of the apparatus of protein synthesis (ribosomes and RNAs).

We can define the age of an erythrocyte depending upon the staining process. The youngest stage of the erythrocyte, *reticulocyte*, stains basic still having protein-sinthesising organelles. The relatively young stages take both acidic and basic dyes, while the mature ones are acidophilic (due to hemoglobin). On average the age of an erythrocyte is 120 days. And then the destruction of old erythrocytes in spleen and liver starts, where their hem content is used for production of new hemoglobin of new erythrocytes and, globulin protein for formation of bilirubin that passes out with urea and uric acid.

Leukocytes

White blood cells are cells of peripheral blood of vertebrates and man showing active locomotion and different morphological characteristic and biological role. In general they are spherical, and their number varies from $3.8–9.0\times10^9$ in 1 l of blood. These cells are characterized by good defined pseudopodium locomotion (chemotaxis). They take part in the defense mechanism either by direct participation i.e. by phagocytosis of microbes or indirectly, producing antigen-antibody reaction.

Classification of leukocytes:

1. Granulocytes (contain granules).
2. Agranulocytes (granules are absent).

Granulocytes

They can be classified on the basis of their staining.

Eosinophils. Granules have acidophilic color, hence their name. They are very large in size. Their diameter in fresh blood is 9–10 μm and in preparation — 12–14 μm. They comprise 1–5% of total leukocytes. Their cytoplasm contains two types of granules: oxyphilic and azurophilic. Oxyphilic granules are oval or polygonal in shape and their size varies from 0.5–1.5 μm. The basic protein of these granules is rich in arginine (amino acid). Azurophilic granules are circular with size 0.1–0.5 μm having homogenous or granulated ultra structure. They contain acid phosphatase and are characterized by extremely large amounts of enzyme peroxidase, thus helping in lysis like lysosomes. The peroxidase unlike other enzymes is located only in the central regions and thus staining the peripheral zone of acidophil relatively darker than other regions. The number of these granules gradually

decreases during the process of specialization of eosinophils. According to their developmental process, three stages can be marked out: eosinophils with segmented nuclei; eosinophils with non-segmented nuclei; eosinophils with rod-shape nuclei. The segmented nuclei are usually bilobed but very rare may be trilobed.

Their nucleus has heterochromatin, and nucleolus is not visible. They show active chemotaxis to certain complexes. Their ratio to other leukocytes rises greatly with certain aggregated disorders, worm infestations and consequently plays an important role in the immune system, phagocytosis and antigen-antibody complex inactivation and also inactivation of certain inflammatory substances e.g., histamine. So they may be important in limiting the effects of these substances on the tissues. The eosinophils show chemotaxis due to stimulus affected on them by labrocytes (mast cells).

The amount of eosinophils periodically oscillates with rhythmic changes of day and night; being more at night. This process is regulated by hormones of hypophysis and suprarenal glands.

Basophiles. Their diameter in fresh blood is 9 μm and in preparation between 11–12 μm. They comprise 0.5–1% of total leukocytes in the blood, with a count of 25–200/ml. Their distinguishable feature is the presence of large conspicuous basophilic granules, varying in number (from 10–100%) and sizes of 0.5–1.2 μm. The granules are membrane-bounded vesicles with densely stained contents showing a variety of crystalline, lamellar and granular inclusions.

Heparin, histamine and several other inflammatory substances are contained in the granules, closely resembling those of labrocytes. These substances are associated with polysaccharides. Since granules are positive to carbohydrate stains e.g., azure A and PAS stain with which they are *metachromatic* i.e. they stain in different color from that of natural color of the dye. Their life span in circulation is very long. Except for the specific basophilic granules the basophiles may also contain certain azurophilic granules. The nucleus is usually irregular but not segmented, unlike those of other granulocytes and the cell contains all other organelles.

The major function of basophiles is the regulation of histamine and heparin metabolism. They contain characteristic enzyme histamindecarboxylase. Histamine and heparin play an important role in the regulation of blood coagulation. They participate in immunological reactions along with inflammatory processes. During the entry of antigens in the body antibody

IgE binds to the basophiles which cause break down of the cell granules and release of histamine and heparin. Histamine causes increase in blood-tissue barrier, widening of vessels and formation of edema. However, phagocytotic activity of basophiles is low. Though basophiles represent mast cells in their granular content, it is an altogether different line of cell as shown by their reactions with monoclonal antibodies and different cell development.

Neutrophils. NeutrophiIs — polymorphonuclear leukocytes form the largest proportion of leukocytes (47–72% in adults) with a count of $4.0–9.0\times10^9$ /ml. In fresh blood their diameter is 7–9 μm, while in specimens around 10–12 μm. Within the cytoplasm the numerous granules give a variety of color shades ranging from violet and pink when stained with Romanovsky — Giemza, commonly employed in hematology. Number of granules may vary from 50–200. The granules are not present throughout the cytoplasm leaving a small area on the periphery of the cell, which contains microtubules, responsible for the amoeboid movement of the cell. Electron-microscopic analysis shows that granules are heterogeneous in size, shape and content. Two major categories can be distinguished according to their origin, development and contents:

l. *Non-specific or primary granules*, which are formed early in neutrophil genesis. In mature neutrophils they comprise 10–20% of total granules with size of 0.4 to 0.8 μm. These are relatively large spherical lysosomes containing myeloperoxidase, acid phosphatase, β-glucouronidase etc. With light microscopy they stain strongly with neutral red and azure dyes and called azurophilic granules.

2. *Specific or secondary granules* are formed later; assume a wide range of shapes including rods, spheres and ellipsoid comprising of 80–90% of total granules in the mature neutrophils. Their size varies from 0.1–0.4 μm. Their chemical composition characterizes the presence of specific enzymes with the absence of lysosomal enzymes and peroxidase. They contain specific enzymes like basic phosphatase (alkaline phosphatase), aminopeptidase, lactoferrin and antibacterial agents. Cell organelles are poorly developed but large amounts of lipid and glycogen inclusions.

In mature neutrophils the nucleus is characterized by multilobulated structure with up to five-six joined segments by a narrow nuclear strand, called segmented neutrophils, comprising about 60–65% of total neutrophils. Less mature cells have fewer lobes, the earliest to be released, under normal

conditions are juvenile in which the nucleus is non-segmented crescent (S-shaped of horse-shoe-shaped) and comprise of 1–6% of total neutrophils. Under clinical conditions, even earlier formations of neutrophils (metamyelocytes) with round or bean-shaped nuclei may be released from the bone marrow. The study of neuthrophils nucleus is important for clinical diagnosis as in mature cells the nucleus have irregular lobes, which in females shows the presence of Barr bodies (sex chromatin). Normally 3% of all neutrophils show the presence of Barr's bodies.

Neutrophils form an important component in the immune system; they can engulf microbes and particles in the circulation and, after migrating between the endothelial cells lining capillaries or venules, can perform local phagocytosis in the extravascular tissues, wherever it is need. The engulfing of antigens is followed by digestion through fusion of the phagocytic vacuole, first with the specific granules, the pH being reduced, to 4.0 by active transport of protons, then the non-specific granules finish the process of killing the bacteria.

Phagocytosis, or the release of granules, may be stimulated by the presence of antibodies attached to the surface of neutrophils, which can bind, specifically to target antigens. These antibodies coating the antigenic target may also promote phagocytosis. Their number varies; often increasing during acute bacterial infection they may either circulate freely in the blood and comprise "Circulating pool" or may adhere to the walls of post capillary venules and comprise "Marginal pool" from where they may reenter the circulation during demand. They have very short life (up to 7.5 hrs).

The phagocytic activity of neutrophils is qualitatively related, to the percentage of phagocytic cells and phagocytic index (quantity of particles absorbed by one cell). Neutrophils produce reolin — a specific substance which decreases DNA synthesis of granular cells and have a regulating effect on the differentiation and proliferation of the leukocytes. The life span is 8 days out of which they stay in circulation only for 8–12 hrs and after this leukocytes migrate to the connective tissue matrix, engaging in defensive activity.

Agranulocytes

Monocytes. Monocytes are the largest of agranulocytes, being 9–12 μm in fresh blood and 18–20 μm in preparation, comprising 6–8% of total

leukocytes, with a count of 100–700/ml. The nucleus, which is euchromatin large, the cytoplasm forms a wide rim around the nucleus, near of which lies a prominent Golgi complex and vesicles stainable with neutral red. Ultrastructurally many lysosomes are seen to be present, together with some peripheral rough endoplasmic reticulum. Mitochondria are quite abundant. The cytoplasm of the monocyte is less basophilic than that of lymphocytes and stains light blue while towards the periphery with dark blue.

The cytoplasm is characterized by digitated processes and presence of phagocytic and pinocytic vacuoles and vesicles respectively.

All the above mentioned properties of the monocytes are similar to that of macrophages and hence they together comprise the *mononuclear phagocytic system*, the time of activity of the cells in blood varies between 36 to 104 hrs. During pathological conditions monocytes may transform into macrophages and as a result large amounts of lysosomes, phagosomes and phagocytic vacuoles forms in them.

Lymphocytes. These cells are second most numerous types of the leukocytes, forming 20–35% of their total number. Their size varies from 4.5 to 10.0 μm. Like other leukocytes they are also found in the extravascular tissue, and they are remarkable in being formed in large numbers outside the bone marrow as well as within it, hence constituting a widely distributed lymphoid system.

According to electron microscopic studies 4 different types of cells have been differentiated as follows: 1) small dark lymphocytes; 2) small light lymphocytes; 3) medium lymphocytes; 4) plasmocytes or lymphoplasmocytes (large lymphocytes).

Small light lymphocytes. They comprise about 70–75% of the total lymphocytes. Diameter is 4.5–6.0 μm. They contain rounded densely staining nucleus surrounded by a very narrow rim of cytoplasm. The nucleus shows a condensed chromatin towards the periphery like a band. The cytoplasm appears lighter due to poorly developed organelles, where the organelles are mainly restricted in the region around the nucleus.

Small dark lymphocytes. They comprise 12–13% of the lymphocytes with diameter of up to 7.0 μm. The nucleus comprises a dense chromatin and large nucleolus. The cytoplasm around the nucleus is relatively darker due to presence of big quantities of ribosomes, remaining organelles are poorly developed. These cells are freely motile when they settle solid surface, and can pass between endothelial cells to exit from or enter into the

vascular system. They may make extensive migrations within the various tissues including the epithelia and even passing into the body secretions like the saliva.

Medium lymphocytes. Medium lymphocytes comprise 10–12% of the lymphocytes with diameter of 7–10 μm. Their nucleus is round with a digitated processed nuclear membrane. The chromatin is loosely arranged and usually found near the nuclear membrane. The nucleolus is well developed. Since these cells are comparatively more metabolically active, they have very well developed organelles, with small amounts of lysosomes.

Large lymphocytes. Large lymphocytes comprise 1–2% of the lymphocytes in blood with diameter more 10 μm. In the circulation it is a mixture of very immature cells (lymphoblasts) capable of cell division to produce small lymphocytes, and maturing or mature cells which became functionally active after stimulation of the immune system, e.g., by the presence of antigens. Both the lymphocytes and maturing cells are actively engaged in synthesizing proteins, and thus contain a nucleus, which is at least in part euchromatic and a basophilic cytoplasm with numerous polyribosome clusters. The life span of lymphocytes ranges from a few days to many years, and so we can distinguish between short-lived and long-lived lymphocytes. Lymphocytes can be divided into several functional and maturational classes, but two major groups exist in the circulation called "B"- and "T"-lymphocytes ("B"- and "T"-cells), according to the histories of their cell lineage.

The lymphocytes, together with the phagocytes of the mononuclear phagocytotoc system, are responsible for the defensive reactions of the body, the former by non-phagocytic interactions of various kinds, the latter mainly by phagocytosis.

β-Lymphocytes. These cells are involved in production of antibodies specifically binding to foreign chemical substances called antigens to inactivate them or cause their destruction. Antibodies may circulate freely in the body fluid, (soluble antibodies) or may be secondarily attached to a variety of defensive cells (homocytotropic antibodies) to enhance their activities or to enable them to carry out a wide range of functions. Chemically, antibodies (or immunoglobulins) are proteins, with a relative molecular mass of 1,500–950,000. Each antibody molecule consists of four polypeptide chains; two of them are long (about 15 nm), called heavy chains and two shorter or light chains. One end of the molecule is highly variable in its amino acid

sequences and since it is responsible for binding to the specific antigens. The other end of the molecule is much less variable and can attach to certain cells if they possess specific receptors on their surfaces. At present, five classes of antibodies are distinguishable in blood plasma:

— IgG — major circulating antibodies;

— IgM — formed early in the immune response;

— IgA — present in secretions of the body, the saliva and fluids of the alimentary tract;

— IgE — homocytotropic cell antibody found on the surface of other cells;

— IgD — participates in the antigens of the immune system.

The antibodies can behave in various ways; they can agglutinate antigens by forming cross-links between them so rendering them inactive. After binding an antigen they may also bind to "complement proteins", which causes puncturing of cell membrane of the antigen. Antibodies may also coat an antigen (opsonization) causing macrophages to be stimulated in the region for phagocytotic activity β-lymphocytes. It can be activated to divide and transform into antibody-secreting plasmocytes by antigenic stimulation of cytophilic IgG bound to their surfaces. T-lymphocytes are also activated in the same manner. Some cells may be very long living ones and are called memory cells.

In pregnancy some IgG is transferred across the placenta, conferring a measure of passive immunity on the fetus, but in the case of Rh-factor incompatibility it brings about destruction of Rh^+ fetal erythrocytes. When circulating antibodies bind to antigens, they form immune complexes, may cause pathological damage to the vascular system and other tissues of the body by causing to attack cell membranes, thus causing vascular disease.

T-lymphocytes. These cells include a number of subclasses, all derived from stem lymphocytes originating in the bone marrow, but later differentiating in the thymus and passing into various other lymphoid organs. They participate in cellular and humoral immunity.

T-lymphocytes responses can be loosely divided into effector and controlling action. Effector's responses are direct and indirect attacks against antigens while controlling responses are induction or suppression of immune responses in other lymphocytes, namely B- and T-cells engaged in effector responses. Effector cells are divided into two major classes, those

which are responsible for cytotoxic killing of virus-infected, tumor or transplanted or bacterial cells, and other cells which give rise to combination of protect actions by not lymphocyte cells.

1. *Cytotoxic T-cells* cause cell death in a number of different ways, including the release of toxic lysosomal proteins (perforins) able to lysis the cell membranes of other cells.

2. *Delayed type hypersensitivity related T-cells* release lymphokins a variety of soluble proteins help in causing chemotaxic in macrophages to the antigens, stimulation of their phagocytotic activity, activation of lymphocyte B- and T- cell proliferation.

3. *Helper T-cells* help in proliferation and secretions of antibodies by the B-lymphocytes.

4. *Suppressor T-cells* help in suppression of the defensive activities of B- and T- cells.

5. *Natural killer cells* constitute a pool of defensive elements, which appear to have similar to cytotoxic T-cells function, although they lack some typical lymphocyte features.

Platelets

They are called thrombocytes, are relatively small (2–4 μm) irregular or oval discs present, in large number 180×10^9 to 320×10^9 in 1 ml of blood. Each platelet shows an outer clear zone *(hyalomere)* and an inner basophilic granular zone *(granulomere)*. Each platelet is non-nuclear and membrane-bounded entity. The plasma membrane bears a thick glycoprotein-rich coat, responsible for its adhesive properties. Beneath the surface is a band of about 10 microtubules, which run around the perimeter of the cell, and determine its shape. They contain actin, myosin and other proteins related to cell contraction. The cytoplasm contains almost all organelles including some narrow tubular channels and invaginations of the plasma membrane and various membrane-bounded vesicles. These vesicles are of three types: α-, δ- and λ-types. Alpha granules are the most prominent, with diameters of 500 nm, containing derived growth factor, fibrinogen and other substances. Delta granules contain serotonin, taken up from blood plasma, calcium ions, ADP, ATP and pyrophosphate. They measure around 300 nm. Lambda granules measure around 250 nm and contain lysosomal enzymes.

According to Romanowsky staining method we can divide platelets into different types:

1. *Juvenile* — with basophilic hyalomeres and small amounts of azurophilic granules.

2. *Mature* — with weak oxyphilic hyalomere and higher azurophilic granular content.

3. *Senile* — with dark purple granules and blue-purple cytoplasm.

4. *Degenerative* — with gray-bluish hyalomere and gray-purple granules.

5. *Giant cells* — the size of which is 2–3 times more than the normal, with pink hyalomere and purple granules.

Platelets are important in homeostasis and other functions. When a vessel is damaged, the platelets form a platelet plug by aggregating together and later agglutination between them. The α-granules along with other factors released from the damaged vessel causes the precipitation of fibrin filament into fibrin clot, to which more platelets adhere and later by their contraction produces a clot retraction which pulls the adhering wails of the vessels together limiting further any blood leakage. After repair of blood vessels starts clot removal with the help of complicated enzymatic activities. During clotting the platelet releases factors like phospholipids, 1ipoprotein and enzymes like thrombokinase, peptidase, phosphatase and catalase.

Hemogramm. In medicine the clinical analysis of blood plays an important role. During analysis the chemical constituency of blood, quantity of erythrocytes, leukocytes, hemoglobin, erythrocyte resistance, speed of sedimentation etc. are determined. In adults the formed elements of blood are located in standard proportions with one another, called hemogramm or blood formula. The leukocyte formula (about the percentage of leukocytes in blood) has a special significance.

LYMPH

Lymph is a pale yellow fluid of protein nature, circulating in lymphatic capillaries and vessels. It consists of plasma and formed elements. Its plasma is chemically quite similar to blood plasma, but contains fewer proteins. Enzymes like diastase, lipase and glycolytic enzymes are present. Plasma contains neutral fats, simple sugars, NaCl, NaCO, Ca, Mg and iron.

Formed elements are 98% lymphocytes and monocytes (and other leukocytes). It contains extreme small amounts of erythrocytes. Lymph is formed in lymphatic capillaries of tissues and organs, where under the effect of different factors like osmotic and hydrostatic pressure from the tissues various components of the lymph plasma are released. From the capillaries lymph flows to peripheral lymphatic vessels and from there to nodes and peripheral lymphatic vessels, and from there to nodes and through ducts into blood vessels. The chemical composition keeps on changing. We divide lymph into peripheral lymph, i.e. the one present up to the lymph nodes; secondary lymph i.e. the one present after the lymph nodes; central lymph i.e. the one present in the thoracic duct and right lymphatic duct. The process of lymph formation is interrelated to the movement of water and other substances from blood to intercellular spaces and formation of tissue fluid.

Lecture 4

SUPPORTING CONNECTIVE TISSUES

Connective tissue is the term traditionally applied to a basic type of tissue of mesodermal origin, which provides structural and metabolic support for other tissues and organs throughout the body. Connective tissues usually contain blood vessels and mediate the exchange of nutrients, metabolites and waste products between tissues and the circulatory system. The traditional term "connective tissue" thus does justice to the wide range of functions of this type of tissue and it is now probably more appropriate to use the term "supporting tissue".

Supporting tissues occur in many different forms with diverse physical properties. In most organs loose supporting tissues act as a biological packing material between cells and other tissues with more specific functions. Dense forms of supporting tissue provide tough physical support in the epidermis of the skin, comprise the robust capsules of organs such as the liver and spleen and are the source of great tensile strength in ligaments and tendons. Cartilage and bone — the major skeletal components — are merely rigid forms of supporting tissue. Supporting tissues also have important metabolic roles such as storage of fat (white adipose tissue) and the regulation of body temperature in the newborn (brown adipose tissue). Some of supporting tissue cells constitute a major part of the body. The processes of tissue repair are largely a function of supporting tissues. All supporting connective tissues have two major constituents: cells and extracellular material. The extracellular material determines the physical properties of each type of supporting tissue. Extracellular material consists of a matrix of organic material called ground substance within which a variety of fibers are embedded. A group of structural glycoproteins are the third constituent of the

extracellular matrix and mediate the interaction of cells with the other constituents.

Cells of connective tissue. The cells of supporting tissue may be divided into three types according to their basic function.

1. *Cells which are responsible for synthesis and maintenance of the extracellular material*. These cells are termed fibroblasts and are derived from precursor cells in primitive supporting tissue (mesenchyme). Fibroblasts with additional contractile function are found in some tissues and are known as myofibroblasts.

2. *Cells which are responsible for the storage and metabolism of fat.* These cells are known as adipocytes and may collectively form adipose tissue.

3. Cells with protective and immune functions. This group of cells includes the mast cells and tissue macrophages as well as all types of white blood cells.

Connective tissue cells. The cells that make up the resident cell population are relatively stable; they typically exhibit little movement and can be regarded as permanent residents of the tissue. These resident cells include:

— fibroblasts and closely related cell type;
— macrophages;
— plasma cells;
— mast cells;
— adipose cells;
— pigment cells;
— adventitional cells.

Fibroblasts are responsible for the synthesis of collagen, elastic and reticular fibers, and complex carbohydrates of the ground substance. Research suggests that a single fibroblast is capable of all the extracellular matrix components production.

Differon is a variety of the cells in process of cellular differentiation: stem cells, semi stem cells, non-specialized fibroblasts, specialized fibroblasts, fibrocytes and also myofibroblasts and fibroclasts.

Non-specialized fibroblast. This is a round shaped cell with the size of 20–25 μm, with small processes, developed from mesenchyme. Cytoplasm is rich with RNA, much free ribosomes. The endoplasmic reticulum, Golgi complex, mitochondria have bad development since these cells have a low level of the protein synthesis and its secretion. Non-specialized fibroblasts are capable to mitotic division.

Specialized fibroblast. This is a large active cell by size 40–45 μm. The nucleus is light, oval shaped, contains 1–2 nucleoli. Weakly basophilic cytoplasm contains well developed organelles of protein synthesis. The active synthesis of RNA, collagen and elastic fibers, glycosaminoglycans and proteoglycans for ground substance and fibers formation occurs in specialized fibroblasts. One of the hydrolytic enzymes — collagenaze — splits nonmature collagen inside cells and regulates the intensity of protein secretion on a cellular level. Peripheral part of cytoplasm of the specialized fibroblasts contains microfilaments consisting of actin and myosin proteins, providing cells movement.

Fibrocytes. These cells are spindle-shape with wing-shaped processes, contains the small number of organelles, vacuoles, lipids and glycogen. The collagen synthesis and synthesis of the other materials are much reduced.

Those known as fibroblasts can change in *myofibroblasts*, which are functionally like smooth muscle cell, but have well developed endoplasmic reticulum. The myofibroblast contains bundles of longitudinally disposed actin filaments. The myofibroblast differs from the smooth muscle cell in that it lacks a surrounding basal lamina (smooth muscle cells are surrounded by a basal or external lamina). Also, it usually exists as an isolated cell, although its processes may contact the processes of other myofibroblasts. The myofibroblast is implicated in wound contraction, a natural process that results in closure of a wound in which loss of tissue has occurred. The myofibroblasts play an important role in the uterus at development of pregnancy.

Fibroclasts are the cells with high phagocytic and hydrolytic activity; occur in connective tissue at a period of organ involution. It takes part in intercellular substance resorption and contains numerous lysosomes.

The supporting tissues not only contain cells responsible for their synthesis, maintenance and metabolic activity, but also a variety of cells with protective and immune functions.

Macrophages. Connective tissue macrophages, also known as tissue histiocytes, are derived from the blood cells called monocytes. Monocytes migrate from the bloodstream into the connective tissue where they differentiate into macrophages.

The macrophage nucleus is irregular with heterochromatin typically clumped under the nuclear envelope. The cytoplasm contains few mitochondria and a variable amount of free ribosomes and rough endoplasmic

reticulum. Lysosomes are abundant but their number is much reduced in actively phagocyting cells, lysosomes are later regenerated by the Golgi apparatus. The macrophage cytoplasm contains an assortment of phagosomes and residual bodies. Actively phagocyting cells exhibit irregular cytoplasmic projections or pseudopodia, which are involved in amoeboid movement and phagocytosis. In addition to their role as tissue scavengers, macrophages play an important role in immune mechanisms since they are often the first cells to make contact with antigens. Macrophages process antigenic material in some way before presenting it to lymphocytes; lymphocytes are then stimulated to undergo specific immune response. Macrophages involved in this way are described as antigen presenting cells. As a result of various immune mechanisms, antigenic material may become combined or coated with substances such as antibodies and complement, which are then collectively known as toxic. Other substances such as lymphokines, which are released during the immune response, act directly upon macrophages to increase greatly their metabolic and phagocytic activity. The wandering category of the protecting and immune cells includes all the remaining members of the white blood cells series. Although leukocytes are usually considered as a constituent of blood, their principal site of activity is outside the blood circulation, particular within loose supporting tissues. Leukocytes are normally found only in relatively small numbers but in response to tissue injury and other disease processes their number increases greatly. The supporting tissues of those regions of the body which are subject to the constant threat of pathogenic invasion, such as the gastrointestinal and respiratory tracts, contain a large population of leukocytes, even in the absence of overt disease.

Plasma cells are a prominent constituent of loose connective tissue where antigens tend to enter the body (gastrointestinal and respiratory tracts). Their number is greatly increased in area of chronic inflammation. Once derived from its precursor, B-lymphocyte, a plasma cell has only limited migratory ability and somewhat short life span of 10 to 30 days. The plasma cell is a relatively large, ovoid cell (7–10 μm) with a considerable amount of cytoplasm. The cytoplasm displays strong basophilia because of an extensive rER with associated ribosomes and has a characteristic unstained area next to the nucleus. This unstained area represents the Golgi complex, which is usually prominent because of its relatively large size and association with secretory vesicles.

The nucleus is spherical, small and eccentrically placed. The nucleus has a "cartwheel" appearance, because most of the chromatin granules are arranged in a regular manner against the nuclear membrane and only a few present toward the center. It exhibits large clumps of peripheral heterochromatin alternating with clear area of euchromatin.

The plasma cells are the major producer of circulatory antibodies.

Mast cells. The mast cells are found in all supporting tissues but are particular prevalent beneath the skin, gastrointestinal lining, serous lining of the peritoneal cavity and around blood vessels. Their major constituents and functions are very similar to those of blood basophiles. Whether or not they are derived from circulating basophiles is in dispute, but the must cells are long living with an ability to proliferate within the tissues.

The mast cells are large, ovoid, connective tissue cells with a spherical nucleus and cytoplasm filled with large, intensely basophilic granules. Several primary substances found inside granules are:

— histamine, which increase the permeability of small blood vessels, causing edema in the surrounding tissue;

— heparin, a sulfated glycosaminoglycan, which is an anticoagulant, it can block numerous coagulation factors.

The mast cell degranulation results in the release of histamine and other vasoactive mediators, which induce the immediate hypersensitivity (anaphylactic) response (typical allergic rhinitis and asthma) and anaphylactic shock. The mast cells are not readily identified in routine histological sections due to the water solubility of their densely basophilic granules, which tend to be lost during preparation. Thus special techniques of fixation, embedding and staining must be employed. With suitable staining, however, the characteristic feature of the mast cells is an extensive cytoplasm packed with large granules which are nevertheless smaller in size, though more numerous than those of basophiles. When stained with certain blue basic dyes such as toluidine blue, the granules bind to the dye changing its color to red. This property is known as metachromasia. The granules are liberated from the cell by exocytose when stimulated during an inflammatory or allergic response.

The cell surface exhibits numerous microvilli and folds. The cytoplasm contains a few rounded mitochondria and small amounts of rough endoplasmic reticulum and Golgi complex. The non-segmented nucleus has less condensed chromatin than that of basophiles.

Ground substance. Ground substance occupies the space among the cells and fibers and is a viscous, clear substance with a slippery feel. Ground substance derived its name from being an amorphous transparent material, which has the properties of a semi-fluid gel. Tissue fluid is loosely associated with ground substance, thereby forming the medium for passage of molecules throughout supporting tissues and for the exchange of metabolites with the circulatory system. Ground substance consists of mixture of long, non-branched polysaccharide chains of seven different types, each composed of repeating disaccharide units. One of the disaccharide units is usually a hyaluronic acid and the other — an amino sugar thus giving rise to the modern term glycosaminoglycans (GAGs). These were formerly called mucopolysaccharides. The glycosaminoglycans are acidic (negative-charged) due to the presence of hydroxyl-, carboxyl- and sulphate-side groups on the disaccharide units. Ground substance consists predominately of proteoglycans, very large macromolecules composed of a core protein to which glycosaminoglycans molecules are covalently bound. Glycosaminoglycans (GAGs) are long-chained polysaccharides composed of repeating disaccharides units. They are named for glucosamine, a hexosamine sugar that is present in each disaccharide.

GAGs are highly negatively charged because of the sulfate and carboxyl groups located on many of the sugars, hence their proper for staining with basic dyes. The gel-like composition of the ground substance permits rapid diffusion of water-soluble molecules, but inhibits movement of large molecules and bacteria.

Hyaluronic acid is the predominant GAG in the loose supporting tissues and is the only one without sulphate-side group. The other GAGs (chondroitinsulphate, dermatansulphate, heparansulphate, heparinsulphate and keratansulphate) differ from hyaluronic acid in that they are covalently linked to a variety of protein molecules to form proteoglycans (formerly known as mucoproteins). These proteolglycans are huge molecules consisting of 90–95% carbohydrate. Further, the proteoglycans may form non-covalent links with hyaluronic acid chains to form even larger molecular complexes. Unlike many proteins, GAG molecules are not flexible enough to form globular aggregates, but remain in an expanded form, thus occupying a huge volume for relatively small mass. In addition, their highly charged side groups render them extremely hydrophilic thus attracting a large volume of water and positive ions, particular sodium, which constitute extracellu-

lar fluid. The extracellular fluid imparts the characteristic turgor of supporting tissue.

The extracellular matrix is a complex structural network that includes fibrous proteins, proteoglycans, and several glycoproteins.

THE FIBERS OF SUPPORTING CONNECTIVE TISSUE

The fibrous components of supporting tissue are of two main types: collagen (including reticular, which was formerly considered a separate fiber type) and elastic. **Collagen** is the main fiber type found in most supporting tissues and is the most abundant protein in the human body. Its most notable function is the provision of tensile strength. Collagen is secreted into the extracellular matrix in the form of tropocollagen which consists of three polypeptide chains (alpha chains) bound together to form a helical structure 300 nm long and 1.5 nm in diameter. In the extracellular matrix, the tropocollagen molecules polymerize to form collagen. Nineteen different types of collagen have now been delineated on the basis of morphology, amino acid composition and physical properties.

Type 1 collagen is found in fibrous supporting tissue, the dermis of the skin, tendon, ligaments and bone, cornea and sclera of eye, artery wall, in a variable arrangement from loose to dense according to the mechanical support required. The tropocollagen molecules are aggregated to form fibrils, strengthened by numerous intermolecular bands. Parallel collagen fibrils are further arranged into strong bundles 2–10 nm in diameter, which confer great tensile strength to the tissue. These bundles are visible with the light microscope.

Type 2 collagen is found in hyaline and fibrous cartilage, vitreous body and consists of fine fibrils which are dispersed in the ground substance.

Type 3 collagen makes up the fiber type known as reticular, which was previously thought to represent a separate species of fiber because of its affinity for salts and it is found in fetus derma.

Type 4 collagen forms a non-fibrillar network that provides structural cohesion to the basal lamina.

Collagen Fibers Formation

The production of fibrillar collagen involves a series of events within the fibroblast that leads to production of *procollagen*, the precursor of the collagen molecule. Production of the actual fibril occurs outside the cell and involves enzymatic activity at the plasma membrane to produce the collagen molecule, followed by assembly of the molecules into fibrils in the extracellular matrix under guidance by the cell.

1. ***Molecular level of collagen fiber organization***. Collagen molecule consists of three polypeptide chains of procollagen (α-chain), which form triplets. Polypeptide chains are produced by polyribosomes of the rER and newly synthesized polypeptides are simultaneously discharged into the cisternae of the rER and Golgi apparatus. Each chain contains sets from three different amino acids, which are normally repeated. The first amino acid can be any, the second one — proline or lysine, the third — glycine. The procollagen moves to the exterior part of the cell by means of exocytosis of secretory vesicles. Note that ascorbate (vitamin C) is required for the function of prolylhydroxylase and lysilhydroxylase; without posttranslational hydroxylation of proline and lysine the hydrogen bonds essential to the final structure of the collagen molecule cannot form.

2. ***Extracellular level of collagen fibers organizations*** presents aggregated lengthwise molecules of collagen, which have transverse connection by means of hydrogen bonds. In the beginning protofibril is formed and 5–6 protofibrils form microfibril.

3. ***Fibrillar level*** — formation of fibril bundles at participation of glycosaminoglycans and proteoglycans. Fibril bundles have transverse alternation of dark and light areas by size 64 nm. Striation is conditioned by location of polar amino acid in collagen molecule. The thickness of fibrils is 50–100 nm.

4. ***Fiber level***. Fibrils unite in collagen fiber by thickness 150 nm.

Reticular fibers form the delicate branched "reticular" supporting meshwork in highly cellular tissues, which is known reticular tissue, such as the endocrine glands, liver, bone marrow and lymphoid organs. In such organs, a fine network of branching fibers ramifies throughout the parenchyma usually anchored to a dense, collagenous capsule and septum, which traverse the tissue. In their composition type 3 collagen and big amount of carbohydrates are affiliated, which are synthesized by reticular cells and form three-dimensional network. Reticular fibers

are usually poorly stained in common preparations, but being covered by succharides sheets are able to absorb metallic silver by which they are stained black. Reticulin is the earliest type of collagen fiber to be produced during the development of all supporting tissues and is also present in varying quantities in most mature supporting tissues.

Elastic fibers are a rubber-like material which is arranged as fibers and/or discontinuous sheets in the extracellular matrix particularly of the skin, lung and blood vessels where it confers the properties of stretching and elastic recoil. Elastin is the globular protein and is synthesized by fibroblasts in a precursor form known as tropoelastin, which undergoes polymerization in the extracellular tissues. Elastin contains amino acids derivatives (desmosine and isodesmosine) which participate in stabilizations of the elastin molecular structure and provide an ability to stretching.

Deposition of elastin in the form of fibers requires the presence of microfibrils of the structural glycoprotein fibrillin which become incorporated around and within the elastic fibers.

The structural glycoproteins. The structural glycoproteins are a group of molecules composed principally of protein chains bound to branched polysaccharides. The structural glycoproteins include two fibril-forming molecules, fibrillin and fibronectin, and a number of non-filamentous proteins including laminin, entactin and others which function as links between cells and extracellular matrix. Fibrillin forms microfibrils 8–12 nm in diameter which, in certain specialized situations e.g., the mesangium of the kidney, appear to enhance adhesion between other extracellular constituents. As mentioned before, fibrillin is a constituent of elastic fibers where it appears to play a role in the orderly deposition of the fibers. Fibroelastin plays a part in controlling the deposition and orientation of collagen in extracellular matrix and the binding of the cell to the extracellular material. Cell membranes incorporate a group of transmembrane protein complexes called integrins, which act as cell adhesion molecules. One of these, the fibroelastin receptor, establishes bonds within the cell to the actin filaments of the cytoskeleton and binds with fibronectin externally. The fibronectin in turn binds with collagen and glycosaminoglycan. Heparinsulphate thus is establishing structural continuity between the cytoskeleton and the extracellular matrix.

Laminin is a major component of basement membranes binding with specific cell adhesion molecules so as to form links between cell mem-

branes and other constituents of the basement membrane. Entactin, another non-fibrillar protein, has the function of binding laminin to type 4 collagen in basement membranes.

CONNECTIVE TISSUE WITH THE SPECIAL PROPERTIES

Adipose tissue. Most supporting tissues contain cells, which are adapted for the storage of fat; these cells, called adipocytes, are derived from primitive mesenchyme. Adipocytes are found in isolation or in clumps throughout loose supporting tissues or may constitute the main cell type as in adipose tissue. Stored fat within adipocytes is derived from three main sources: dietary fat circulating in the bloodstream as chylomicrons, triglycerides synthesized in the liver and transported in the blood, and triglycerides synthesized from glucose within adipocytes. Adipose tissue is often regarded as an inactive energy store; however, it is an extremely important participant in general metabolic processes in that it acts as a temporary store, of substrate for the energy-deriving processes of almost all tissues. Adipose tissue, therefore, generally has a rich blood supply. The rate of fat deposition and utilization within adipose tissue is largely determined by dietary intake and energy expenditure, but a number of hormones and the sympathetic nervous system profundity influence the fat metabolism of adipocytes.

There are two main types of adipose tissue:

White adipose tissue comprises up to 20% of total body weight in normal, well-nourished male adults and up to 25% in females. It is distributed throughout the body particular in the deep layers of the skin. In addition to being an important energy storage, white adipose tissue acts as a thermal insulator under the skin and functions as a cushion against mechanical shock in such sites as around the kidneys. Fat stored in adipocytes accumulates as lipid droplets, which fuse to form a single large droplet which distends and occupies most of the cytoplasm. The adipocyte's nucleus is compressed and displaced to one side of the stored lipid droplet and the cytoplasm is reduced to a small rim around the periphery. In routine histological sections, the lipid content of adipocytes is extracted during tissue processing leaving a large, unstained space within each cell. Note the minute dimensions of blood capillaries compared with the size of the surrounding adipocytes.

Brown adipose tissue is highly specialized type of adipose tissue found in newborn mammals and some hibernating animals, where it plays an important part in body temperature regulation. Only small amounts of brown adipose tissue are found in human adults and, although previously though to contribute little to thermoregulation, there is now increasing evidence that (at least in some individuals) brown adipose tissue may play a role in burning off excess energy thus preventing obesity. Unlike the metabolism of other tissues, in brown adipose cells the process of electron transport is readily uncoupled from the phosphorylation of ADP to form ATP. The energy derived from oxidation of lipids, and energy released by electron transport in the uncoupled state, is dissipated as heat, which is rapidly conducted to the rest of the body by the rich vascular network of brown adipose tissue. Using these metabolic processes, neonatal humans and other mammals utilize brown adipose tissue to generate body heat during the vulnerable period after birth. Brown adipose tissue undergoes involution in early infancy and in adult humans is found only in odd sites such as around the adrenal gland and great vessels. The production of heat by brown adipose tissue is controlled directly by the sympathetic nervous system.

Mucous connective tissue. This is a transient type of tissue which appears in the normal development and differentiation of the connective tissues. It occurs also as Wharton's jelly in the umbilical cord, where it does not differentiate further. Cellular composition is presented mainly by mucocytes — the stellate fibroblast-like cells. The ground substance is soft and jelly-like, gives a mucin reaction, and stain metachromatically with toluidine blue. It contains a delicate meshwork of fine collagenous fibers and hyaluronic acid.

Pigmented connective tissue is a tissue containing much pigmented cells — melanocytes. It is situated in area of the skin, the nipple, the scrotum, near the anus, in the choroid and iris of the eye.

DENSE CONNECTIVE TISSUE

Dense connective tissues are characterized by the close packing of their fibers. Cells are proportionally fever than in loose connective tissues, and there is less amorphous ground substance. In the areas where tensions are exerted in all directions, the fiber bundles are interwoven and without regular orientation and the tissues are termed irregularly. In structures subject to

tension in one direction, the fibers have an orderly parallel arrangement and the tissues are designated regularly arranged. In most regions collagenous fibers are the main component, but in a few ligaments elastic fibers predominate.

Irregularly arranged. This tissue occurs in sheets, its fibers interlacing to form a coarse, tough network. Although course of collagenous fibers are the main component, elastic and reticular fibers are present also in small numbers. Dense irregular connective tissue forms the basis of most fascias, the dermis of the skin, the fibrous capsules of some organs, including the testis, liver, lymph nodes, and the fibrous sheaths of the bone (periosteum) and cartilage (perichondrium).

Regularly arranged. This tissue contains fibers which are densely packed and lie parallel to each other, forming structures of great tensile strength. This group includes tendons, ligaments, and aponeuroses.

In tendons, the collagenous fibers, or *primary tendon bundles*, run parallel courses. Each fiber or bundle is composed of a large number of fibrils. Fibroblasts, or *tendon cells*, are the only cell type present, and in longitudinal sections of tendon they are aligned in rows between the collagenous fibers. Each primary bundle is covered by a small amount of loose connective tissue, termed the *endotendineum*. Generally, several primary bundles are grouped together into secondary bundles or fascicles bounded by the connective tissue, the *peritendineum*. The tendon, composed of a number of fascicles, is sheathed by thick connective tissue called the *epitendineum.*

Lecture 5

SKELETAL CONNECTIVE TISSUE

CARTILAGE

Cartilage, like other regions of the connective tissue compartment, consists of cells, fibers, and ground substance. The last named, however, has physical properties, which give the tissue an elastic firmness, rendering it capable of withstanding a considerable degree of pressure and shear. Cartilage contains about 70–80% of water, 10–15% of organic material and 4–7% of salts.

In the adult body, cartilage covers the joint surfaces of bones, and it forms the sole skeletal support of the larynx, trachea, bronchi, and certain other structures. According to the nature and visibility of the fibril elements cartilage is subdivided into three varieties: 1) *hyaline,* 2) *elastic*, and 3) *fibrous.* Among these, hyaline cartilage is the most widely distributed type.

The cells. The cartilage cells, chondrocytes, occupy chambers known as lacunae. The cells appear irregularly shaped and shrunken away from the walls of their lacunae in most light microscope preparations because their cytoplasm contains glycogen and lipids which are dissolved by routine techniques. Although the cell surface is irregular and has short processes extending into depressions in the matrix, there is no obvious space between the cell and its surrounding matrix. Chondrocytes cytoplasm contains, in addition to glycogen and lipid, usual characteristics of a secreting cell. Chondrocytes and their lacunae vary in shape in relation to their position within the cartilage. Chondrocytes are often arranged in groups, which represent the offspring of a parent cartilage cell. Such groups, termed isogenous groups, are found particularly in costal and tracheal cartilages. In embryonic cartilage,

the cells are randomly distributed and variable in shape. Some have processes and resemble the mesenchymal cells from which they are derived. Intercellular matrix is relatively scant. Three types of chondrocytes are distinguished in isogenous groups.

Chondrocytes of the 1st type are characterized by high nuclear-cytoplasm correlation, presence of mitochondria and free ribosomes in cytoplasm. These cells divide mitotically and are a source to isogenous group regeneration.

Chondrocytes of the 2nd type are characterized by reduction of nuclear cytoplasm correlation, reduction of the DNA synthesis, and high level of the RNA synthesis, intensive development of granular endoplasmic reticulum and Golgi apparatus. Chondrocytes of the 2nd type produce GAG and proteoglycans in matrix.

Chondrocytes of the 3rd type are characterized by the lowest nuclear cytoplasm correlation, good development of granular endoplasmic reticulum. These cells save the ability of formation and secretion of proteins, but reduce the synthesis of glycosaminoglycans.

The intercellular substance. Like other connective tissue matrixes, cartilage matrix is highly hydrated. Sixty to eighty per cent of the net weight of cartilage is water.

Because of the proteoglycans hyaline cartilage contains a high concentration of bound sulfate groups, ground substance stains with basic dyes and with hematoxylin. Thus, the basophily and metachromasia seen in stained sections of cartilage provide information on the distribution and relative concentration of sulfated proteoglycans (chiefly of chondroitin sulfate). The highest concentration of these substances occurs straight around lacunae. This ring of intensely staining matrix is called the *capsule* or *territorial matrix.* The matrix more removed from the immediate vicinity of chondrocytes has a lower concentration of sulfated proteoglycans and stains less intensively. These areas are designated as the *interterritorial matrix*.

The apparently homogeneous intercellular substance contains fine collagenous fibers, which are masked with ground substance of similar refractive index. The intercellular substance contains chondromucoids, which are composed of glycosaminoglycan complexes containing chondroitin sulfate. There is also present some keratin sulfate. It is insignificant in amount at birth but increases with age and may reach relatively high levels in senile, degenerating cartilage.

Hyaline cartilage is composed of cells and an extracellular matrix of ground substance and connective tissue fibers, which have irregular direction, proteoglycans, and hyaluronic acid. Hyaline cartilage is characterized by matrix containing type II collagen fibers. It forms the costal cartilage and the cartilage of the nose, larynx, trachea, and bronchi. It is also a major component of the epiphyseal cartilage of growing long bones. In fetus, nearly all of the skeleton is first laid down as hyaline cartilage and is replaced later by osseous tissue in the formation of the bones. Hyaline cartilage is always invested by a layer of dense fibrous connective tissue, the perichondrium, except free surfaces of joint cartilage.

The perichondrium is a dense connective tissue composed of cells that are undistinguishable from fibroblasts. During active growing, the perichondrium appears to be divided into an inner cellular layer, which gives rise to new cartilage cells, and outer fibrous layer. There are some exceptions to the general rule that hyaline cartilage is surrounded by perichondrium. They include areas where cartilage forms a free surface, as in the joint surfaces, and areas where cartilage makes direct contact with bone, as in the nasal and costal cartilage and sites of bone formation. In this area, proliferation of chondrocytes within the cartilage lacunae provides new cells for *interstitial growth*.

Elastic cartilage is characterized by elastic fibers in addition to the matrix material of hyaline cartilage. Elastic cartilage appears more yellow and opaque than hyaline cartilage in fresh condition because of the large number of elastic fibers and interconnecting sheers of elastic material in its matrix. These branch and course are all directions to form a dense network of anastomotic and interlacing fibers. In the peripheral layers, the fibers are thin and in the deeper portions they are thicker and more closely packed. The ground substance also contains collagenous fibrils, particularly in the subperichondrial region. Elastic fibrils are assembled just peripherally to the cells and traverse the matrix as an elastic network. Growth of the cartilage takes place interstitially and subperichondrially. Calcification of elastic cartilage occurs very rarely, if at all.

Elastic cartilage occurs in the external ear, the auditory (Eustachian) tube, the epiglottis of the larynx, and some laryngeal cartilages. The cartilage in all of these locations is surrounded by perichondrium, similar to that found around most hyaline cartilages. Unlike hyaline cartilage, which calcifies with aging, the matrix of elastic cartilage does not calcify during the aging process.

Fibrous cartilage. Fibrous cartilage is a combination of dense collagenous fibers with cartilage cells and a scant cartilage matrix. It is generally not circumscribed by the perichondrium. The relative proportions of cells, fibers, and matrix vary greatly. The chondrocytes are dispersed among the collagen fibers, singly, in rows, and in isogenous groups.

Fibrous cartilage is found in considerable amounts in the intervertebral discs, pubic symphisys, joint discs of the sternoclavicular and temporomandibular joints, menisci of the knee joint, and certain places where tendons attach to the bones. Because the joint cartilage has numerous fibrils with the 640A-cross banding, they have a hyaline-like matrix and other hyaline cartilage characteristics.

DEVELOPMENT AND GROWTH

Cartilage is a part of the connective tissue spectrum. Like all the other connective tissues, it originates within the mesenchymal compartment of the embryo. The blastema of a future cartilagenous mass is firstly recognized as an area of mesenchymal cells concentration resulting from cells proliferation and enlargement. The cells in the inferior of the precartilage blastema show marked cytoplasmic basophilia resulting from an increase in rough-surfaced endoplasmic reticulum, and they are known as chondroblasts. They form the collagenous fibrils and the ground substance of the matrix. As the cells of the central region continue to form more matrix, they become separated from each other and become the chondrocytes, whereas those of the periphery continue as chondroblasts. The amino acids, such as glycine and proline, are synthesized into peptide chains in the presence of the ribosomes of the rough-surfaced endoplasmic reticulum and are then transported to the Golgi complex. The synthesized proteins and polysaccharides are then combined in the Golgi region to form the chondromucoproteins, which are secreted by the cells. Growth of cartilage takes place in twoways: 1) formation of new cartilage by chondroblasts at the surface, known as *appositional growth*, and 2) expansion of the internal mass of cartilage by division of chondrocytes, known as *interstitial growth*.

In *appositional growth*, chondroblasts of the perichondrium multiply, and some form cartilage matrix as described above whereas others remain as a part of the chondroblasts population. In the *interstitial growth*, cartilage cells are divided into two, and the daughter cells may divide again, each

isogenous group representing the progeny of a single parent cell. The cells become separated from each other, each surrounded by its own capsule and matrix. Interstitial growth occurs mainly in young cartilage and gradually ceases. Further growth is being chiefly appositional (subperichondrial).

Nutrition of Cartilage

Cartilage is devoid of vascular and lymphatic channels; hence, nutrition is entirely by diffusion. The matrix permeability even to coarse particles has been demonstrated. Injection of indigo carmine into the circulation leads to a deposition of the colored particles within the matrix and the lacunae.

Age changes. The poor nutrition is probably responsible for certain degenerative changes found in old cartilage, especially in cartilage of considerable thickness. The deeper portions of the cartilage show areas, extending through many cell territories, where the homogeneous matrix is replaced by closely packed coarse fibers. On aging, cartilage loses its translucency and bluish white color and appears yellowish and cloudy. This change is due to a decrease of acid mucopolysaccharides and an increase in non-collagenous proteins. Calcification is likewise of common occurs in old cartilage and is usually associated with degenerative changes of the cartilage cells. Calcification of cartilage is a normal process during bone formation.

BONE (OSSEOUS TISSUE)

Osseous tissue is a rigid form of connective tissue and is normally organized into definite structures, the bones. Bone contains about 70% of inorganic materials, calcium phosphate mainly. They form the skeleton, serve for the attachment and protection of the soft parts, and by their attachment to the muscles, acts as levers, which bring about body motion. Bone is also a storage place for calcium long, which can be withdrawn when needed to maintain a normal level of calcium in blood.

Organic and Inorganic Components of Bone

Bone is composed of cells and an intercellular matrix of organic and inorganic substances. The organic fraction consists of collagen and an amorphous component of glycosaminoglycans (protein-polysacharides) contain-

ing chondroitin sulfate. The matrix is acidophilic in stained sections, in contrast to the basophilic reaction of cartilage matrix. This is due to the high content of collagen and low content of chondroitin sulfate in bone. The collagen of bone is generally type 1. The inorganic component of bone is responsible for its rigidity and may constitute up to two-thirds of the fat-free dry weight. It is composed chiefly of calcium phosphate and calcium carbonate, with small amounts of magnesium hydroxide, fluoride, and sulphate. The composition varies with age and with a number of dietary factors. X-ray diffraction studies show that the minerals are present as crystals having an apatite pattern or structure. More specifically, they are hydroxyapatites. There are two types of the bones — *non-lamellar (woven)* and *lamellar bone*. They differ on structural and physical characteristics, which are conditioned mainly by extracellular substance construction.

Cells of bone tissue. Four designated cells are associated with bone tissue: osteoprogenitor cells, osteoblasts, osteocytes, and osteoclasts. Each undergoes transformation from a less mature form to a more mature form in relation to functional activity. In contrast, the osteoclast originates from a different cell line and is responsible for bone resorption, an activity associated with bone remodeling.

Osteoprogenitor cell. These cells are found on the external and internal surface of bone. They comprise the periosteal cells that form the innermost layer of the periosteum and the endosteal cells that line the marrow cavities, the osteonal (Haversian) canals, and the perforating (Volkmann's) canals. Osteoprogenitor cells can divide and proliferate. In growing bones, osteoprogenitor cells appear as flattened cells with lightly staining, elongate or ovoid nuclei and slightly basophilic cytoplasm. Electron micrographs reveal profiles of rough endoplasmic reticulum and free ribosomes as well as a small Golgi apparatus and other organelles. Osteoprogenitor cell differentiates into more active secretory cells, the osteoblasts.

Osteoblasts. Like its close relatives, the fibroblast and the chondroblast, the osteoblast is a secretion of the cell that retains the ability to divide. It secretes both the collagen and the ground substance that constitute the initial dismineralized bone, or ***osteoid.*** The osteoblast is also responsible for the calcification of the matrix. The calcification process appears to be initiated by the osteoblast through the secretion into the matrix with its small, 50 to 250 nm, membrane-limited matrix vesicles. The vesicles are rich in alkaline phosphatase and are actively secreted only during the period in

which the cell produces the bone matrix. These cells have cuboidal or polygonal shape and a rounded nucleus, a prominent nucleolus, and cytoplasm which is basophilic. The nucleus is often shifted toward one side of the cells, and close to it. One can frequently seen a clear zone which is the negative image of the enlarged Golgi complex. Electron micrographs disclose numerous mitochondria, a well developed rough endoplasmic reticulum, an active Golgi complex with numerous vesicles, and microtubules. The basophilia of the osteoblasts is due to the abundance of rough-surfaced endoplasmic reticulum, characteristics of cells engaged in protein synthesis. It has been shown by studies in which there labeled precursors of the matrix, i.e., the collagen fibers and the glycosaminoglycans.

Osteoblasts respond to mechanical stimuli to mediate the changes in bone growth and bone remodeling. As osteoid deposition occurs, the osteoblast is eventually surrounded by osteoid matrix then becomes an osteocyte.

Osteocytes. These cells are responsible for maintaining the bone matrix. Osteocytes can synthesize new matrix as well as participate in matrix degradation, aiding to maintain the homeostasis of blood calcium.

Each osteocyte occupies a space, or ***lacuna***, that conforms to the shape of the cell. Osteocytes extend cytoplasm processes through the canaliculi in the matrix to contact processes of neighboring cells by means of gap junctions. Osteocytes exhibit less cytoplasm basophilia than osteoblasts, badly developed organelles.

Osteoclasts. Osteoclasts are large, multinucleated giant cells of hemopoietic origin found at sites where bone is being removed. They are often found in depressions in the bone.

The number of nuclei in osteoclasts varies greatly (from 3 to several groups of ten). The cytoplasm has a foam-like or vacuolated appearance and gives a variable staining reaction. It is less basophilic than the osteoblast cytoplasm and, in some cells, particularly in the other ones. They become slightly acidophilic. Electron micrographs show a paucity of granular endoplasmic reticulum, but reveal clusters of free ribosomes. The mitochondria are relatively short and they are most numerous in the cytoplasm facing the bone. There are multiple paired centrioles corresponding to the number of nuclei and also numerous Golgi complexes. Vacuoles of various sizes are abundant like lysosomes, which often have granules.

The portion of the cell in direct contact with the bone can be divided into two parts: a central region containing numerous plasma membrane infillings

forming microvillous-like structures, called the *ruffled border*; and a ring-like perimeter of cytoplasm, the *clear zone*, that demarcates the bone area being resorbed. Production of CO_2 which meets environmental H_2O results in H_2CO_2 formation and mineral salts removement. Further enzymatic activity helps organic matrix resorption. Internal to the ruffled border and in close proximity are numerous mitochondria and lysosomes. The fact that osteoclasts are often present in depressions when bone is being resorbed supports the view that they have an important role in the process. An increase in the number of osteoclasts when resorption is stimulated by intravascular injections of parathyroid hormone (PTH) also supports this view.

In regions of cartilage resorption, as in stages of endochondrial bone development, there are multinucleated cells with some characteristics of osteoclasts. They are known as chondroclasts, because they are associated with cartilage. They form by fusion of chondrocytes after the last are released from their lacunae.

General Structure of Bones

Lamellar bone. Bones are covered with a periosteum except in areas where they join with another bone. In the latter case, the joint surface is covered by cartilage. The periosteum consists of an outer fibrous layer that resembles other dense connective tissues and an inner, more cellular layer that contains the osteoprogenitor cells. In general, the collagen fibers of the periosteum are arranged parallel to the surface of the bone in the form of capsule.

A characteristic feature of adult bone tissue is its lamellar structure, with the cells and fibers organized in layers or lamellae. Mature bone is largely composed of cylindrical units called *osteons* or *Haversian systems*. In a cross section of the bone, the central canals are seen to be surrounded by a varying number of concentric lamellae and accompanying bone cells. The concentric lamellae of intercellular substance, the cells, and the central canal form the osteon or Haversian system. The whole bone does not, however, consist of such concentric systems. In the periphery, the lamellae run parallel with the surface and form a relatively thin outer layer of the bone. These are the outer circumferential lamellae. A few similarly arranged inner circumferential lamellae separate the osteons from the marrow cavity. Finally, the interval between the osteons is occupied by more irregular layers of bone, which constitute the interstitial lamellae.

Compact bone thus consists of branching and anatomizing concentric tubular lamellae, with intervals filled in by internal more parallel circumferential lamellae. The perforating canals (Volkmann's canals), which pierce the bone from its outer and inner surface and continue with the central canals are not lined by concentric lamellae.

Electron micrographs show that the osteocytes and their processes do not rest directly on the mineralized matrix but are separated from the walls of their lacunae and canaliculi by an amorphous coat. It probably serves as an extra medium by which substances can be exchanged between the cells and the blood vessels present in the Haversian canals. The osteocytes where bone-forming cells (osteoblasts) become imprisoned in the bone as it was deposited. Osteoblasts and osteoprogenior cells also persist as an incomplete lining of the central and perforating canals and are present in the endosteum and inner layer of the periosteum.

When the collagenous fibrils are studied in preparations, the fibrils are found in delicate fascicles coursing parallel with one another within a single lamella. They follow a helical course in each lamella with differences in slope and direction of alternate lamellae. This is apparently the reason why transverse sections of osteons show the concentric lamellae alternately striated and punctuated. In the former, the fibrils are coursing circularly at the level of the section and are cut lengthwise. In the punctuated lamellae, the fibrils are parallel, to the long axis of the Haversian system at the level of the section and are cut across.

Non-lamellar (woven) bone. Although the bone of all vertebrae consists of collagen, ground substance, calcium salts, and a permeating system of spaces occupied by cells and their processes, different samples of bone differ in the manner in which their constitution is combined. Thus, the skeletons of fish, amphibians, and birds differ from each other and from those of mammals, and the bone of man shows structural changes during ontogenesis. The human embryonic skeleton consists of coarsely bundled woven bone; i.e., the collagenous fibers are in coarse bundles and are irregularly dispersed. Non-lamellar bone is also referred to bundle or woven bone because of the interlacing arrangement of the collagen fibers. Non-lamellar bone (immature bone) forms more rapidly than lamellar (mature) one. Although lamellar bone is clearly the major bone type in the adult, and non-lamellar bone is the major bone type in the developing fetus, areas of immature bone are present in adults, especially where bone is being remod-

eled. Areas of immature bone are also seen regularly in the alveolar sockets of the adult oral cavity and where tendons insert into bones, in tooth sockets, bony sutures, osseous labyrinth. The first bone formed during repair of fractures is of the woven type.

Development and Growth of Bone

According to the embryological origin, there are two types of bone development, *intramembranous* and *intracartilagenous or endochondral*. The bone of extremities and those parts of the axial skeleton that bear weight (e.g., vertebrae) develop by endochondrial ossification. The flat bones of the skull and face, the jaw, and the collar-bone develop by intramembranous *ossification*.

It should be kept in mind, however, that the fundamental process of bone deposition is the same in both types. In intracartilagenous bone formation, there is simply the additional feature of the removal portions of the cartilage preparatory to the deposition of the bone.

Intramembranous bone formation. As the development of the flat bones of the skull involves only intramembranous bone formation, it is an excellent place to study the structural features of the deposition of osseous tissue uncomplicated by changes in cartilage. The first evidence of intramembranous ossification occurs around the eighth week of gestation in humans. Some of the pale-staining, elongate mesenchymal cells within the mesenchyme migrate and aggregate in specific areas, the sites where bone is destined to form. This condensation of cells within the mesenhymal tissue is the membrane referred to in the term intramembranous ossification. As the process continues, the newly organized tissue at the presumptive bone site becomes more vascularized, and the aggregated mesenchymal cells become larger and rounded. The cytoplasm of this cells change from eosinophilic to basophilic, and a clear Golgi area becomes evident. This cytological changes result in the differentiated osteoblast, which then secretes collagen and other components of the bone matrix (osteoid). The osteoblasts within the bone matrix become increasingly separated from one another as the matrix is produced, but they remain attached by thin cytoplasmic processes.

With time, the matrix becomes calcified, and the interconnecting cytoplasm processes of the bone-forming cells, now termed osteocytes, are

contained within canaliculi. Concomitantly, most of the surrounding mesenchymal cells in the membrane proliferate, giving rise to a population of osteoprogenitor cells. Some of the osteoprogenitor cells come into apposition with the initially formed spicules, become osteoblasts, and add more matrix. By this process, called *appositional growth*, the spicules enlarge and become joined in a trabecular network with the general shape of the developing bone. Through continued mitotic activity, the osteoprogenitor cells maintain their numbers and thus provide a constant source of osteoblasts for growth of the bone spicules.

Endochondral (intracartilagenous) ossification. Endochondrial ossification also begins with the proliferation and aggregation of mesenchymal cells at the site of the future bone. Initially, a hyaline cartilage model with the general shape of the bone is formed. The increase in the length of the cartilage model is attributed to interstitial growth. The increase in its width is largely due to the addition of cartilage matrix produced by new chondrocytes that differentiate from the chondrogenic layer of the perichondrium surrounding the cartilage mass.

The first sign of ossification is the appearance of a cuff of the bone around the cartilage model. At this stage, the perichondrial cells in the midregion of the cartilage model no longer give rise to chondrocytes. Instead, osteoblasts are produced. Thus, the connective tissue surrounding this portion of the cartilage is no longer functionally as perichondrium, it is now called periosteum. As a result of these changes, a layer of bone is formed around the cartilage model. This bone can be classified as either periosteal bone, because of its location, or intramembranous bone, because of its method of development. In case of long bone, a distinctive cuff of periosteal bone, the *bony collar*, is established around the cartilage model in the diaphyseal portion of the developing bone.

The chondrocytes in the midregion of the cartilage model become hypertrophic. As the chondrocytes enlarge, their surrounding cartilage matrix is desorbed, forming thin irregular cartilage plates between the hypertrophic cells. The hypertrophic cells begin to synthesize alkaline phosphatase and surrounding cartilage matrix undergoes calcification. The calcified cartilage matrix inhibits diffusion of nutrients, causing death of chondrocytes in the cartilage model. With the death of chondrocytes, much of the matrix breaks down, and neighboring lacunae become confluent, producing an increasingly large cavity. While these events are occurring, one of the several blood

vessels grows through the thin diaphyseal bony collar to vascularize the cavity.

The cells from the periosteum migrate with the penetrating blood vessels and some of the primitive periosteal cells become osteoprogenitor cells in the cavity. As the calcified cartilage breaks down and is partially removed, some remains as irregular spicules. When the osteoprogenitor cells come in apposition to the remaining calcified cartilage spicules, they become osteoblasts and begin to lay down bone (osteoid) on the spicule framework. Thus, the bone formed in this manner may be described as endochondral bone.

As the diaphyseal marrow cavity enlarges, a distinct zonation can be recognized in the cartilage at both ends of the cavity. This remaining cartilage, referred to as *methoepiphyseal cartilage*, exhibits distinct zones:

Zone of reserve cartilage, which exhibits no cellular proliferation or active matrix production.

Zone of proliferation, which is adjacent to the zone of reserve cartilage in the direction of the diaphysis. In this zone, the cartilage cells undergo division and organize into distinct columns.

Zone of hypertrophy, which contains greatly enlarged cartilage cells. The cytoplasm of these cells is clear. The matrix is compressed into linear bands between columns of hypertrophied cartilage cells.

Zone of calcified cartilage, in which the enlarged cells begin to degenerate and the matrix become calcified.

Zone of desorption, which is the zone nearest the diaphysis. The calcified cartilage here is in direct contact with the connective tissue of the marrow cavity.

Lecture 6

MUSCLE

In multicellular organisms, some cells are specialized to enable movement of tissues or organs. These cells may function as single contractile units, named the myoepithelial cells surrounding the acini of some exocrine glands and the pericytes, which invest the smallest blood vessels. Alternatively, contractile cells may be aggregated to form muscles for the movement of large structures such as the skeleton or the visceral. Recent evidence, partly based on the study of the contractile mechanisms of some unicellular organisms, suggests that there is a similar contractile mechanism in all cells. This mechanism consists of fibril proteins arranged in an organized manner in the cytoplasm and linked by intermolecular bonds. Contraction results from the rearrangement of the intermolecular bonds with the utilization of chemical energy.

Two principal types of muscle are recognized:

Striated muscle, in which the cells exhibit cross-striations at the light microscope level. Striped muscle tissue is further subclassified on the basis of its location: *skeletal muscle* and *cardiac muscle*.

Smooth muscle, in which the cells do not exhibit cross-striations.

There are known 5 histogenetic types of the muscle development: 1) *mesenchymal* (smooth muscle of visceral organs); 2) *epidermal* (myoepithelial cells), 3) *neural* (sphincter and dilator muscles of pupil), 4) *coelomic* (cardiac) and 5) *somatic* (skeletal). The first three types refer to smooth muscle, the fourth and fifth — to striped muscle.

Myoepithelial cells develop from the skin ectoderm, are situated in sweat, mammary, salivary and the other glands. They have stellate shape and cover secretory parts and small excretory ducts of the glands. They are also named the basket cells. Nucleus and organelles are situated in the central part of cell and in the processes — contractile apparatus.

Myocytes of neural origin develop from cells of neural germ of the optic cup wall. They are included in two muscles of the iris — sphincter and dilator muscles of the pupil.

Smooth muscle. This type of muscle forms the muscular component of visceral structures, such as blood vessels, the gastrointestinal tract, the uterus and the urinary bladder. Since the visceral muscle is under inherent autonomic and hormonal control, it is described as involuntary muscle. As the arrangement of contractile proteins does not give the histological appearance of cross-striations, the name smooth muscle is also commonly applied.

In contrast to the skeletal muscle, which is specialized for relatively forceful contractions of short duration and under fine voluntary control, the visceral muscle is specialized for continuous of relatively low force producing diffuse movements resulting in contraction of the whole muscle mass rather than contraction of individual motor units. Contractility is an inherent property of visceral muscle, occurring independently of neurological innervations often in a rhythmic and wave-like fashion. Superimposed on this inherent contractility are the influences of the autonomic nervous system, hormones and local metabolites, which modulate contractility to accommodate changing functional demands. For example, the smooth muscle of the intestinal wall undergoes continuous rhythmic contractions, which result in waves of constriction passing along the bowel. This activity is enhanced by parasympathetic stimulation and influenced by a variety of hormones released in response to changes in the nature and volume of the gut contents.

The cells of smooth muscle are relatively small with only a single nucleus. They are bound together in irregular branching fascicles, the arrangement varying considerably from one organ to another according to functional requirements. Smooth muscle fibers are elongated, spindle-shaped cells with tapered ends which may occasionally be bifurcated. Smooth muscle fibers are generally shorter than skeletal muscle fibers and contain only one nucleus, which is elongated and centrally located in the cytoplasm at the widest part of the cell. However, depending on the contractile state of the fibers at fixation, the nuclei may sometimes appear to be spiral-shaped. Smooth muscle fibers are bound together in irregular, branching fascicles and these fascicles, rather than individual fibers, are the functional contractile units. Within the fascicles, individual muscle fibers are arranged roughly parallel to each other with the thickest part of one cell lying against the thinnest parts of adjacent cells.

The contractile proteins of smooth muscle are not arranged in myofibrils as in skeletal and cardiac muscle, thus smooth muscle cells are not striated, giving rise to the common name of smooth muscle. In many tubular visceral structures such as the ileum, smooth muscle is disposed in layers with the cells of one layer arranged at right angles to those of the adjacent layer. This arrangement permits a wave of contraction to pass down the tube propelling the content forward; this action is called peristalsis.

At high magnification the plasma membrane contains significant for Ca ions uptaking numerous flask-shaped invaginations. In some areas these are irregular in shape and size and may be involved in pinocytosis. In other areas, the invaginations are regular in shape and distribution and are called caveolae. The membrane system contains some elements which represent a poorly developed Golgi apparatus and rough endoplasmic reticulum, but good development of smooth endoplasmic reticulum. Other vesicular and tubular structures are seen near the plasma membrane, often in association with caveolae; these probably constitute a system analogous to the sarcoplasmic reticulum of skeletal muscle with the caveolae being analogous to the tubule system. Parallel myofilaments occupy most of the cytoplasm analogous to the thin (actin) filaments of skeletal muscle. Thick (myosin) filaments have also been demonstrated using special techniques. The mechanism of smooth muscle contractility is therefore believed to the basically similar to that of skeletal muscle. Dense bodies (best seen at low magnification) are a feature of visceral muscle and are involved in maintaining the longitudinal orientation of the contractile filaments. The narrow intercellular spaces are almost uniform width but at numerous sites the plasma membranes of adjacent cells form specialized cell junctions. Nexus (gap) junctions mediate spread of excitation throughout visceral muscle. Junctions resembling the desmosomes of epithelia provide anchorage points for contractile proteins and desmin, the intermediate filament of smooth muscle.

Skeletal muscle. They are responsible for the movement of the skeleton and organs such as the globe of the eye and the tongue. Skeletal muscle is often referred to voluntary muscle since it is capable of voluntary (conscious) control. The arrangement of the contractile proteins gives rise to the appearance of prominent cross-striations in some histological preparations and hence the name striated muscle is often applied to skeletal muscle.

Skeletal muscles have a wide variety of morphological forms and modes of action, nevertheless all have the same basic structure. Skeletal muscle is

composed of extremely elongated, multinuclear contractile cells, often described as muscle fibers, bound together by collagenous supporting tissue. Individual muscle fibers vary considerably in diameter from 10–100 mm and may extend throughout the whole length of a muscle reaching up to 35 cm in length.

Skeletal muscle contraction is controlled by large motor nerves, individual nerve fibers branching within the muscle to supply a group of muscle fibers, collectively described as a motor unit. Excitation of any one motor nerve results in simultaneous contraction of all fibers of the corresponding motor unit. The vitality of skeletal muscle fibers is dependent on the maintenance of their nerve supply which, if damaged, results in atrophy of the fibers. Skeletal muscle contains highly specialized stretch receptors known as neuromuscular spindles.

The individual muscle fibers are grouped together into elongated bundles called fascicle with delicate supporting tissue called endomysium occupying the spaces between individual muscle fibers. Each fascicle is surrounded by loose collagenous tissue called perimysium. Most muscles are made up of many fascicle and the whole muscle mass is invested in a dense collagenous sheath called the epimysium. Large blood vessels and nerves enter the epimysium and divide to ramify throughout the muscle in the perimysium and endomysium.

The size of the fascicle reflects the function of the particular muscle concerned. Muscles responsible for fine, highly controlled movements, e.g., the external muscles of the eye, have small fascicle and a relatively greater proportion of perimysial supporting tissue. In contrast, muscles responsible for gross movement only, (e.g., the muscle of the buttocks), have large fascicle and relatively little perimysial tissue. Within each muscle, the supporting tissue component contains both collagen and elastic fibers acting as a flexible skeleton to which individual muscle fibers and fascicle are anchored. This connective tissue becomes continuous with that of the tendons and muscle attachments, which distribute and direct the motive forces of the muscle to bone, skin etc. as appropriate. Skeletal muscle fibers are extremely elongated, non-branched cylindrical shape with numerous flattened nuclei located at fairly regular intervals just beneath the sarcolemma (plasma membrane). During embryological development, certain mesenchymal cells in each myotome differentiate into long, mononuclear skeletal muscle precursors called myoblasts, which then proliferate by mitosis. Sub-

sequently, the myoblasts fuse end to end forming progressively elongated multinuclear myofibrils, which may eventually contain up to 100 nuclei.

Synthesis of the contractile proteins begins after myoblast fusion and myosymplast formation, the proteins being laid down initially in the central axis of the myofibril. The nuclei being displaced peripherally as more contractile protein are formed. Most of the process of muscle development is completed by the time of birth along with the innervations. Thereafter, growth occurs by increase in bulk of the muscle cell cytoplasm.

Mature muscle fibers are highly differentiated and, if damaged, have very limited capacity for repair and regeneration. Nevertheless, a few myoblasts (*satellite cells*) persist after maturity; they are capable of mitosis and appear to play some part in repair of muscle after injury. The main structural-functional unit of the skeletal muscle is a myon, formed by myosymplast surrounded by endomysium. Each myosymplast is surrounded by sarcolemma, consisting of basal membrane and plasmolemma of symplast. Myosatellitocyte abuts to symplast surface and are situated between the last and basal membrane.

Regular cross-striations are the characteristic features of skeletal muscle fibers and can be seen in longitudinal sections. The striations of a skeletal muscle fiber composed of alternating broad light I-bands (isotropic in polarized light) and dark (anisotropic) A-bands. Fine dark lines called Z-lines (Zwischenscheiben) can be seen bisecting the light I-bands. Note the nucleus at the extreme periphery of the cell. Each myofibril has prominent regular cross-striations arranged in register with those of the other myofibrils and corresponding to the I-, A-bands and Z-lines seen in light microscopy. The Z-bands are the most electron-dense, and divide each myofibril into numerous contractile units, called sacromeres, arranged end to end.

The arrangement of the contractile proteins (myofilaments) may be seen in each sacromere. The dark A-band is bisected by the lighter H-band (Heller), which is bisected by a denser M-line (Mittelscheibe). Irrespective of the degree of contraction of the muscle fiber, the A-band remains constant in width. In contrast, the I- and H-bands narrow during contraction and the Z-bands are drawn closer together. These findings are explained by the sliding filament theory.

Mitochondria and numerous glycogen granules provide a rich energy source in the scanty cytoplasm between the myofibrils. The mature muscle

cell contains little rough endoplasmic reticulum; it contains, however, a smooth membranous system, which is involved in activation of the contractile mechanism.

The arrangement of myofilaments in the sarcomere. The sarcomere consists of two types of myofilaments, thick filaments and thin filaments. Each type remains constant in length irrespective of the state of contraction of the muscle. The thick filaments, which are composed mainly of the protein myosin, are maintained in register by their attachment to a disk-like zone represented by the M-line. Similarly the thin filaments, which are composed mainly of the protein actin, are attached to a disk-like zone represented by the Z-line. The I- and H-bands, both areas of low electron-density, represent areas where the thick and thin filaments do not overlap one another. The widely accepted sliding filament theory proposes that under the influence of energy released from ATP, the thick and thin filaments slide over one another, thus causing shortening of the sarcomere.

The transverse tubular system, or *T-system*, consists of numerous tubular invaginations of the plasma membrane; each of one is called a *T-tubule.* T-tubules penetrate to all levels of the muscle fiber and are located between adjacent terminal cisternae at the A- and I-bands junctions.

Between the T-tubules, a second membrane system derived from smooth endoplasmic reticulum. The sacroplasmic reticulum forms a membranous network, which embraces each myofibril. On either side of each tubule, the sarcoplasmic reticulum exhibits a flattened cisternae arrangement, each pair of *terminal cisternae* and a tubule forming a triad near the junction of the I- and A-bands of each sarcomere. Calcium ions are concentrated within the lumen of the sarcoplasmic reticulum. Depolarization of the sarcolemma of the muscle fiber is rapidly disseminated throughout the sarcoplasm by the tubule system. This promotes the release of calcium ions from the sarcoplasmic reticulum into the sarcoplasm surrounding the myofilaments. Calcium ions activate the sliding filament mechanism resulting in muscle contraction. In the vicinity of the junction of the A- and I-bands (and depending on the state of contraction) are tubular *triads*, each comprising a central flattened tubule of the T-system and a pair of terminal cisternae of the sarcoplasmic reticulum. Within the A-bands can be seen tubular elements of the sarcoplasmic reticulum connecting the terminal cisterns. Likewise within the I-bands, similar though less regular longitudinal tubular profiles of sarcoplasmic reticulum are seen. The conducting system of: “slow-twitch”

(red) fibers as shown here is more regular than that of "fast-twitch" (white) fibers where this pattern is more difficult to discern. Note the distribution of mitochondria, regularly arranged between the sarcomeres within the I-bands in immediate association with that part of the actin and myosin filaments, which interact during the process of contraction.

The mode of activity of skeletal muscle varies from one part of the body to another. Some muscles are required to contract almost continuously than others, such as the extra-ocular muscles, make rapid short-lived movements. In humans, distinction between these types cannot be made on gross examination of the muscle. In domestic poultry, however, the extremes are easily identified by a difference in colors; for example, leg muscles are red and flight (breast) muscles are white. Correspondingly, "slow-twitch" and "fast-twitch" muscle fiber types can be demonstrated by nerve stimulation studies. The metabolic requirements of each fiber type differ markedly, the slow red fibers mainly relying on aerobic metabolism and the fast white fibers using predominantly anaerobic pathways. Most muscles actually contain a mixture of these extreme fiber types as well as an intermediate type.

Aerobic (type 1) muscle fibers are small in cross section and contain a large content of myoglobin, an oxygen-storage molecule analogous to hemoglobin, which accounts for the red color of such fibers. In addition these fibers have a rich blood supply. In contrast, anaerobic (type 2) muscle fibers are large in cross-section, contain few mitochondria and relatively little myoglobin; they also have a relatively poor blood supply. These muscle fibers are, however, rich in glycogen and glycolytic enzymes. These characteristics account for the "white" color of such fibers. Anaerobic fibers predominate in muscles responsible for intense but sporadic contraction such as the biceps and triceps of the arms.

Cardiac muscle. The cardiac muscle exhibits many structural and functional characteristics intermediate between those of skeletal and smooth muscle and provides for the continuous, rhythmic contractility of the heart. Like the skeletal muscle, its contractions are strong and utilize a great deal of energy, and like the smooth muscle the contractions are continuous and initiated by inherent mechanisms though modulated by external autonomic and hormonal stimuli.

Cardiac muscle cells (cardiomyocytes) are surrounded by a thin basement membrane or external lamina, which binds the cells to adjacent cells

and supporting tissue. There are two types of cardiomyocytes: conducting and contractile.

Contractile cardiomyocytes are essentially long, cylindrical cells, formed muscle fibers, with one or at most two nuclei, centrally located within the cell. Between the muscle fibers, delicate colagenous tissue analogous to the endomysium of skeletal muscle supports the extremely rich capillary network necessary to meet the high metabolic demands of strong continuous activity.

Cardiac muscle fibers have an arrangement of contractile proteins similar to that of skeletal muscle and are consequently striated in a similar manner. However, this is often difficult to visualize with light microscopy due to the irregular branching shape of the cells and their myofibrils. Cardiac muscle fibers also have a system of tubules and endoplasmic reticulum analogous to that of skeletal muscles. In the case of the cardiac muscle, however, there is a slow leak of calcium ions into the cytoplasm from the endoplasmic reticulum after recovery from the proceeding contraction; this causes a succession of automatic contractions independent of external stimuli. The rate of this inherent rhythm is then modulated by external autonomic and hormonal stimuli.

Between the ends of adjacent cardiac muscle cells are specialized intercellular junctions called *intercalated discs* which not only provide points of anchorage for the myofibrils but permit extremely rapid spread of contractile stimuli from one cell to another. Thus, adjacent fibers are caused to contract almost simultaneously, thereby acting as a functional syncitium. In addition, a system of highly modified cardiac muscle cells constitute the pacemaker regions of the heart and ramify throughout the organ as the Purkinje system thus coordinating contraction of the myocardium as a whole in each cardiac cycle. The sarcomeres of the cardiac muscle have an identical banding pattern to that of the skeletal muscle. The sarcomeres are not, however, arranged into single columns making up cylindrical myofibrils as in skeletal muscle, but form a branching myofibrillar network continuous in three dimensions throughout the cytoplasm. The branching columns of sarcomeres are separated by sarcoplasm containing rows of mitochondria and endoplasmic reticulum. The great abundance of mitochondria in the cardiac muscle reflects the enormous metabolic demands of continuous cardiac muscle activity. Conduction of excitatory stimuli to the sarcomeres of the cardiac muscle is mediated by a system of tubules and endoplasmic reticu-

lum essentially similar in arrangement to that of the skeletal muscle. The tubules, however, ramify throughout the cardiac muscle cytoplasm at the Z- lines and their origins are seen as indentations in the sarcolemma, which thus has a somewhat scalloped outline. Intercalated discs are specialized transverse junctions between cardiac muscle cells at sites where they meet end to end; they always coincide with the Z-lines. Intercalated discs bind the cells, transmit forces of contraction and provide areas of low electrical resistance for the rapid spread of excitation throughout the myocardium. The intercalated discs are an interdigitated junction, zonal adherence and nexus. The predominant type of contact resembles the zonal adherence of epithelial junction complexes but is more extensive and less regular. The actin filaments at the ends of terminal sarcomeres insert into the zonal adherence and thereby transmit contractile forces from cell to cell. Desmosomes occur less frequently and provide anchorage for intermediate filaments of the cytoskeleton. Gap (nexus) junctions are present mainly in the longitudinal portions of the interdigitations and are sites of low electrical resistance through which excitation passes from cell to cell.

The size of conducting cardiomyocytes is more than contractile; the cytoplasm contains all organelles, small mitochondria. The cell membrane does not form T-system. Conducting cardiomyocytes unite in fibers with each other not only by end, but also by lateral surface. Interdigitations, nexuses and desmosomes in intercalated disks occur less.

Lecture 7

NERVOUS TISSUE

The function of the nervous system is to receive stimuli from both the internal and external environments which are then analyzed and integrated to produce appropriate, coordinated response in various effector organs. The nervous system is composed of an intercommunicating network of specialized cells called neurons, which constitute most sensory receptors, the conducting pathways, and the sites of integration and analysis.

The functions of the nervous system depend on a fundamental property of neurons called excitability. As in all cells, the resting neuron maintains an ionic gradient across its plasma membrane thereby creating an electrical potential. Excitability involves a change in membrane permeability in response to appropriate stimuli, such as the ionic gradient is reversed and the plasma membrane becomes depolarized; a wave of depolarization, known as an action potential, then spreads along the plasma membrane. At synapses, the sites of intercommunication between adjacent neurons depolarization of one neuron causes it to release chemical transmitter substances, neurotransmitters, which initiate an action potential in the adjacent neuron.

Within the nervous system, neurons are arranged to form pathways for the conduction of action potentials from receptors to effector organs via integrating neurons. Neurotransmitters not only mediate neuron-to-neuron transmission, but also exhibit the property of excitability. The effector organs of voluntary nervous pathways are generally skeletal muscle whilst, those of involuntary pathways are usually smooth muscle, cardiac muscle and muscle-like epithelial cells (myoepithelial cells) within some exocrine glands. The nervous system is divided anatomically into the central nervous system (CNS) comprising the brain and spinal cord, and the peripheral

nervous system (PNS) which constitutes all nervous tissue outside the CNS. Functionally, the nervous system is divided into the somatic nervous system, which is involved in voluntary functions, and the autonomic nervous system, which exerts control over many involuntary functions. Historically, however, the entire nervous system merely consists of variations in the arrangement of neurons and its supporting tissues.

NEURON

Despite great variation in size and shape in different parts of the nervous system, all neurons have the same basic structure. The neuron consists of a large cell body containing the nucleus surrounded by cytoplasm known as the perikaryon. Processes of two types extend from the cell body, namely a single axon and one or more dendrites. Dendrites are highly branched, tapering processes, which either end in specialized sensory receptors (as in primary sensory neurons), or form synapses with adjacent neurons from which they receive stimuli. In general, dendrites function as the major sites of information input into the neuron.

Although neurons show the greatest variation in size and shape of any group of the cells in the body, they fall into three general categories:

1. ***Sensory neurons*** convey impulses from receptors to the CNS. Processes of these neurons are included in somatic afferent and visceral afferent nerve fibers.

2. ***Motor neurons*** convey impulses from the CNS or ganglia to effector cells. Processes of these neurons are included in somatic efferent and visceral efferent nerve fibers.

3. ***Interneurons***, also called ***intercalated neurons***, form a communicating and integrating network between the sensory and motor neurons.

Neurons are classified on the basis of number of processes extending from the cell body:

Multipolar neurons have one axon and two or more dendrites, which project from the cell body.

Bipolar neurons have one axon and one dendrite, which arise from the pole of the cell body opposite to the origin of the axon. Such neurons act as receptor neurons for the senses of smell, sight and balance and are located in the retina, spiral ganglion of the cochlea and other.

Unipolar neurons which have one process are not located in a human body.

Pseudounipolar neurons, has a single dendrite and the axon arise from a common stem of the cell body and then T-divide. This stem is formed by the fusion of the first part of the dendrite and axon of a bipolar type of neuron during embryological development. The vast majority of the pseudounipolar neurons are located in the dorsal root ganglia and cranial nerve ganglia.

Each neuron has a single axon arising from a cone-shaped portion of the cell body called the axon hillock. The axon is a cylindrical process up to one meter in length terminating on other neurons or effector organs by way of a variable number of small branches which end in small swellings. Action potentials arise in the cell body as a result of integration of afferent (incoming) stimuli. Action potentials are then conducted along the axon to influence other neurons or effector organs. Axons are commonly referred to as nerve fibers. As a general rule, neuron impulses are conveyed along dendrites towards the nerve cell body (afferent) whilst axons usually convey impulses away from the nerve cell body (efferent).

Neurons are terminally differentiated cells and are completely incapable of cell division and replacement in the event of cell death. However, regeneration of axons and dendrites can occur in the event of damage, provided the neuron cell body remains viable.

Ultrastructure of the Neuron

The nucleus of the neuron is large, round or ovoid and usually centrally located within the perikaryon. Reflecting the intense metabolic activity of the neuron (and consequent need to replace damaged enzymes), the chromatin is completely dispersed and the nucleolus is a conspicuous feature. The cytoplasm of the cell body contains large aggregations of rough endoplasmic reticulum, which correspond to the Nissle substance of light microscopy. The rough endoplasmic reticulum extends into the dendrites, but not into the axon hillock or axon. Rough endoplasmic reticulum is much more prominent feature of large neurons, such as somatic motor neurons, than in smaller neurons such as those of the autonomic nervous system. A diffuse Golgi apparatus is found adjacent to the nucleus. Smooth endoplasmic reticulum is not a prominent feature of the perikaryon but tubules, cisternae

and vesicles are prominent in the axon and dendrites. The mitochondria of the perikaryon are numerous and have the usual rod-like appearances; those of the axon are extremely slender and elongated.

Neurons are metabolically very active and expend lots of energy in maintaining ionic gradients across the plasma membrane. Neuron syntheses neurotransmitter substances or their precursors in the perikaryon from where they are transported along the axon to the synapse to be released when appropriately stimulated.

Numerous intermediate filaments (neurofilaments) and microtubules are arranged in parallel bundles throughout the perikaryon and along the length of the axon and dendrites. The intermediate filaments provide structural support and the microtubules are involved in axonal transport of neurotransmitter substances, enzymes, membrane and other cellular constituents.

Almost all structural and functional protein molecules are synthesized in the perikaryon. These molecules are distributed to the axons and dendrites via axonal transport system. However, contrary to the common view that the perikaryon is the only site of protein synthesis, recent studies provide evidence of local synthesis of axonal proteins in some large nerve terminals. These discrete areas within the axon terminals possess biochemical and molecular characteristics of active protein synthesis. Protein synthesis is modulated by neuronal activity.

Axonal transport. Axonal transport serves as a mode of intercellular communication, carrying molecules and information along the microtubules and intermediate filaments from the axon terminal to the perikaryon and from the perikaryon to the axon terminal. Axonal transport is described as follows:

Anterograde transport carries material from the perikaryon to the periphery.

Retrograde transport carries material from the axon terminal and the dendrites to the perikaryon.

The transport system may also be distinguished by the rate at which substances are transported:

A slow transport system conveys substances from the cell body to the terminal bouton at the speed of 0.2 to 4 mm/day.

A fast transport system conveys substances in both directions at a rate of 20 to 400 mm/day.

NEUROGLIA

Within the NS, supporting cells are designated *neuroglia*, or *glial cells*. Neuroglia execute supporting, delimitating, trophic, secretory and protective functions.

Four principal types of neuroglia are recognized: oligodendrocytes, astrocytes, ependymal cells and microglia.

Oligodendrocytes are the NS equivalent of the Schwann cells of the peripheral nervous system and are responsible for the elaboration of myelin sheaths in the NS. Astrocytes are highly branched cells, which pack the interstices between the neurons, their processes and oligodendrocytes. They provide mechanical support as well as mediating the exchange of metabolites between neurons and the vascular system. Astrocytes also play an important role in repair of nerve tissue after injury or damage by disease. Microglia's cells are the NS representatives of the monocyte-macrophage system and have protective and immune functions. Ependymal cells make up a specialized epithelium, which lines the brain ventricles and spinal canal.

Astrocytes. Classical heavy metal impregnation methods identify the presence of star shaped neuroglia, the astrocytes. These cells, which are the most numerous glial cells in grey matter, have long branching processes, which occupy most of the interneuron spaces. In grey matter, many of the astrocyte processes end in terminal expansions adjacent to the non-synaptic regions of neurons. Other processes of the same astrocytes terminate upon the basement membranes of capillaries, these perivascular feet covering most of the surface of the capillary basement membranes.

Astrocytes are the largest of the neuroglial cells. Two kinds of astrocytes are identified:

Protoplasmic astrocytes, which are more prevalent in gray matter. These astrocytes have numerous, short, branching cytoplasmic processes.

Fibrous astrocytes, which are more prevalent in white matter. These astrocytes have fever processes, and they are relatively straight.

Both types of astrocytes contain prominent bundles of intermediate filaments. The filaments are more numerous in the fibrous astrocytes, however, hence the name. Astrocytes processes extend between the blood vessels and neurons. The ends of the processes expand, forming *end feet* that cover large areas of the outer surface of the vessels or of the axolemma. It is now thought that astrocytes play a role in the movement of metabolites and

wastes to and from neurons and regulate ionic concentrations in the intercellular compartment of the neurons. They also have a role in the blood-brain barrier. Protoplasmic astrocytes at brain and spinal cord surfaces extend their processes to the basal lamina of the pia mater to form the *glia limitans*, a relatively impermeable barrier surrounding the CNS.

Oligodendrocytes. It is now known that oligodendrocytes are the cells responsible for myelination of axons in the CNS and the dendrites previously described are the short pedicles that connect the cell body to the myelin sheaths. Each oligodendrocyte gives the several tongue-like processes that find their way to the axon, forming an internodal segment of myelin. In fact, a single oligodendrocyte can contribute to the myelination of up to 50 axons, which may belong to the same or different fiber tracts. The mechanism of myelin sheath formation is very similar to that of Schwann cells in peripheral nerve. Oligodendrocytes are thus the predominant type of neuroglia in white matter as well as being abundant in grey matter. Oligodendrocytes also aggregate closely around nerve cell bodies in the grey matter, where they are thought to have a support function analogous to that of the satellite cells, which surround nerve cell bodies in peripheral ganglia. Myelin sheath formation begins in the CNS of the human embryo at about 4 months gestational age with the formation of most sheaths at least commenced by about the age of one year. From this time, successive layers continue to be laid down with final myelin sheath thickness being achieved by the time of physical maturity. Three types of oligodendrocytes are described, namely light, medium and dark oligodendrocytes according to their staining density with special light microscopic methods and electron microscopy. Light oligodendrocytes are capable of cell division and are highly active in myelin sheath formation and thus predominate in the fetus and neonate, whereas dark oligodendrocytes are main form in the mature CNS. Medium oligodendrocytes represent the immature form involved in myelin sheath growth and maturation. Some light and medium forms are found in the mature CNS suggesting that there is some slow constant cell turnover and capacity for remyelination should the need arise (e.g., after demyelinating diseases such as multiple sclerosis). Reflecting their intense biosynthetic activity, light oligodendrocytes are relatively large cells, with dispersed nuclear chromatin and prominent nucleoli. The cytoplasm contains numerous ribosomes, microtubules and a large Golgi apparatus. In contrast, the dark oligodendrocyte is smaller with a condensed nucleus.

Ependymal cells. Ependymal cells form the simple epithelial lining of the fluid-filled cavities of the CNS (brain ventricles and spinal canal). cuboidal or low columnar in shape, the cells are tightly bound together at their luminal surfaces by the usual epithelial junction complexes. Unlike other epithelia, however, ependymal cells do not rest on a basal membrane but, rather, the bases of the cells taper then break up into fine branches which ratify into an underlying layer of processes derived from astrocytes. At the apical surface, there is variable number of cilia, which may be involved in propulsion of cerebrospinal fluid within the ventricles. The latter are involved in absorbing cerebrospinal fluid. Microvilli are also present and probably have absorptive and secretory functions. The modified ependymal cells and associated capillaries are called *choroid plexus*.

Microglia. Microglia are small cells, relatively few in number, derived from cells of mesenchymal origin which invade the CNS at a late stage of fetal development. Microglia has small irregular nuclei and relatively little cytoplasm which form fine, highly branched processes. In consequence, they are difficult to indentify in conventional preparations for light microscopy. In response to tissue damage, microglia transforms into large amoeboid phagocytic cells which are thus considered to be the CNS representatives of the macrophage-monocyte defense system. Under normal circumstances, lymphocytes and other leukocytes do not enter the brain substance proper. Considerable numbers of macrophages are however present in the space surrounding the CNS capillaries but separated from the CNS compartment proper by the perivascular feet of astrocytes.

MYELINATED AND NON-MYELINATED NERVE FIBERS

In the peripheral nervous system all axons are enveloped by highly specialized cells called Schwann cells, which provide both structural and metabolic support. In general, small diameter axons (e.g., those of the autonomic nervous system and small pain fibers) are simply enveloped by the cytoplasm of Schwann cells; these nerve fibers are said to be ***non-myelinated (unmyelinated)***. Large diameter fibers are wrapped by a variable number

of concentric layers of the Schwann cell plasma membrane forming a myelin sheath; such nerve fibers are said to be ***myelinated***.

Formation of the Non-Myelinated Nervous Fibers

The Schwann cells are elongated in parallel to the long axis of the axons, and the axons fit into grooves in the surface of the cell. The lips of the groove may be open, exposing a portion of the axolemma (axon plasma membrane), the cell membrane of the axon, to the adjacent external lamina of the Schwann cell, or the lips may be closed, forming a *mesaxon.*

A single axon or a group of axons may be enclosed in a single invagination of the Schwann cell surface. Schwann cells in the PNS may have 20 or more grooves, each containing one or more axons. In the ANS, it is common for bundles of unmyelinated axons to occupy a single groove. Each Schwann cell extends for only a short distance along the nerve tract and at its termination is continued by another Schwann cell with which it interdigitates closely end to end.

In the CNS, unmyelinated fibers have no specific cellular support but are indirectly supported by the mass of surrounding nerve cell processes and support cells called astrocytes.

Formation of the Myelinated Nervous Fibers

Myelinated axons are surrounded by a lipid-rich layer, which is called the myelin sheath.

Within the central nervous system myelination is similar to that in the peripheral nervous system except myelin sheath is formed by cells, called oligodendrocytes.

In peripheral nerves, myelination begins with the invagination of a single nerve axon into a Schwann cell; a mesaxon is then formed. As myelination proceeds, the mesaxon rotates around the axon thereby enveloping the axon in concentric layers of Schwann cell cytoplasm and plasma membrane. The cytoplasm is then excluded so that the inner leaflets of plasma membrane fuse with each other and the axon becomes surrounded by multiple layers of membrane, which together constitute the myelin sheath. Each Schwann

cell is covering only a segment of the axon. Between the Schwann cells there are short intervals at which the axon is not covered by a myelin sheath. The single segment of myelin produced by each Schwann cell is termed an internode. The myelin sheath is segmented because is formed by numerous Schwann cells arrayed sequentially along the axon. The junction where two adjacent Schwann cells meet is devoid of myelin. This site is called the *node of Ranvier.*

Myelin is rich in lipid because as a Schwann cell winds around the axon, its cytoplasm extruded from space between the opposing layers of the plasma membranes. Electron micrographs, however, show small amounts of cytoplasm in several locations:

— *the inner collar of Schwann cell cytoplasm,* between the axon and the myelin;

— *the Schmidt—Lanterman clefts*, small island within successive lamellae of the myelin;

— *the perinodal cytoplasm*, at the node of Ranvier;

— *the outer collar of perinuclear cytoplasm*, around the myelin.

These areas of cytoplasm are what light microscopics identified as the Schwann sheath. Cytoplasm of the clefts contains lysosomes, occasional mitochondria and microtubules, as well as cytoplasmic inclusions, or dense bodies. The number of Schmidt—Lanterman clefts correlates with the diameter of the axon. Larger axons have more clefts.

Synapses and Neuromuscular Junctions

Synapses are highly specialized intercellular junctions, which link the neurons of each nervous pathway. Similar intercellular junctions link neurons and their effector cells such as muscle fibers, where neurons synapse with skeletal muscle they are referred to as neuromuscular junctions or motor end plates. Individual neurons intercommunicate via a widely variable number of synapses depending on their location and function within the nervous system. Classically, the axon of one neuron synapses with the dendrite of another neuron forms *axodendritic synapse*. But axons may synapse with the cell bodies of the neurons — *axosomatic synapse*, or other axons — *axoaxonae synapse*. And dendrite of one neuron may synapse with the dendrite of another neuron — *dendrodendritic synapse*.

Chemical synapses. For a given synapse, the conduction of an impulse is unidirectional but the response may be either excitatory or inhibitory depending on the specific functional nature of the synapse and its location. The mechanism of conduction of the nerve impulse involves the release from one neuron of a chemical neurotransmitter, which then diffuses across a narrow intercellular space to induce excitation or inhibition in the other neuron or effector cell of that synapse. Neurotransmitters mediate their effects by interacting with specific receptors incorporated in the apposing plasma membrane.

The chemical nature of neurotransmitters and the morphology of synapses are highly variable in different parts of the nervous system, but the principles of synaptic transmission and the basic structure of synapses are similar throughout the nervous system. The axon is responsible for propagating the stimulus terminals at a bulbous swelling or terminal part; this is separated from the plasma membrane of the opposed neuron or effector cell by narrow intercellular gap of uniform width (20–30 nm) called the synaptic cleft. The terminal parts are not myelinated. These parts contain mitochondria and membrane-bound vesicles of neurotransmitter substance known as synaptic-vesicles approximately 50 nm in diameter.

Although many types of neurotransmitter substance occur in the CNS, only two types are known in the peripheral nervous system: acetylcholine and noradrenalin (norepinephrine). Acetylcholine precursors, acetate and choline, are synthesized in the perikaryon and transported to the synapse where they are conjugated. Noradrenalin synthesis takes place in both the perikaryon and the terminal part. Synaptic vesicles derived by budding from the smooth endoplasmic reticulum of the axon. Synaptic vesicles tend to aggregate towards the presynaptic membrane and, on arrival of an action potential, release their contents into the synaptic cleft by exocytosis. The neurotransmitter diffuses across the synaptic cleft to stimulate receptors in the postsynaptic membrane. Associated with synapses there are a variety of biochemical mechanisms such as hydrolytic and oxidative enzymes, which inactivate the released neurotransmitter between successive nerve impulses. The cytoplasm beneath the postsynaptic membrane often contains a network of fine fibrils, the postsynaptic web, which may be associated with desmosome-like structures in maintaining the integrity of the synapse.

Electrical synapses, which are common in invertebrates, contain gap junctions that permit movement of ions between cells and consequently permit the direct spread of electrical current from one cell to another. These synapses do not require neurotransmitters for their function. Mammalian equivalents of electrical synapses include gap junctions in smooth muscle and cardiac muscle cells.

Motor end plates. The motor end plates of skeletal muscle have the same basic structure as other synapses with the addition of several important features. Firstly, one motor neuron may innervate from a few to more than a thousand muscle fibers depending on the precision of movement of the muscle; the motor neuron and the muscle fibers which it supplies together constitute a motor unit. The terminal part of the axon of a motor neuron is seen dividing into several branches, each terminating as a motor end plate on a different skeletal muscle fiber near its mid-point. The axonal branch loses its myelin sheath and divides to form a cluster of small bulbous swelling (terminal part) or the muscle fiber surface.

The motor end plate occupies a recess in the muscle cell surface, described as the sole plate, and is covered by an extension of the cytoplasm of the last Schwann cell surrounding the axon. The external lamina (basement membrane) of the Schwann cell merges with that of the muscle fiber and the delicate collagenous tissue investing the nerve (endoneurium) becomes continuous with the endomysium of the muscle fiber. Each of the terminal swelling of the cluster making up the motor end plate has the same basic structure. But the postsynaptic membrane of the neuromuscular junction is deeply folded to form parallel secondary synaptic clefts. The overlying presynaptic membrane is also irregular and the cytoplasm immediately adjacent contains numerous synaptic vesicles. The remaining cytoplasm of the terminal bulb contains many mitochondria and a considerable amount of rough endoplasmic reticulum. The sole plate of the muscle fiber also contains a concentration of mitochondria and an aggregation of muscle cell nuclei.

The neurotransmitter of somatic neuromuscular junctions is acetylcholine, the receptors for which are concentrated at the margins of the secondary synaptic clefts. The hydrolytic enzyme acetylcholinesterase is presented deeper in the clefts and is involved in deactivation of the neurotransmitter between successive nerve impulses.

Peripheral Nervous Tissues

Peripheral nerves are anatomical structures which may contain any combination of afferent or efferent nerve fibers of either the somatic or autonomic nervous systems. The cell bodies of fibers coursing in peripheral nerves are either located in the CNS or in ganglia in peripheral sites. Each peripheral nerve is composed of one or more bundles (fascicles) of nerve fibers. Within the fascicles, each individual nerve fiber, with its investing Schwann cell, is surrounded by a delicate packing of loose vascular supporting tissue called endoneurium. Each fascicle is surrounded by a condensed layer of robust collagenous tissue called the epineurium binds the fascicles together and is condensed peripherally to form a strong cylindrical sheath. Peripheral nerves receive a rich blood supply via numerous penetrating vessels from surrounding tissues and accompanying arteries. Larger vessels course longitudinally in the perineurium and epineurium with a rich capillary network in the endoneurium. Extensive anastomosis ensures adequate supply under normal circumstances although this can be put at risk during surgical procedures if too great a length of nerve is dissected from surrounding structures.

SENSORY RECEPTORS

Sensory receptors are the nerve endings or specialized cells, which convert stimuli from the external or internal environments into afferent nerve impulses. The impulses pass into the CNS where they initiate appropriate voluntary or involuntary respond. No classification system for sensory receptors has yet been divided which adequately incorporates either functional or morphological features. A widely used functional classification divides sensory receptors into three groups: exteroreceptors, proprioreceptors and interoreceptors.

Exteroreceptors are those which respond to stimuli from outside the body and include receptors for touch, light pressure, deep pressure, dermal pain, temperature, smell, taste, sight and hearing.

Proprioreceptors are located within the skeletal system and provide conscious and unconscious information about orientation, skeletal position, tension and movement. Such receptors include the vestibular apparatus of the ear, tendon organs and neuromuscular spindles.

Interoreceptors respond to stimuli from the viscera and include the chemoreceptors of blood, vascular (pressure) baroreceptors, the receptors for the state of distention of hollow viscera such as the gastrointestinal tract and urinary bladder, and receptors for such nebulous senses as visceral pain, hunger, thirst, well-being and malaise.

The structure of the receptors involved in some of these sensory modalities is poorly understood. Sensory receptors may be classified morphologically into two groups, simple and compound. Simple receptors are merely free, branched or non-branched nerve endings such as those responsible for dermal pain and temperature; they are rarely visible with the light microscope unless special staining methods are employed. Compound receptors involve organization of associated non-neural tissues to complement the function of the neural receptors. The degree of organization may range from mere encapsulation to highly arranged such as in the eye and ear. By tradition, the eye, ear and receptors for the senses of smell and taste are described as the organs of special sense.

Free nerve endings. Free nerve endings are the simplest forms of sensory receptor, merely consisting of numerous small terminal branches of afferent nerve fibers. Such free nerve endings are found in supporting tissues throughout the body provided a variety of relatively sensory modalities, such as temperature, touch and pain. The afferent fibers are of relatively small diameter with slow rates of conduction. Although some of these fibers are myelinated, the nerve endings are devoid of myelin. In the skin, free nerve endings are found along the dermo-epidermal junction. Some exhibit a terminal expansion, which is intimately associated with non-neuronal cells, called Merkel cells scattered in the basal layers of the epidermis. The adjacent Merkel cell cytoplasm contains vesicles with ultrastructural features similar to those found in synapses, but no neurotransmitter. Merkel nerve endings are served by large-diameter myelinated fibers and are thought to the responsible for the sensation of touch. A variety of different arrangements of free nerve endings are also incorporated in the follicles of the fine and coarse hairs acting as touch receptors.

Meissner's corpuscles. Meissner's corpuscles are small, encapsulated, sensory receptors found in the dermis of the skin, particularly of the fingertips, soles of the feet, nipples, eyelids, lips and genitalia. They are involved in the reception of light discriminatory touch, the degree of discrimination depending on the proximity of receptors to the other. Meissner's corpuscles

are oval in shape and are usually located in the dermal papillae immediately beneath the epidermis. The receptors consist of a delicate collagenous tissue capsule surrounding a mass of plump, oval cells arranged transversely and probably representing specialized Schwann cells. Non-myelinated branches of large myelinated sensory fibers ramify throughout the cell mass in a helical manner.

Pacinian corpuscles. Pacinian corpuscles are large encapsulated sensory receptors responsive for pressure of coarse touch, vibration and tension, and are found in the deeper layers of the skin, ligaments and joint capsules, in some viscera and in some erogenous areas. Pacinian corpuscles range from 1–4 mm in length and in section have the appearance of an onion. These organs consist of a delicate capsule enclosing many concentric lamellae of flattened cells (probably modified Schwann cells) separated by interstitial fluid spaces and delicate collagen fibers. Towards the center of the corpuscle the lamellae become closely packed and the core contains a single large unbranched unmyelinated nerve fiber with several club-like terminals, which becomes myelinated as it leaves the corpuscle. Distortion of the Pacinian corpuscle produces an amplified mechanical stimulus in the core, which is transuded into an action potential in the sensory neuron.

Neuromuscular spindle. Neuromuscular spindles are stretch receptor organs within skeletal muscles, which are responsible for the regulation of muscle tone via the spinal stretch reflex. These receptors are particularly numerous in muscles involved in fine, precision movements such as the intrinsic muscles of the hand and the external muscles of the eye. Neuromuscular spindles are encapsulated, lymph filled, spindle-shaped structures up to 6 mm long but less than 1 mm in diameter. They lie parallel to the muscle fibers, embedded in endomysium or perymysium. Each spindle contains 2–10 modified skeletal muscle fibers called intrafusal fibers, which are much smaller than skeletal muscle fibers proper (extrafusal fibers). The intrafusal fibers have a central non-striated area in which their nuclei tend to be concentrated. Two types of intrafusal fibers are recognized. In one type, the central nuclear area is dilated, these fibers are being known as nuclear bag fibers. In the other type, there is no dilatation and the nuclei are arranged in a single row, giving rise to the name nuclear chain fibers. The sensory receptors are stimulated by stretching of the intrafusal fibers which occurs when the extrafusal muscle mass is stretched. This stimulus evokes reflex contraction of the extrafusal muscle fibers via large (alpha) motor

neurons of a simple two-neuron spinal reflex arch. Contraction of the extrafusal muscle mass thus removes the stretch receptors and equilibrium is restored.

The sensitivity of the neuromuscular spindle is modulated by higher centers via small (gamma) motor neurons arising from the extrapyramidal system. These gamma motor neurons innervate the striated portions of the intrafusal fibers thus controlling their state of contraction. Contraction of intrafusal fibers increases the sensitivity of the intrafusal receptors to stretching of the extrafusal mass.

Lecture 8

THE NERVOUS SYSTEM

The nerves of the peripheral nervous system (PNS) are made of the nerve fibers that carry sensory and motor (effector) information between the organs and tissues of the body and the brain and spinal cord. The central nervous system (CNS) consists of the brain and spinal cord, each of which can be divided macroscopically into grey matter and white matter areas. Grey matter contains almost all the neuron cell bodies and their associated fibers (axons). White matter consists of tracts of nerve fibers at which a substantial number of the axons are myelinated.

The cell bodies of peripheral nerves may be located in the central nervous system or in paravertebral or peripheral ganglia. Ganglia contain clusters of neuronal cell bodies and nerve fibers leading to and from them. The cell bodies in ganglia may belong to sensory neurons (somatic and visceral afferents), and their distribution is restricted to specific locations, or they may belong to postsynaptic "motor" neurons of the autonomic nervous system (ANS).

The cell bodies of motor neurons that innervate skeletal muscle (somatic efferent) are located in the brain, the brain stem, and the spinal cord. The axons leave CNS and travel in peripheral nerves to the skeletal muscles that they innervate. A single neuron conveys impulses from the CNS to the effector organ.

In the ANS, a chain of two neurons connects the CNS to smooth muscle, cardiac muscle, and glands (visceral efferents). The preganglionic neurons of the ANS have their cell bodies in specific locations in the CNS. Their axons leave the CNS and travel in peripheral nerves to synapse with postganglionic neurons in peripheral ganglia.

In the sensory system, both the somatic and the visceral afferent components, a single neuron connects the receptor, through a sensory ganglion, to the spinal cord or brain stem. Sensory ganglia are located along the dorsal roots of the spinal nerves and in association with cranial nerves number 5, 7, 8, 9 and 10.

The CNS is an array of various types of cell assembled to carry out their specific functions. Groups of nerve cell bodies carrying out similar functions are termed *nuclei.* If linearly arranged (as in the spinal cord), such cell groups may be termed *columns*. Groups of nerve fibers interconnecting these neuronal groups are called myelinated tracts from the white matter of the CNS. When neuronal cell bodies occupy at the surface of the brain, these areas are termed cortical regions or cortex. The CNS is covered with connective tissue and supported with a special fluid, the cerebrospinal fluid (CSF).

The brain and spinal cord are enclosed by the dura mater, the pia mater and arachnoid. The dura mater consists of dense fibrous tissue. The pia mater consists of fibrous tissue and contains the blood vessels that send branches into the nerve tissue. The arachnoid contains delicate strands of connective tissue covered with a layer of flat or low cuboidal cells which extends over the outer pail surface. The layer of cells is epithelia and lines the subarachnoid spaces. The arachnoid is nonvascular.

THE SENSITIVE (SPINAL) GANGLIA

The sensitive ganglia lie on running dorsal root of the spinal cord or craniocerebral nerves. Pseudounipolar neurons of the sensitive ganglia are situated in the groups on periphery of the organ. Center of the ganglia consists of processes of these cells. The dendrites go in composition of the sensitive part of mixed cerebrospinal nerves on periphery and end there by receptors. Axons form dorsal roots of the spinal cord and conduct nerve impulses in its grey matter or in the medulla oblongata.

Neurons of the spinal cord are surrounded by the layer of neuroglial cells, which are called *mantle gliocytes*. They possibly spot on round nuclei of the cells surrounding perikaryon of the neuron. The thin connective tissue layer covers the glial layer of the neuron perikaryon outside.

THE SPINAL CORD

The spinal cord consists of two simmetrical parts separated from each other in front by deep ventral fissure, in back — the connective tissue septum. In cross section, the spinal cord exhibits a butterfly-shaped grayish tan inner substance, the grey matter surrounding the central canal, and a whitish peripheral substance, the white matter. The white matter contains only myelinated and unmyelinated axons traveling to and from other parts of the spinal cord and to and from the brain. Functionally related bundles of axons in the white matter are called *tracts*.

The gray matter contains neuronal cell bodies and their dendrites, along with axons and neuroglia. Functionally related groups of nerve cell bodies in the grey matter are called nuclei. Among neurons of the spinal cord it is possible to select the following types of the cells:

— *rootlet neurons (motor)*, whose axons leave the spinal cord in composition with its ventral roots;

— *fascicular neurons (sensitive),* whose axons pass in white matter by fascicles and form conducting ways;

— *internal (intercalated) neurons,* whose axons end by synapses within grey matter of the spinal cord.

The dorsal grey column contains three major nuclear groups specialized for the reception of the sensory impulses carried into the spinal cord by the axons of dorsal root ganglia. The sensory information available to the organism via the first order sensory neurons of the dorsal root ganglia is carried to second order sensory neurons and is distributed widely throughout the CNS.

In central regions of the cord intermediate zone contains motor neurons of the visceral system.

Dorsal horn. In composition of dorsal horn there are distinguished: spongy substance, substantia gelatinosa, nucleus proper and nucleus thoracic.

Spongy substance is formed by the broad loop-forming glial skeleton, which contains numerous small internal neurons.

The glial cells dominate in *substantia gelatinosa*, neurons here are small, and their amount is small. The neurons of the spongy substance, substantia gelatinosa and internal cells realize relationship between sensitive cells of the spinal ganglia and motor cells of the ventral horns, abridging reflex arches.

Nucleus proper is situated in the center of the dorsal horn. It contains the intercalated neurons, whose axons transfer through anterior white commissure and move to opposite side (commissural fibers) of the spinal cord into lateral column of the white matter. The axons are included in *tractus spino-cerebellaris ventralis* and *tractus spino-thalamicus*, which are directed in the cerebellum and thalamus.

Nucleus thoracic consists of large intercalated neurons, which axons come out in the lateral column of the white matter of the same side (associate fibers) and in composition of the *tractus spino-cerebellaris dorsalis* ascend to the cerebellum.

Lateral horn. The medial intermediate nucleus and the lateral intermediate nucleus are distinguished in the lateral horns. Axons of the medial intermediate nucleus neurons join the *tractus spino-cerebellaris ventralis* on the same side. Axons of the lateral intermediate nucleus come out of spinal cord together with somatic motor fibers in composition of the ventral roots.

Ventral horn. The ventral horn contains many motor cells (ventral or anterior horn cells) sized 100–140 μm, arranged in columns in relations to the portion of the body musculature that they innervate. Their axons form main mass of the ventral roots. Motor neurons providing fibers to trunk musculature are more medially disposed in ventral grey matter, and motor neurons to muscles of the limbs are located laterally. Certainly its motor neurons are the largest neurons in the spinal cord. Their dendrites portions, which are generally multipolar, extend several millimeters up or down the cord to receive a variety of signals from locate (spinal) interneurons as well as axons from distant (e.g., cortical) parts of the CNS.

Axons leave the grey matter and form more superficial white matter. The white matter is thus composed of myelinated and few unmyelinated nerve fibers and neuroglia, along with blood vessels and inward continuations of the pia mater.

Axons of the fascicular neurons of the grey matter come out into the white matter and form the own fiber bundles of the white matter directly adjoining the gray matter.

THE CEREBELLAR CORTEX

The cerebellum is the central organ of balance and coordination of movements. The surface of the cerebellum is composed of grey matter, the cor-

tex, which envelops the white matter. Three layers are distinguished in the cerebellar cortex:

— the outer or *molecular layer* containing a few cells and unmyelinated nervous fibers;

— the middle or *ganglionic layer* containing a single row of large flask-shaped cells, the cells of Purkinje;

— the inner or *granular layer* containing a few types of cells and myelinated nervous fibers.

Below the granular layer there is an area of white matter containing the fibers that carry signals to or from the neuronal machinery of the cortex.

In the ***ganglionic layer*** Purkinje cells are situated only in one row. The Purkinje cell possesses several main dendrites, which enter the molecular layer and form a remarkably rich arborization extending to the surface. The dendrite arborization is fan-shaped, extending at right angles to the laminas. The axon is given of from the end of the cell opposite to the dendrites and passes into the granular layer. Here axon collaterals may turn back to enter another part of the cerebellar cortex, but the main axon continues on to neurons deep within the cerebellum that forms the deep cerebellar nuclei. The influence of the Purkinje axons on the neurons of the deep cerebellar nuclei is thought to be inhibitory. It is the neurons of the deep cerebellar nuclei that have axons leaving the cerebellum to provide the cerebellar output to other regions of the nervous system.

Molecular layer contains two main types of neurons: basket cells and stellate cells.

Basket cells are situated in lower one third of the molecular layer. There are small cells by size 10–20 μm. Their fine, long dendrites are branched in planes, located transverse to gyris. The long axon always goes across gyri and parallel surface on Purkinje neurons. They will return collaterals to perikaryon of Purkinje cells and together with others fibers form around perikaryons the typical structure — a basket of the nervous fibers. Activation of the axon of the basket cells causes the inhibition of the Purkinje cells.

Stellate cells lie above basket cells and distinguish two types. Minor stellate neurons have thin short dendrites and bad-branched axons, forming synapses with the dendrites of the Purkinje cells. Large stellate neurons have long, good-branched dendrites and axons. Branched axons join with the dendrites of the Purkinje cells and are included in baskets. Basket and stellate cells of the molecular layer form the system of the intercalated

neurons transmitting nervous impulses on the dendrites and perikaryons of the Purkinje cells in planes transverse gyri.

Granular layer is rich with neurons. Perikaryon of *granule cell*, or *grain-cell* is about 5–8 μm, and contains large spherical nucleus. Dendrites of the granule cells have synapses with the mossy fibers and form specific structures, called *glomerules of cerebellum.* The major input of nerve fibers to the cerebellum arrives in the granule cell layer. The cell nuclei belong to small neurons, the granule cells, and the glomerules are the regions where the granule cell dendrites receive impulses from axons arriving from the cerebellum. The incoming fibers, which are highly branched, are called mossy fibers. Each granule cell possesses three to six short dendrites for the reception of the mossy fiber input.

The granule cell sends its fine, unmyelinated axon to ascend into the molecular layer, where it divides into two branches running longitudinally along the laminas and terminating in dendrites of Purkinje cells. These are the parallel fibers of the molecular layer. They thus run at right angles to and through the dendrite expansions of the Purkinje cells, and their cross sections, together with the terminal dendritic arborizations of the Purkinje cells, give the molecular layer its punctate appearance. During their course in the molecular layer, the parallel fibers make synaptic contact (known to be excitatory) with dendrites from a number of different Purkinje cells.

The Purkinje dendrite also receives excitatory input from climbing fibers, many of which are recurrent collaterals from neurons of the deep cerebellar nuclei. These climbing fibers, which entwine the dendritic trunk, have a powerful excitatory influence on the Purkinje cell.

Other granule cell axons are known to make contact with smaller neurons, the basket cells, which are located in the region of the Purkinje layer. These cells are so named because their axons form a basket like skeins around the bodies and initial axonal segments of the Purkinje cells. They are thus well disposed to exert an inhibitory influence on Purkinje cell activity.

Secondary types of the granular layer of the cerebellum are inhibitory *large stellate cells. There d*istinguished the two types of these cells: with the short and with the long axons. Neurons with the short axons are laid near ganglionic layer. Its branched dendrites are spread in the molecular layer and form synapses with the parallel fibers — axons of the granule cells. Axons trend into granular layer to the glomerules of cerebellum and end by synapses on the branched dendrites of the granule cells more proxi-

mal than synapses of the mossy fibers. Excitation of the stellate cells can block impulses entering from mossy fibers. A few stellate neurons with the long axons have good-branched dendrites in granular layer. Their axons enter the white matter. These cells are supposed to provide the connection between the different regions of cerebellum cortex.

The third type of cells is spindle-shaped horizontal cells. They are mostly located between the granular and ganglionic layers. They have a small elongated perikaryon with the bilateral long horizontal dendrites, which reach ganglionic and granular layers. Their axons spread collaterals into the granular layer and go to the white matter.

Myeloarchitecture of the cerebellum. Afferent fibers, incoming in the cortex of cerebellum, represented by two types of fibers: mossy and climbing. Mossy fibers are a part of the *olivo-cerebellar* and *ponto-cerebellar* tracts. Their stimulating activity towards the Purkinje cells is mediated by the granule cells. Climbing fibers, most probably, enter the cerebellum cortex with the *spino-cerebellar* and *vestibulo-cerebellar* tracts. They cross the granular layer, reach the Purkinje cells and run along with their dendrites making synapses on their surface. The excitatory stimulus of the climbing fibers is conducted directly to the Purkinje neurons. The degeneration of Purkinje cells results in coordination disorders.

Therefore the excitatory stimulus that reaches the cerebellum cortex is conducted to the Purkinje cells through climbing fibers or through parallel fibers of granule cells. The inhibitory function is managed by the stellate neurons of the molecular layer, basket cells and large stellate neurons of the granular layer. The axons of the first two types of cells run across the gyri and inhibit the activity of Purkinje cells by limiting their excitation to narrow discrete areas of the cortex.

THE CEREBRAL CORTEX

The cerebral cortex contains, in addition to nerve fibers, neuroglia, blood vessels, the bodies of nearly 14 billion neurons.

Cytoarchitecture of the Cerebral Cortex

The chief types of neurons found in the cortex are pyramidal cells, stellate or granule cells, horizontal cells, polymorphous.

The pyramidal cells are characterized by a pyramid-shaped perikaryon with an apical dendrite directed toward the surface of the brain and an axon leaving the base of perikaryon to course into the white matter as a projection or association fiber. Sizes of these cells vary from 10 till 140 μm. Pyramidal cells have long triangular perikaryon the top of which is directed to the cortex surface. Dendrites leave top and lateral surfaces of the perikaryon finishing in different layers of the grey matter. The axons branched, leave the basis of the pyramidal cells and pass in white matter. The axons of the pyramidal cells provide the principle output of the cortex.

The granular or stellate cells are characterized by their relatively small size, numerous dendrites coursing in various directions, and a relatively short axon. Many of the axons providing an input to the cortex are thought to end on their dendrites. The horizontal cells, found mostly in the outer layer, are characterized by their horizontally disposed dendrites and axons, which presumably serve to interconnect neighboring cortical regions.

In a section cut perpendicular to the cortical surface, the most striking aspect of the cerebral cortex is the lamination of its cellular components in layers horizontal to the surface. The neocortex is characterized by a laminated appearance in which six layers can be identified.

1. The outermost *molecular layer* made up chiefly of cell processes and of small horizontal cells.

2. The *external granular layer,* which contains small pyramidal and stellate neurons with size about 10 μm.

3. The *pyramidal layer* is the broadest. It has a good development in the precentral gyrus and composed chiefly of relatively large pyramidal cells plus many granule cells. Pyramidal cells reach 10–40 μm. Axons of these cells form myelinated associative and commissural nerve fibers coming in white matter.

4. The *internal granular layer* is made up chiefly of the stellate or granule cells. It has a good development in some areas, for example, visual cortex, and nearly is absent in the precentral gyrus.

5. The *ganglionic layer* is formed by the giant pyramidal cells, which were described firstly by anatomist Betz. These cells reach 120–140 μm in the length and about 80 μm in the width. Axons of the giant pyramidal cells form main part of the *cortico-spinal* and *cortico-nuclear* tracts.

6. The *multiform layer*, containing neurons of widely varying shape.

The functional organization of some cortical areas receiving sensory stimuli (e.g., the cortex at the back of the head, which receives signals from the visual system, involves columns of neurons oriented vertical to the cortical surface.

The thickness of the various cell layers differs considerably in different areas of the cerebral cortex. Some areas exhibit such marked modification in the layers of cells that they are known as a heterotypic, in contrast with homotypic areas, which show all of the six layers. On the basis of differences in structure and functions, the cortex has been mapped into a number of areas. In some regions, the cytoarchitecture of a particular area of cortex corresponds quite precisely to the functional modality known to be processed in that cortical region. This is true, for example, in cortical regions concerned with vision and with hearing. In other case, cortical regions of different function have virtually identical cytological arrangements.

The input to the cortex comes from a great variety of sources. Many of the sensory modalities have representation in discrete cortical regions, which provide surface representations for the different body parts. Other cortical regions are concerned with the initiation and control of motor activities. These regions are also somatotropically organized, each part of the body being represented in a discrete area of cortex. In the human, there are additional substantial cortical areas, called association areas, which have connections with the motor and sensory regions of the cortex. These association areas provide additional orders of circuitry to assist in the analysis of sensory input and the programming of motor output.

Myeloarchitecture of the cerebral cortex

Among nervous fibers of the cerebral cortex there are recognized: *associative*, connecting separate areas of the one hemisphere; *commissural*, connecting two hemispheres; *projectional*, connecting cerebral cortex and nuclei of the inferior segments of the central nervous system. The nervous fibers of the cerebral cortex form the following layers:1) lamina tangencialis, 2) lamina disfibrosa, 3) lamina suprastriata, 4) stria externa, 5) lamina interstriata, 6) stria interna and 7) lamina infrastriata.

The structure of the cerebral cortex vary too much so detailed study of its cellular composition and run of the fibers is a subject of a special course.

Lecture 9

THE ORGANS OF SPECIAL SENSES. THE EYE

The eyeball (bulbus oculi) is essentially a spherical structure, light proof except its transparent anterior surface (the cornea), which contains a system of refracting media convex surfaces. They transmit light rays reflected from outside objects and bring them to focus on a photosensitive surface (the neural retina) in the form of a small inverted image. The photosensitive cells (rods and cones) on which the images fall thereby initiate nervous activity which, when amplified, coordinated and integrated by other excitable cells of the retina, is relayed over fibers of the optic nerve to the brain. Focus is accomplished by changing the curvature of one of the refracting bodies (the lens) through alteration of the tension exerted on it by the mechanism from which it is suspended (the ciliary body). Out of the lens and perpendicular to the optic axis (direction of light transmission) is an adjustable diaphragm (the iris), the adjustable aperture of which (the pupil) regulates the amount of light admitted.

The eyeball is formed by the three sheets: *fibrous* (sclera and cornea), *vascular* and *internal* (sensory). In the eyeball there distinguished three main functional apparatuses: *refracting* (cornea, fluid of the anterior and posterior chambers of the eye, lens, vitreous body), *accommodative* (iris, ciliary body) and *receptor* (retina).

TUNICA FIBROSA

Sclera. The sclera is composed of dense fibrous connective tissue, the thickest at the posterior pole (about 1 mm) and gradually thinner until it is only 0.3 mm thick at the insertion of the recti muscles. The sclera proper

is a dense network of collagenous fiber bundles running parallel to the surface. Near the cornea and around the optic nerve the bundles of fibers are disposed chiefly in an equatorial direction; elsewhere, they cross and interlace. Numerous delicate elastic fibers are interspersed with the collagenous fibers, particularly at the periphery of the bundles. The cellular component consists chiefly of flattened fibroblasts located between the fiber bundles.

Cornea. The zone of transition between the cornea and sclera known as the limbus, is about 1mm in width and has histological features differing from the remainder of the cornea.

The corneal epithelium is stratified scaled, five or six cell layers in thickness. The deepest cells are columnar with the base of each cell resting on a basal lamina that is tenuously attached to the thick underlying Bowman's (basement) membrane. The cells of the remaining layers range from polyhedral to very flat.

None of the corneal cells lose their nuclei or normally undergo keratinization. They are attached to each other by desmosomes.

Bowman's membrane appears homogeneous structure less under the light microscope. However, electron micrographs show that it contains a network of relatively fine collagenous fibers having an irregular arrangement. The basal lamina at the anterior border of this basement membrane is sharply defined from the corneal epithelium whereas the fibers of the posterior border blend with the superficial lamellae of the corneal stroma.

The substantia propria (corneal stroma) forms about 90 % of the cornea and is composed of connective tissue fibers and cells. The characteristic transparency of the cornea is related, in part, to the pattern of its ultrastructural components. The predominant structural elements are collagenous fibrils arranged in layers, or lamella, which course parallel with the surface of the cornea. Electron micrographs show strictly parallel course fibrils within any one lamella, but those of adjacent lamellae differ in direction. The fibrils of each lamella, as well as the different lamellae themselves, are also held together by a mucopolysaccharide matrix rich in chondroitin sulfate, keratosulfate and hyaluronic acid. These macromolecules apparently contribute to corneal transparency.

Most of the connective tissue cells of the corneal stroma are modified fibroblasts. They are located between and parallel to the lamellar collagen-

ous fibers and are flat. The fibroblasts have branched processes that often come into close apposition with neighboring cell processes.

The endothelium is continuos at the margins of the anterior chamber with a less tightly knit epithelium that forms a network over the anterior surface of the iris stroma.

The basement membrane of the endothelium is prominent and separates the latter from the corneal stroma. Traditionally it has been termed Descemet's membrane. It appears homogeneous and highly refractive under the light microscope. It consists of basal lamina reticular fibers (collagenous type III) and elastic fibers.

The cornea proper is entirely devoid of blood vessels, deriving its nutrition from the anterior chamber and the superficial marginal plexus of vessels from the limbus.

The cornea has a rich sensory nerve supply derived from the ophthalmic division of the trigeminal nerve.

A zone about 1 mm wide known as the limbus is transitional between the cornea and the adjacent sclera and conjunctiva. The surface cells retain the characteristics of those of the cornea, but the basal cells become smaller. Here also the corneal stroma loses its regular arrangement, the fiber bundles becoming irregular like those of the sclera. Some elastic fibers are also found.

The only blood vessels, which nourish the cornea, are found in the limbus.

Descemet's membrane and the corneal endothelium become thinner as they approach the scleral meshwork of the iris angle.

TUNICA VASCULOSA

The tunica vasculosa comprises the choroid, ciliary body and iridial stroma and is characterized by the presence of numerous blood vessels and pigment cells.

Choroid. It forms the posterior part of the uvea. Superficially, the choroid is separated from the sclera by a potential space, the perichoroidal space. Internally, the choroid is intimately related to the pigment epithelium layer of the retina. When the retina is detached, the pigment epithelium remains adherent to the choroid.

Histologically, the choroid may be divided into four layers:

1. *The suprachoroid* (lamina suprachoroidea) the superficial layer of the choroid, consists of loosely arranged collagenous and elastic fibers which course obliquely backward from choroid to sclera, bridging the perichoroidal space. Within the meshwork of fibers, there are occasional fibroblasts, some histiocytes and numerous chromatophores that contain black — brown melanin granules.

2. *The vessel layer* (stratum vasculosa) is sometimes regarded as being subdivided into an outer layer of large vessels and an inner layer of medium-sized vessels. Choroidal stroma occupies the spaces between the vessels. Its structure resembles that of the suprachoroid, but the stellate chromatophores have longer and more slender processes.

3. *The capillary layer* (lamina choriocapillaris) is the only layer not continued forward into the ciliary body, contains vessels which supply nutrition for the outer layers of the retina. Its capillaries form a network.

The interspaces of the capillary net are filled in with stroma of delicate collagenous and elastic fibrils which become continuous with that of the vessel layer. Toward the vessel layer, pigment cells are lacking. On the inner aspect of the capillary layer, a condensation of elastic fibrils forms the outer lamella of the lamina vitrea.

4. *The lamina vitrea* (glassy membrane) has traditionally been considered as the innermost layer of the choroid. It is about 2 to 2.5 nm thick with a thin outer lamella composed of slender collagenous fibers and a plexus of elastic fibers. Electronic micrographs show that the basement membrane portion is similar to that in other regions where it consists of a basal lamina and a reticular lamina.

THE CILIARY BODY

In meridian section, the ciliary body has a triangular form. Its outer surface is separated from the sclera by the perichoroidal space. Its inner surface faces the vitreous body and lens and it displays two zones. The posterior two thirds appear darkly pigmented and are relatively smooth. This is orbiculus ciliaris or pars plana. The anterior one third bears some 70

to 80 radially arranged pale ridges, the ciliary processes; this region is the corona ciliaris (pars plicata).

The ciliary body represents a forward continuation of all elements of the choroid except the capillary layer, plus epithelial layers continued from the retina as the pars ciliares retina.

The outermost layer constitutes the suprachoroid and ciliary muscle. Reference has been made to the presence of smooth muscle fibers in the suprachoroid. In the ciliary body, this smooth muscle forms a mass of very appreciable bulk. According to the directions in which they are disposed, three sets of fibers are distinguished in the ciliary muscle:

a) the *meridional* fibers (longitudinal fibers) begin in the suprachoroid in front of the equator and, increasing in number, form bundles, which run into the scleral spur;

b) the *radial* fibers lie internal to the meridional fibers. They are intermingled with connective tissue elements, which become continuous anteriorly with the meshwork of the iris angle;

c) the *circular* fibers are continuous with the radial fibers and lie at the inner edge of ciliary body. These fibers course in a circular direction around the ciliary body just posterior to the root of the iris.

The blood vessels of the ciliary muscle run in the interstitial tissue and are of small caliber.

The vessel layer underlying the muscles is similar in structure to that of the choroid, except that there are fewer chromatophores and more collagenous fibers, and the vessels consist for the most part of veins.

The *lamina vitrea* of the ciliary body is a continuation of the vitreous lamina of the choroid and it contains the same structures: an outer elastic lamella and an inner lamella that is the basement membrane of the pigment epithelium. In the ciliary region, however, an added layer of the connective tissue is interposed between the two lamellae. The inner lamella, or basement membrane, continues onto the iris.

The pigment epithelium of the ciliary body is a forward continuation of the pigment epithelium layer of the retina.

The ciliary epithelium represents a continuation of the neural retina. Electron micrographs show cytological characteristics commonly found in epithelial cells that are active in transport, such as numerous infillings of the basal plasma membrane and the presence of a fenestrated type of the endothelium in the capillaries of the ciliary processes.

THE IRIS

The iris is a thin circular diaphragm placed directly in front of the lens. It forms a distensible aperture, the pupil, located slightly to the nasal side of its center. Its peripheral border (ciliary border, iris root) is attached to the anterior surface of the ciliary body; its pupillary border rests on the lens. The iris divides the space between the cornea and the lens into the anterior chamber of the eye ahead and the posterior chamber behind.

Like the ciliary body, the iris consists of structural continuations from both the tunica vasculosa and the tunica interna. Five layers can be distinguished. The endothelium, anterior border layer and the vessel layer (stroma) form the uveal portion of the iris. Dilator pupil muscles and a pigment epithelium represent a forward continuation of the *tunica interna* and constitute the *pars iridica retinae*.

1. *The endothelium* of the anterior surface of the iris is continuous with the endothelium of the iris angle and, in turn, with that of the cornea.

2. *The anterior border layer* lies immediately under the endothelium and is the layer which determines the color of the iris. It is essentially a condensation of the iris stroma but has fewer collagenous fibers and no blood vessels. It is formed principally of chromatophores — branched connective tissue cells containing granules of yellowish brown pigment. The color of the iris depends upon the thickness of the anterior border layer and the degree of pigmentation of its cells.

3. *The vessel layer* consists of a great number of blood vessels imbedded in a loose stroma of delicate collagenous fibrils, with some elastic fibers and a number of stromal cells. Most of the latter are pigmented (chromatophores). Within the stroma of the iris, the arteries course radially and spirally.

The anterior surface of the iris shows a division into two regions, an inner pupillary zone and an outer ciliary zone, which differ in structure and frequently also in color.

In the stroma of pupillary zone there is a circular band of smooth muscle fibers, the sphincter of the pupil. Their contraction reduces the diameter of the opening. Contraction of the dilator of the pupil produces dilation of the pupil.

Together with the ciliary muscle, the sphincter and dilator of the pupil comprise the intrinsic muscles of the eye.

4. *The posterior border layer* does not differ on the anterior border layer construction.

5. *The ciliary epithelium*, as it nears the root of the iris, acquires increasing amounts of pigment.

THE RETINA

The retina forms the *pars optica retina* of the *tunica interna* and is the part of the eye which transduces the stimulus of light into nerve impulses, resulting in the sensation of vision.

The retina consists of an outer layer, the pigment epithelium, and an inner layer, the neural retina.

The neural retina consists of nine layers:

1) layer of rods and cones contains outer and inner segments of photo receptors;

2) external limiting membrane (apical boundary of Muller's cells);

3) outer nuclear layer cell bodies of rods and cones;

4) outer plexiform layer;

5) inner nuclear layer;

6) inner plexiform layer;

7) ganglion cell layer;

8) nerve fibers layer (axons of ganglion cells);

9) internal limiting membrane (basal lamina of Muller's cells).

Retina represents a chain of three radially located neurons:

1) photoreceptor (rod and cone cells);

2) associative (horizontal, bipolar and amacrine cells);

3) ganglionic (ganglion cells).

1. ***The layer of rods and cones***

The rod cells. The apical protrusions of outer and inner segments of these cells are slender cylindrical elements, 40 to 60 nm in length and about 2 nm in diameter.

Electron micrographs show that the outer segment is made up of hundreds of flattened membranous sacs. The membranes of the sacs contain molecules of visual pigment, which absorb the light and undergo spatial

chemical conformational changes that lead to the production of generator potential by the photoreceptor cell.

The base of the outer segment is connected with the inner segment by a slender stalk or cilium which contains nine peripheral doublets of microtubules. The outer portion of the inner segment, identified as an "ellipsoid" by light microscopy, contains mitochondria. The inner portion of each inner segment contains the Golgi complex and granular and smooth endoplasmic reticulum. The rod cells synthesize new proteins in the inner segments. These proteins are conducted by the ciliary stalks to the bases of the outer segments where groups of new sacs are formed by infillings of the cell membrane. The older sacs are displaced outward as new sacs are built below, and they are eventually shed from the tips of the rods into the region of the pigment epithelium.

Chemical studies have shown that rod pigment, visual purple, consists of vitamin A aldehyde, now known as retinal combined with a protein known as rodopsin. When a pigment molecule is exposed to light, there is a steric change in the form of the retinal and the relationship between retinal and its combined protein is broken. This leads to a change in electrical potential in the cell that results in the formation of a membrane generator potential (signal) which is released at the synaptic contact of the rod basal process to the dendrites of a bipolar neuron.

The cone cells. The cones are flask-shaped, having a relatively short and conical outer segment and a relatively broad and bulbous inner segment. The region that connects the cone inner segment to the cone body is short and it is not constricted as it is in the rods.

Electron microscopic studies show that the cone outer segments are composed of sacs somewhat like those of the rods. The cone sacs differ, however, in that they remain attached to the cell plasmolemma from which they arise and are less closely packed, and they become progressively smaller in diameter along the length of the cone.

The cone outer segment is attached to the cone inner segment by a ciliary stalk that is similar in structure to that in the rod.

In some species, the inner segment often contains a characteristic oil droplet or accumulations of glycogen just beneath the connecting cilium. Otherwise it resembles the rod inner segment in having a region of closely packed mitochondria followed by a region containing the Golgi complex and smooth and rough endoplasmic reticulum.

The visual pigment of the cones is associated with the sacs of the outer segment, as it is in the rods. In the cones, the pigment is known as iodopsin and it consists of retinal combined with a coneopsin. The cones respond to light of relatively high intensity, and they function for visual acuity and for color perception. Detection of different colors apparently depends upon the presence of different pigments in the cones, each apparently absorbing light most efficiently at red, blue or green wavelengths.

2. ***The external limiting membrane*** is really not a discrete membrane, but rather, as seen in electron micrographs, a region of junction complexes between the outer ends of the supporting neuroglial (Muller) cells and the adjoining photoreceptor cells.

3. ***The outer nuclear layer*** consists of rod and cone cell bodies containing rod cells and cone cells nuclei, respectively. The cone nuclei are located close to the outer junctional zone, and with the exception of the region of the fovea, they are limited to a single row. The rod nuclei are more numerous than cone nuclei, except in the fovea, and are distributed in several layers.

4. ***The outer plexiform layer*** is composed chiefly of the basally directed axons of rod and cone cells, the dendrites of bipolar cells, and processes of horizontal cells. These are in synaptic relationship with the processes of rod and cone acting as presynaptic components in transferal of the photoreceptor generator potential to the bipolar cells where it is converted to membrane potential. The processes of rods are indented, enclosing the dendrites of one or more bipolar cells and several horizontal cells in an enclosed synaptic cleft.

5. ***The inner nuclear layer*** is thinner than the outer nuclear layer but resembles it in general appearance. It contains the nuclei of the bipolar neurons, nuclei of association neurons known as horizontal cells and amacrine cells and nuclei of the supporting Muller's cells. In general, the nuclei of this layer are arranged in three zones: an outer one of horizontal cell nuclei; a middle one of bipolar cell nuclei; and an inner one in which amacrine cell nuclei predominate. Muller's cells are the most obvious glial population of the neural retina. Their processes course among receptor and other cell bodies and processes, extending from the inner to outer limiting membranes. They are probably physically supportive in function.

6. ***The inner plexiform layer*** consists of the processes of the amacrine cells, the axons of the bipolar cells and the profusely branched dendrites of the ganglion cells.

7. ***The ganglion cell layer*** is composed of multipolar ganglion cells, among which there are scattered neuroglial cells. Branches of the retinal blood vessels are also present. The ganglion cells are variable in size with clear round nuclei containing one or more prominent nucleoli.

8. ***The nerve fibers layer*** consists of the axons of the ganglion cells. These nonmyelinated fibers are arranged in bundles, which run parallel to the inner surface of the retina and converge at the optic disk to form the optic nerve. Between the bundles there are numerous fibrous neuroglia cells (spider cells) and rows of Muller's cell processes. The retinal blood vessels are also present in this layer.

9. ***The internal limiting membrane*** is formed by the apposition of the expanded inner ends of processes of Muller's cells and by their basal lamina.

Near the posterior pole of the eye, the human neural retina undergoes a localized modification of its layers and shows a funnel-shaped depression. This region has a yellowish color when viewed in gross specimens; hence, it is named the macula lutea, or yellow spot. The inner layers of the retina are spread apart and deviate from the center of the region, leaving a small pit known as the fovea centralis. There the photoreceptors consist only of cones. In the center of the fovea the cones are extremely slender and closely packed. This appears to explain partly the high visual acuity of the fovea.

Peripheral to the fovea region, rod receptors begin to appear among the cones and they gradually increase in number until three to four rods intervene between individual cones in area peripheral to the macula.

The retina pigment epithelium (RPE) is a single layer of cuboidal cells possessing cylindrical sheaths on their apical surface that are associated with the tip of the photoreceptor processes of the adjacent rod and cone cells. Numerous melanin granules are present in many of these processes. The RPE serves several important functions:

— absorption of light to prevent reflection and resultant glare;

— a major component of the blood-retina barrier;

— restoration of photosensitivity (resynthesis of visual pigment);

— phagocytosis and disposal of membraneus discs from rods and cones.

The lens. The lens is a transparent and somewhat plastic biconvex epithelial body situated between the iris and the vitreous body. Its posterior surface has a greater convexity than the anterior surface. Three structural components make up the lens, the capsule, the anterior epithelium and the lens substance.

The capsule consists of the basal lamina and the reticular lamina surrounding the lens. It is of varied thickness in different parts of the lens but always the thinnest at the posterior pole. On either surface zone is concentric with the equator serves for the insertion of the zonular fibers of the suspensor ligament.

The anterior epithelium is a single layer of cuboidal cells on the anterior lens surface, just under the capsule. The posterior epithelial cells have been greatly modified to form the primitive lens fibers during embryonic development. At the equator, or margin, the cells are elongated and arranged meridionally in rows. This is the region where new lens fibers are constantly being formed during lens growth, and the cells themselves may be regarded as young lens fibers.

The lens substance consists of elongated prismatic lens fibers. Succeeding fibers are formed superficially by mitosis, elongation and differentiation of epithelial cells at the equator of the lens. These fibers are arranged meridionally in concentric layers. The older and deeper fibers lose their nuclei but the epithelial cells of the equator region continue to multiply and differentiate into new lens fibers. As a result, the concentric layers show varying degrees of differentiation. Individual fibers can be identified more readily in the outer part of the lens, known as the cortex, than in the inner part, sometimes called the nucleus of the lens.

Electron micrographs show that the epithelial cells of the equatorial region have numerous interdigitations and occasional desmosomes. The lens, like the cornea, is avascular and totally dependent for its nutrition upon the circulating intraocular fluid and transport by its own cells.

The zonula ciliaris. The zonula ciliaris (zonula of Zinn, suspensor ligament) is a system of delicate collagenous fibers which form a fairly thick band radiating from the equatorial zone of the lens capsule to the inner surface of the ciliary body. Many of the fibers arise from the orbiculus ciliaris and sweep forward over the surface of the ciliary body to the lens capsule. Others come from the corona ciliaris.

The zonular fibers are inserted on the lens capsule in two main zones: in front of the equator and just behind it. Fibers, which insert on the anterior capsule, are thicker.

The vitreous body. The vitreous body (VB) is the transparent jelly-like substance that fills the vitrous chamber in the posterior segment of the eye, The VB is loosely attached to the surrounding structures. The main its

portion is a homogenous gel containing approximately 99% of water, collagen, GAG (principally hyaluronan) and a small population of cells called hyalocytes. Fibroblasts and microphages could be in the periphery of VB. The hyaloid canal (Cloquet's canal), remnant of the pathway of the hyaloid artery of the developing eye, runs through the center of VB from the optic disc to the posterior lens capsule.

The eyelids. The eyelids are essentially movable folds of the skin which protect the eye both from injury and from excessive light. Each lid is covered by a thin skin, which on the posterior surface is modified to form a transparent mucous membrane, the conjunctiva. The form of the lid is maintained by a tough fibrous tarsal plate. In the connective tissue between this and the anterior surface are the palpebral fibers of the orbicularis oculi muscle. Associated with the free margin of the lid are the eyelashes and certain small glands.

The skin is very thin. It is provided with many delicate downy hairs, which are associated with the small sebaceous glands. Numerous small sweat glands and pigment cells are also present. The subcutaneous layer is a loose connective tissue, rich in elastic fibers but containing no fat. It is loosely adherent to the underlying muscle.

The tarsal plate is a curved plate of dense fibrous connective tissue with some elastic fibers. Embedded in the tarsal plate are a number of simple branched alveolar glands, Meibomian glands. Each Meibomian gland consists of a long straight central duct surrounded by numerous alveoli, which open into it. The ducts are lined by simple cuboidal epithelium and open on to the lid margin by a series of minute orifices.

The conjunctiva consists of the epithelium and the connective tissue substantia propria. At the lid margin, the epithelium has stratified scaly character of the epidermis with which it is continuous. Over the tarsal plates, it becomes reduced to two layers of cells; the surface cells are tall columnar and the deeper ones, low cuboidal.

The substantia propria consists of a thin layer of fine connective tissue fibers in which a profuse infiltration of lymphocytes occurs.

Accessory lacrimal glands, compound serous tubuloalveolar glands with distended lumina, are located on the inner surace at the upper eyelids and in the fornix at the lacrimal sac. They produce tears that moisten the cornea and pass to the nasolacrimal duct. Tears contain antibacterial and UV-protective agents.

Lecture 10

THE ORGANS OF SPECIAL SENSES. THE ORGAN OF HEARING AND EQUILIBRIUM

The ear contains a series of receptors specialized for hearing and also for the perception of the head position and head movement.

The special apparatus of reception is mounted within fluid — filled spaces deep within the temporal bone. The complex of interconnecting channels containing these receptors is called the inner ear. The receptors for position and motion need no access to the external environment, but the mechanism for hearing requires a chamber, the middle ear, across which sound waves from the air are transmitted to the fluid spaces of the inner ear. In addition, a channel is required for the passage of sound waves from external environment to the middle ear; this passageway is a part of the external ear.

The external ear. The external ear consists of the part we see, the auricle or pinna, plus the external auditory canal (meatus). The auricle contains an irregular shaped plate of elastic cartilage except the region known as the lobule. The skin of the auricle is of thin epidermal type, contains numerous hairs and sebaceous glands; the skin of the posterior surface also has some sweat glands.

In its final form, the external auditory canal (meatus) is a slightly S-shaped channel leading to the middle ear and separated from the latter by the tympanic membrane. The wall of the outer portion of the canal contains elastic cartilage while that of the inner portion is formed by a part of the temporal bone. Both portions of the canal are lined by skin which continuous from the auricle.

The skin, which lines the cartilaginous portion of the auditory canal contains stiff hair, which guard from the entrance of foreign objects. It also

contains sebaceous glands associated with the hair. Simple coiled tubular glands are present and they open directly to the surface of skin by long narrow ducts, known as ceruminous glands because they contribute wax, or cerumen, to the ear which consist of secretion from both types of glands plus desquamated epithelial cells.

The cells of the ceruminous glands are columnar in shape and they contain numerous brown pigment granules and fat droplets.

The skin of the bony portion of the auditory canal is thinner than that of the cartilaginous portion. The hair of this portion are fine; they and sebaceous glands are present only on the superior wall of the canal.

The middle ear. The middle ear consists of an extensive air-filled space in the temporal bone known as the tympanic cavity. This cavity is ventilated by the auditory (Eustachian) tube. The tympanic cavity is a laterally compressed chamber, composed of the middle ear proper, the atrium. The cavity is continuous posteriorly, via the tympanic antrum with the mastoid cells, which are air-filled spaces in the mastoid process of the temporal bone.

The lateral wall of the middle ear cavity is formed almost entirely by the tympanic membrane.

The bone-supported inner wall of the middle ear cavity bears a rounded eminence, the promontory, which marks the position of the first, or basal, coil of the underlying cochlea. Somewhat above and behind this there is an oval aperture, the oval window on the membrane of which fits the base of the stapes. Behind and below the promontory there is a funnel-shaped recess, which leads to a second aperture in the bone, the round window, which is closed by thin secondary tympanic membrane.

Lining the tympanic cavity, and investing all the structures contained within, there is a mucous membrane, the tympanic mucosa. It consists of a thin connective tissue layer partly covered by simple squamous epithelium and partly by pseudostratified epithelium, which is composed of ciliated columnar cells interspersed with secretory cells. The secretory portion of this epithelium is thought to be the source of fluid in middle ear; the ciliated cells may play part in removal of this fluid.

The tympanic membrane is a thin, rather rigid, semitransparent structure in which three layers can be distinguished. The outer cutaneous layer is composed of very thin skin stratum germinativum and a thin stratum corneum. The inner layer consists of a single layer of squamous epithelial cells

on a sparse lamina propria. Between the two surface membranes is the substantia propria, which forms the main mass of the tympanic membrane. It consists of two layers of tendon-like collagenous fiber bundles. The fibers of the outer layer are disposed in a radial manner. The inner fibers course in a circular direction and are most numerous near the periphery. Both layers are lacking in the upper parts flaccid portion of the membrane.

The auditory ossicules, malleus, incus and stapes, are composed of compact bone with interstitial lamellae interspersed with osteons. Their joint surfaces are covered with a thin layer of hyaline cartilage and the bases of the stapes, manubrium and malleus have patches of hyaline cartilage. The middle ear serves the important function of amplifying the weak forces of sound waves that move the eardrum to provide larger force vibrations at the foot plate of the stapes in the oval window.

The auditory (Eustachian) tube. It is flattened canal leading from the anterior wall of the tympanic cavity to the nasopharynx. In its upper extent, near the middle ear, it is surrounded by a bony wall. Below this osseous part, the reinforcing wall is formed partly by a cartilaginous plate and partly by fibrous connective tissue.

The mucosa, which lines the auditory tube, consists of the connective tissue lamina propria covered with ciliated columnar epithelium. In the bony part, the mucosa is thin and firmly united to the underlying bony wall. The epithelium is of low ciliated columnar type. In the cartilaginous part of the tube, the mucosa is loose and the epithelium is of the pseudostratified ciliated variety. Goblet cells occur near the pharyngeal opening, as well as tubuloacinar mucous glands.

The auditory tube serves as a mean of ventilating the middle ear. Normally collapsed, the tube is opened during chewing and swallowing to allow pressure equilibration between throat and middle ear. Unfortunately, it also can be a route for spread of infection between these two regions.

The internal ear. The internal ear is contained in the petrosus part of the temporal bone and consists of an interconnected series of bonny-walled chambers and passages containing similarly shaped membranous sacs and canals. They are known as the osseous labyrinth and the membranous labyrinth, respectively. Intervening between the two is a space, the perilymphatic space, which contains the fluid called perilymph. Within the membranous labyrinth there is also a fluid, the endolymph.

The osseous labyrinth. The osseous labyrinth consists of an ovoid central chamber, the vestibule, from which three semicircular canals and the cochlea are given off. The osseous vestibule of the inner ear is separated from the osseous middle ear by a plate of bone, which is pierced by the oval window and the round window.

The three osseous semicircular canals are arranged with their respective planes perpendicular to each other. Thus, there are two vertical canals and one horizontal canal. The two vertical ones superior (anterior) and inferior (posterior) canals; the horizontal one is known as the lateral, or external canal. Gust after leaving the vestibule, each canal has a dilation known as the ampoule.

The central chamber of the osseous vestibule of each ear is continuous anteriorly with a spiral cavity, which constitutes the bony cochlea. The latter is a spiral chamber, which houses the organ of Corti.

The membranous labyrinth. The membranous labyrinth consists of connected series of sacs and canals whose walls are formed of a fibrous connective tissue lined internally by simple squamous epithelium of ectodermal origin. In general, the membranous labyrinth has the same form as the osseous labyrinth in which it is contained. However, that part enclosed within the osseous vestibule is divided into two sacs. The largest one, the utricle, is an elliptical sac form, which is given off the membranous semicircular canals form. In front of the utricle there is the smaller spherical saccule, which connects by a short and narrow canal, the ductus reuniens, with the membranous cochlea, cochlear duct. The utricle and the saccule are connected by the utriculosaccular duct the two parts of which converge and continue backward through the vestibular aqueduct as the slender endolymphatic duct.

The membranous labyrinth only partially fills the spaces within the osseous labyrinth.

In certain regions, the wall of the membranous labyrinth is considerably modified to form the true sensory areas. In them, the epithelium takes on a special complexity and among its cells the fibers of the vestibulocochlear nerve terminate. There are six such neuroepithelial areas in each labyrinth: one in each sac, the macula utriculi and the macula sacculi; one in each ampoule of semicircular canal, the cristae ampullares; and one in the cochlear duct, the organ of Corti.

These regions project from the wall of the membranous labyrinth into the endolymphatic space.

The vestibule. The *maculae* represent local epithelial thickenings of the membranous walls. The epithelium is columnar and composed of supporting and hair cells derived their name from organized boundle of rigid projections at their apical surface.

The supporting cells are tall columnar elements resting on the basal lamina. Their apical portions extend to the lumen and form a support for the hair cells. Their lateral borders are irregular and difficult to follow in sections. The nuclei are oval in shape. Electron micrographs show that the cytoplasm contains the usual organelles plus numerous secretory granules and an abundance of microtubules. At the edge of the macula, the supporting cells show a gradual transition into the simple squamous epithelium characteristic of the remainder of the membranous labyrinth.

The hair cells are epithelial mechanoreceptors. Two types have been described. The first is *a flask-shaped cell* embraced over its entire inferior aspect by a single large nerve terminal. The second is a *cylindrical cell* contacted by a series of nerve endings only around its base.

In the apical portion of each hair cell, there is a dense terminal web, which is usually described as a cuticular plate. Extending from this region, there are long tapering processes composed of bundles of non-motile projections (called stereocilia) and one conventional cilium (a kinocilium).

The gelatinous polysacharide material (otolithic membrane) overlies the maculae. Its outer surface contains crystalline bodies of calcium carbonate and a protein — otoliths.

The hair cells are thought to alter their resting potential when their hairs are displaced by the action of linear movement or of gravity on the overlying otolithic membrane because of inertia.

This cytological mechanism provides the brain with information both on the position of the head in space and on linear head movements.

Certain special features are present in the structure of the *cristae ampullares*, the sensory areas, which are found in each ampulla. Here the membranous wall is thickened to form a ridge, placed transversely to the long axis of the canal. This ridge consists of the connective tissue tunica propria, containing many nerve fibers and blood vessels, surrounded by a specialized columnar epithelium.

The epithelium of the crista consists of sustentacular cells and hair cells that are remarkably similar to those described above for the maculae. The cupula is seen over the surface of the cells. This is a gelatinous structure, which is separated from the epithelium by a narrow space containing endolymph. The long tapering hairs of the cells pass through this space and penetrate for some distance into the cupula. The hairs in the middle of the crista stand perpendicular to its surface, whereas those at the border are inclined toward the median plane of the cupula.

Fibers of the vestibular nerve terminate in the epithelium of the crista in the same manner as they do in the maculae. The naked axons pierce the basement membrane and form contacts around each of the two types of hair cells similar to those in the maculae.

A change in the speed of rotation of the cupula in two or more of the semicircular canals is resulting of movement of the hairs embedded in its base. The underlying nerve endings are then signaled and the brain thus receives information regarding the speed and direction of rotation of the head.

The cochlea. The osseous cochlea consists of a conical axis of spongy bone, the modiolus, around which winds a spiral bony canal. Along the outer wall of the canal, opposite the osseous spiral lamina is a projection of thickened periosteum, the spiral ligament. A connective tissue membrane the membranous spiral lamina, bridges the space intervening between the spiral ligament and the osseous spiral lamina. Thus, the osseous canal of the cochlea is divided into two spirally parallel parts, an upper scala vestibuli and lower scala tympani.

From the thickened periosteum covering the upper surface of the osseous spiral lamina, a thin membrane, the vestibular membrane, extends obliquely outward to the upper part of the spiral ligament. This membrane forms the roof of a triangular-shaped canal known as the cochlear duct (scala media).

The scala vestibuli begins at the oval window, and the scala tympani ends at the round window.

The scala vestibuli and the scala tympani are perilymph-containing spaces that communicate with each other at the apex of the cochlea through a small channel called the helicotrema.

The upper or vestibular wall is formed by the vestibular membrane, which consists of a thin central lamina of connective tissue covered on either side by simple squamous epithelium.

The outer wall of the cochlear duct is formed by the spiral ligament. The outer portion of the ligament, that is adjacent to the osseous wall, is composed of dense fibrous connective tissue while that of the inner portion consists of more loosely arranged connective tissue. The lateral or outer wall of the scala media is bordered by a unique epithelium, the stria vascularis. It is responsible for production and maintenance of endolymph. The stria vascularis encloses a complex capillary network and contains three types of cells: 1) marginal involved in K+ transport; 2) intermediate pigment-containing; 3) basal.

The lower or tympanic wall of the cochlear duct has an extremely complex structure. It is formed by the outer part of the osseous spiral lamina and the whole of the membranous spiral lamina.

A thickening of periosteal connective tissue along the upper border of the osseous spiral lamina forms an elevation known as the limbus. The limbus has upper and lower projections known, respectively, as the vestibular lip and the tympanic lip. The connective tissue of the limbus is firm and unusually cellular. Lateral to the point of attachment of the vestibular membrane to the limbus the latter is covered by columnar epithelium, the surface of which bears a cuticular formation continuous with the tectorial membrane.

Continued directly from the tympanic lip is the basilar membrane, which extends outward to the crista basillaris of the spiral ligament. The basilar membrane consists of fine straight unbranched fibers, embedded in a sparse homogeneous ground substance.

The organ of Corti. On the upper surface of the basilar membrane, an arrangement of the cochlear duct epithelial cells forms a complex structure, which is the sensory part of the organ of hearing. This structure is the spirally disposed organ of Corti — the cochlear receptor.

The organ of Corti extends the entire length of the cochlear duct with the exception of a short distance at either end. The specialized cells which provide the components listed are border cells, inner phalangeal cells, inner hair cells, inner and outer pillar cells, outer phalangeal cells (*cells of Deiters*), outer hair cells and outer border *cells of Hensen*. The last named are continuous with the *cells of Claudius*, which extend over the remainder of the basilar membrane to the spiral ligament and are underlined by Böttcher's cells.

The inner border cells are slender columnar elements, which rest on the tympanic lip and form a single row along the inner side of the inner cells. Their surfaces are provided with a cuticle.

The inner hair cells are larger than the outer hair cells. They form a single row occupying only the upper part of the epithelial layer. The rounded base of each cell rests on the adjacent supporting phalangeal cells. The surface of this cell has a number of stereocilia which are in contact with the tectorial membrane.

The inner phalangeal cells are arranged in a row along the inner surface of the inner pillar cells. Their bases rest on the basilar membrane. The nucleus lies in the lower portion of the cell, which is continued as a slender process to the surface completely surrounding inner hair cells.

The inner and outer pillar cells each consist of a broad curved base which contains the nucleus, and an elongated body or pillar which contains a stout bundle of closely packed microtubules. The thickened end of the pillar away from the base is known as the head. The head of the outer pillar presents a convexity on its inner side, which fits into a corresponding concavity on the head of the inner pillar, the heads of opposite pillar thus "articulating" with each other. There are thus formed by the pillars a series of arches enclosing a triangular canal, the inner tunnel or Corti's tunnel. This is crossed by delicate nerve fibers.

The outer phalangeal cells (cells of Deiters) are the supporting elements for the outer hair cells, one for each cell, like the hair cells, therefore, their number varies in different region of the cochlear duct (three ranks in the basal pars of coil to five ranks at the apex of cochlear).

Each outer phalangeal cell is an elongated element with its basal portion resting on the basal lamina and surrounding only basal portion of the hair cells. Their slender apical process extends between the hair cells and flatten near the apical end at the hair cell forming complete surrounding plate. Tightly bounded to one another and to the hair cells these apical ends form the reticular lamine that seals extracellular endolymph from intercellular cortilymph.

The outer hair cells are columnar in shape and their apical surfaces bear a number of short sensory hairs, which are in contact with the tectorial membrane. The base of each sell, supported by a phalangeal cell, contains the nucleus and a granular cytoplasm.

Electron micrographs show that the hairs are straight projecting rods surrounded by a typical plasma membrane. The lateral border of the apical

end of the hair cell is in contact with apical end of its supporting cells by occluding junctions, which separate endolymph from perilymph.

The hairs project from the surface in a regular pattern and have a contact with the tectorial membrane.

The cells of Hensen are tall columnar elements, which form the outer border cells of the organ of Corti. They are arranged in several rows on the basilar membrane lateral to the outer phalangeal cells. The base of each cell is narrower than its upper part, which contains the nucleus. The outer cells decrease in height and pass into the cells of Claudius.

The cells of Claudius are cuboidal in shape and have a clear cytoplasm. They line the outermost portion of the basilar membrane.

The tectorial membrane consists of extracellular material that is continuous with a cuticle-like covering of the columnar cells of the limbus region of the cochlea. It is apparently formed by certain connective tissue cells of the limbus region. After extending over the limbus region it becomes thickened into a striated gelatinous structure which extends outward as far as the cells of Hensen. In the living condition the tectorial membrane is in contact with the processes of the hair cells and the processes of these cells are stimulated by movements of the tectorial membrane which are related to vibrations in the endolymph.

PHYSIOLOGY OF THE AUDITORY MECHANISM

Sound vibrations, which impinge upon the tympanic membrane, will cause vibrations at the same frequency as the incident sound waves. The movement consequently imparted to the auditory ossicules will serve to move the base of the stapes in and out of the oval window at the same frequency as that of the stimulating sound. Since, the fluid perilymph on the other side of the oval window lies in a chamber with rigid bony walls and is itself incompressible, it follows that the inward movement of the stapes will produce a pressure within the perilymph, which can be relived only by a compensating outward movement of the secondary tympanic membrane covering the round window.

It could travel the length of the scala vestibuli and pass by way of the slender helicotrema to the perilymph of the scala tympani. This would cause a displacement of the basilar membrane toward the scale tympani; consequently, the pressure is transmitted to the perilymph of the scala tympani and released at the round window. Thus a sound vibration of a given frequency would cause movements of the basilar membrane of equal frequency.

Because the hair cells are held within a framework mounted on the basilar membrane and their hairs are in contact with the overlying tectorial membrane, movements of the basilar membrane will cause the hairs to bend. This bending constitutes the effective stimulus for the hair cells, with consequent activity, which is translated into nerve impulses in the associated nerve fibers.

It is known that damage to structures at the base of the cochlea leads to a loss of hearing for high tones and that the damage near the apex affects reception of lower tones. Because the length of the basilar membrane is shorter near the base of the cochlea and longer near the apex, it has been assumed that the basilar membrane vibrates in a specific for different sound frequencies.

It has also been suggested that the outer hair cells are particularly concerned with determining the intensity of sound and the inner hair cells with pitch discrimination.

THE ORGAN OF SMELL

The sense of smell is perceived in a restricted specialized portion of the mucosa in the upper part of each nasal cavity. This olfactory mucosa contains nerve cell bodies, which provide the mechanism for olfactory reception on their exposed ends and send an axon from their basal end to the first olfactory way station in the brain — the olfactory bulb.

The epithelium of olfactory mucosa is pseudostratified columnar, and is considerably thicker than that of the respiratory region. The surface cells are of two kinds: sustentacular cells and olfactory cells. The **sustentacular** cells are the more numerous. Each cell consists of:

— a superficial portion which is shaped like a stout cylinder and contains pigment and granules arranged in longitudinal rows;

— a middle portion which contains an oval nucleus;

— a thin filamentous process which extends from the nuclear portion down between the cells of the deeper layers.

The luminal surface of the cell is covered with microvilli.

The **olfactory** cells lie between the sustentacular cells. Their nuclei are spherical, lie at different levels, and most of them are more deeply placed than those of the sustentacular cells. From the nuclear portion of each cell a delicate process extends to the surface, where it is expanded in a minute knob (sometimes called the olfactory vesicle). From this terminal knob several cilia arise, each from a typical basal body. Near their base these are typical cilia, but more distantly their long narrow extensions contain only two microtubules. The cilia do not project vertically but are flattened against the mucosal surface. The mucosal surface and the cilia are constantly bathed by the product of special tubular glands (the glands of Bowman), which underlie the olfactory mucosa and deliver their products to its surface.

From the opposite pole of the olfactory cell a longer process extends centrally, which, as a centripetal nerve fiber of one of the olfactory nerves to terminate in the olfactory bulb. The olfactory cell is thus seen to be of the nature of a bipolar ganglion cell with a short peripheral transformed dendrite and a longer central process axon. Between the basal portions of the olfactory cells and the basal processes of the sustentacular cells are small irregular cells of unknown function termed basal cells.

The basement membrane supporting this epithelium is not well-developed. The underlying stroma consists of loosely arranged collagenous fibers, delicate elastic fibers and connective tissue cells. Embedded in the stroma are the numerous simple branched tubular glands.

THE ORGAN OF TASTE

The organ of taste consists of specialized taste buds located mainly within the mucosa of the tongue but also within parts of the palate and the pharynx.

The taste bud is an ovoid epithelial structure embedded in the epithelium and connected with the surface by means of a minute canal, called a taste pore. In light microscope preparations there can be distinguished three varieties of cells:

1) a few relatively small cells scattered along the basal and lateral borders of the taste bud;

2) columnar cells with fairly dark staining round or oval nuclei;

3) columnar cells with oval nuclei and light staining cytoplasm.

The light cells have been described as the taste receptor elements and the dark cells as supporting (sustentacular) cells.

It is known that different taste buds are specialized for the perception of salty, sweet, sour or bitter tastes, and that these tastes are better perceived on certain parts of the tongue than on the others. Substances must be in solution to be tasted, and the amounts required to stimulate sensation are much greater than for the sense of smell.

Lecture 11

THE ENDOCRINE SYSTEM

The endocrine system produces various secretions called *hormones* that serve as effectors to regulate the activities of various cells, tissues, and organs in the body. Its functions are essential in maintaining homeostasis and coordinating body growth and development. The function of the endocrine system is similar to that of the nervous system; both communicate information to peripheral cells and organs. Communication in the endocrine system is through hormones, which are carried to their destination via connective tissue spaces and the vascular system. The nervous and endocrine systems are functionally interrelated. Both systems may act simultaneously on the same target cells and tissues, and some nerve cells secrete hormones.

The close interrelationship of the autonomic and endocrine systems, both structural and functional, is exemplified by the hypothalamus. Though conveniently considered separately, the autonomic, diffuse neuroendocrine and endocrine systems are really a single neuroendocrine regulator of the metabolic activities and internal environment, providing conditions in which it can function successfully. There are, in addition to endocrine glands and diffuse-endocrine system, other hormone producing cells which form minor components of other systems.

Cells of the endocrine system release over 100 hormones and hormonally active substances that are chemically divided into three classes of compounds:

1. ***Steroids***, cholesterol-derived compounds, are synthesized by cells of the ovaries, testes, and adrenal cortex. These hormones are released into the bloodstream and transported to target cells with the help of plasma proteins or specialized carrier proteins such as *androgen-binding protein.*

2. ***Small peptides, proteins, and glycoproteins*** are synthesized and secreted by cells of the hypothalamus, pituitary gland, thyroid gland, parathyroid gland, pancreas and scattered enteroendocrine cells of the gastrointestinal tract and respiratory system. Hormones in this group, when released into the circulation, dissolve readily in the blood and do not require special transport proteins.

3. ***Amino acid analogues and derivatives***, including the catecholamines (norepinephrine and epinephrine), are synthesized and secreted by many neurons as well as cells of the adrenal medulla. Also included in this group of compounds are thyroid hormones, the indicated amino acids that are synthesized and secreted by the thyroid gland. When released into the circulation, catecholamines dissolve readily in the blood, in contrast to thyroid hormones, which bind to serum proteins and a specialized carrier protein, *thyroxin-binding protein*.

The first step in hormone action on a target cell is its binding to a specific hormone receptor. Two groups of hormone receptors have been identified:

1. ***Cell surface receptors***, which interact with peptide hormones or catecholamines that are unable to penetrate the cell membrane.

2. ***Intracellular receptors***, which are localized within the cell (mainly within the nucleus), are used by steroids and thyroid hormones that can easily penetrate both plasma and nuclear membranes.

We can classify endocrine system under the following basic components:

I. Central regulatory formation of endocrine system:
1) hypothalamus (neurosecretory nuclei);
2) pituitary gland (hypophysis);
3) epiphysis.

II. Peripheral endocrine glands:
1) thyroid gland;
2) parathyroid gland;
3) suprarenal gland: a) cortex, b) medulla.

III. Organs having both endocrine and non-endocrine functions:
1) gonads: a) testis, b) ovary;
2) placenta;
3) pancreas.

IV. Solitary hormone producing cells:

1) solitary hormone producing APUD-cells of nervous origin (Amine Precursor Uptake and Decarboxilation);

2) solitary hormone producing cells (of non-nervous origin).

HYPOTHALAMUS

Hypothalamus is the higher centre of control and coordination of the endocrine system. It controls and interprets all the visceral functions of the organism and unites the endocrine mechanism of regulation with the nervous regulation and especially with the sympathetic and parasympathetic parts of the vegetative nervous system. The hypothalamus contains special neurosecretory cells; they are aggregated to form nuclei (30 pairs) which are classified into anterior, middle (medio-basilar and tuberal) and posterior groups. In the hypothalamus region an median eminence is present in which the neurogemal centre of the hypothalamo-hypophysial system forms. It is formed of ependyma (of individual specialized cells which differentiate to form tanicytes). The tanicytes are characterized by branched processes which contact with the primary capillaries of the hypophysial portal system. The hypothalamo-adenohypophysial system accumulates neurohormones called adenohypotropins which pass into the portal system of the hypophysis. The hypothalamo-neurohypophysial system accumulates nonapeptides which are released into the blood. The anterior group of nuclei of the hypothalamus contains two main nuclei:

1. *Nuclei supraoptic* are formed by large cholinergic neurosecretory cells which contain secretory granules both in the perikaryon and in the processes. The axons of these cells pass through the median eminence and the infundibulum of hypophysis into the posterior lobe of the hypophysis, here they end in terminal buds of Herring on the blood capillaries. These cells produce neurohormone — *vasopressin* also called *antidiuretic hormone* which controls reabsorption of water by renal tubules.

2. *Nuclei paraventricular* — are composed of a central and peripheral part, while the central part is formed of large cholinergic neurosecretory cells whose axons pass into the posterior lobe of the hypophysis. The peripheral part is made up of small adrenergic neurosecretory cells whose axons pass into the medial eminence. The cells of the central part release hormone *oxytocin* which regulates smooth muscles contraction of the uterus and mammary gland.

In the medial group of nuclei small adrenergic cells are present which produce *adenohypophysotropin* neurohormones, by means of which the hypothalamus regulates the activities of the adenohypophysis. These hormones are low molecular oligopeptides which are divided into *liberins (releasing factors)* which stimulate the activity of the anterior and medial lobe of the pituitary, and *statins (inhibitory factors)* which inhibit their activity. Some of the important nuclei lie in the region of the tuber cinereal (nucleus arcuatus, nucleus ventromedialis and nucleus dorsomedialis). The principle areas of production of liberins and statins includes both ventromedial and arcuate nuclei, small peptidoadrenergic cells of the paraventricular nuclei and analogous cells of the grey periventricular matter, preoptic zone of the hypothalamus and suprachiasmatic nuclei.

Hypothalamus controls the visceral activates of organs by two mechanisms:

— through its regulation of hypohysial activity it is called transadenohypophysial regulation;

— by sending efferent impulses to control sympathetic and parasympathetic, nervous system it is called parahypophysial regulation.

HYPOPHYSIS (PITUITARY GLAND)

The pituitary gland and the hypothalamus, the portion of the brain to which the hypophysis is attached, are morphologically and functionally linked in the endocrine and neuroendocrine control of other endocrine glands. Because they play central roles in a number of regulatory feedback systems, they are often called the "master organs" of the endocrine system.

There are distinguished 3 lobes in the hypophysis: anterior, medial and posterior. But the pitutary gland is studied according to the following classification:

1. *The anterior lobe (adenohypophysis)*, the glandular epithelial tissue.

2. *The posterior lobe (neurohypophysis*), the neural secretory tissue.

The anterior lobe of the pituitary gland consists of three parts: *pars distalis* or *pars anterior*, *pars intermedia* and *pars tuberalis*.

Neurohypophysis includes the pars posterior or pars nervosa, infundibulum and median eminence.

Adenohypophysis is highly vascular and consists of epithelial cells of varying size and shape arranged in cords or irregular follicles, separated from

each other by vascular sinusoids, and supported by a loose connective tissue. Each trabecula (cord) is formed of glandular epithelial cells (adenocytes).

The cells within the pars anterior vary in size, shape and staining properties. The cells are arranged in cords. Using mixtures of acidic and basic dyes, histologists identified two groups and three types of cell according to their staining reaction. *Chromophils* (1st group) are arranged in periphery of trabeculae and also stain intensively due to presence of secretory granules in their cytoplasm. Chromophilic cells (40%) are divided into *acidophils* and *basophils.* Another type of cells arranged in middle part of trabecula does not stains intensively and called *chromophobes* (60%).

All known hormones of the anterior lobe of the pituitary gland are small proteins or glycoproteins. This important fact has led to definite identification of specific cell types by immunocytochemistry.

I. **Acidophils**

1. *Somatotropes (growth hormone (GH) cells).* They are ovoid and usually grouped along sinusoids: they are largest and most abundant class of adenohypophysial chromophils, secreting the protein *somatotropin (GH)*. Somatotropes are medium-sized, oval cells exhibit round, centrally located nuclei. Ultrastructurally are seen to contain numerous electron dense, spherical secretory phase, relatively small amount of granular endoplasmic reticulum. The presence of eosinophilic vesicles in their cytoplasm by a diameter near 350–400 nm, classifies them into acidophil cell type. Two hypothalamic hormones regulate the release of GH from somatotropes: growth hormone-releasing hormone (GHRH) and somatostatin.

2. *Lactotropes* (PRL cells, mammotropes) constitute 15 to 20 % of the parenchymal cells in the anterior lobe of the pituitary gland. These are large, polygonal cells with oval nuclei, secreting the polypeptide hormone prolactin (PRL) that is dominant in pregnancy and hypertrophies during lactation. Their granules are largest among hypophysial cells (about 500–600 nm in diameter).Their size is bigger in pregnant and lactating females than that in non-pregnant females. The granules are evenly dense, ovoid or fuse with lysosomes to form autophagic vacuoles which degrade unused granules. In active cells granular endoplasmic reticulum and the Golgi complex are prominent. Secretion of PRL is under inhibitory control by dopamine, the catecholamine producing by hypothalamus. During pregnancy and lactation these cells undergo hypertrophy and hyperplasia, causing the pituitary gland to increase a size.

II. Basophils or B-cells

The granules of basophils contain glycoproteins being material for hormones biosynthesis. The cells have relatively large sizes.

1. *Corticotropes (adrenocorticotropic hormone (ACTH) cells)* constitute 15 to 20% of the parenchymal cells in the anterior lobe of the pituitary gland. These polygonal, medium size cells with round and eccentric nuclei produce a precursor molecule of ACTH, known as *propriomelanocortin.* Propriomelanocortin is cleaved by proteolytic enzymes within the corticotrope into several fragments, namely *ACTH, ß-lipotropic hormone, melanocyte-stimulating hormone, ß-endorphin and enkephalin.* These cells are irregular in shape and size and have short dendritic processes which are inserted among other neighboring cells. Their granules are also small (about 200 nm) in diameter, along with this are present vesicular endoplasamic reticulum and a well developed Golgi complex.

2. *Thyrotropes (thyroid-stimulating hormone (TSH) cells)*. They constitute about 5% of the parenchymal cells in the anterior lobe of the pituitary gland. These large polygonal cells with round and eccentric nuclei produce TSH (*thyrotropic hormone, thyrotropin*). They are arranged in clusters towards the adenohypophysial centre. Their granules, peripheral and irregular, are less electron dense than in other basophils, being 100–150 nm in diameter and the smallest granules among adenohypophysial cells. TSH acts on the follicular cells of the thyroid gland stimulating production of thyroglobulin and thyroid hormones.

3. *Gonadotropes (follicle-stimulating and luteinizing hormone (FSH and LH) cells)* constitute about 10% of the parenchymal cells in the anterior lobe of pituitary gland. These small oval cells with round and eccentric nuclei produce both FSN and LH. However, immunocytochemical studies indicate that some gonadotropes may produce only one hormone or the other one. Both FSH and LH play an important role in male and female reproduction.

Chromophobes. They constitute the majority of the cells of the adenohypophisis. They appear to consist of a number of cells of different types, including degranulated secretory cells of the all types, the stem cells capable of giving rise to chromatophils, and follicular cells containing numbers of lysosomes and forming cells clusters around cysts of various sizes.

Pars Intermedia

Pars intermedia is constituent of the anterior pituitary gland, being derived embryologically from the cells lining the cavity of Rathke's pouch. The parenchymal cells of the pars intemedia surround colloid-filled follicles. The cells lining these follicles appear to be derived from various secretory cells. The pars intermedia contains basophils and chromophobes.

The function of the pars intermedia cells in human remains unclear. It is known that basophils have scattered vesicles in their cytoplasm that contains either a- or ß-endorphin. In frogs, the basophils produce melanocyte-stimulating hormone (MSH), which stimulates pigment production in melanocytes and pigment dispersion in melanophores, and assigned to the APUD series secretory cells. In humans, MSH is not a distinct, functional hormone. Because MSH is found in the human pars intermedia in small amounts, the basophils of the pars intermedia are assumed to be corticotropes.

Pars Tuberalis

This part is remarkable for its large number of blood vessels. It is a highly vascular region containing veins of the hypothalamohypophyseal system. The parenchymal epithelial cells are arranged in small clusters of cords in association with the blood vessels. Groups of squamous cells and small follicles lined with cuboidal cells are scattered in this region. These cells often show immunoreactivity for ACTH, FSH, and LH. From pars tuberalis trabeculae penetrate in the anterior lobe, in some cells of trabeculae some basophilic granules are found. But secretion from their cells only starts only after signals from neurons, in the form of releasing factors.

Posterior Lobe of the Pituitary Gland (Neurohypophysis)

The posterior lobe of the pituitary gland consists of the *pars nervosa* and *pars infundibulum.*

The *pars nervosa* contains nonmyelinated axons and their nerve endings of approximately 100,000 neurosecretory neurons whose cells bodies lie in the *supraoptic* and *paraventricular* nuclei of the hypothalamus. In neuro-

hypophysis hormones (vasopressin and oxytocin) are stored and secreted from big peptidocholinergic neurosecretory cells of anterior lobe of hypothalamus. Axons of this neurosecretory cells end as big terminals, called *Herring body*, contacting with capillaries. The axons form the hypothalamohypophyseal tract and are unique in two respects. First, they do not terminate on other neurons or target cells but end in close proximity to the fenestrated capillary network of the *pars nervosa.* Second, they contain secretory vesicles in all parts of the cells.

Neurohypophysis is mainly made up of ependymocytes. They have spinous processes on their body and are called pituicytes. Processes of pituicytes end on adventitial cells or basal membranes of capillaries.

PINEAL GLAND (EPYPHYSIS)

The pineal gland (because of its pine cone-shaped structure) is an neuroendocrine gland that regulates rhythmic changes and periodicity (daily body rhythm, ovarian menstrual cycle). The capability of epyphysis to regulate rhythmic functions is accentuated by its capacity to release hormones under the stimulation of light.

The organ is covered with a thin connective tissue capsule from which the septae pass dividing the whole organ into lobules. There are two types of cells found in the parenchyma:

1) *pinealocytes* and 2) *gliocytes.*

Pinealocytes are the chief cell of the pineal gland and are found towards the centre of the lobules. They are large neurosecretary cells arranged in clumps of cords, containing a large nucleus with prominent nucleoli and lipid droplets within their cytoplasm. From their bodies long processes similar to dendrites arise, which undergo branching and intermingle with the processes of gliocytes. The processes are usually directed towards capillaries and undergo contact with them. The cells contain both granular and agranular endoplasmic reticulum, mitochondria and Golgi complex. An organelle of unusual structure made up of groups of microfibrils and perforated lamellae may be present (so called canaliculate lamellar bodies). The terminal buds of the processes of these cells contain monoamines and polypeptide hormones. There are two types of pinealocytes: 1) light ones, which contain light homogenous cytoplasm and 2) dark ones, which contain acidophilic inclusions. These two cells are the functional diversity of a

single cell. The cell also contains well developed ribosomes and polysomes. The pinealocytes are separated from one another by neuroglial cells that resemble astrocytes in structure. Their processes are directed towards the interlobular connective tissue septa.

The *interstitial (glial) cells* constitute about 5% of the cells in the gland. They have staining and ultrastructural features that closely resemble those of astrocytes and are reminiscent of the pituicytes of the posterior lobe of the pituitary gland.

In addition of the two cells types, the human pineal gland is characterized by the presence of calcified concretions *(corpora arenacea or brain sand)*. Concretions increase in number with age and appear to be derived from precipitation of calcium phosphates and carbonates on carrier proteins that are released into the cytoplasm when the pineal secretion is exocytosed.

The pinealocytes form quiet heterogenous population synthesising approximately 40 types of regulatory peptides and also biologically active amines — serotonin and melatonin. Synthesis and release of the latter depends on the lightening rate: increases during darkness and decreases during light. Production of its methabolic precursor serotonin takes place intensively during light and is reduced during darkness. Melatonin inhibits hypothalamic secretion of gonadoliberin (GnRH) that prevents precoxious puberty. At adults melatonin controls pigment exchange, reproductive functions, circadian and seasonal rythms, the processes of division and differentiation, expresses antitumor activity. Deficient serotonin level in brain tissues is the pathogenic factor of depression, while increase in seratonin concentration causes emotional rise. Among regulatory peptides of epiphysis luliberin, thyroliberin (TTRh), hormones regulating mineral exchange (factor increasing potassium level in blood).

ADRENAL GLANDS

The adrenal (suprarenal) gland is a paired yellowish organ formed by the union of two independent hormones producing glands, comprising of cortex and medulla of different origin. The adrenal glands secrete both steroid hormones and catecholamines.

The adrenal glands are covered with a connective tissue capsule from which trabeculae extend into the parenchyma, carrying blood vessels and

nerves. Connective tissue capsule is divided into two layers — the outer (dense one) and inner (loose one). The secretory parenchymal tissue is organized into cortical and medullary regions. Under the capsule is a thin striated layer of small epithelial cells, whose division participates in regeneration of cortical zona glomerulosa.

The adrenal cortex shows three cellular zones on the basis of arrangement of its cells. The three cellular zones of the glands are:

— ***Zona glomerulosa***, the narrow outer zone that constitutes up to 15% of the cortical volume.

— ***Zona fasciculata***, the thick middle zone that constitutes nearly 80% of the cortical volume.

— ***Zona reticularis***, the inner zone that constitutes only 7% of the cortical volume but is thicker than the glomerulosa because of its more central location.

Zona glomerulosa is found in outer region (subcapsular zone) of the gland. It is formed of small, polyhedral cells (endocrinocytes) which are arranged in closely packed ovoid clusters and curved columns. The spherical nuclei of the cells appear closely packed and stain densely, scanty basophilic cytoplasm contains sparse lipid droplets and displays many microtubules, large mitochondria and agranular endoplasmic reticulum, free ribosomes and some rER. The Golgi apparatus is very well developed. The mitochondria is characterised by *lamellae (shelf-like) cristae.* A rich network of fenestrated sinusoidal capillaries surrounds each cell cluster.

The cells of the zona glomerulosa secrete hormones *mineralocorticoids*, the compounds that function in the regulation of sodium and potassium homeostasis and water balance. The principal secretion, aldosterone (95%), acts on the distal tubules of the nephron in the kidney, the gastric mucosa, and the salivary and sweat glands to stimulate resorption of sodium at these sites. The zona glomerulosa is under feedback control of the *renin-angiotenzin-aldosterone system*.

Between the zona glomerulosa and fasciculata a narrow layer of nonspecialized cells is found. This layer is called intermediate or s*udanophobic zone*. The division of cells in this layer participates in the regeneration of zona fasciculata and zona reticularis.

Zona fascilulata consists of large and polyhedral cells with basophilic cytoplasm arranged in straight columns, one or two cells thick, that are

separated by sinusoidal capillaries. The cells of the zona fasciculata have a lightly staining spherical nuclei and possescharacteristics typical of steroid-secreteng cells, i.e. a highly developed smooth ER and mitochondria with tubular cristae, well-developed Golgi apparatus. Numerous profiles of rough ER may give a slight basophilia to some parts of the acidophilic in general cytoplasm. Numerous lipid droplets contain neutral fats, fatty acids, cholesterol, phospholipids that are precursors for steroid hormons.

Among with light colored cells there are found dark colored cells which contain small amount of lipids, but high amount of ribonucleic protein. Well developed agranular endoplasmic reticulum and granular endoplasmic reticulum are found in the dark cells. The light and dark cells represent different functional entities of the endocrinocytes. The dark cells are concerted with the formation of enzymes responsible for corticosteroids synthesis. After synthesis of steroids the cell becomes light in color and prepares for the release of secretion into the blood.

Cells of this zone produce *glucocorticoids* — corticosterone, cortizon and cortisol (hydrocortizon), so called because of their role in regulating gluconeogenesis (glucose synthesis) and glucogenesis (glucose polymerization). Glucocorticoids act on many different cells and tissues to increase the metabolic availability of glucose and fatty acids, both of which are immediate sources of energy. Within this broad function they may have different, even opposite effects in different tissues.

In the *liver*, glucocorticoids stimulate conversion of amino acids to glucose, stimulate the polymerization of glucose to glycogen, and promote the uptake of amino acids and fatty acids.

In the *adipose tissue*, glucocorticoids stimulate the break-down of lipids to glycerol and free fatty acids.

In *other tissues*, they reduce the rate of glucose use and promote the oxidation of fatty acids.

In cells such as *fibroblasts*, they inhibit protein synthesis and even promote protein catabolism to provide amino acids for conversion to glucose in the liver.

High concentration of glucocorticoids causes destruction of lymphocytes and eosinophils in the blood, causing lymphocytopenia and erytrocytopenia which causes changes in inflammatory responses of body.

ACTH regulates secretion of the zona fasciculata.

Zona reticularis contains branching inter connected columns of round smaller, than those of zona fasciculata and their nuclei are more deeply stained. They are arranged in anastomosing cords separated by fenestrated capillaries. Cells cytoplasm consists of large deposits of agranular endoplasmic reticulum, lysosomes and pigment bodies. The cells have relatively few lipid droplets. Both dark and light cells are seen. Dark cells have abundant large pigment granules, and deeply staining nuclei are evident. The *cristae* of mitochondria are *tubular* in shape. The cells secrete sex hormones — progesterone, estrogen and androgen. However, the formation of testosterone and other androgenic hormones dominate over the development of female hormones. The cells also secrete some glucocorticoids, in much smaller amount than those of the zona fasciculata. The principal glucocorticoid secreted is cortisol.

Between the reticular zone and the medulla is found a zone of highly acidophilic cells called X-zone, which are remains of fetal cortex.

The adrenal medulla is the catecholamine-secreting portion. It lies deep and forms the center of the gland.

Medulla is composed of groups and columns of chromaffinocytes separated by the sinusoidal blood capillaries. The chromaffin cells (chromaffinocytes) are modified neurons and synthesise and expel epinephrine (adrenalin) and norepinephrine (noradrenalin) into the capillaries.

There are two types of chromaffinocytes depending upon their secretions. Light colored ones called epinephrocytes, dark colored ones norepinephrocytes. The cytoplasm of the cell is filled with secretory granules of 100–500 nm in diameter. The granules are filled with protein-catecholamine. In noradrenalin containing cells, the vesicles are round or ellipsoid while in adrenalin containing cells, the vesicles are paler. Norepinephrocytes contain only large dense core vesicles and secrete norepinephrine. Epinephrocytes contain vesicles that are smaller, more homogenous, and less dense. These cells secrete epinephrine.

THYROID GLAND

The gland has a thin connective tissue capsule that extends and divides it into masses of irregular form and size. The structural-functional units of the gland are follicles which are spherical vesicles of varying size with a cavity in the centre. If the thyroid gland is highly active, the walls of the

follicles form many branched infoldings and the contour of the follicle become stellated. In the cavity colloid is present whose principle component is a large iodinated glycoprotein called thyroglobulin. The space between the follicles is filled by a stroma made up of delicate connective tissue containing numerous capillaries and lymphocapillar network. In these septums compactly arranged groups of epitheliocytes along with lymphocytes and labrocytes are found.

A thyroid follicle is a roughly spherical cyst-like compartment with a wall formed by a simple cuboidal or low columnar epithelium — ***follicular cells***. These *principal cells* are responsible for the production of thyroid hormones. The follicles contain a gel-like mass called *colloid.* The apical surfaces of the follicular cells form microvillae and are in contact with the colloid, and the basal surfaces rest on a typical basal lamina. Between neighboring cells well developed polydesmosomal contacts are formed and in mature follicles lateral interdigitated contacts among the cells of the follicles are found.

The cell shows well developed organelles especially those concerned with the protein synthesis: granular endoplasmic reticulum, Golgi apparatus, lysosomes, peroxisomes, mitochondria microtubules, microfilaments etc. The synthesis and release of thyroid hormone takes place in few phases.

1. *Production phase* starts with the absorption of amino acid tyrosine along with iodine and other mineral ions, carbohydrates and water through the basal lamina from blood. In the endoplasmic reticulum formation of glycoprotein thyroglobulin takes place which is paced into secretory vesicles along with carbohydrate components in the Golgi complex. The vacuoles travel to the luminal surface where they release thyroglobulin into follicular cavity by exocytosis. Iodine is absorbed from blood by the cells as iodide ions, but as tyrosine molecule can only join to atomic iodine, the iodide ion is converted into iodine (active form of iodide) atom by enzyme peroxydase. This process takes place on the apical surface of the cell. Here tyrosine residue joins with one atom of iodine to form *monoiodotyrosine*, and then with another to form *diiodothyrosine* which joins in pair to form *tetraiodotyronine (thyroxin)*. Along with thyroxin compound *triiodotyronine* forms which is an active form of thyroxin.

2. *Resorption phase* starts with resorption of colloid and takes place under different degrees depending upon the amount and continuity of the

activity of the gland. If activity is high, the cells become columnar, increase the number and size of their microvillae and develop pseudopodia on the surface. Follicular cells take up thyroglobulin from the colloid by a process of receptor-mediated endocytosis. Large endocytotic vesicles called colloidal resorption droplets are present at this stage in the apical region of the follicular cells. They gradually migrate to the basal surface of the cells, where they fuse with lysosomes. Thyroglobulin is then degraded by lysosomal proteases into constituent amino acids and carbohydrates tetra-, triiodotyronine, di- and monoiodotyrosine. If TSH levels remain high, the amount of colloid in the follicles is reduced because it is synthesized, secreted, iodinated, and resorbed too rapidly to accumulate.

3. *Release of tetraiodotyronine and triiodotyronine into the circulation and recycling process.* Tetra- and triiodotyronine cross the basal membrane and enter the blood and lymphatic capillaries. Most of the released hormones are immediately bound to either a specific plasma protein, *thyroxin-binding protein.* Only the follicular cells are capable of producing tetraiodotyronine, whereas most triiodotyronine, which is five times more active than tetraiodotyronine, is produced through conversion from tetraiodotyronine by organs such as the kidney, the liver, and the heart.

In humans, thyroid hormones are essential to normal growth and development. Both hormones regulate cell and tissue basal metabolism and heat production. In normal pregnancy both tetraiodotyronine and triiodotyronine cross the placental barrier and are critical in the early stages of brain development.

Parafollicular cells are called calcitoninocytes. These big cells are of irregular roundish or polyhedral shape, with oval eccentric nuclei. They lie between the follicular cells within their basement membrane. They may, however, lie between adjoining follicular cells, but do not reach the lumen. They may also be arranged in groups within the connective tissue septa. Parafollicular cells do not absorb iodine but synthesise neuroamines noradrenalin and serotonin by mean of decarboxylation of tyrosine and 5-hydroxytryptophan with formation of hormone called calcitonin and somatostatin. The cytoplasm is filled with acidophilic secretory granules. The cell shows well developed endoplasmic reticulum (granular), Golgi complex, mitochondria. Those which contain small but strongly osmiophilic granules release calcitonin. Those which contain large but weakly osmiophilic granules release somatostatin and calcitonin behaves as a physiologic antagonist

to PTH. Calcitonin lowers blood calcium levels by suppressing the resorptive action of osteoclasts and promotes calcium deposition in bones by increasing the rate of osteoid calcification. Secretion of calcitonin is regulated directly by blood calcium levels. High levels of calcium stimulate secretion, low levels inhibit it. Secretion of calcitonin is unaffected by the hypothalamus and pituitary gland.

PARATHYROID GLAND

Each parathyroid gland is surrounded by a thin connective tissue capsule that separates it from the thyroid. Septa extend from the capsule into the gland to divide it into poorly defined lobules and to separate densely packed cords of cells. The connective tissue is more evident in the adult, with the development of fat cells that increase with the age. Within the gland a network of reticular fibers supports specializing epithelial cells. Between neighboring epitheliocytes well developed desmosomal contacts and interdigitation are present. There are two types of cells:

Principal (chief cells), the more numerous of the parenchymal cells of the parathyroid, are responsible for parathormone (PTH) secretion. They are small, polygonal cells with centrally located nucleus. Cytoplasm contains densely collected ribosomes toward the periphery, that shows a very high activity state of the cell in protein synthesis. Golgi complex highly developed with flattened vesicles and cisternae, giving rise to secretory granules 150–200 nm. Mitochondria are abundant and have an elongated form with transversely arranged cristas. The chief cells are divided into *light and dark* cells depending upon their state of activity and glycogen content.

The light cells cytoplasm possesses lipofuscin-containing vesicles, large accumulation of glycogen, and lipid droplets. The light and dark cells are not the independent types of parathyrocytes but represent their age and functional stages.

Oxyphil cells are found in less number and appear only a little before puberty. Their cytoplasm is a distinctly acidophilic and is densely packed with mitochondria. True secretory granules are absent, and cells are not known to have secretory role. Cytoplasmic inclusion bodies consist of occasional lysosomes, lipid droplets, and glycogen distributed among the mitochondria.

Parathyroid glands are not under any regulation of hypophysis for its secretions but works on the basic of concentration of calcium in blood. It's activity increases under hypocalcification and decreases with hypercalcification. Parathyrocytes carry receptors which can directly measure the amount of calcium in the blood.

The functional significance of this gland lies in the regulation of calcium and phosphate levels metabolism. It produces PTH which causes the release of calcium from bones into the blood i.e. hyper calcification and demoralization of bones.

PTH functions at several sites:

— *bone resorption* is stimulated by PTH. The hormone activates osteolysis by osteoclasts during which calcium and phosphate are both released from calcified bone matrix into the extracellular fluid;

— *kidney excretion* of calcium is decreased by PTH stimulation of tubular reabsorption, thus conserving calcium;

— *urinary phosphate excretion* is increased by PTH secretion, thus lowering phosphate concentration in the blood and extracellular fluids;

— *kidney conversion* of vitamin D_3 to hormonally active form;

— *intestinal absorption of calcium* is increased under PTH influence. Vitamin D_3, however, has a greater effect than PTH on intestinal absorption of calcium.

The parathormone is essential for life. Care must be taken during thyroid ectomy 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 level falls.

Lecture 12

THE LYMPHATIC SYSTEM

The lymphatic system consists of groups of cells, tissues, and organs that monitor body surfaces and internal fluid compartments and react to the presence of potentially harmful substances. Lymphocytes are the definitive cell type of the lymphatic system and are the effector cells in the response of the immune system to harmful substances. Included in this system are the *diffuse lymphatic tissue, lymphatic nodules, lymph nodes, spleen, bone marrow, and thymus*. The various lymphatic organs and lymphatic tissues are often collectively referred to as the immune system.

Lymphatic tissues serve as sites where lymphocytes proliferate, differentiate, and mature. In addition, in the thymus, bone marrow, and *gut-associated lymphatic tissue (GALT)*, lymphocytes are "educated" to recognize and destroy specific antigens. These now ***immunocompetent cells*** can distinguish ***"self"*** molecules, normally present within an organism, and ***"nonself"***, foreign molecules.

All living tissues are subject to the constant threat of invasion by disease-producing foreign agents and microorganisms (pathogens): bacteria, viruses, fungi, protozoa and multicellular parasites, which may enter via breaches in the skin or linings of the gut, respiratory and urogenital tracts. Three main lines of defense have consequently involved:

— protective surface mechanisms;
— non-specific tissue defense;
— specific immune responses.

Protective surface mechanisms. In humans, these provide the first line of defense. The skin constitutes a relatively impenetrable barrier to most

micro-organisms unless breached by injury such as abrasion or burning. The serous-mucous surfaces of the body, such as the conjunctiva and oral cavity, are protected by a variety of antibacterial substances including the enzyme lysozyme, secreted in tears, and saliva. The respiratory tract is protected by a layer of surface mucus. The maintenance of an acidic environment in the stomach, vagina and to a lesser extent the skin, inhibits the growth of pathogens in these sites. When such defenses fail, the two other main types of defense mechanisms are activated.

Non-specific tissue defenses consist of physical barriers. Damage to tissues usually excites a non-specific response called inflammation (which may be acute or chronic) aimed at removal of any dead tissue and foreign matter, replacement of lost tissue by scar and in some cases regeneration of normal tissue. Acute inflammation is characterized by vascular changes including capillary dilatation, enhanced permeability and increased blood flow resulting in the production of fibrin-rich inflammatory exudates. Neutrophils and later macrophages migrate into the damaged tissues, their phagocytic activity removing tissue debris, deleterious foreign material and pathogens. Phagocytosis is often greatly enhanced by involvement of immunological mechanisms. Destroyed tissue is initially replaced by proliferation of capillary-rich granulation tissue which is then slowly replaced by fibroblast proliferation and collagen deposition with the eventual formation of a relatively avascular and acellular fibrous scar. Some tissues, such as the liver, the skin and the epithelial linings are able to regenerate to greater or lesser degree. If the tissue damage is minimal, phagocytic activity may be accompanied by mild vascular, exudative and reparative response or occur without any response at all. Complement is a series of plasma proteins constituting an enzyme cascade analogous to the clotting system. The cascade can be activated via either the classical pathway (usually involving antigen-antibody complexes, or the alternative pathway (direct activation by certain organisms).The various peptides and proteins so generated mediate a diverse range of processes including vascular changes (dilatation and increased permeability), cell lysis, opsonization (coating of organisms with complement which enhances phagocytosis by leukocytes), leukocytes attraction (chemotaxis) and activation. The alternative pathway probably envolved first as a non-specific defense system with the classical pathway envolving later as an effector mechanism linked to the immune response.

If the nonspecific responses fail, the immune system provides ***specific immune responses*** that target individual invades. The highly specific immune system depends upon the recognition of exogenous materials as being foreign to the body, any particular foreign substance so recognized being known as an antigen. Some normal body components may also act as antigens in autoimmune reactions. This results in the activation of the immune system with the purpose of neutralizing or destroying the antigen. Lymphocytes play the central role in the immune response. The process is highly antigen-specific but usually depends on phagocytic cells of the monocyte-macrophage system in presentation of antigen to lymphocytes. Components of the non-specific defense system (i.e. complement, neutrophils and macrophages) are frequently employed in final destruction of antigen. As any one micro-organism is usually made up of many different antigens, the immunological response may involve a combination of responses. The cells of the immune system, particularly lymphocytes, are scattered throughout the body either as isolated cells, as non-encapsulated aggregations in the gastrointestinal, respiratory and other tracts (mucosa associated lymphoid tissue or MALT) or within the lymphoid organs namely the thymus, lymph nodes and spleen.

The immune system is traditionally divided into two branches namely cell-mediated immunity and humoral immunity corresponding to two types of lymphocytes identified by early immunological research. ***B-lymphocytes*** have variable lifespan and are involved in the production and secretion of the various circulating *antibodies*, also called *immunoglobulins*, the immune proteins associated with ***humoral immunity*** that act directly on an invading agent. Each B-lymphocyte reacts only with a single antigen or type of antigenic site that it has been genetically programmed to recognize. The reaction of a B-lymphocyte with antigen complex activates the cell. Activated B-lymphocytes are transformed into plasmoblasts that proliferate and then differentiate into:

— ***plasma cells***, which synthesize and secrete a specific antibody;

— ***memmory B-cells***, which respond more quickly to the next encounter with the same antigen.

In ***cell-mediated immunity***, ***T-lymphocytes*** function as cytotoxic cells directly killing abnormal body cells. T-lympocytes attack and destroy virus infected host cells or foreign cells. Cell-mediated immunity is important in

the defense against viral, fungal, and mycobacterial infections, as well as tumor cells. Cell-mediated immunity is also responsible for transplant rejection. Lymphocyte subtypes such as T-helper and T-suppressor cells control B- and T-cell responses. T-lymphocytes have a long lifespan. Antigen presenting cells (APC) of various types including macrophages and β-lymphocytes control the activation of the T- cells. ***NK-cells (natural killer)*** develop from the same precursor cell as B- and T-cells and are named for their ability to kill certain types of transformed cells. During their development, they are genetically programmed to recognize transformed cells. Following this recognition, they release ***perforins and fragmentins***, substances that create channels in the cell's plasma membrane and cytoplasm, which induces them to self-destruction (a process known as apoptosis) and lysis.

BONE MARROW

The bone marrow is the central hemopoietic organ and a human adult organism contains both red and yellow bone marrow.

Red bone marrow. The red bone marrow is normally restricted to the space of spongy bones and in epiphysis of long bones. It has dark red color and semi-fluid consistence. The red bone marrow consists of developing blood cells in different stages of development and a network of reticular cells and fibers that serve as a supporting framework for the developing blood cells and vessels. As an individual grows, the amount of red bone marrow does not increase in proportion to bone growth. In later stages of growth and in the adult, when the rate of blood cells formation has diminished, the tissue in the marrow cavity consists mostly of fat cells; it is then called *yellow marrow.*

The bone marrow consists of blood vessels, specialized units of blood vessels called *sinuses*, and a sponge-like network of hemopoietic cells. In sections, the hemopoietic cells appear to lie in "cords" between sinuses or between sinuses and bone. The sinus wall consists of endothelial lining (simple squamous epithelium), a basal lamina, and an outer adventitial cells layer. The adventitial cell, also called a reticular cell, sends sheet-like extensions into the substance of the hemopoietic cords, which provide some support for the developing blood cells. In addition, adventitial cells produce reticular fibers. They also play a role in stimulating the differentiation of

developing progenitor cells into blood cells. When blood cells formation and the passage of mature blood cells into the sinuses are active, adventitial cells and the basal lamina become displaced by mature blood cells as they approach the endothelium to enter the sinus from the bone marrow cavity and form aperture. Each blood cell must squeeze through such aperture to enter the lumen of a sinus.

In active red bone marrow, the cords of hemopoietc cells contain predominantly developing blood cells and megakaryocytes. The cords also contain macrophages, mast cells, and some adipose cells. Although the cords of hemopoietic tissue appear to be unorganized, specific types of blood cells develop in "nests" or hemopoietic islands.

It is known that maturing erythrocytes accumulate hemoglobin composed of *heme* (iron-bearing part) and *globin*. In the bone marrow erythroblasts gather around macrophage that had accumulated iron. Glycoproteins take up position around differentiating erythrocytes, the volume of these glycoproteins decreases during erythrocytes maturation. As a result of iron and protein accumulation amount of hemoglobin increases in cytoplasm of erythroblasts therefore the color changes of cytoplasm from the basophilic to acidophilic occurs. Erythrocytes are mature, their mobility rate and the level of penetration into circulation increases.

The sets of myeloblasts are situated farther from the sinusoid wall and surrounded by proteoglycans that promote formation and accumulation of the specific granules in cytoplasm of maturing granulocytes. Histochemical composition of granules determines the further differentiation of granulocytes to eosinophils, basophils, and neutrophils. When mature, the granulocytes migrate to the sinus and enter the bloodstream.

Megakaryocytes are located adjacent to the sinus wall, and they discharge their platelets directly into the sinus through apertures in the endothelium.

Yellow bone marrow. Inactive bone marrow is called the yellow bone marrow. It is the chief form of the bone marrow in the diaphysis of long bone. About half of the bone marrow space is occupied by adipose tissue and half by hemopoietic tissue. Cytoplasm of adipocytes contains pigment *lipochrome* that gives yellow color. The yellow bone marrow retains its hemopoietic potential, however, and when necessary, as after severe loss of blood, it can revert to red bone marrow, both by extension of the hemopoietic tissue into the yellow bone marrow and by repopulation of the yellow bone marrow by circulating stem cells.

THYMUS

The thymus is a large flattened lymphoid organ located in the upper anterior mediastinum and lower part of the neck. The thymus is most active during childhood after which it undergoes slow involution. In the embryo, the thymus originates from epithelial outgrowth of the ventral wing of the third pharyngeal pouch on each side. These merge in the midline forming a single organ comprising two lobes subdivided into numerous fine lobules. The epithelium develops into a sponge-like structure containing a labyrinth of interconnecting spaces which become colonized by lymphocytes derived from haemopoietic tissue elsewhere in the developing embryo. Towards the centre of the lobule, the epithelial framework has a coarser structure with smaller interstices and a much smaller lymphocyte population so that, on microscopic examination, the gland lobule has a highly cellular outer cortex and a less cellular central medulla which is continuous throughout the gland.

The epithelial cells of the thymus not only provide a mechanical supporting framework for the lymphocyte population but in the cortex tend to envelop the lymphocytes performing some kind of "nurse" function promoting T-cell differentiation, proliferation and maturation. Furthermore, the epithelial cells secrete at least three different hormones and a variety of other humor factors which regulate T-cell maturation and proliferation within the thymus and in other lymphoid organs and tissues.

The prime functions of the thymus include:

— development of immunocompetent T-lymphocytes from lymphocytes derived from bone marrow. This involves differentiation of the two T-cell subsets: T-helper cells and T-cytotoxic/suppressor cells;

— proliferation of premature T-cells in order to supply the circulating lymphocyte pool in peripheral tissues;

— development of immunological self tolerance;

— secretion of hormones and other soluble factors which regulate T-cell maturation, proliferation and function within the thymus and peripheral tissues. Thymic hormones also regulate the development of peripheral lymphoid organs and tissues. There are at least three polypeptides with hormonal characteristics namely thymulin, thymopoetin and thymosin alpha 1;

— hemopoiesis is an important function of the thymus during fetal development.

The thymus possesses a thin connective tissue capsule from which trabeculae extend into the parenchyma of the organ. The capsule and trabeculae contain blood vessels, lymphatic vessels, and nerves. The trabeculae establish domains in the thymus called thymic lobules. The inner surface of the thymic capsule and trabeculae is invested by a continuous layer of thymic epithelial cells resting on a basement membrane.

The outer portion of the lobule, the ***thymic cortex***, is markedly basophilic because of the closely packed developing T-lymphocytes with their intensely staining nuclei. These T-lymphocytes occupy spaces within an extensive meshwork of *epithelioreticular cells*. Macrophages are also dispersed among the cortical cells.

Epithelioreticular cells have features of both epithelial and reticular cells. They provide a framework for the developing T-cells; thus, they correspond to the reticular cells and their associated reticular fibers in other lymphatic tissues and organs. Reticular connective tissue cells and their fibers, however, are not present in the thymic parenchyma. Epithelioreticular cells exhibit certain features characteristics of epithelium, such as intercellular junctions and intermediate filaments. At the ultrastructural level, the epithelial cells are found to have typical desmosomes at their points of contract and the cytoplasm contains bundles of intermediate filaments of the protein keratin.

The epithelial framework of the medulla is relatively coarse and bulky, the interstices being much smaller than those of the cortex and therefore accommodating fewer lymphocytes.

Six types of epithelioreticular cells are recognized on the basis of function: three types in the cortex and three types in the medulla.

Type I epithelioreticular cells are located at the boundary of the cortex and the connective tissue capsule. They also surround the adventitia of the cortical blood vessels. These types of cells serve to separate the thymic parenchyma from the connective tissue of the organ and isolate developing T-cells from the connective tissue of the organ.

Type II epithelioreticular cells are located within the cortex. Type II cells compartmentalize the cortex into isolated areas for the developing T-cells and involved in thymic cells education.

Type III epithelioreticular cells are located at the boundary of the cortex and medulla. Like type I cells, type III epithelioreticular cells create a

functional barrier, in this case between the cortex and medulla. Like type II cells, they involved in thymic cells education.

Macrophages reside within the thymic cortex and are responsible for phagocytosis of T-cells that do not fulfill thymic educational requirements. These T-cells are programmed to die before leaving the cortex.

While the epithelioreticular cells of the thymic cortex play an important role in the development of immunocompetent T-cells, recent evidence shows that T-cells at the different stages of differentiation control the microarchitecture of the epithelioreticular cells. The developing lymphocytes and epithelioreticular cells thus influence each other during T-cells development.

The ***thymic medulla***, the inner portion of the parenchyma, contains a large number of epithelioreticular cells and loosely packed T-cells. The medulla stains less intensely than the cortex because it contains mostly large lymphocytes. Like the cortex, the medulla also contains three types of epithelioreticular cells.

Type IV epithelioreticular cells are located between the cortex and the medulla close to type III cells. In cooperation with type III cells they create the barrier at the corticomedullary junction.

Type V epithelioreticular cells are located throughout the medulla. They provide the cellular framework of the medulla and compartmentalize groups of lymphocytes.

Type VI epithelioreticular cells form the most characteristic feature of the thymic medulla, the ***Hassall's corpuscles***. Thymic corpuscles are isolated masses of closely packed, concentrically arranged type VI epithelioreticular cells that exhibit flattened nuclei. Cytoplasm of these cells contains keratohyalin granules, bundles of cytoplasmic intermediate filaments, and lipid droplets. The cells are joined by desmosomes. Thymic corpuscles are unique, antigenically distinct, and functionally active multicellular components of the medulla. Although the function of thymic corpuscles is not fully understood, histochemical studies show that they produce thymic hormones (thymosin and thymopoietin). Hassal's corpuscles first appear in fetal life and increase in number there after. Some authorities maintain that macrophages filled with debris from degenerate cortical lymphocytes become incorporated in the Hassal's corpuscles and the number and size of the corpuscles increase at times of intense lymphocytes destruction. Although the thymus is usually considered a specially T-cell organ, occasional mature B-

lymphocytes, B-cell germinal centers and rarely plasma cells can be found in the thymus particularly in children.

Lymphocytes reaching the thymic cortex are prevented from contact with antigen by a physical barrier called the ***blood-thymus barrier***. The components that constitute the blood-thymus barrier between the T-cells and the lumen of cortical blood vessels from the lumen outward, are:

Lining ***endothelium of the capillary wall***. It is highly impermeable to macromolecules. The underlying ***basal lamina*** of endothelial cells and occasional ***pericytes*** are also part of the capillary wall.

Macrophages in the surrounding perivascular connective tissue. Antigenic molecules that escape from the capillary lumen into the cortical parenchyma may be phagocytosed by macrophages residing in this tissue.

Type I epithelioreticular cells with their ***basal lamina*** provide further protection to the developing T-cells.

During fetal life, the thymus is populated by multipotential lymphoid stem cells that originate from the bone marrow. Stem cell maturation and differentiation into immunocompetent T-cells is called ***thymic cell education***. This process is characterized by the expression and deletion of specific surface CD (clusters of differentiation) antigens. The cortical T-lymphocytes are presented with self and foreign antigens by type II and III epithelioreticular cells. If the lymphocytes recognize self identification molecules (MHC, histocompatibility) and self and foreign antigen, it will survive ***(positive selection)***. If not, the cell will die. Cells that pass the positive selection test leave the cortex and enter the medulla. Here, they undergo another selection process in which cells that recognize "self" antigen displayed by self molecules of histological compatibility are eliminated ***(negative selection)***. The cells that survive become either cytotoxic T-lymphocytes or helper T-lymphocytes. This stage is called the ***single-positive stage***. Now the cells leave the thymus by passing from the medulla into the blood circulation. The process of thymic cell education is promoted by substances secreted by epithelioreticular cells, including interleukins, colony stimulating factors, interferon, thymosin, and thymopoietin.

Involution of the thymus. The thymus continues to grow from birth until puberty but after this it slowly involutes. Involution involves two distinct processes: fatty infiltration and lymphocyte depletion. Adipocytes first begin to appear at birth, their numbers slowly rising until puberty when the rate of fatty infiltration increases markedly. The process involves the sup-

porting tissue component of the organ which is mainly located around the blood vessels. Consequently, fatty infiltration of the interlobular septa occurs first, spreading out into the cortex and later the medulla. Lymphocyte numbers begin to fall from about one year of age, the process continuing thereafter at a constant rate. Despite this, the thymus continues to provide a supply of mature T-lymphocytes to the circulating pool and peripheral tissues. Lymphocyte depletion results in collapse of the epithelial framework. However, cords of epithelial cells persist and continue to secrete thymic hormones throughout life. The normal process of slow thymic involution associated with the age is called the ***age involution***. It should be distinguished from acute thymic involution which may occur in response to severe disease and metabolic stress associated with pregnancy, lactation, infection, surgery, malignancy and other systemic insults. ***Stress or accidental involution*** is characterized by greatly increased lymphocyte death and is probably mediated by high levels of corticosteroids. The borders between thymic cortex and medulla disappear. Thus the size and activity of the adult thymus is often underestimated if examined after a prolonged disease.

LYMPH NODES

Mature lymphocytes are distributed throughout the body where they are arranged in aggregations which exhibit various degrees of structural organization. Isolated lymphocytes are found in most loose supporting tissues and among epithelial cells, particularly the epithelium of the gastrointestinal and respiratory tracts; in addition, large non-encapsulated aggregations of lymphocytes are found in the walls of these tracts. Most lymphocytes are, however, located in encapsulated, highly organized structures called lymph nodes, which are interposed along the larger regional vessels of the lymph vascular system. Four interrelated functions occur within the lymph nodes:

1. Non-specific filtration of particulate matter and microorganisms from lymph by the phagocytic activity of macrophages, thus preventing exogenous material from reaching the general circulation.

2. Interaction of circulating lymphocytes with antigen-containing lymph as it is filtered through the narrow confines of the node; initiation of an immune response may require the involvement of antigen presenting cells.

3. Aggregation, activation and proliferation of B-lymphocytes in response to appropriate antigenic stimulation, this leads to plasma cell formation and antibody production.

4. Aggregation, activation and proliferation of T-lymphocytes with induction of cytotoxic immune responses after appropriate antigenic stimulation.

The lymph nodes are small bean-shaped organs situated in the course of the regional lymphatic vessels so that lymph draining back to the bloodstream passes through one or more lymph nodes. Two types of lymphatic vessels serve the lymph node:

— *afferent lymphatic vessels* convey lymph toward the node and enter it at various points on the convex surface of the capsule;

— *efferent lymphatic vessels* convey lymph away from the node and leave at the hilum, a depression on the concave surface of the node that also serves as the entrance and exit for blood vessels and nerves.

Relatively inactive nodes are only a few millimeters long, increasing greatly in size when mounting an active immunological response. The outer part of the lymph node is highly cellular and is known as the cortex while the central area is less cellular and is known as the medulla. The lymph node is encapsulated by dense connective tissue passed trabeculae which extend from the capsule into the substance of the node, forming a gross framework. The parenchyma of the lymph node is divided into the *cortex* and the *medulla.* The cortex forms the outer portion of the node except at the hilum. It consists of a dense mass of lymphatic tissue which forms *cortical zone* or *lymphatic nodules* and *paracortical zone* situated at the border of cortex and medulla. The medulla of the lymph node includes *sinuses* and *medullary cords.* Reticular tissue composed of reticular cells and reticular fibers forms a fine supporting meshwork fine or delicate strome. Afferent lymphatic vessels divide into several branches outside the lymph node, pierce the capsule to drain into a narrow space called the ***subscapu1ar sinus*** extending between capsule and convex surface of the lymphatic nodules. From here, a labyrinth of channels called sinuses passes towards the medulla.

Trabecular sinuses that originate from the subcapsular sinuses extend through the cortex along the trabeculae (between lymphatic nodules and trabeculae) and drain into the ***medullary sinuses*** that are located between a medullary cords or medullary cord and trabeculae. The medullary sinuses converge upon the hilum in the concavity of the node. Lymph drains from

the hilum into one or more efferent lymphatic vessels. Lymphatic sinuses are not open spaces. Particularly in the medulla, macrophage processes, along the reticular fibers surrounded by reticular cell processes, form a meshwork that retards the free flow of lymph and enhances its filtration. Antigenic material is trapped by this mechanical filter and then phagocytosed by macrophages.

The lymphocytes of the superficial cortex are mainly arranged in spherical lymphoid follicles and these are the major sites in which B-lymphocytes localize and proliferate. Traditionally, lymphoid follicles have been classified as "primary follicles" if a central pale area is absent and "secondary follicles" if such an area is present. The pale central areas are the sites of B-lymphocyte proliferation and are thus termed germinal centers.

The deep cortical zone or paracortex consists mainly of T-lymphocytes which are never arranged as follicles. The medullary cords mainly contain B-lymphocytes and plasma cells involved in immunoglobulin synthesis. The number of cortical lymphoid follicles and the depth of the paracortex vary greatly according to the immunological state of the particular lymph node and the individual as a whole. A predominantly cytotoxic immunological response is associated with paracortical thickening whereas a predominantly humoral response is shown by the appearance of many cortical follicles with pale germinal centres.

Lymphoid follicle and germinal centre. Drawing a lymphoid follicle with a less intensely stained germinal center, the more intensely stained periphery is described as the lymphocyte corona. B-lymphocytes are the predominant cell type in the lymph node follicles along with a small number of helper T-lymphocytes (mainly located in the corona) and various types of immunological accessory cells. The B-lymphocytes in the superficial cortex are mainly natural B-cells and B-memory cells. B-cell activation within the follicles results in blast transformation, the cells are increasing in cytoplasmic RNA content prior to cell division. The center of the follicle is less intensely stained than the corona because the cells are not only much larger but also less closely packed together. The product of follicular B-lymphocyte activation and proliferation is an expanded population of B-memory cells and not the formation of plasma cells. It was formerly thought that the germinal centers were the site of proliferation and differentiation of antibody-secreting plasma cells but this is now believed to occur as a result of T- and B-lymphocytes interaction in the paracortex with the

antibody-secreting cells migrating directly to the medullary cords where they are conveniently situated for secretion of antibody into the efferent lymph. At the periphery of the germinal centers is a capsule-like structure of flattened stromal cells called germinal centre bordering cells open at its superficial aspect. This appears to prevent the entry of circulating T-cells to the germinal center. Immunological accessory cells of various types are found in the superficial cortex, all appearing to be involved in antigen processing.

The reticular meshwork of lymphatic tissues and organs (except the thymus) consists of cells of mesenhymal origin and reticular fibers and ground substance produced by those cells. The cells of the reticular meshwork appear as stellate or elongated cells with an oval euchromatic nucleus and a small amount of acidophilic cytoplasm. These cells can take up dyes and colloidal materials. Electron microscopy, immunocytochemistry, and autoradiography indicate few populations of the following cells:

Reticular cells synthesize and secrete type III collagen (reticular fibers) and the associated ground substance that forms the supporting delicate stroma. Elongated cytoplasmic processes of these cells wrap around the bundles of reticular fibers, effectively isolating these structural components from the parenchyma of the lymphatic tissue and organs.

Follicular dendritic cells have multiple, thin hair-like branching cytoplasmic processes that interdigitate between B-lymphocytes in the germinal centers. The antigen-antibody complexes adhere to the dendritic cytoplasmic processes by means of the antibody's receptors, and the cells can retain antigen on its surface for weeks, months or years. Although this mechanism is similar to the adhesion of antigen-antibody complexes to macrophages, the antigen is not generally endocytosed, as it is by the macrophages. *Macrophages* may reside in the lymphatic parenchyma, but often send pseudopods into sinuses through endothelial discontinuities (coastal microphages) and monitor the lymph as it percolates through the sinuses. They are less efficient in antigen-presentation, but instead have an immense capacity for endocytosis and digestion of internalized materials.

Natural killer cells have an ability to kill target cells without previous sensitization, are found in small numbers in the follicles and in appearance resemble large lymphocytes. They appear to play a role in surveillance for tumor and virus-infected cells.

Paracortical zone. T-lymphocytes are the main cell type in the paracortical zone. Circulating T-lymphocytes of both helper and cytotoxic (sup-

pressor) enter the lymph node in the arterial blood then migrate through the endothelial walls of venules into the paracortical zone.

The T-lymphocytes of the paracortical zone are surrounded by interdigitating dendritic macrophages. *Dendritic cells* are unique bone marrow-derived antigen-presenting cells (APCs). They are much more efficient in antigen presentation than other APCs expressing an exceptionally high level of MHC and costimulatory molecules necessary fpr activation of T-cells monitoring the local environment for foreign substances that they then process and present to antigen specific T-cells.

Dendritic cells produce glycoproteins that impact the humoral regulation of lymphocytogenesis. The glycoproteins of the paramembranic layer are able to absorb and preserve the antigen on their cytoplasmic membranes. They induce the proliferation of T-lymphocytes. The proliferation of T-cells, blast-transformation and the differentiation of T-killers occurs in the paracortical zone. T- and B-lymphocytes enter the lymph node through the postcapillar venulae.

Medullary cords. The medullary cords largely contain plasma cells and their precursors although a few macrophages and T- lymphocytes may also be present. As in the cortex, the cells of the medullary cords are supported by a reticulin framework. The dominant cell population of the medullary cords is plasmocytes and their smaller precursors, the plasmoblasts. B-lymphocyte activation, proliferation and maturation of antibody-secreting cells appear to occur in the paracortex rather than in the superficial cortical follicles. Plasmocytes synthesize antibodies which are carried from the node to the general circulation via efferent lymphatic vessels. Some mature plasma cells probably also migrate from the node likewise.

SPLEEN

The spleen is a large lymphoid organ situated in the left upper part of the abdomen. It receives a rich blood supply via a single artery, the splenic artery, and is drained by the splenic vein into the hepatic portal system.

Because the spleen filters blood as the lymph nodes filter lymph, it functions in both the immune and hemopoietic systems.

Immune system functions of the spleen include:

— antigen presentation and initiation of immune response;

— activation and proliferation of B- and T-lymphocytes;

— production of antibodies against antigen present in circulating blood;

— removal of macromolecular antigens from the blood;

— proliferation of lymphocytes and differentiation of B-lymphocytes and plasma cells, as well as secretion of antibodies, occur in the white pulp of the spleen; in this regard, the white pulp is the equivalent of other lymphatic organs.

Hemopoietic functions of the spleen include:

— removal and destruction of senescent, damaged, and abnormal erythrocytes and platelets;

— retrieval of iron from erythrocyte hemoglobin;

— formation of erythrocytes during early fetal life;

— storage of blood, especially red blood cells, in some species.

The spleen has both morphologic and immunologic filtering functions. In addition to large numbers of lymphocytes, it contains specialized vascular spaces or channels, a meshwork of reticular cells and reticular fibers, and a rich supply of macrophages.

On macroscopic examination of the cut surface, the spleen appears to consist of discrete white nodules, a so-called ***white pulp***, embedded in the red matrix called the ***red pulp***. White pulp appears as circular or elongated whitish gray areas.

The spleen has a thin but dense fibroelastic outer capsule covered by mesothelium from which short trabeculae extend into the parenchyma of the organ. The capsule is thickened at the hilum and is continuous with supporting tissue which unsheathes the larger blood vessels entering and leaving the organ. The connective tissue of the capsule and trabeculae contains myofibroblasts. These controctile cells also produce extracellular connective tissue fibers. The human spleen normally retains relatively little blood, but it has the capacity for construction by means of contractile cells in the capsule and trabeculae.

The hilum, located on the medial surface of the spleen, is the site for the passage of the splenic artery and vein, nerves, and lymphatic vessels. Branches of the splenic artery enter the trabeculae as the trabecular arteries and then enter the pulp as pulpar arteries. Within the white pulp, the branch of the splenic artery is called the *central artery*. The central artery sends branches to the white pulp itself and to the sinuses at the perimeter of white pulp, called *marginal sinuses*. The central artery continues into the red pulp,

where it branches into several relatively straight arterioles called *penicillar arterioles*. The penicillar arterioles then continue as arterial capillaries. Some arterial capillaries are surrounded by aggregations of macrophages and are thus called *sheathed capillaries*. Sheathed capillaries then empty directly into the reticular meshwork of the splenic cords rather than connecting to the endothelium-lined splenic sinuses. Blood entering the red pulp in this manner percolates through the cords and is exposed to the macrophages of the cords before returning to the circulation by squeezing through the wall of the splenic sinuses. This type of circulation is known as *open circulation*. Other way, some of the blood from the sheathed capillaries passes directly to the splenic sinuses of the red pulp. This type of circulation is known as *closed circulation*.

Open circulation exposes the blood more efficiently to the macrophages of the red pulp. The blood collected in the sinuses drains to tributaries of the trabecular veins that converge into larger veins and eventually leaves the spleen by the splenic vein.

The lymphatic vessels originate in the white pulp near the trabeculae and constitute a route for lymphocytes leaving the spleen. The white pulp consists of lymphatic tissue, mostly lymphocytes. Lymphocytes that aggregate around the central artery constitute the ***periarterial lymphatic sheath*** (PALS) or ***periarterial zone***. In cross section it appears circular and may resemble a lymphatic nodule, but the presence of the central artery distinguishes the PALS from typical lymphatic nodules found in other sites. The periarterial zone is chiefly territory of T-lymphocytes. This zone may be considered a thymus-depended one similar to the paracortical zone of the lymph node. Nodules appear as localized expansions of the PALS and displace the central artery so that it occupies an eccentric position. The nodules usually contain ***germinal centers***, which, as in other lymphatic tissues, develop as B-cells proliferate following their activation. In humans, germinal centers develop within 24 hrs after antigen exposure and may become extremely large and visible with the naked eye. These enlarged nodules are called *splenic nodules* or Malpighian corpuscles.

At the follicle periphery is a narrow zone of small lymphocytes called the ***mantle zone*** (corona) beyond which is a broader ***marginal zone*** of less densely packed larger lymphocytes supported by a framework of reticulum fibers. Mantle zone consists of densely packed small B-lymphocytes and little amount of T-lymphocytes, as well as plasmocytes and macrophages.

Marginal zone is transitional area between the white and red pulp. It consists of predominantly T- and B-lymphocytes and single macrophages surrounded by marginal sinusoid capillaries.

Periarterial lymphatic vagina is accumulation of B-lymphocytes and plasmocytes along the penicillar arteriole of spleen. Small T-lymphocytes occupy periphery of the vagina.

Splenic red pulp. Red pulp has a red appearance in the fresh state because it contains large numbers of red blood cells. The role of the red pulp is primarily blood filtration, i.e., removal of particulate material, macromolecular antigens, and aged, abnormal, or damaged blood cells and platelets from the circulating blood. Essentially, red pulp consists of *splenic sinuses* separated by *splenic cords*. The splenic cords consist of the loose meshwork of reticular cells and reticular fibers that contains large numbers of erythrocytes, macrophages, lymphocytes, plasma cells, and granulocytes. Splenic macrophages phagocytose damaged red blood cells. The spleen has an ability to decrease the stability rate of the old or damaged erythrocytes. Such erythrocytes are phagocyted by red pulp macrophages. The hemoglobin of phagocyted erythrocytes is destroyed; bilirubin and iron-containing transferin are excreted to circulation. The iron from destroyed red blood cells is transported to red bone marrow used in the formation of new red blood cells. Transferin is captured by bone marrow macrophages which provide growing erythrocytes with iron. Bilirubin is transported to the liver and becomes a component of bile.

Spleen is a depot of blood, it also accumulates platelets. Old platelets are destroyed here.

Mucosa-associated lymphoid tissue (MALT). Lymphoid tissue is distributed throughout the gastrointestinal tract either as a diffuse lymphocytic infiltrate or as large discrete non-encapsulated aggregations such as the tonsils and intestinal Peyer's patches. In the large aggregations, follicles may form with germinal centers similar to those of lymph nodes. Smaller follicular aggregations and diffuse lymphocytic infiltrates are also seen in the tracheo-bronchial tree and urogenital tract. The total mass of lymphoid tissue in the gastrointestinal and respiratory tracts is enormous. Lymphocytes and other free cells of this tissue are found in the lamina propria (subepithelial tissue) of these tracts. This mass of lymphoid tissue is now considered to be a lymphoid organ in its own right and is collectively known as mucosa-associated lymphoid tissue

(MALT). These cells are strategically located to intercept antigens and initiate an immune response. Following contact with antigen, they travel to regional lymph nodes, where they undergo proliferation and differentiation. Progeny of these cells then return to the lamina propria as effector of B- and T-lymphocytes.

The importance of diffuse lymphatic tissue in protecting the body from antigens is indicated by:

— the regular presence of large number of plasma cells, especially in the lamina propria of the gastrointestinal tract, a morphological indication of local antibody secretion;

— the presence of large numbers of eosinophils, also frequently observed in the lamina propria of the intestinal and respiratory tracts, an indication of chronic inflammation and hypersensitivity reactions.

The larger aggregations function in a manner analogous to lymph nodes, sampling antigenic material entering the discrete B- and T-cell zones as well as antigen-processing accessory cells. The diffusely scattered lymphocytes seen in the lamina propria of the gut and respiratory tree are mainly B-lymphocytes some of which mature into antibody-secreting plasma cells. These cells concentration, called ***lymphatic nodules*** or ***lymphatic follicles***, are sharply defined but not encapsulated. A lymphatic nodule consist chiefly of small lymphocytes and is called a *primary nodule*. A *germinal center* located in the central region of the nodule appears lightly stained. The germinal center develops when a lymphocyte that has recognized an antigen returns to a primary nodule and undergoes proliferation. The lighter staining is due to the large lymphocytes (lymphoblasts and plasmoblasts) that it contains. The presence of germinal center represents a cascade of events that includes proliferation of lymphocytes, differentiation of plasma cells, and antibody production. The number of macrophages in the germinal center often increases following a period of intense response to an antigen.

Considerable numbers of lymphocytes are also found within the epithelium of the small and large intestines and are present in particularly large numbers in the epithelium overlying Peyer's patches. These lymphocytes are almost exclusively T-cells and the majority are of the suppressor/cytotoxic subset with most of the rest being natural killer cells.

The epithelium overlying all MALT aggregations is specialized for the sampling of luminal contents for antigen and acts as the equivalent of the

lymph node. The amount of MALT is maximal during childhood undergoing progressive atrophy in adulthood. The whole MALT organ probably acts as an integrated unit. Then antigen is encountered, it is carried to local MALT tissue or regional lymph nodes where it evokes the relevant immunological response. Lymphocytes, antibodies and probably even plasma cells then pass via the general circulation to the gastrointestinal and respiratory mucosa where lymphocytes and plasma cells await antigenic challenge. IgA is secreted either by diffusing directly through the lining epithelium or in specific secretions such as saliva, tears and in milk during lactation.

The lymphoid aggregates of the respiratory tract are similar though generally smaller than those of the gut and are covered by similar antigen sampling and transport cells. As in the gut there is no afferent lymphatic but efferent lymphatic drain lymph to the regional nodes and activated lymphocytes derived from the respiratory tract aggregations have a predilection for specifically homing respiratory mucosa.

Lecture 13

THE CARDIOVASCULAR SYSTEM

The cardiovascular system is a transport system that carries blood and lymph to and from the tissues of the body. The constitutive elements of these fluids include cells, nutrients, waste products, hormones, and antibodies. The cardiovascular system includes the heart, blood vessels and lymphatic vessels.

The circulatory system mediates the continuous movement of all body fluids, its principal functions being the transport of oxygen and nutrients to the tissues and transport of carbon dioxide and other metabolic waste products from the tissues. The circulatory system is also involved in temperature regulation and the distribution of molecules such as hormones, and cells such as those of the immune system the circulatory system has two functional components: the blood vascular system and the lymph vascular system. Blood vessels provide the route by which blood circulates to and from all parts of the body. The heart pumps the blood through the arterial system under significant pressure; blood is returned to the heart under low pressure with the assistance of negative pressure in the thoracic cavity during inspiration and compression of the veins by skeletal muscle. The blood vessels are arranged so that blood delivered from the heart quickly reaches a network of narrow, thin-walled vessels, the blood capillaries, within or proximity to the tissues in every part of the body.

In the capillaries, a two-directional exchange of fluid occurs between the blood and tissues. The fluid, called blood filtrate, carrying oxygen and metabolites, passes through the capillary wall. In the tissues, these molecules are exchanged for carbon dioxide and waste products. Most of the fluid reenters the distal or venous end of the blood capillaries. The remaining

fluid enters lymphatic capillaries as lymph and is ultimately returned to the bloodstream through the system of lymphatic vessels that join the blood system at the junction of the internal jugular veins with the subclavian veins. Normally, many of the white blood cells conveyed in the blood leave the blood vessels to enter the tissues. This occurs at the level of postcapillary venules.

Arteries are the vessels that deliver blood to the capillaries. The smallest arteries, called arterioles, are functionally associated with the networks of capillaries into which they deliver blood. The arterioles regulate the amount of blood that enters these capillary networks. Together, the arterioles, associated capillary network, and postcapillary venules form a functional unit called the microcirculatory or microvascular bed of that tissue. Veins, beginning with the postcapillary venule, collect blood from the microvascular bed and carry it away.

THE HEART

The heart is a muscular pump that maintains unidirectional blood flow. It contains four chambers, the right and left atria and right and left ventricles. Valves guard the exit at the chamber, preventing backflow of the blood. An interatrial septum and an interventricular septum separate the right and left side of the heart.

The wall of the heart is composed of three layers: the inner one — the endocardium, the middle one — myocardium and the outer or serous one — the epicardium.

The endocardium consists of the inner layer of the endothelium (simple squamous epithelium) with its thick basal lamina and subendothelial connective tissue, a middle layer of connective tissue and smooth muscle cells, and deeper layer of connective tissue (subendocardial layer), which is continuous with the connective tissue of the myocardium and contains thick elastic, collagenous and reticular fibers. The impulse-conducting system of the heart is located in the subendocardial layer of the endocardium. Also it contains the blood vessels. But predominantly the endocardium is supplied by nutrients from the chambers of the heart.

There are the heart valves between the heart atria and the ventricles, and the ventricles and the large vessels. The heart valves are composed of the dense connective tissue with the overlying endocardium. Their surfaces

being invested with a thin endothelial layer continuous with that of the heart chambers and great vessels. At the attached margins of each valve, the lamina fibrosa becomes condensed to form a fibrous ring (valve anulus) and the rings of the four valves together form a central fibrous cardiac "skeleton" which is continuous with the collagenous tissue of the myocardium, endocardium and epicardium. The mitral and tricuspid leaflets are connected to the papillary muscles by collagenous strands, the chordae tendinae, which also merge with the fibrous lamina of the valve leaflet.

The myocardium consists of closely connected to one another striated cardiac muscle cells forming functional fibers. The layers of the loose connective tissue with the vessels and nerves are situated between myocardial muscle elements. The cardiac muscle can contract in a rhythmic manner without any direct stimulus from the nervous system. The electrical impulses are generated at the sinoatrial node or pacemaker of the heart, a group of specialized cardiac muscle cells located near the junction of the superior vena cava and the right atrium. *Pacemaker-cells (P-cells)* are characterized as polygonal-shaped small (no more than 8–9 mm in diameter) cells with the low developed irregularly arranged myofibrils and sarcoplasmic reticulum; T-system is absent, but there are a lot of pinocytotic vesicles and caveoli. Because of non-stable resting potencial P-cells spontaneously initiates an impulse that spreads along the cardiac muscle fibers of the atria and along internodal tracts composed of modified cardiac muscle fibers. The impulse is then picked up by the transitional cells at the atrioventricular node and conducted across the fibrous skeleton to the ventricles by the atrioventricular bundle of His. The bundle divides into smaller right and left bundle branches and then into subendothelial branches commonly called *Purkinje fibers*. Represented in this conducting system cells convey impulses from transitional cells to contractile cardiomyocytes. Transitional cells are thin (thinner than working cardiomyocytes) elongated cells containing irregular myofibriles more in number than in P-cells, but less than in working cardiomyocytes. They can contain short T-tubules. Purkinje fibers are the largest cells of the myocardium (more than 15 μm in diameter). Almost total absence of T-systems, thin irregular peripherally situated myofibrils, excentrical position of the nuclei, a lot of glycogen inclusions are the characteristics of the Purkinje fibers.

Atrial cardiomyocytes possessing well developed rough endoplasmic reticulum and Golgi apparatus participate in the natriuretic factor synthesis.

The latter can increase the water and salts removing from the organism, able to enhance hematocrit and to decrease arterial pressure. Atrial natriuretic factor is accumulating in the cytoplasm of the atrial cardiomyocytes as specific electronically dense granules 300–400 nm in diameter.

The ***epicardium*** or the visceral layer of the pericardium is formed by thin (0.3–0.4 mm — no more) connective tissue lamina closely connected with the myocardium and outer covering mesothelial cells.The mesothelial cells secrete a small amount of serous fluid which lubricates the movement of the epicardium on the parietal pericardium. Epicardial connective tissue is represented by superficial collagenous fibers layer, elastic fibers layer, deep layer of the collagen fibers and deep collagenous-elastic layer. The blood vessels and nerves that supply the heart lie in the epicardium and are surrounded by adipose tissue that cushions the heart and pericardial cavity. In the pericardium the connective tissue basis is developed better than in the epicardium and contains more elastic fibers.

THE VESSELS

The wall of any vessel has a common basic structure and is composed of three layers called tunics.

The innermost layer of the vessel, ***tunica intima***, consists of: a) a single layer of squamous epithelial cells, the endothelium; b) the basal lamina of endothelial cells; and c) the subendothelial layer, consisting of loose connective tissue and occasional smooth muscle cells.

The middle layer, ***tunica media***, consists primarily of circumferentially arranged layers of smooth muscle cells. Variable amounts of elastin, reticular fibers and proteoglycans are interposed between the smooth muscle cells. The sheets or lamellae of elastin are fenestrated and are arranged in circular concentric layers. All of the extracellular components of the tunica media are produced by the smooth muscle cells.

The outermost connective tissue layer, tunica adventitia, is composed primarily of longitudinally arranged collagenous tissue and few elastic fibers. These connective tissue elements gradually merge with the loose connective tissue surrounding the vessels. The tunica adventitia ranges from relatively thin in most of the arterial system to quite thick in the venules and veins, where it is the major component of the vessel wall. In addition, the

tunica adventitia of large arteries and veins contains a system of vessels, called vasa vasorum, that supply blood to the vascular walls themselves, as well as a network of autonomic nerves, called nervi vascularis that control contraction of the smooth muscle cells in the vessels walls.

Histologically, the various types of arteries and veins are distinguished from each other on the basis of the thickness of the vascular wall and differences in the composition of the layers that principally depends on the hemodynamic conditions (speed of blood flow and pressure).

The arteries

Traditionally, the arteries are classified into three types on the basis of quantitative ratio of the muscle cells and elastic fibers in their middle layer:

— elastic arteries;
— muscular arteries;
— mixed arteries.

There is an influenced by hemodynamic conditions gradual transition in structure and function between the three types of arterial vessel rather than an abrupt demarcation. In general, the amount of elastic tissue decreases as the vessels become smaller and the smooth muscle component assumes relatively greater prominence.

Elastic Arteries

The largest arteries, the aorta and pulmonary arteries, convey blood from the heart to the systemic and pulmonary circulations, respectively. They are classified as elastic arteries. The pressure generated by contraction of the ventricles during systole moves the blood through the elastic arteries and along the arterial tree. Simultaneously, it also causes the wall of the large elastic arteries to distend. The distention is limited by the network of collagenous fibers in the tunica media and tunica adventitia. During the relaxation phase (diastole) of the cardiac cycle, when no pressure is generated by the heart, the recoil of the distended elastic arteries serves to maintain arterial blood pressure and the flow of blood within the vessels.

The tunica intima of the elastic arteries is relatively thick and consists of endothelial lining with its basal lamina, subendothelial connective tissue and an inconspicuous internal elastic membrane. Typically flat and elongated endotheliocytes are oriented with their long axes parallel to the direction of

blood flow and are joined by tight junctions and gap junctions. Endothelial cells possess rodlike inclusions, called *Weibel-Palade bodies*, containing *von Willebrand factor* (coagulating factor VIII). Studies indicate that most von Willebrand factor is synthesised by arterial endothelial cells and secreted into the blood. But endothelial cells playing an important role in blood homeostasis provide the maintenance of a selective permeability barrier, maintenance of a nonthrombogenic barrier by producing anticoagulants and antithrombogenic substances (but damaged endothelial cells release prothrombogenic agents). Modulation of blood flow and vascular resistance (vasoconstrictors and dilators), regulation and modulation of immune responses (control of interaction with lymphocytes), hormonal synthesis (various growth stimulating and inhibiting factors, conversion of angiotensin I to angiotensin II), modification of the lipoproteins by oxidation are the endothelial functions too.

The subendothelial layer of the connective tissue with both elastic and collagen fibers is characterized by presence of the smooth muscle cells as the main cell type. These muscle cells are contractile and secrete extracellular ground substance as well as collagen and elastic fibers. Occasional macrophages may also be present.

The tunica media of elastic arteries is the thickest of the three layers and consists of elastin in the form of fenestrated sheets or lamellae between the muscle cells layers. These lamellae are arranged in concentric layers, and fenestrations in the lamellae facilitate the diffusion of substances within the arterial wall. The smooth muscle cells are arranged in a low-pitch spiral relative to the long axis of the vessel. Smooth muscle cells synthesize the collagen, elastin and other molecules of the extracellular matrix. Fibroblasts are not present in the tunica media.

The tunica adventitia in elastic arteries is usually less than half of the thickness of the tunica media. It consists of loose network of collagen and elastic fibers, fibroblasts and macrophages as the principal cells, blood vessels *(vasa vasorum)* and nerves *(nervi vascularis)*.

Muscular Arteries

Muscular arteries have the same basic structure as elastic arteries but the elastic tissue is reduced.

The tunica intima is relatively thinner and consists of the endothelial lining with its basal lamina, a sparse subendothelial layer of the connective

tissue, and a prominent internal elastic membrane. In some muscular arteries, the subendothelial layer is so scanty that the basal lamina of the endothelium appears to make contact with the internal elastic membrane. In histological sections, the internal elastic membrane usually appears as a well-defined, undulating or wavy structure because of contraction of smooth muscle. The thickness of the tunica intima varies with the age and other factors. In young children, it is very thin; in young adults it accounts for about one sixth of the total wall thickness; in older adults, tunica intima may be expanded by lipid deposits, often in the form of irregular "fatty streaks".

The tunica media of muscular arteries consists of smooth muscle cells amid collagen fibers and relatively little elastic material. As in elastic arteries, there no fibroblasts in this layer: the smooth muscle cells are arranged in spiral fashion to help maintain blood pressure. They possess an external (basal) lamina except at the sites of gap junctions and produce extracellular collagen, elastin, and ground substance.

The tunica adventitia of muscular arteries consists of fibroblasts, collagen fibers, elastic fibers and in some vessels scattered adipose cells. Compared with elastic arteries, the tunica adventitia of muscular arteries is relatively thick, about the same thickness as the tunica media. Collagen fibers are principal extracellular component. However, a concentration of elastic material immediately adjacent to the tunica media is often present as such constitutes the external elastic membrane. Nerves and small vessels travel in the adventitia and give off branches that penetrate into the tunica media in the large muscular arteries as the vasa vasorum.

Mixed arteries

There are no sharp difference between elastic arteries and muscular arteries. Some of these arteries have features that intermediate between the two types. These arteries, as well as their main branches, the brachiocefalic, common carotid, subclavian, and common iliac arteries, are clasified as mixed or muscular-elastic arteries.

Their tunica intima traditionally consists of endothelium with its basement membrane, subendothelial layer and a prominent internal elastic membrane, wich becomes apparent. Generally, in the region of transition, the amount of elastic material decreases, and smooth muscle cells become the predominant constituent of the tunica media. In many instances, a recognizable external elastic membrane is also evident. Together with the joining

them elastic elements of the tunica media (fibers and fenestrated membranes) they form elastic supporting skeleton, which helps flexibility during stretchening and prevents collups during compression. In tunica adventitia two sublayers can be identified: inner — solitary bundles of smooth muscle cells containing, and outer — including longitudinal and oblique collagen and elastic fibers, connective tissue cells, vasa vasorum and nervi vascularis.

Small arteries and *arterioles* are distinguished from one another by number of smooth muscle cell layers in the tunica media. By definition, arterioles have only one or two layers of smooth muscle; a small artery has up to about eight layers. Typically, the tunica intima of a small artery has an internal elastic membrane, whereas this layer may or may not be present in the arteriole. The endothelium in both is essentially similar to endothelium in other arteries, except the gap junctions may be found between endothelial and the smooth muscle cells of the tunica media. At last, the tunica adventitia is thin, ill-defined sheath of the connective tissue that blends with the connective tissue in which these vessels travel.

Arterioles serve as flow regulators for the capillary beds. In the normal relationship between an arteriole and a capillary network, contraction of the smooth muscle in the wall of an arteriole increases the vascular resistance and reduces or shuts off the blood going to the capillaries. The slight thickening of the smooth muscle at the origin of the capillary bed from an arteriole is called the precapillary sphincter. Most arterioles can dilate by 60–100% from their resting diameter, and they can maintain up to 40% constriction for a long time. Therefore, a large decrease or increase in vascular resistance has a direct effect on blood flow and systemic arterial pressure. This regulation directs blood to where it may be most needed. For instance, during strenuous physical exertion, such as running, blood flow to skeletal muscle increases by dilation of arterioles, and blood flow to the intestine is reduced by arteriolar constriction. After ingestion of large meal, however, the reverse is true.

Capillaries

The capillaries are the smallest diameter blood vessels, often smaller than the diameter of an erythrocyte. The capillaries form blood vascular networks that allow fluids containing gases, metabolites, and waste products to move through their thin walls. The capillary structure varies in dif-

ferent tissues and organs. On the basis of their morphology, three types of capillaries are described: *continuous* (or somatic), *fenestrated* (or visceral), and *discontinuous* (or sinusoidal) capillaries.

The ***continuous capillaries*** are typically found in the muscle, the lung, and the CNS. Their wall consists of the endothelial layer with the basement membrane *(tunica intima)*, pericytes *(tunica media)*, and scattered adventitial cells *(tunica adventitia)*. Occluding junctions are typical for endotheliocytes. Numerous pinocytotic (transport) vesicles underlie both the luminal and basal plasma membrane surfaces. Pericytes (rouget cells) may be associated with the endothelium. The pericyte, when present, intimately surrounds the capillary, with branching cytoplasmic processes, and is enclosed by basal lamina. The pericytes display features of a relatively unspecialized cell with a large nucleus rich in heterochromatin. It is derived from the same precursor cell that forms endothelial cells in new vessels. It can also give rise to smooth muscle cells during vessels growth.

The ***fenestrated capillaries*** are typically found in endocrine glands and sites of fluid and metabolite absorption, such as the gallbladder and intestinal tract. They are characterized by fenestrations, 80–100 nm in diameter, which provide channels across the capillary wall. Fenestrated capillaries also have pinocytotic vesicles. Some researches suggest that fenestrations are formed when a forming pinocytotic vesicle spans the narrow cytoplasmic layer and simultaneously opens on the opposite surface. Fenestration may have a thin, non-membranous diaphragm across its opening with a central thickening, it may be remnant of the glycocalix formerly enclosed in the pinocytotic vesicle.

The fenestrated capillaries in the gastrointestinal tract and gallbladder have fewer fenestrations and a thicker wall when no absorption is occurring. When absorption takes place, the walls thin, and the number of pinocytotic vesicles and fenestrations increases rapidly.

The ***discontinuous capillaries*** (sinusoidal capillaries, or sinusoids) are typically found in the liver, spleen, and bone marrow. They are larger in diameter and more irregularly shaped than other capillaries. Structural features of these capillaries vary from organ to organ: it can include specialized cells (stellate sinusoidal macrophages — Kupffer cells and vitamin A-storing lipocytes in the liver; endothelial cells exhibit a unique spindle shape with gaps between the neighboring cells (in the spleen); the basal lamina underlying the endothelium may be partially or even completely absent.

Arteriovenous (AV) anastomoses or shunts allow blood to bypass capillaries by providing direct routs between arteries and veins. AV shunts are commonly found in the skin of fingertips, nose, and lips and in the erectile tissue of the penis and clitoris. The arterioles of AV shunts is often coiled, has a relatively thick smooth muscle layer, is enclosed in a connective tissue capsule, and is richly innervated. Contrary to the ordinary precapillary sphincter, contraction of the arteriole smooth muscle of the AV shunts sends blood to a capillary bed; relaxation of the smooth muscle sends blood to a venule, bypassing the capillary bed. AV shunts serve in thermoregulation at the body surface. Closing AV shunts in the skin causes blood flow through the capillary bed, enhancing heat loss. Opening an AV shunt reduces the blood flow to the skin capillaries, conserving body heat. Ultimately, AV shunts participate in organs blood-filling, venous blood stream stimulation, arterialization of the venous blood, and also at violations of blood circulation and formation of pathological processes. There are two groups of anastomoses: 1) true, which are thrown pure arterial blood, and 2) false, which carry mixed blood. First group of true shunts may possess different appearence — short straight tubes, loops, brunched connections. According to their structure true anastomosis can be subdivided in the simple AV shunts and AV shunts, which are served by special contractile structures (described above). The second group — false anastomosis, or semishunts, are formed by short and sick (until 30 μm) capillaries, which because of the permitted exchange throw in the venules not completely arterial blood.

Venules

The postcapillary venules receive blood from capillaries and have diameter as small as 0.2 mm or slightly larger. They possess the endothelial lining with its basal lamina and pericytes relatively capillaries more number. The endothelium of postcapillary venules is the principal site of action of vasoactive agents such as histamine and serotonin. Response to these agents results in extravasation of fluid and emigration of white blood cells from the vessel during inflammation and allergic reactions. Postcapillary venules of lymph nodes also participate in the transmural migration of lymphocytes from the vascular lumen into the lymphatic tissue. The postcapillary venules in the lymph nodes are also called high endothelial venules (HEVs) because

of the prominent cuboidal appearance of their endothelial cells and their ovoid nuclei.

In the wall of collecting venules having diameter 30–50 μm sparse smooth muscle cells are present, and tunica adventitia is more prominent.

Muscular venules are located distal to the postcapillary venules in the returning venous network and have a diameter up to 1 mm. Whereas postcapillary venules have no true tunica media, the muscular venules have one or two layers of smooth muscle that constitute tunica media. These vessels also have a thin tunica adventitia.

Veins

The tunics of veins are not as distinct or well defined as the tunics of arteries. Although veins have three layers, also designated tunica intima, tunica media, and tunica adventitia, these layers are not as distinct as they are in the arteries. The veins and arteries usually travel together, and, typically, veins have thinner walls than their accompanying arteries, and the lumen of the veins is larger than that of the artery. Many veins, especially those that convey blood against gravity, contain valves that allow blood to flow in only one direction, back towards the heart. The valves are semilunar flaps consisting of a thin connective tissue core covered by endothelial cells. Traditionally, the veins are divided into:

— fibrous (amuscular) veins;

— muscular veins with bad, intermediate and good development of the muscle elements.

Fibrous (amuscular) veins with a highly atypical structure are present in the dura mater, retina, placenta, trabeculae of the spleen, bones. Such veins differ by very thin wall and tunica media absence. They are passive to blood movement inside because of the blood easy going out due to the gravity or close attachement of their wall connective tissue to the surrounding dense structural elemets of the corresponding organs.

Muscular veins with bad developed muscle elements

The tunica intima consists of the endothelium with its basal lamina, and a thin subendothelial layer of the connective tissue.

The tunica media contains circularly arranged solitary or forming rings smooth muscle cells with interspersed collagen and elastic fibers.

The tunica adventitia is typically thicker than tunica media and consists of collagen fibers and networks of elastic fibers.

Muscular veins with intermediate developed muscle elements

The tunica intima consists of the endothelium with its basal lamina, a thin subendothelial layer with occasional longitudinally arranged smooth muscle cells scattered in the connective tissue elements.

The tunica media contains several layers of circularly arranged smooth muscle cells with interspersed collagen and elastic fibers. In addition, longitudinally arranged smooth muscle cells may be present just beneath the tunica adventitia.

The tunica adventitia is typically thicker than tunica media and consists of collagen fibers and networks of elastic fibers.

Muscular veins with well developed muscle elements show the three tunics. Valves are a characteristic feature of these vessels and are most numerous in the inferior portion of the body, particularly the legs, to prevent retrograde movement of blood because of gravity.

The tunica intima of these veins consists of the endothelial lining with its basal lamina, a small amount of subendothelial connective tissue, and longitudinal bundles of smooth muscle cells.

The tunica media is relatively thin and contains circumferentially arranged smooth muscle cells, collagen fibers, and some fibroblasts.

The tunica adventitia of large veins is the thickest layer of the vessel wall. Along with the usual collagen and elastic fibers and fibroblasts, the tunica adventitia also contains longitudinally disposed bundles of smooth muscle cells.

Lymphatic Vessels

In addition to blood vessels, another set of vessels circulates fluid, called lymph, through most parts of the body. These lymphatic vessels serve as adjuncts to the blood vessels. Unlike the blood vessels, which convey blood to and from tissues, the lymphatic vessels are unidirectional, conveying fluid only from tissues. The smallest lymphatic vessels are called lymphatic capillaries. They are especially numerous in the loose connective tissues under the epithelium of the skin and mucous membranes. The lymphatic capillaries begin as “blind-ended” flattened tubes in the microcapillary beds.

The lymphatic capillaries are anastomosing to one another and penetrating organs (excluding brain, spleen, placenta, bone marrow, sklera and lens of eyeball, and also epithelial tissue and cartilage). Because of their greater permeability, lymphatic capillaries are more effective than blood capillaries in removing protein-rich fluid from the intercellular spaces. Lymphatic vessels also serve to convey proteins and lipids that are too large to cross the fenestrations of the absorptive capillaries in the small intestine.

Lymphatic capillaries converge into increasingly larger vessels, called lymphatic vessels. They ultimately unite to form two main channels than empty into the blood vascular system by draining into the large veins in the base of the internal jugular and subclavian veins. The largest lymphatic vessels, draining most of the body and emptying into the veins on the left side, is the thoracic duct. The other main channel is the right lymphatic trunk.

Before lymph is returned to the blood, it passes through the lymph nodes, where it is exposed to the cells of the immune system. Thus, the lymphatic vessels also serve as an integral component of the immune system.

Lymphatic capillaries are essentially tubes of endothelium that, unlike the typical blood capillary, lack a continuous basal lamina. This incomplete basal lamina can be correlated with their high permeability. Anchoring filaments extend between the incomplete basal lamina and the perivascular collagen. These filaments may help maintain the patency of the vessels during times of increased tissue pressure, as in inflammation.

As lymphatic vessels become larger, the wall becomes thicker. The increasing thickness is because of connective tissue and bundles of smooth muscle. Lymphatic vessels possess valves that prevent backflow of the lymph, thus aiding unidirectional flow. There are a lot of similarities in the structure of the lymphatic vessels and veins wall structure because of the similar hemodynamic conditions.

There is no central pump in the lymphatic system. Lymph moves sluggishly, driven primarily by compression of the lymphatic vessels by adjacent skeletal muscles.

Lecture 14

THE ORAL CAVITY AND ASSOCIATED STRUCTURES

The digestive system consists of the alimentary canal and its principal associated organs, namely, the tongue, teeth, salivary glands, pancreas, liver, and gallbladder.

The lumen of the alimentary canal is physically and functionally external to the body. As it passes through the alimentary canal, food is broken down physically and chemically so that the degraded products can be absorbed into the body. The various segments of the alimentary canal are morphologically specialized for specific aspects of digestion and absorption.

After preliminary maceration, moistening, and formation into a *bolus* by the actions of the structures of the oral and salivary glands, food passes rapidly through the pharynx to the esophagus. The rapid passage of food through the pharynx keeps it clear for the passage of air. The food passes more slowly through the gastrointestinal tract, and during its transit through the stomach and small intestine, the major alterations associated with digestion, solubilization, and absorption occur. Absorption occurs chiefly through the wall of the small intestine. Undigested food and other substances within the alimentary canal, such as mucus, bacteria, desquamated cells, and bile pigments, are excreted as feces.

The alimentary mucosa is the surface across which most substances enter the body. The alimentary *mucosa* performs numerous functions in its role as an interface between the body and the environment. These include the following:

Secretion. The lining of the alimentary canal secretes, at specific sites, digestive enzymes, hydrochloric acid, mucin, and antibodies.

Absorption. The epithelium of the mucosa absorbs metabolic substrates, e.g., the breakdown products of digestion, as well as vitamins, water, electrolytes, recyclable materials such as bile components and cholesterol, and other substances essential to the functions of the body.

Barrier. The mucosa serves as a barrier to prevent the entry of noxious substances, antigens, and pathogenic organisms.

Immunologic protection. Lymphatic tissue within the mucosa serves as the body's first line of immune defense.

THE ORAL CAVITY

The oral cavity consists of the mouth and its structures, which include the tongue, teeth and their supporting structures (peridontium), major and minor salivary glands, and tonsils.

The *oral cavity* is divided into the *vestibule* and the *oral cavity proper.* The *vestibule* is the space between the lips, cheeks, and teeth. The *oral cavity proper* lies behind the teeth and is bounded by the hard and soft palates superiorly, the tongue and the floor of the mouth interiorly, and the entrance to the oropharynx posteriorly.

The oral cavity is lined with the masticatory mucosa, the lining and the specialized mucosa.

Mucosa is represented usually by epithelial lamina, connective tissue lamina propria and smooth muscle muscularis mucosa. But in the oral cavity it is peculiar and consists of epithelium and connective tissue lamina propria. According to the functions of the oral cavity the epithelium is characterized as *stratified keratinized and nonkeratinized.*

The ***masticatory mucosa*** is found on the gingiva (gums) and the hard palate. It has a *keratinized* and, in some areas, a *parakeratinized* stratified squamous epithelium. Parakeratinized epithelium is similar to keratinised epithelium except that the superficial cells do not lose their nuclei and their cytoplasm does not stain intensely with eosin. The nuclei of the parakeratinized cells are pyknotic (highly condensed) and remain until the cell is exfoliated. The keratinized epithelium of the masticatory mucosa resembles that of the skin but lacks a stratum lucidum. The underlying lamina propria consists of a thick papillary layer of loose connective tissue that contains blood vessels and nerves, some of which send bare axon endings into the

epithelium as sensory receptors, and some of which end in *Meissner's corpuscles*. Deep to the lamina propria is a reticular layer of more dense connective tissue.

As in the skin, the depth and number of connective tissue papillae contribute to the relative immobility of the masticatory mucosa, thus protecting it from frictional and shearing stress. At the midline of the hard palate, in the *palatine raphe, the* mucosa adheres firmly to the underlying bone. The reticular layer of the lamina propria blends with the periosteum, and thus there is no submucosa. The same is true of the gingiva. Where there is a submucosa underlying the lamina propria on the hard palate, it contains adipose tissue anteriorly (fatty zone) and mucous glands posteriorly (glandular zone) that are continuous with those of the soft palate. In the submucosal regions, thick collagenous bands extend from the mucosa to the bone.

Lining mucosa is found on the lips, cheeks, alveolar mucosal surface, floor of the mouth, inferior surfaces of the tongue, and soft palate. At these sites it covers striated muscle (lips, cheeks, and tongue), bone (alveolar mucosa), and glands (soft palate, cheeks, inferior surface of the tongue). The lining mucosa has fewer and shorter papillae so that it can adjust to the movement of its underlying muscles.

Generally, the epithelium of the lining mucosa is nonkeratinized, although in some places it may be parakeratinized. The epithelium of the vermilion border of the lip (the reddish portion between the moist inner surface and the facial skin) is keratinized. The nonkeratinized lining epithelium is thicker than keratinized epithelium. It consists of only three layers:

— *stratum basale,* a single layer of cells resting on the basal lamina;

— *stratum spinosum,* which is several cells thick;

— *stratum superficiale,* the most superficial layer of cells, also referred as the *surface layer* of the mucosa.

The cells of the mucosal epithelium are similar to those of the epidermis of the skin and include keratinocytes, Langerhans' cells, melanocytes, and Merkel's cells.

The lamina propria contains blood vessels, nerves that send bare axon endings into the basal layers of the epithelium, and encapsulated sensory endings in some papillae. The sharp contrast between the numerous deep papillae of the alveolar mucosa and the shallow papillae in the rest of the lining mucosa allows easy identification of the two different regions in a histologic section.

A distinct submucosa underlies the lining mucosa except on the inferior surface of the tongue. This layer contains large bands of collagen and elastic fibers that bind the mucosa to the underlying muscle; it also contains the many minor salivary glands of the lips, tongue, and cheeks. Occasionally, sebaceous glands not associated with a hair follicle are found in the submucosa just lateral to the corner of the mouth and in the cheeks opposite the molar teeth. They are visible to the eye and are called *Fordyce spots*. The submucosa contains the larger blood vessels, nerves, and lymphatic vessels that supply the subepithelial neurovascular networks in the lamina propria throughout the oral cavity.

Specialized mucosa is restricted to the dorsal surface of the tongue, where it contains papillae and taste buds.

The Tongue

The *tongue* is a muscular organ projecting into the oral cavity from its inferior surface. *The lingual* (i.e., pertaining to the tongue) *muscles* are both extrinsic (having one attachment outside of the tongue) and intrinsic (confined entirely to the tongue, without external attachment). The striated muscle of the tongue is arranged in bundles that generally run in three planes, with each arranged at right angles to the other two. This arrangement of muscle fibers allows enormous flexibility and precision in the movements of the tongue, which are essential to human speech as well as to its role in digestion and swallowing. This form of muscle organization is found only in the tongue, which allows easy identification of this tissue as lingual muscle. Variable amounts of adipose tissue are found among the muscle fiber groups.

Numerous mucosal irregularities and elevations called *lingual papillae* cover the dorsal surface of the tongue anterior to the sulcus terminalis. The lingual papillae and their associated taste buds constitute the ***specialized mucosa*** of the oral cavity. Four types of papillae are described: *filiform, fungiform, circumvallate,* and *foliate.*

Filiform papillae are the smallest and most numerous in humans. They are conical, elongated projections of connective tissue that are covered with highly keratinized stratified squamous epithelium. This epithelium does not contain taste buds. The papillae serve only a mechanical role.

Fungiform papillae, as the name implies, are mushroom-shaped projections located on the dorsal surface of the tongue. They project above the

filiform papillae, among which they are scattered, and are just visible with the naked eye as small spots. They tend to be more numerous near the tip of the tongue. *Taste buds* are present in the stratified squamous epithelium on the dorsal surface of these papillae.

Circumvallate papillae are large, dome-shaped structures that reside in the mucosa just anterior to the sulcus terminalis. The human tongue has 8 to 12 of these papillae. Each papilla is surrounded by a moat-like invagination lined with stratified squamous epithelium that contains numerous taste buds. The ducts of the *lingual salivary glands (von Ebner's glands)* empty their serous secretion into the base of the moats. This secretion presumably flushes material from the moat to enable the taste buds to respond rapidly to changing stimuli.

Foliate papillae consist of parallel low ridges separated by deep mucosal clefts, which are aligned at right angles to the long axis of the tongue. They occur on the lateral edge of the tongue. In aged individuals, the foliate papillae may not be recognized; in younger individuals, they are easily found on the posterior lateral surface of the tongue and contain many taste buds in the epithelium of the facing walls of neighboring papillae. Small serous glands empty into the clefts. In some animals, such as the rabbit, foliate papillae constitute the principal site of aggregation of taste buds.

In histologic sections, taste buds appear as oval, pale-staining bodies that extend through the thickness of the epithelium. A small opening onto the epithelial surface at the apex of the taste bud is called the *taste pore*.

Three principal cell types are found in taste buds:

1. ***Neuroepithelial (sensory) cells*** are the most numerous cells — elongated and extending from the basal lamina of the epithelium to the taste pore, through which the tapered apical surface of each cell extends microvilli. Near their apical surface they are connected to neighboring neuroepithelial or supporting cells by tight junctions. At their base they form a synapse with the processes of afferent sensory neurons. The turnover time of neuroepithelial cells is about 10 days.

2. ***Supporting cells*** are less numerous, also elongated and extending from the basal lamina to the taste pore. Like neuroepithelial cells, they contain microvilli on their apical surface and possess tight junctions, but they do not synapse with the nerve cells. The turnover time of supporting cells is also about 10 days.

3. ***Basal cells*** are small cells located in the basal portion of the taste bud, near the basal lamina. They are the stem cells for the two other cell types.

In addition to those associated with the papillae, taste buds are also present on the glossopalatine arch, the soft palate, the posterior surface of the epiglottis, and the posterior wall of the pharynx down to the level of the cricoid cartilage.

Taste buds react to only four stimuli: sweet, salty, bitter, and acid. In general, taste buds at the tip of the tongue detect sweet stimuli, those immediately posterolateral to the tip detect salty stimuli, and those more posterolateral detect acid or sour testing stimuli. Taste buds on the circumvallate papillae detect bitter stimuli.

The *lingual tonsil* is located in the lamina propria of the root or base of the tongue. It is found posterior to the sulcus terminalis. The lingual tonsil contains diffuse lymphatic tissue with lymphatic nodules containing germinal centers. Epithelial crypts usually invaginate into the lingual tonsil. However, the structure of the epithelium may be difficult to distinguish because of the extremely large number of limphocytes that normally invade it. Between nodules, the lingual epithelium has the characteristics of lining epithelium. Mucous lingual salivary glands may be seen within the lingual tonsil and may extend into the muscle of the base of the tongue.

Teeth and Supporting Tissues

Teeth are a major component of the oral cavity and are essential for the beginning of the digestive process. Teeth are embedded in and attached to the alveolar processes of the maxilla and mandible. Children have 10 *deciduous (primary, milk) teeth* in each jaw, on each side. Over a period of years, usually beginning at about age 6 and finishing at about age 12 to 13, deciduous teeth are gradually replaced by 16 *permanent (secondary) teeth* in each jaw. Incisors, canines, and premolars have one root each, except for the first premolar of the maxilla, which has two roots. Molars have three and, on rare occasions, four roots. All teeth have the same basic structure, however.

Teeth are made up of three specialized tissues: 1) enamel, 2) dentin, and 3) cementum.

Enamel is an acellular mineralized tissue that covers the crown of the tooth. Once formed it cannot be replaced. Enamel is a unique tissue be-

cause unlike bone, which is formed from connective tissue, it is a mineralized material derived from the epithelium. Enamel is more highly mineralized and harder than any other mineralized tissue in the body. The enamel that is exposed and visible above the gum line is called the clinical crown; the anatomic crown describes all of the tooth that is covered by enamel, some of which is below the gum line. Enamel varies in thickness over the crown and may be as thick as 2.5 mm on the cusps (biting and grinding surfaces) of some teeth. The enamel layer ends at the *neck* or *cervix* of the tooth at the *cementoenamel junction*; the root of the tooth is then covered by *cementum*, a bone-like material. *Dentin* lies deep to the enamel and cementum.

Enamel is the hardest substance in the body; it consists of 96% to 98% hydroxyapatite. The nonstoichiometric carbonated calcium hydroxyapatite enamel crystals that form the enamel are arranged as rods that measure 4 μm wide and 8 μm high. Each enamel rod spans the full thickness of the enamel layer from the dentinoenamel junction to the enamel surface. When examined in cross section at higher magnification, the rods reveal a keyhole shape; a ballooned part or the head is oriented superiorly and the tail is directed inferiorly toward the root of the tooth. The enamel crystals are primarily oriented parallel to the long axis of the rod within the head, and in the tail they are oriented more obliquely. Due to the S-shaped rods at the tooth sections Shreger's lines are observed corresponding to the light beams reflection or absorption from the surface. The limited spaces between the rods are also filled with enamel crystals. Another striations observed on enamel rods (contour lines of Retzius) may represent evidence of rhythmic growth of the enamel in the developing tooth. A wider line of hypomineralization is observed in the enamel of the deciduous teeth. This line, called the neonatal line, marks the nutritional changes that take place between prenatal and postnatal life.

Although the enamel of an erupted tooth lacks cells and cell processes, it is not a static tissue. It is influenced by substances in saliva, the secretion of the salivary glands, which are essential to its maintenance. The substances in saliva that affect teeth include: *digestive enzymes, antibacterial enzymes, antibodies and inorganic (mineral) components.*

Mature enamel contains very little organic material. Despite its hardness, enamel can be decalcified by acid-producing bacteria acting on food products trapped on the enamel surface. This is the basis of the initiation of *dental caries.* Fluoride added to the hydroxyapatite complex makes the

enamel more resistant to acid demineralization. The widespread use of fluoride in drinking water, toothpaste, pediatric vitamin supplements, and mouthwashes significantly reduces the incidence of dental caries.

Enamel is produced by ameloblasts of the enamel organ, and dentin by odontoblasts of the adjacent mesenchyme.The ***enamel organ*** is an epithelial formation that is derived from ectodermal epithelial cells of the oral cavity. The onset of tooth development is marked by proliferation of the oral epithelium to form a horseshoe-shaped cellular band of tissue, the dental lamina, in the adjacent mesenchyme where the upper and lower jows will develop. At the site of each future tooth, there is a further proliferation of cells that arise from the dental lamina, resulting in a rounded, cellular, bud-like outgrowth, one for each tooth, that projects into the underlying mesenchymal tissue. This outgrowth, referred to us as the ***bud stage***, represents the early enamel organ (the 1st tooth germ). Gradually, the rounded cell mass enlarges and then a concavity develops at the site opposite where it arose from the dental lamina induced by formation in mesenchyme the 2nd tooth germ — ***dental pappila***. The enamel organ is now reffered to as being in the ***cap stage***. Further growth and development of the enamel organ results in the ***bell stage***. At this stage the enamel organ consists of four recognizable cellular components:

— ***outer enamel squamous epithelium***, forming the convex surface;

— ***inner columnar enamel epithelium***, forming the concave surface;

— ***stratum intermedium***, a cell layer that develops internal to the inner enamel epithelium;

— ***stellate reticulum***, a stellate appearanced cells and occupying the inner portion of the enamel organ

Simultaneously with dental papilla formation, the 3rd tooth germ occurs from mesenchyme and surrounds the basis of dental papilla — ***dental sac***. Splited into two layers, dental sac gives use for cementum (inner layer) and periodontal ligament (outer layer).

The outer mesenchymal cells within the “bell” adjacent to the inner enamel epithelial cells become columnar and have an epithelial type appearance. They will become ***odontoblasts*** and form the dentin of the tooth. The inner dental papilla cells transform into fibroblasts and form connective tissue basis of future tooth pulp. The inner enamel epithelial cells of the enamel organ will become ***ameloblasts***. Along with the cells of the *stratum intermedium*, they will be responsible for enamel production. At the early

stage, just prior to dentinogenesis and amelogenesis, the dental lamina degenerates, leaving the developing tooth primordium detached from their site of origin.

Dental enamel is formed by matrix-mediated biomineralization process known as ***amelogenesis***. The major stages of amelogenesis are:

— ***matrix production***, or ***secretory stage***. In the formation of mineralized tissues of the tooth, dentin is produced first. Then, partially mineralized enamel matrix is deposited directly on the surface of the previously formed dentin. The cells producing this partially mineralized organic matrix are called ***secretory-stage ameloblasts***. As do osteoblasts in bone, these cells produce an organic proteinaceous matrix by activity of the rough endoplasmic reticulum (rER), Golgi apparatus, and secretory granules. The secretory-stage ameloblasts continue to produce enamel matrix until the full thickness of the future enamel is achieved.

— ***matrix maturation***. Maturation of the partially mineralized enamel matrix involves the removal of organic materials as well as continued influx of calcium and phosphate into the maturing enamel. The cells involved in this second stage of enamel formation are called ***maturation-stage ameloblasts***. Maturation-stage ameloblasts differentiate from secretory-stage ameloblasts and function primarily as a transport epithelium, moving substances into and out of the maturing enamel. Maturation-stage ameloblasts undergo cyclical alterations in their morphology the correspond to cyclical entry of calcium into the enamel (Retzius lines formation).

Secretory-stage ameloblasts are polarized columnar cells that produce enamel. At the apical pole of each ameloblast is a process, Tomes' process, which is surrounded by the developing enamel. A claster of mitochondria in the base of the cell accounts for the eosinophilic staining of this region in hematoxylin and eosin stained paraffin sections. Adjacent to the mitochondria is the nucleus; in the main column of cytoplasm are the rER, Golgi, secretory granules, and other cell elements. Junctional complexes are present at both apical and basal parts of the cell. They maintain the integrity and orientation of the ameloblasts as they move away from the dentoenamel junction. Actin filaments joined to these junctional complexes are envolved in moving the secretory-stage ameloblast over the developing enamel. The rod produced by the ameloblast follows in the wake of the cell. Thus, in mature enamel, the direction of the enamel rod is a record of the path taken earlier by the secretory-stage ameloblast.

At their base, the secretory-stage ameloblasts are adjacent to a layer of enamel organ cells called the stratum intermedium. The plasma membrane of these cells, especially at the base of the ameloblasts, contains alkaline phosphatase, an enzyme active in calcification. Stellate enamel organ cells are external to the stratum intermedium and are separated from the adjacent blood vessels by a basal lamina. They are responsible for the nutrition of the secretory ameloblaste and by this way the thikness of enamel is limited becaus the limited number of the stellate enamel organ cells.

Maturation-stage ameloblasts transport substances needed for enamel maturation. The histologic feature that marks the cycles of maturation-stage ameloblasts is a striated or ruffled border. Maturation-stage ameloblasts with a striated border occupy approximately 70% of a specific cycle, and those that are smooth-ended, approximately 30% of a specific cycle. There is no stratum intermedium in the enamel organ during enamel maturation; stellate *papillary cells* are adjacent to the maturation-stage ameloblasts.

The maturation-stage ameloblasts and the adjacent papillary cells are characterized by numerous mitochondria. Their presence indicates cellular activity that requires large amounts of energy and reflects the function of maturation-stage ameloblasts and adjacent papillary cells as a transporting epithelium.

Recent advances in the molecular biology of ameloblast gene products have revealed the enamel matrix to be highly heterogenous. It contains proteins encoded by a number of different genes. The principal proteins in the extracellular matrix of the developing enamel are:

— *amelogenins* — important in establishing and maintaining the spacing between enamel rods in early stages of enamel development;

— *ameloblastins* — produced by ameloblast at the early secretory to late maturation stages and are believed to guide the enamel mineralization process by controlling elongation of the enamel crystals.

— *enamelins* — enamel proteinases (ameloproteases-I) are responsible for degradation of amelogenins in muturing enamel (distributed throughout the enamel layer).

— *tuftelins,* acidic enamel proteins located near the dentinoenamel junction that participate in the nucleation of enamel crystal. Tuftelins are present in *enamel tufts and* account for hypomineralization.

The maturation of the developing enamel results in its continued mineralization, so that it becomes the hardest substance in the body. Amelogenins

and ameloblastins are removed during enamel maturation. Thus, mature enamel contains only enamelins and tuftelins. The ameloblasts degenerate after the enamel is fully formed, at about the time of tooth eruption through the gum.

The development of tooth as described above accounts only for the formation of crown. At the periphery of the enamel organ in the future neck region inner and outer enamel epithelia come together forming epithelial root sheath of Hertwig. It grows downward toward the root and stimulates dentinoblastogenesis providing shape of developing root. Root development occurs shortly before tooth eruption and gradually progresses as the crown emerges through gingiva. The epithelial sheath of Hertwig disappears only when the root is formed completely.

Cementum covers the root of the tooth that fits into its *socket* or *alveolus* in the maxilla or mandible. *Cementum* is a thin layer of bone-like material that is secreted by *cementocytes,* cells that closely resemble osteocytes. Like bone, cementum is 65% mineral. The lacunae and canaliculi in the cementum contain the cementocytes and their processes, respectively. They resemble those structures in bone that contain osteocytes and osteocyte processes.

Unlike bone, cementum is avascular. Also, the canaliculi in the cementum do not form an interconnecting network. A layer of *cementoblasts* (cells that resemble the osteoblasts of the surface of growing bone) is seen on the outer surface of the cementum, adjacent to the *periodontal ligament.*

Collagen fibers that project out of the matrix of the cementum and embed in the bony matrix of the socket wall form the bulk of the periodontal ligament. These fibers are another example of Sharpey's fibers. In addition, elastic fibers are also a component of the periodontal ligament. This mode of attachment of the tooth in socket allows slight movement of the tooth to occur naturally. It also forms the basis of orthodontic procedures used to straighten teeth and reduce malocclusion of the biting and grinding surfaces of the maxillary and mandibular teeth. During corrective tooth movements, the alveolar bone of the socket is resorbed and resynthesized, but the cementum is not.

Dentin is a calcified material that forms most of the tooth substance and lies deep to the enamel and cementum. It contains less hydroxyapatite than enamel, about 70%, but more than is found in bone and cementum. Dentin

is secreted by odontoblasts that form an epithelial-like layer over the inner surface of the dentin, i.e., the surface that is in contact with the pulp. Like ameloblasts, odontoblasts are columnar cells that contain a well-developed rER, a large Golgi apparatus, and other organelles associated with the synthesis and secretion of large amounts of protein. The apical surface of the odontoblasts is in contact with the forming dentin; junctional complexes between the odontoblasts at that level separate the dentinal compartment from the pulp compartment.

The layer of odontoblasts retreats as the dentin is laid down, leaving odontoblast processes embedded in the dentin in narrow channels called dentinal tubules. The tubules and processes continue to elongate as the dentin continues to thicken by rhythmic growth. The rhythmic growth of dentin produces certain "growth lines" in the dentin (incremental lines of von Ebner and thicker lines of Owen) that mark significant developmental times such as birth (neonatal line) and when unusual substances such as lead are incorporated into the growing tooth. Study of growth lines has proved useful in forensic medicine.

Predentin is the newly secreted organic matrix, closest to the cell body of the odontoblast, which in the organic matrix are similar to those of the bone, predentin contains two unique proteins involved in the mineralization process: dentin phosphoprotein (DPP) and dentin sialoprotein (DSP).

An unusual feature of the secretion of collagen and hydroxyapatite by odontoblasts is that Golgi vesicles believed to contain calcium attach to these precursors, giving rise to structures called ***abacus bodies***. Abacus bodies become more condensed as they mature into secretory granules. This specific pathway of mineralization combined with radial arrangement of matrix fibers results in occurence of nonmineralized interglobular spaces in mantle dentine in tooth crown and Tom's layer in root of tooth.

Dentin is the first mineralized component of the tooth to be deposed. The outermost dentin, which is referred to as mantle dentin, is formed by subodontoblastic cells that produce small bundles of radially arranged collagen fibers (von Korff's fibers). The odontoblasts differentiate from cells at the periphery of the dental papilla. The progenitor cells have the appearance of typical mesenchymal cells; i.e., they contain little cytoplasm. During their differentiation into odontoblasts, the cytoplasmic volume and organelles characteristic of collagen-producing cells increase. The cells form a layer at the periphery of the dental papilla, and they secrete the organic matrix of

dentin, or predentin, at their apical end (away from the dental papilla). As the predentin thickens, the odontoblasts move or are displaced centrally. A wave of mineralization follows the receding odontoblasts; this mineralized product is the dentin. As the cells move centrally, the odontoblastic processes elongate; the longest are surrounded by the mineralized dentin. In newly formed dentin, the wall of the dentinal tubule is simply the edge of the mineralized dentin. With time, the dentin immediately surrounding the dentinal tubule becomes more highly mineralized; this more mineralized sheath of dentin is referred to as the ***peritubular dentin***. The remainder of the dentin is called the ***intertubular dentin.***

The **central pulp cavity** is the space within a tooth that is occupied by ***dental pulp,*** a loose connective tissue that is richly vascularized and supplied by abundant nerves. The pulp cavity takes the general shape of the tooth. The blood vessels and nerves enter the pulp cavity at the tip (apex) of the root, at a site called the ***apical foramen***. The blood vessels and nerves extend to the crown of the tooth where they form vascular and neural networks beneath and within the outer layer of odontoblasts and intermediate layer of periodontoblasts. Some bare nerve fibers also enter the proximal portions of the dentinal tubules and contact odontoblast processes. The odontoblast processes are believed to serve a transducer function in transmitting stimuli from the tooth surface to the nerves in the dental pulp. In teeth with more than one cusp, *pulpal horns* extend into the cusps and contain large numbers of nerve fibers. More of these fibers extend into the dentinal tubules than at other sites. Because dentin continues to be secreted throughout life, the pulp cavity decreases in volume with age.

Supporting tissues of the teeth include the alveolar bone of alveolar processes of the maxilla and mandible, periodontal ligaments, and gingiva.

The *alveolar bone proper,* a thin layer of compact bone, forms the wall of the alveolus and is the bone to which the periodontal ligament is attached. The rest of the alveolar process consists of supporting bone. The surface of the alveolar bone proper usually shows regions of bone resorption and bone deposition, particularly when a tooth is being moved. Periodontal disease usually leads to loss of alveolar bone, as does the absence of functional occlusion of a tooth with its normal opposing tooth.

The *periodontal ligament* is the fibrous connective tissue joining the tooth to its surrounding bone. The periodontal ligament provides for tooth attachment (fixation), tooth support, bone remodeling (during movement of

a tooth), proprioception, tooth eruption. It contains areas of both dense and loose connective tissue. The dense connective tissue contains collagen fibers and fibroblasts that are elongated parallel to the long axis of the collagen fibers. The fibroblasts are believed to move back and forth, leaving behind a trail of collagen fibers. Periodontal fibroblasts also contain internalized collagen fibrils that are digested by the hydrolytic enzymes of the cytoplasmic lysosomes. These observations indicate that the fibroblasts not only produce collagen fibrils but also resorb collagen fibrils, thereby adjusting continuously to the demands of tooth stress and movement. The loose connective tissue in the periodontal ligament contains blood vessels and nerve endings. In addition to fibroblasts and thin collagenous fibers, the periodontal ligament also contains thin, longitudinally disposed *oxytalan fibers.*

The *gingiva* is a specialized part of the oral mucosa located around the neck of the tooth commonly called the gums. It is firmly attached to the teeth and to underlying alveolar bony tissue. The gingiva is composed of two parts:

Gingival mucosa, which is synonymous with the masticatory mucosa, was described above *junctional epithelium* or *attachment epithelium* adheres firmly to the tooth. A basal lamina-like material is secreted by the junctional epithelium and adheres firmly to the tooth surface. The cells then attach to this material via hemidesmosomes. The basal lamina and the hemidesmosomes are together referred to as the *epithelial attachment.* In young individuals, this attachment is to the enamel; in older individuals, where passive tooth eruption and gingival recession expose the roots, the attachment is to the cementum. Above the attachment of the epithelium to the tooth, a shallow crevice called the ***gingival sulcus***, is lined with ***crevicular epithelium*** that is continuous with the attachment epithelium. The term ***periodontium*** refers to all the tissues involved in the attachment of a tooth to the mandible and maxilla. These include the crevicular and junctional epithelium, the cementum, the periodontal ligament, and the alveolar bone.

Salivary Glands

The major salivary glands consist of the paired parotid, submandibular, and sublingual glands. The parotid and the submandibular glands are actually located outside the oral cavity; their secretions reach the cavity by ducts.

The parotid gland is located subcutaneously, below and in front of the ear in the temporal region of the head, and the submandibular gland is located under the floor of the mouth, in the submandibular triangle of the neck. The sublingual gland is located in the floor of the mouth anterior to the submandibular gland.

The minor salivary glands are located in the submucosa of different parts of the oral cavity. They include *the lingual, labial, buccal, molar, and palatine glands.*

Each salivary gland arises from the developing oral cavity epithelium. Initially, the gland takes the form of a solid cord of cells that enters the mesenchyme. The proliferation of epithelial cells eventually produces highly branched epithelial cords with bulbous ends. Degeneration of the innermost cells of the cords and bulbous ends leads to their canalization. The cords become ducts, and the bulbous ends become *secretory acini.*

The major salivary glands are surrounded by a capsule of moderately dense connective tissue from which septa divide the secretory portions of the gland into lobes and lobules. The septa contain the larger blood vessels and excretory ducts. The connective tissue associated with the groups of secretory acini blends imperceptibly into the surrounding loose connective tissue. The minor salivary glands do not have a capsule. Numerous lymphocytes and plasma cells populate the connective tissue surrounding the acini in both the major and minor salivary glands.

The basic secretory unit of salivary glands, the *salivon,* consists of the acinus, intercalated duct, and excretory duct. The *acinus* is a blind sac composed of secretory cells. The term *acinus* (from Latin — berry or grape) refers to the secretory unit of the salivary glands. The acini of salivary glands contain either ***serous*** *cells* (protein secreting), *mucous cells* (mucin secreting), or both. The relative frequencies of the three types of acini are a prime characteristic by which the major salivary glands are distinguished. Thus, three types of acini are described:

— *serous acini,* which contain only serous cells and are generally spherical;

— *mucous acini,* which contain only mucous cells and are usually more tubular;

— *mixed acini,* which contain both serous and mucous cells. In routine H&E preparations, mucous acini have a cap of serous cells that are thought to secrete into the highly convoluted, intercellular space between the mu-

cous cells. Because of their appearance in histologic sections, such caps are called *serous demilunes (half-moon)*.

Serous demilunes are artifacts of the traditional fixation method. Recent electron microscopic studies now challenge this classical interpretation of demilune. Rapid freezing of the tissue reveals that both mucous and serous cells are aligned in the same row to surround the lumen of the secretory acinus. No serous demilune is found. Sections prepared from the same specimen by conventional methods show swollen mucous cells with enlarged secretory granules. The serous cells form typical demilunes and are positioned in the peripheral region of the acinus with slender cytoplasmic processes interposed between the mucous cells. These findings indicate that the demilune observed in light or electron microscopy is an artifact of the routine fixation method. The process of demilune formation can be explained by the expansion of mucinogen, a major component of secretory granules, during routine fixation. This expansion increases the volume of the mucous cells and displaces the serous cells from their original position, thus creating the demilune effect. A similar phenomenon is sometimes seen in the intestinal mucosa, in which swollen goblet cells displace adjacent absorptive cells.

Serous cells are protein-secreting cells wich have a pyramidal shape, with a relatively wide basal surface facing the basal lamina and a small apical surface facing the lumen of the acinus. They contain large amounts of rER, free ribosomes, a prominent Golgi apparatus, and numerous spherical secretory granules. As in most protein-secreting cells that store their secretions in zymogen granules, the granules are located in the apical cytoplasm. Most other organelles are located in the basal or perinuclear cytoplasm. In H&E sections, the basal cytoplasm of the serous cell stains with hematoxylin because of the rER and free ribosomes, whereas the apical region stains with eosin, in large part because of the secretory granules. The serous cell may display infoldings of the plasma membrane and basolateral folds in the form of processes that interdigitate with similar processes of adjacent cells. The serous cells are joined near their apical surface by junctional complexes to neighboring cells of the acinus.

Mucous cells are mucin-secreting cells and as in other mucus-secreting epithelia, the mucous cells of the mucous salivary acini undergo cyclic activity. During part of the cycle, mucus is synthesized and stored within the cell as mucinogen granules. When the product is discharged after hormonal

or neural stimulation, the cell begins to resynthesize mucus. After discharge of most or all of the mucinogen granules, the cell is difficult to distinguish from an inactive serous cell. However, most mucous cells contain large numbers of mucinogen granules in their apical cytoplasm, and because the mucinogen is lost in H&E-stained paraffin sections, the apical portion of the cell usually appears empty. In TEM preparation, the rER, mitochondria, and other components are seen chiefly in the basal portion of the cell; this part of the cell also contains the nucleus, which is typically flattened against the base of the cell. In rapid-freeze preparations, cells are rounded and clearly isolated from each other. The nuclei are round and centrally located. The apical portion of the mucous cell contains numerous mucinogen granules and a large Golgi apparatus, in which large amounts of carbohydrate are added to a protein base to synthesize the glycoprotein of the mucin. Mucous cells possess apical junctional complexes, the same as those seen between serous cells.

Myoepithelial cells are contractile cells with numerous processes that embrace the basal aspect of the acinar secretory cells. They lie between the basal plasma membrane of the epithelial cells and the basal lamina of the epithelium. Myoepithelial cells also underlie the cells of the proximal portion of the duct system. In both locations, the myoepithelial cells are instrumental in moving secretory products toward the excretory duct. Myoepithelial cells are sometimes difficult to identify in H&E sections. The nucleus of the cell is often seen as a small round profile near the basement membrane. The contractile filaments srain with eosin and are sometimes recognized as a thin eosinophilic band adjacent to the basement membrane.

Salivary ducts

The lumen of the salivary acinus is continuous with that of a duct system that may have as many as three sequential segments. These are referred to as:

— ***intercalated duct***, which leads from the acinus;

— ***striated duct***, so-called because of the presence of "striations," the infoldings of the basal plasma membrane of the columnar cells that form the duct;

— ***excretory ducts***, which are the larger ducts that empty into the oral cavity.

The degree of development of the intercalated duct and striated ducts varies, depending on the nature of the acinar secretion. Serous glands have well-developed intercalated ducts and striated ducts tnat modify the serous secretion by both absorption of specific components from the secretion and secretion of additional components to form the final product. Mucous glands, in which the secretion is not modified, have very poorly developed intercalated ducts that may not be recognizable in H&E sections. Moreover, they do not display striated ducts.

Intercalated ducts are lined by low cuboidal epithelial cells that usually lack any distinctive feature to suggest a function other than that of a conduit. However, the cells of intercalated ducts possess carbonic anhydrase activity. In serous-secreting glands and mixed glands, they have been shown to secrete bicarbonate ion into the acinar production and absorb chloride ion from the acinar production. As noted above, intercalated ducts are more prominenet in those salivary glands that produce a watery serous secretion. In mucous-secreting salivary glands, the intercalated ducts, when present, are short and difficult to identify.

Striated ducts are lined by a simple cuboidal epithelium that gradually becomes columnar as it approaches the excretory duct. The infoldings of the basal plasma membrane are seen in histoilogic sections as "striations". Longitudinally oriented, elongated mitochondria are enclosed in the infoldings. Basal infoldings associated with elongated mitochondria are a morphologic specialization associated with reabsorption of fluid and electrolites. The striated duct cells also have numerous basolateral folds that are interdigitated with those of adjacent cells. The nucleus typically occupies a central (rather than basal) location in the cell. Striated ducts are the sites of reabsorption of Na^+ from the primary secretions and secretion of $K^+$and HCO_3^- into the secretions. More Na^+ is reabsorbed than K^+ is secreted, so the secretion becomes hypotonic. When secretion is very rapid, more Na^+ and less K^+ appear in the final saliva because the reabsorption and secondary secretion systems cannot keep up with the rate of primary secretion. Thus, the saliva may become isotonic to hypertonic. The diameter of striated ducts often exceed that of the secretory acinus. Striated ducts are located in the parenchyma of the glands (they are intralobular ducts) but may be resurrounded by small amounts of connective tissue in which blood vessels and nerves can be seen running in parallel with the duct.

Excretory ducts travel in the interlobular and interlobar connective tissue and constitute the principal ducts of each of the major gland. They ultimately connect to the oral cavity. The epithelium of small excretory ducts is simple cuboidal. It gradually changes to pseudostratified columnar or stratified cuboidal. As the diameter of the duct increases, stratified columnar epithelium is often seen, and as the ducts approach the oral epithelium, stratified squamous epithelium may be present. The parotid duct (Stensen's duct) and the submandibular duct (Wharton's duct) travel in the connective tissue of the face and neck, respectively, for some distance from the gland before penetrating the oral mucosa.

The paired serous **parotid glands** are the largest of the salivary glands. The parotid duct travels from the gland, which is located below and in front of the ear, to enter the oral cavity opposite the second upper molar tooth, secretory units in the parotid are serous and surround numerous, long, narrow intercalated ducts. Striated ducts are large and conspicuous. Large amounts of adipose tissue often occur in the parotid gland; this is one of its distinguishing features. The facial nerve (cranial nerve VII) passes through the parotid gland; large cross sections of this nerve may be encountered in routine H&E sections of the gland and are useful in identifying the parotid. Mumps, a viral infection of the parotid gland, can damage the facial nerve.

The large, paired, mixed **submandibular glands** are located under either side of the floor of the mouth, close to the mandible. A duct from each of the two glands runs forward and medially to a papilla located on the floor of the mouth just lateral to the frenulum of the tongue. Some mucous acini capped by serous demilunes are generally found among the predominant serous acini. Intercalated ducts are less extensive than in the parotid gland.

The **sublingual glands**, the smallest of the paired major salivary glands, are located in the floor of the mouth anterior to the submandibular glands. Their multiple small sublingual ducts empty into the submandibular duct as well as directly onto the floor of the mouth. Some of the predominant mucous acini exhibit serous demilunes, but purely serous acini are rarely present. Intercalated ducts and striated ducts are short, difficult to locate, or sometimes absent. The mucous secretory units may be more tubular than purely acinar.

The parotid and submandibular glands have relatively long ducts that extend from the secretory portion of the gland to the oral cavity. The sublingual ducts are relatively short.

The *minor salivary glands* are located in the submucosa of the oral cavity. They empty directly into the cavity via short ducts and are named for their location i.e., buccal, labial, lingual, and palatine.

Saliva includes the combined secretions of all the major and minor salivary glands and mostly is produced by the salivary glands. A smaller amount is derived from the gingival sulcus, tonsillar crypts, and general trunsudation from the epithelial lining of the oral cavity. One of the unique features of saliva is the large and variable volume produced. The volume (per weight of gland tissue) of saliva exceeds that of other digestive secretions by as much as 40 times. The large volume of saliva produced is undoubtedly related to its many functions, only some of which concern digestion.

The salivary glands produce about 1,200 ml of saliva a day. Saliva has numerous functions relating to metabolic and nonmetabolic activities. These include:

1. Moistening the oral mucosa.

2. Moistening dry foods to aid swallowing.

3. Providing a medium for dissolved and suspended food materials that chemically stimulate taste buds.

4. Buffering the contents of the oral cavity, because of its high concentration of bicarbonate ions.

5. Digesting carbohydrates with the digestive enzyme α-amylase that breaks 1–4 glycosidic bonds and continues to act in the esophagus and stomach.

6. Controlling the bacterial flora of the oral cavity by use of lysozyme (muramidase), an enzyme that lyses the murainic acid in certain bacteria (e.g., staphylococci).

7. Endocrine function is provided by presence of hormone-like biologically active substances — insuline, parotine, factor of nerves growth, factor of epithelium growth, mortality factor and some others.

Saliva is a source of calcium and phosphate ions essential for normal tooth development and maintenance. Calcium and phosphate in the saliva are essential for the mineralization of newly erupted teeth and for repair of precarious lesions of the enamel in erupted teeth. In addition, saliva serves several other roles in protecting the teeth. Proteins in saliva cover the teeth with a protective coat called the acquired pellicle. Antibodies and other

antibacterial agents retard bacterial action that would otherwise lead to tooth decay. Patients whose salivary glands are irradiated, as in the treatment of salivary gland tumors, fail to produce normal amounts of saliva; these patients typically develop caries. Anticholinergic drugs used to treat some forms of heart disease also significantly reduce salivary secretion, leading to dental caries.

Saliva performs immunologic functions containing antibodies, salivary *immunoglobulin A (IgA)*. IgA is synthesized by plasma cells in the connective tissue surrounding the secretory acini of the salivary glands.

Saliva contains chiefly water, proteins and glycoproteins (enzymes and antibodies), and electrolytes. It has a high potassium concentration that is approximately seven times that of blood, a sodium concentration approximately one tenth that of blood, a bicarbonate concentration approximately three times that of blood, and significant amounts of calcium, phosphorus, chloride, thiocyanate, and urea. Lysozyme and α-amylase are the principal enzymes present.

The **tonsils** consist of aggregations of lymphatic nodules that are clustered around the posterior opening of the oral and nasal cavities. Lymphatic tissue is organized into a *tonsillar ring (Pirogov's or Waldeyer's ring)* of immunologic protection located at the shared entrance to the digestive and respiratory tracts. This lymphatic tissue surrounds the posterior orifice of the oral and nasal cavities and contains aggregates of lymphatic nodules that include:

— *the palatine tonsils,* or simply the *tonsils,* which are located at either side of the entrance to the oropharynx between the palatopharyngeal and palatoglossal arches;

— *the tubal tonsils,* which are located in the lateral walls of the nasopharynx posterior to the opening of the auditory tube;

— *the pharyngeal tonsil,* or *adenoid,* which is located in the roof of the nasopharynx;

— *the lingual tonsil,* which is located at the base of the tongue on its superior surface.

Lecture 15

THE ESOPHAGUS AND GASTROINTESTINAL TRACT

The portion of the alimentary canal that extends from the proximal part of the esophagus to the distal part of the anal canal is a hollow tube of varying diameter. This tube has the same basic structural organization throughout its length. Its wall is formed by four distinctive layers. From its lumen outward, they are:

— *mucosa,* consisting of a lining epithelium, an underlying connective tissue called the *lamina propria,* and the *muscularis mucosae,* composed of smooth muscle;

— *submucosa,* consisting of dense irregular connective tissue;

— *muscularis externa,* consisting in most parts of two layers of smooth muscle.

— *serosa*, or adventitia. A serous membrane consists of a simple squamous epithelium, the mesothelium, and a small amount of underlying connective tissue. *Adventitia* consisting only of connective tissue is found where the wall of the tube is directly attached or fixed to adjoining structures (i.e., body wall and certain retroperitoneal organs).

The structure of the esophagus and gastrointestinal tract varies considerably from region to region; most of the variation occurs within the mucosa. The epithelium differs throughout the alimentary canal and is adapted to the specific function of each part of the tube. The mucosa has three principal functions: *protection, absorption,* and *secretion.*

The **epithelial barrier** separates the external luminal environment of the tube from the tissues and organs of the body. The barrier aids in protection of the individual from the entry of antigens, pathogens, and other noxious substances. In the esophagus, a stratified squamous epithelium pro-

vides protection from physical abrasion by ingested food. In the gastrointestinal portion of the alimentary tract, tight junctions between the simple columnar epithelial cells of the mucosa serve as a selectively permeable barrier. These epithelial cells transport products of digestion and other essential substances such as water through the cells and into the extracellular space beneath the tight junctions.

Effective absorption of digested nutrients, water, and electrolytes is possible because of projections of the mucosa and submucosa into the lumen of the digestive tract. These surface projections greatly increase the surface area able for absorption and vary in size and orientation. They consist of the following structural specializations:

1. *Plicae circulares* are circumferentially oriented submucosal folds present along most of the length of the intestine.

2. *Villi* are mucosal projections that cover the entire of the small intestine, the principal site of absorption of the products of digestion.

3. *Microvilli* are tightly packed, microscopic projections of the apical surface of intestinal absorptive cells. They further increase the surface available for absorption.

In addition, the *glycocalyx* consists of glycoproteins that project from the apical plasma membrane of epithelial absorptive cells. It provides additional surface for adsorption and includes enzymes secreted by the absorptive cells that are essential for the final steps of digestion of proteins and sugars. The epithelium selectively absorbs the products of digestion both for its own cells and for transport into the vascular system for distribution to other tissues.

Secretion is carried out largely by glands distributed throughout the length of the digestive tube. The various secretory products provide mucus for protective lubrication, as well as buffering of the tract lining and substances that assist in digestion, including enzymes, hydrochloric acid, peptide hormones, and water. The mucosal epithelium also secretes antibodies that it receives from the underlying connective tissue.

The glands of the alimentary tract develop from invaginations of the luminal epithelium and include:

a) *mucosal glands* that extend into the lamina propria.

b) *submucosal glands* that either deliver their secretions directly to the lumen of mucosal glands or via ducts that pass through the mucosa to the luminal surface.

c) *extramural glands* that lie outside the digestive tract and deliver their secretions via ducts that pass through the wall of the intestine to enter the lumen. The liver and the pancreas are extramural digestive glands that greatly increase the secretory capacity of the digestive system. They deliver their secretions into *duodenum,* the first part of the small intestine.

The **lamina propria** contains glands, vessels that transport absorbed substances, and components of the immune system. The mucosal glands extend into the lamina propria throughout the length of the alimentary canal. In addition, in several parts of the alimentary canal (e.g., the esophagus and anal canal), the lamina propria contains aggregations of mucus-secreting glands. In general they lubricate the epithelial surface to protect the mucosa from mechanical and chemical injury.

In segments of the digestive tract in which absorption occurs, principally the small and large intestines, the absorbed products of digestion diffuse into the blood and lymphatic vessels of the lamina propria for distribution. Typically, the blood capillaries are of the fenestrated type and collect most of the absorbed metabolites. In the small intestine lymphatic capillaries are numerous and receive some absorbed lipids and proteins.

The lymphatic tissues in the lamina propria function as an integrated immunologic barrier that protects against pathogens and other antigenic substances that could potentially enter through the mucosa from the lumen of the alimentary canal. The lymphatic tissue is represented by d*iffuse lymphatic tissue* consisting of numerous lymphocytes and plasma cells, located in the lamina propria, and lymphocytes transiently residing in the intercellular spaces of the epithelium; *lymphatic nodules* with well-developed germinal centers; *eosinophils, macrophages,* and sometimes *neutrophils.* The diffuse lymphatic tissue and the lymphatic nodules are referred to as *gut-associated lymphatic tissue (GALT).*

The ***muscularis mucosae,*** the deepest portion of the mucosa, consists of smooth muscle cells arranged in an inner circular and outer longitudinal layer. Contraction of this muscle produces movement of the mucosa, forming ridges and valleys that facilitate absorption and secretion. This localized movement of the mucosa is independent of the peristaltic movement of the entire wall of the digestive tract.

The **submucosa** consists of a dense, irregular connective tissue layer containing blood and lymphatic vessels, a nerve plexus, and occasional glands. The nerve cell bodies of parasympathetic ganglia and their postganglionic

nerve fibers represent in the submucosa the *enteric nervous system,* the third division of the autonomic nervous system. This system is primarily responsible for innervating the smooth muscle layers of the alimentary canal and can function totally independent of the central nervous system. In the submucosa, the network of unmyelinated nerve fibers and ganglion cells constitute the *submucosal plexus (Meissner's plexus).*As noted, glands occur occasionally in the submucosa in certain locations. For example, they are present in the esophagus and the initial portion of the duodenum. In histologic sections, the presence of these glands often aids in identifying the specific segment or region of the tract.

Muscularis externa in most parts of the digestive tract consists of two, concentric and relatively thick layers of the smooth muscle. The cells in the inner layer form a tight spiral, described as a *circularly oriented layer;* those in the outer layer form a loose spiral, described as a *longitudinally oriented layer.* Located between the two muscle layers is a thin connective tissue layer. Within this connective tissue lies the *myenteric plexus (Auerbacb's plexus)* containing nerve cell bodies (ganglion cells) of postganglionic parasympathetic neurons and neurons of the enteric nervous system, as well as blood vessels and lymphatic vessels.

Contraction of the inner circular layer of the muscularis externa compresses and mixes the contents by constricting the lumen; contraction of the outer, longitudinal layer propels the contents by shortening the tube. The slow, rhythmic contraction of these muscle layers under the control of the enteric nervous system produces *peristalsis*, i.e., waves of contraction. Peristalsis is marked by constriction and shortening of the tube, which moves the contents through the intestinal tract.

A few sites along the digestive tube exhibit variations in the *muscularis externa*. For example, in the wall of the proximal portion of the esophagus (pharyngoesophageal sphincter) and around the anal canal (external anal sphincter), the striated muscle forms a part of the muscularis externa. In the stomach, a third, obliquely oriented layer of smooth muscle is present deep to the circular layer. Finally, in the large intestine, part of the longitudinal smooth muscle layer is thickened to form three distinct, equally spaced longitudinal bands called *teniae coli*.

At several points along the digestive tract the circular muscle layer is thickened to form *sphincters* or *valves.* From the oropharynx distally, these structures include:

Pharyngoesophageal sphincter. Actually, the lowest part of the cricopharyngeus muscle is physiologically referred to as the superior esophageal sphincter. It prevents the entry of air into the esophagus. The inferior esophageal sphincter creates a pressure difference between the esophagus and stomach that prevents reflux of gastric contents into the esophagus.

Pyloric sphincter. Located at the junction of the pylorus of the stomach and duodenum (gastroduodenal sphincter), it controls the release of *chyme,* the partially digested contents of the stomach, into the duodenum.

Ileocecal valve. Located at the junction of the small and large intestines, it prevents reflux of the contents of the colon with its high bacterial count into the distal ileum, which normally has a low bacterial count.

Internal anal sphincter. This, the most distally located sphincter, surrounds the anal canal and prevents passage of the feces into the anal canal from the undistended rectum.

Serosa or **adventitia** constitutes the outermost layer of the alimentary canal.

The *serosa* is a serous membrane consisting of a layer of simple squamous epithelium, called the *mesothelium,* and a small amount of underlying connective tissue. It is equivalent to the visceral peritoneum described in gross anatomy. The serosa is the most superficial layer of those parts of the digestive tract that are suspended in the peritoneal cavity. The serosa is continuous with both the *mesentery* and the lining of the abdominal cavity.

Large blood and lymphatic vessels and nerve trunks travel through the serosa (from and to the mesentery) to reach the wall of the digestive tract. Large amounts of the adipose tissue can develop in the connective tissue of the serosa (and in the mesentery).

Parts of the digestive tract do not possess serosa. These include the thoracic part of the esophagus and portions of structures in the abdominal and pelvic cavities that are fixed to the cavity wall — the duodenum, the ascending and descending colon, the rectum, and the anal canal. These structures are attached to the abdominal and pelvic wall by the connective tissue, the adventitia, which blends with the connective tissue of the wall.

Esophagus

Esophagus is a fixed muscular tube that delivers food and liquid from the pharynx to the stomach.

The *mucosa* that lines the length of the esophagus has nonkeratinized

stratified squamous epithelium. In many animals, however, the epithelium is keratinized, reflecting a coarse food diet. In humans, the surface cells may exhibit some keratohyalin granules, but keratinization does not normally occur. The underlying lamina propria is similar to the lamina propria throughout the alimentary tract; diffuse lymphatic tissue is scattered throughout, and lymphatic nodules are present. The deepest layer of the mucosa, the muscularis mucosae, is composed of longitudinally organized smooth muscle that begins near the level of the cricoid cartilage. It is unusually thick in the proximal portion of the esophagus and presumably functions as an aid in swallowing.

The *submucosa* consists of dense irregular connective tissue that contains the larger blood and lymphatic vessels, nerve fibers, and ganglion cells. The nerve fibers and ganglion cells make up the submucosal plexus (Meissner's plexus). The glands are also present. In addition, diffused the lymphatic tissue and lymphatic nodules are present mostly in the upper and lower parts of the esophagus where submucosal glands are more prevalent.

The *muscularis externa* consists of two muscle layers, an inner circular layer and an outer longitudinal layer. It differs from the muscularis externa found in the rest of the digestive tract in that the upper one third is striated muscle, continuation of the muscle of the pharynx. The striated muscle and the smooth muscle bundles are mixed and interwoven in the muscularis externa of the middle third of the esophagus; the muscularis externa of the distal third consists only of smooth muscle, as in the rest of the digestive tract. A nerve plexus, the myenteric plexus (Auerbach's plexus), is present between the outer and inner muscle layers. As in the submucosal plexus (Meissner's plexus), nerves and ganglion cells are present here. This plexus innervates the muscularis externa and produces peristaltic activity.

As noted, the esophagus is fixed to adjoining structures throughout most of its length; thus its outer layer is composed of adventitia. After entering the abdominal cavity, die short remainder of the tube is covered by serosa, the visceral peritoneum.

The *glands* are present in the wall of the esophagus and are of two types. Both secrete mucus to lubricate and protect the luminal wall, but their locations differ.

The *esophageal glands proper* occur in the submucosa. These small compound, tubuloalveolar glands are scattered along the length of the esophagus but are somewhat more concentrated in the upper half. The

excretory duct is composed of stratified squamous epithelium and is usually conspicuous when present in a section, because of its dilated appearance.

The *esophageal cardiac glands* are named for their similarity to the cardiac glands of the stomach and occur in the lamina propria of the mucosa. They are present in the terminal part of the esophagus and frequently, though not consistently, in the beginning portion of the esophagus**.**

The mucus produced by the esophageal glands proper is slightly acidic and serves to lubricate the luminal wall. Because the secretion is relatively viscous, transient cysts often occur in the ducts. The esophageal cardiac glands produce a neutral mucus. Those glands near the stomach tend to protect the esophagus from regurgitated gastric contents. Under certain conditions, however, they are not fully effective, and excessive reflux results in pyrosis, a condition more commonly known as *heartburn.*

Stomach

Stomach is an expanded part of the digestive tube that lies beneath the diaphragm. It receives the bolus of macerated food from the esophagus. Mixing and partial digestion of the food in the stomach by its gastric secretions produce a pulpy fluid mix called chyme. The chyme then passes into the small intestine for further digestion and absorption.

The stomach is divided histologically into three regions on the basis of the type of gland that each contains:

— *cardiac region (cardia),* the part near the esophageal orifice, which contains the *cardiac glands*;

— *pyloric region (pylorus*), the part proximal to the pyloric sphincter, which contains the pyloric glands;

— *fundic region (fundus*), the largest part of the stomach, which is situated between the cardia and pylorus and contains the fundic gastric glands.

The stomach has the same general structural plan throughout, consisting of the mucosa, submucosa, muscularis externa, and serosa. Examination of the inner surface of the empty stomach reveals a number of longitudinal folds or ridges called *rugae*. They are prominent in the narrower regions of the stomach but poorly developed in the upper portion. When the stomach is fully distended, the rugae, composed of the mucosa and underlying submucosa, virtually disappear. The rugae do not alter total surface area; rather, they serve to accommodate expansion and filling of the stomach.

A view of the stomach's surface with a hand lens shows that smaller regions of the mucosa are formed by grooves or shallow trenches that divide the stomach surface into bulging irregular areas called mamillated areas. These grooves provide a slightly increased surface area for secretion.

At higher magnification, numerous openings can be observed in the mucosal surface. These are the gastric pits, or foveolae. The gastric glands open into the bottom of the gastric pits. Surface mucous cells line the inner surface of the stomach and the gastric pits.

The *epithelium* that lines the surface and the gastric pits of the stomach is simple columnar. The columnar cells are designated surface mucous cells. Each cell possesses a large, apical cup of mucinogen granules, creating a glandular sheet of cells. The mucous cup occupies most of the volume of the cell. It typically appears empty in routine H&E sections because the mucinogen is lost in fixation and dehydration. When the mucinogen is preserved by appropriate fixation, however, the granules stain intensely with toluidine blue and with the periodic acid-Schiff (PAS) procedure.

The nucleus and Golgi apparatus of the surface mucous cells are located below the mucous cup. The basal pan of the cell contains small amounts of rough endoplasmic reticulum that may impart a light basophilia to the cytoplasm when observed in well-preserved specimens.

The mucous secretion from the surface mucous cells is described as visible mucus because of its cloudy appearance. It forms a thick, viscous, gel-like coat that adheres to the epithelial surface; thus, it protects against abrasion from rougher components of the chyme. Additionally, its high bicarbonate concentration protects the epithelium from the acidic content of the gastric juice. The bicarbonate that makes the mucus alkaline is secreted by the surface cells but is prevented from mixing rapidly with the contents of the gastric lumen by its containment within the mucus coat.

The lining of the stomach does not function in absorptive capacity. However, some water, salts, and soluble drugs may be absorbed; alcohol and certain drugs, e.g., aspirin, enter the lamina propria by damaging the surface epithelium.

The ***fundic glands***, also called *gastric glands*, produce the gastric juice of the stomach. The fundic glands are simple, branched, tubular glands that extend from the bottom of the gastric pits to the muscularis mucosae. Located between the gastric pit and the gland below is a short segment known as the isthmus, the site of cell replication. Cells destined to become mucous

surface cells migrate upward in the gastric pits to the stomach surface. Other cells migrate downward, maintaining the population of the fundic gland epithelium. Typically, several glands open into a single gastric pit. Each gland has a narrow, relatively long neck segment and a shorter and wider base or fundic segment. The base of the gland usually divides into two and sometimes three branches that become slightly coiled near the muscularis mucosae. The cells of the gastric glands produce gastric juice (about 2 L/day), which contains a variety of substances. In addition to water and electrolytes, gastric juice contains four major components:

1. Hydrochloric acid (HCl) in a concentration ranging from 150 to 160 mmol/L, which gives the gastric juice low pH (<1.0 to 2.0). It is produced by parietal cells and initiates digestion of dietary protein (it promotes acid hydrolysis of substrates). It also converts inactive pepsinogen into the active enzyme pepsin. Because HCl is bacteriostatic, most of the bacteria entering the stomach with the ingested food are destroyed. However, some bacteria can adapt to the low pH of gastric contents. Helicobacter pylory contains large amounts of urease, the enzyme that hydrolizes urea, in its cytoplasm and on its plasma membrane, creating a protective basis "ammonia cloud" around the bacteria and allowing it to survive.

2. Pepsin, a potent proteolytic enzyme. It is converted from pepsinogen produced by the chief cells by HCl with pH lower than 5. Pepsin hydrolyzes proteins into small peptides by splitting interior peptide bonds. Peptides are further digested into amino acids by enzymes in the small intestine.

3. Mucus, an acid-protective coating for the stomach secreted by several types of mucus-producing cells. The mucus and bicarbonates trapped within the mucous layer maintain a neutral pH and contribute to a so-called physiologic gastric mucosa barrier. In addition, mucus serves as a physical barrier between the cells of the gastric mucosa and the ingested material in the lumen of the stomach.

4. Intrinsic factor, glycoprotein that binds to vitamin B12. It is essential for vitamin B12 absorption, which occurs in the distal part of the ileum.

In addition, gastrin and other hormones and hormone-like secretions are produced by enteroendocrine cells in the fundic glands and secreted into the *lamina propria* where they enter the circulation or act locally on other gastric epithelial cells.

The various cells that constitute the gland are:

— mucous neck cells;

— chief cells;
— parietal cells, also called oxyntic cells;
— enteroendocrine cells;
— undifferentiated cells.

Mucous neck cells are localized in the neck region of the gland and are interspersed with parietal cells. The parietal cells are usually interspersed between groups of these cells. The mucous neck cell is much shorter than the surface mucous cell and contains considerably less mucinogen in the apical cytoplasm. Consequently, these cells do not exhibit a prominent mucous cup. Also, the nucleus tends to be spherical compared with the more prominent, elongate nucleus of the surface mucous cell. The mucous neck cells secrete a soluble mucus compared with the insoluble or cloudy mucus produced by the surface mucous cell. Release of mucinogen granules is induced by vagal stimulation; thus, secretion from these cells does not occur in the resting stomach.

Chief cells are located in the deeper part of the fundic glands and are typical protein-secreting cells. The abundant rER in the basal cytoplasm gives this part of the cell a basophilic appearance, whereas the apical cytoplasm is eosinophilic due to the presence of the secretory granules, also called zymogen granules because they contain enzyme precursors. The basophilia, in particular, allows easy identification of these cells in H&E sections. The eosinophilia may be faint or absent when the secretory granules are not adequately preserved. Chief cells secrete pepsinogen and a weak lipase. On contact with the acid gastric juice, pepsinogen is converted to pepsin, a proteolytic enzyme.

Parietal (oxyntic) cells tend to be most numerous in the upper and middle portions of the neck, among the mucous neck cells, and in deeper part of the gland. They are large cells, sometimes binucleate, and appear somewhat triangular in sections, with the apex directed toward lumen of the gland and the base resting on the basal lamina. The nucleus is spherical, and the cytoplasm stains eosin and other acidic dyes. Their size and distinctive staining characteristics allow them to be easily distinguished from other cells in the fundic glands. When examined with the transmission electron miscope (TEM), parietal cells are seen to have an extensive intracellular canalicular system that communicates with the lumen of the gland. Numerous microvilli project from the surface of the canaliculi, and an elaborate tubulovesicular membrane system is present in the cytoplasm adjacent to

the canaliculi. In an actively secreting cell, the number of microvilli in the canaliculi increases, and the tubulovesicular system reduces significantly or disappears.The membranes of the tubuluvesicular system serve as reservoir of plasma membrane containing active proton pumps. Numerous mitochondria with complex cristae and many matrix granules supply the high levels of energy necessary for acid secretion.

Parietal cells have three different types of membrane receptors for substances that activate HCl secretion: gastrin, histamine H_2, and acetylcholine M_3 receptors.

In human, the intrinsic factor is secreted by parietal cells. The intrinsic factor is a glycoprotein that complexes with vitamin B12 in the stomach and duodenum, a step necessary for subsequent absorption of the vitamin in the ileum.

Enteroendocrine cells are found at every level of the fundic gland, although they tend to be somewhat more prevalent in the base. They are small cells that rest on the basal lamina and do not always reach the lumen secreting their products into the lamina propria.

The names given to the enteroendocrine cells in the older literature were based on their staining with salts of silver and chromium, i.e., enterochromaffin cells, argentaffin cells, and argyrophil cells. Such cells are currently identified and characterized by immunochemical staining for more than 20 peptide and polypeptide hormones and hormone-like regulating agents. The most abundant are: gastrin producing G-cells stimulate gastrine acid and pepsinogen secretion; EC-cells produce serotonin, melatonin, motilin, substances regulating secretory and motor activity.

Cardiac glands are limited to a narrow region of the stomach (the cardia) that surrounds the esophageal orifice. Their secretion, in combination with that of the esophageal cardiac glands, contributes to the gastric juice and also helps protecting the esophageal epithelium against gastric reflux. The glands are tubular, somewhat tortuous, and occasionally branched. They are composed mainly of mucus-secreting cells, with occasional interspersed enteroendocrine cells. The mucus-secreting cells are similar in appearance to the cells of the esophageal cardiac glands. They have a flattened basal nucleus, and the apical cytoplasm is typically filled with mucin granules. A short duct segment containing columnar cells with elongate nuclei is interposed between the secretory portion of the gland and the shallow pits into which the glands secrete. The duct segment is the site at which the surface mucous cells and the gland cells are produced.

Pyloric glands are located in the pyloric antrum. They are brunched, coiled, tubular glands. The lumen is relatively wide, and the secretory cells are similar in appearence to the surface mucous cells, suggesting a relatively viscous secretion. The enteroendocrine cells are found interspersed within the gland epithelium among with the occasional parietal cells.

Surface mucous cells are renewed approximately every 3 to 5 days. The cells of the fundic glands have a relatively long lifespan. The parietal cells have the longest lifespan, approximately 150 to 200 days. The chief and enteroendocrine cells are estimated to live for about 60 to 90 days before they are replaced by new cells migrating downwards from the isthmus. The mucous neck cell, in contrast, has a much shorter lifespan, approximately 6 days.

The **lamina propria** of the stomach is relatively scant and restricted to the limited spaces surrounding the gastric pits and glands. The stroma is composed largely of reticular fibers with associated fibroblasts and smooth muscle cells. Other components include cells of the immune system, namely, lymphocytes, plasma cells, macrophages, and some eosinophils. When inflammation occurs, as is often the case, neutrophils may also be prominent. Occasional lymphatic nodules are also present, usually intruding partially into the muscularis mucosae.

The **muscularis mucosae** is composed of two relatively thin layers, usually arranged as an inner circular and outer longitudinal layer. In some regions a third layer may be present; its orientation tends to be in a circular pattern. Thin strands of smooth muscle cells extend toward the surface in the lamina propria from the inner layer of the muscularis mucosae. These smooth muscle cells in the lamina propria are thought to help outflow of the gastric gland secretions.

The **submucosa** is composed of a dense connective tissue containing variable amounts of adipose tissue and blood vessels, as well as the nerve fibers and ganglion cells that compose the submucosal (Meissner's) plexus. The latter innervates the vessels of the submucosa and the smooth muscle of the muscularis mucosae.

The **muscularis externa** of the stomach is traditionally described as consisting of an outer longitudinal layer, a middle circular layer, and an inner oblique layer. This description is somewhat misleading, as distinct layers may be difficult to discern. As with other hollow, spheroidal organs (e.g., the gallbladder, the urinary bladder, and the uterus), the smooth mus-

cle of the muscularis externa of the stomach is somewhat more randomly oriented than the term "layer" implies. Moreover, the longitudinal layer is absent from much of the anterior and posterior stomach surfaces, and the circular layer is poorly developed in the periesophageal region. The arrangement of the muscle layers is functionally important, as it relates to its role in mixing chyme during the digestive process as well as to its ability to force the partially digested contents into the small intestine. Groups of ganglion cells and bundles of unmyelinated nerve fibers are present between the muscle layers. Collectively, they represent the myenteric (Auerbach's) plexus, which provides innervation of the muscle layers.

The **serosa** of the stomach is as described above for the alimentary canal in general. It is continuous with the parietal peritoneum of the abdominal cavity via the greater omentum and with visceral peritoneum of the liver at the lesser omentum. Otherwise, it exhibits no special features.

Small Intestine

Small intestine is the longest component of the digestive tract, measuring over 6 m, and is divided into three anatomic portions:

Duodenum (25 cm long) is the first, shortest, and widest part of the small intestine. It begins at the pylorus of the stomach and ends at the duodenojejunal junction.

Jejunum (2.5 m long) begins at the duodenojejunal junction and constitutes the upper two fifths of the small intestine. It gradually changes its morphologic characteristics to become the ileum.

Ileum (3.5 m long) is a continuation of the jejunum and constitutes the lower three fifths of the small intestine. It ends at the ileocecal junction, the union of the distal ileum and cecum.

The small intestine is the principal site for the digestion of food and absorption of the products of digestion. Chyme from the stomach enters the duodenum, where enzymes from the pancreas and bile from the liver are also delivered to continue the solubilization and digestion process. Enzymes, particularly disaccharidases and dipeptidases, are also located in the glycocalyx of the microvilli of the enterocytes, the intestinal absorptive cells. These enzymes contribute to the digestive process by completing the breakdown of most sugars and proteins to monosaccharides and amino acids, which are then absorbed. Water and electrolytes that reach the small intestine with the chyme and pancreatic and

hepatic secretions are also reabsorbed in the small intestine, particularly in the distal portion.

The absorptive surface area of the small intestine is amplified by tissue and cell specializations of the submucosa and mucosa.

Plicae circulares (circular folds), also known as Kerckring's valves, are permanent transverse folds that contain a core of submucosa. Each circular fold is circularly arranged and extends about one half to two thirds of the way around the circumference of the lumen. The folds begin to appear about 5 to 6 cm beyond the pylorus. They are most numerous in the distal part of the duodenum and the beginning of the jejunum and become reduced in size and frequency in the middle of the ileum.

Villi are unique, finger-like and leaf-like projections of the mucosa that extend from the theoretical mucosal surface for 0.5 to 1.5 mm into the lumen. They completely cover the surface of the small intestine, giving it a velvety appearance when viewed with the naked eye. They consist of a core of loose connective tissue covered by a simple columnar epithelium. The core of the villus is an extension of the lamina propria, which contains numerous fibroblasts, smooth muscle cells, lymphocytes, plasma cells, eosinophils, macrophages, and a network of fenestrated blood capillaries located just beneath the epithelial basal lamina. In addition, the lamina propria of the villus contains a central, blind-ending lymphatic capillary, the lacteal. Smooth muscle cells derived from the muscularis mucosae extend into the villus and accompany the lacteal. These smooth muscle cells may account for reports that villi contract and shorten intermittently, an action that may force lymph from the lacteal into the lymphatic vessel network that surrounds the muscularis mucosae.

Microvilli of the enterocytes provide the major amplification of the luminal surface. Each cell possesses several thousand closely packed microvilli giving the apical region of the cell a striated appearance, the so-called striated border.

The ***intestinal glands*****,** or crypts of Lieberkuhn, are simple tubular structures that extend from the muscularis mucosae through the thickness of the lamina propria, where they open onto the luminal surface of the intestine at the base of the villi. The glands are composed of a simple columnar epithelium that is continuous with the epithelium of the villi.

As in the stomach, the *lamina propria* surrounds the intestinal glands and contains numerous cells of the immune system (lymphocytes, plasma

cells, mast cells, macrophages, and eosinophils), particularly in the villi. The lamina propria also contains numerous nodules of lymphatic tissue that represent a major component of the GALT. The nodules are particularly large and numerous in the ileum, where they are preferentially located on the side of the intestine opposite the mesenteric attachment. These nodular aggregations are known as aggregated nodules or Peyers patches. In gross specimens, they appear as aggregates of white specks. The *muscularis mucosae* consists of two thin layers of the smooth muscle cells, an inner circular and an outer longitudinal layer. As noted above, strands of smooth muscle cells extend from the muscularis mucosae into the lamina propria of the villi.

The mature cells of the intestinal epithelium are found both in the intestinal glands and on the surface of the villi. They include:

— enterocytes, whose primary function is absorption;

— goblet cells, unicellular mucin-secreting glands;

— paneth cells, whose primary function is to maintain mucosal innate immunity by secreting antimicrobial substances;

— enteroendocrine cells, which produce various paracrine and endocrine hormones;

— M-cells (microfold cells), modified enterocytes that cover enlarged lymphatic nodules in the lamina propria.

Enterocytes are tall columnar cells with a basally positioned nucleus. They are absorptive cells specialized for the transport of substances from the lumen of the intestine to the circulatory system. Microvilli increase the apical surface area as much as 600 times; they are recognized in the light microscope as forming a striated border on the luminal surface. Each microvillus has a core of vertically oriented actin microfilaments that are anchored to villin located in the tip of the microvillus and that also attach to the microvillus plasma membrane by myosin molecules. The actin microfilaments extend into the apical cytoplasm and insert into the terminal web, a network of horizontally oriented contractile microfilaments that form a layer in the most apical cytoplasm and attach to the intracellular density associated with the zonula adherens. Contraction of the terminal web causes the microvilli to spread apart, thus increasing the space between them to allow more surface area exposure for absorption to take place. In addition, contraction of the terminal web may aid in “closing” the holes left in the epithelial sheet by exfoliation of aging cells. Entero-

cytes are bound to one another and to the goblet, enteroendocrine, and other cells of the epithelium by junctional complexes. The tight junctions between the intestinal lumen and the connective tissue compartment of the body establish a barrier and allow selective retention or substances absorbed by the enterocytes. Although the "tightness" of these junctions can vary.

The lateral cell surface of the enterocytes exhibits elaborate, flattened cytoplasmic processes (plicae) that interdigitate with those of adjacent cells. These folds increase the lateral surface area of the cell, thus increasing the amount of plasma membrane containing transport enzymes. During active absorption, especially of solutes, water, and lipids, these lateral plications separate, enlarging the intercellular compartment. The increased hydrostatic pressure from the accumulated solutes and solvents causes a directional flow through the basal lamina into the lamina propria.

In addition to the membrane specializations associated with absorption and transport, the enterocyte cytoplasm is also specialized for these functions. Elongated mitochondria that provide energy for transport are concentrated in the apical cytoplasm between the terminal web and the nucleus. Tubules and cisternae of the smooth endoplasmic reticulum, which are involved in the absorption of fatty acids and glycerol and in the resynthesis of neutral fat, are found in the apical cytoplasm beneath the terminal web.

Enterocytes are also secretory cells, producing enzymes needed for terminal digestion and absorption as well as secretion of water and electrolytes.The secretory function of enterocytes, primarily the synthesis of glycoprotein enzymes that will be inserted into the apical plasma membrane, is represented morphologically by aligned stacks of Golgi cisternae in the immediate supranuclear region and by the presence of free ribosomes and rER lateral to the Golgi apparatus. Small secretory vesicles containing glycoproteins destined for the cell surface are located in the apical cytoplasm, just below the terminal web, and along the lateral plasma membrane.

The small intestine also secretes water and electrolytes. This activity occurs mainly in the cells within the intestinal glands. The secretion that occurs in these glands is thought to assist the process of digestion and absorption by maintaining an appropriate liquid state of the intestinal chyme. Under normal conditions, the absorption of fluid by the villus enterocyte is balanced by the secretion of fluid by the gland enterocyte.

Goblet cells represent unicellular glands that are interspersed among the other cells of the intestinal epithelium and produce mucus. In the small intestine, goblet cells increase in number from the duodenum to the terminal part of the ileum. With the apex of the cell containing a large accumulation of mucinogen granules, the basal portion of the cell resembles a narrow stem. This basal portion is intensely basophilic in histologic preparations because it is occupied by a heterochromatic nucleus, extensive rER, and free ribosomes. Mitochondria are also concentrated in the basal cytoplasm. The characteristic shape, with the apical accumulation of granules and the narrow basal stem, is responsible for the name of the cell, as in a glass "goblet." An extensive array of flattened Golgi cisternae forms a wide cup around the newly formed mucinogen granules adjacent to the basal part of the cell. The microvilli of goblet cells are restricted to a thin rim of cytoplasm (the theca) that surrounds the apical-lateral portion of the mucinogen granules. Microvilli are more obvious on the immature goblet cells in the deep one half of the intestinal gland.

Paneth cells are found in the bases of the intestinal glands. (They are also occasionally found in the normal colon in small numbers; their number may increase in certain pathologic conditions.) They have a basophilic basal cytoplasm, a supranuclear Golgi apparatus, and large, intensely acidophilic, refractile apical secretory granules. These granules allow their easy identification in routine histologic sections. These secretory granules contain the antibacterial enzyme lysozyme, α-defensins, other glycoproteins, and arginine-rich protein, and zinc. Lysozyme digests the cell walls of the certain groups of bacteria. α-Defensins are homologs of peptides that function as mediators in cytotoxic $CD8^+$ T lymphocytes. This antibacterial action and their ability to phagocyte certain bacteria and protozoa suggest that Paneth cells play a role in regulation of normal bacterial flora of the small intestine.

Enteroendocrine cells in the small intestine produce nearly all of the same peptide hormones as they do in the stomach.They are concentrated in the lower portion of the intestinal gland but migrate slowly and can be found at all levels of each villus. Cholecystokinin (CCK), gastric inhibitory peptide (GIP) and motilin are most active regulators of gastrointestinal physiology that are released in this portion of the gut. CCK and secretin increase pancreatic and gallbladder activity and inhibit gastric secretory function and motility. GIP stimulates insulin release in the pancreas, and motilin initiates

gastric and intestinal motility. Although other peptides produced by enteroendocrine cells have been isolated, they are not considered hormones and are therefore called candidate hormones. Enteroendocrine cells also produce at least two hormones, somatostatin and histamine, which act as paracrine hormones, i.e., hormones that have a local effect and do not circulate in the bloodstream. In addition, several peptides are secreted by the nerve cells located in the submucosa and muscularis externa. These peptides, called neurocrine hormones, are represented by vasoactive inhibitory peptide (VIP), bombesin, and the enkephalins.

M-cells are epithelial cells that overlie Peyer's patches and other large lymphatic nodules; they differ significantly from the surrounding intestinal epithelial cells. M-cells have microfolds rather than microvilli on their apical surface, and they take up microorganisms and macromolecules from the lumen in endocytotic vesicles. The vesicles are transported to the basolateral membrane where they discharge their contents into the epithelial intercellular space in the vicinity of $CD4^+$ T-lymphocytes. Thus, substances that gain access to the body from the intestinal lumen via M-cells come into contact with cells of the immune system as they reach the basolateral surface. Antigens that reach lymphocytes in this manner stimulate a response in the GALT.

Intermediate cells constitute most of the cells in the lower half of the intestinal gland. These cells are still capable of cell division and usually undergo one or two divisions before they become committed to differentiation into either absorptive or goblet cells. These cells have short, irregular microvilli with long core filaments extending deep into the apical cytoplasm and numerous macular (desmosomal) junctions with adjacent cells. Small mucinlike secretory granules form a column in the center of the supranuclear cytoplasm.

All of the mature cells of the intestinal epithelium are derived from a single stem cell population. Stem cells are located in the base of the intestinal gland. The zone of cell replication is restricted to the lower half of the gland. A cell destined to become a goblet cell or absorptive cell usually undergoes several additional divisions after it leaves the pool of stem cells. The epithelial cells migrate upward in the intestinal gland onto the villus and are shed at the tip of the villus. Autoradiographic studies have shown that the renewal time for absorptive and goblet cells in the human small intestine is 5 to 6 days.

Enteroendocrine cells and Paneth cells are also derived from the stem cells at the base of the intestinal gland. Enteroendocrine cells appear to divide only once before differentiating. They migrate with the absorptive and goblet cells but at a slower rate. Paneth cells do not migrate; they remain in the base of the intestinal gland near the stem cells from which they are derived. They live for approximately 4 weeks and are then replaced by differentiation of a nearby "committed" cell in the intestinal gland. Cells that are recognizable as Paneth cells no longer divide.

GALT is prominent in the lamina propria of the small intestine. As noted above, the lamina propria of the digestive tract is heavily populated with elements of the immune system: approximately one fourth of the mucosa consists of loosely organized layer of lymphatic nodules, lymphocytes macrophages, plasma cells, and eosinophils. Lymphocytes are also located between epithelial cells. This GALT serves as an immunologic barrier throughout the length of the gastrointestinal tract.

The ***submucosa*** consists of a dense connective tissue and localized sites that contain aggregates of adipose cells. A conspicuous feature in the duodenum is the presence of submucosal glands, also called Brunner's glands.The branched, tubular submucosal glands of the duodenum have secretory cells with characteristics of both zymogen- and mucus-secreting cells. The secretion of these glands has a pH of 8.1 to 9.3 and contains neutral and alkaline glycoproteins and bicarbon ate ions. This highly alkaline secretion probably serves to protect the proximal small intestine by neutralizing the acid-containing chyme delivered to it. It also brings the intestinal contents close to the optimal pH for the pancreatic enzymes that are also delivered to the duodenum.

Tne ***muscularis externa*** consists of an inner layer of circularly arranged smooth muscle cells and an outer layer of longitudinally arranged smooth muscle cells. The main components of the myenteric plexus are located between these two muscle layers. Two kinds of muscular contraction occur in the small intestine. Local contractions displace intestinal contents both proximally and distally, this type of contraction called segmentation. These contractions primarily involve the circular muscle layer. They serve to circulate chyme locally, mixing it with digestive juice and moving it into contact with the mucosa for absorption. Peristalsis, the second type of contraction, involves coordinated action of both circular and longitudinal muscle layers and moves the intestinal contents distally.

The ***serosa*** of the parts of the small intestine that are located intraperitoneally in the abdominal cavity corresponds to the general description.

Large Intestine

Large intestine comprises the cecum with its projecting the vermiform appendix, the colon, the rectum and the anal canal. The colon is further subdivided into the basis of its anatomic location into the ascending colon, transverse colon, descending colon, and sigmoid colon. The four layers characteristic of the alimentary canal are present throughout. However, several distinctive features exist at the gross level:

1. Except for the rectum, the anal canal, and the vermiform appendix, the outer longitudinal layer of the muscularis externa exhibits three thickened, equally spaced bands known as the teniae coli.

2. The external surface of the cecum and colon exhibits sacculations known as haustra that are visible between the teniae. The mucosa has a "smooth" surface; neither plicae circulares nor villi are present.

3. Small fatty projections of the serosa known as omental appendices are visible on the outer intestinal surface.

The **mucosa** of the large intestine contains numerous straight tubular intestinal glands (crypts of Lieberkuhn) that extend through the full thickness of the mucosa. The glands consist of simple columnar epithelium as does the intestinal surface from which they invaginate. Examination of the luminal surface of the large intestine at the microscopic level reveals the openings of the glands, which are arranged in an orderly pattern.

The principal functions of the large intestine are reabsorption of electrolytes and water and elimination of undigested food and waste

The primary function of the columnar absorptive cells is absorption of water and electrolytes. The morphology of absorptive cells is essentially identical to that of the enterocytes of the small intestine. Reabsorption is accomplished by the same Na^+/K^+-activated ATPase-driven transport system as described for the small intestine. Elimination of semisolid to solid waste materials is facilitated by the large amounts of mucus secreted by the numerous goblet cells of the intestinal glands. Goblet cells are more numerous in the large intestine than in the small intestine. They produce mucin that is secreted continuously to lubricate the bowel, facilitating the passage of the increasingly solid contents.

The mucosal epithelium of the large intestine contains the same cell types as the small intestine except Paneth cells, which are normally absent in humans.

Columnar absorptive cells predominate (4:1) over goblet cells in most of the colon, although this is not always apparent in histologic sections. The ratio decreases, however, approaching 1:1, near the rectum where the number of goblet cells increases. Although the absorptive cells secrete glycocalyx at a rapid rate (turnover time is 16 to 24 hrs in humans), this layer has not been shown to contain digestive enzymes in the colon. As in the small intestine, however, Na^+/K^+-ATPase is abundant and is localized in the lateral plasma membranes of the absorptive cells. The intercellular space is often dilated, indicating active transport of fluid.

Goblet cells may mature deep in the intestinal gland, even in the replicative zone. They secrete mucus continuously, even to the point where they reach the luminal surface. Here, at the surface, the secretion rate exceeds the synthesis rate, and "exhausted" goblet cells appear in the epithelium. These cells are tall and thin and have a small number of mucinogen granules in the central apical cytoplasm, an infrequently observed cell type. The caveolated "tuft" cell has also been described in the colonic epithelium; however, this cell may be a form of exhausted goblet cell.

As in the small intestine, all of the mucosal epithelial cells of the large intestine arise from stem cells located in the bottom of the intestinal gland. The lower third of the gland constitutes the normal replicative zone where newly generated cells undergo two to three more divisions as they begin their migration up to the luminal surface where they shed about five days later. The intermediate cell types found in the lower third of the intestinal gland are identical to those seen in the small intestine.

The turnover times of the epithelial cells of the large intestine are similar to those of the small intestine, i.e., about 6 days for absorptive cells and goblet cells and 4 weeks — for enteroendocrine cells. Senile epithelial cells that reach the mucosal surface are shed into the lumen at the midpoint between two adjacent intestinal glands.

Although the ***lamina propria*** of the large intestine contains the same basic components as the rest of the digestive tract, it demonstrates some additional structural features and greater development of some others. These include:

1. The collagen table, a thick layer of collagen and proteoglycans that lies between the basal lamina of the epithelium and that of the fenestrated

absorptive venous capillaries. This layer is as much as 5 μm thick in the normal human colon and can be up to 3 times that thickness in human hyperplastic colonic polyps. The collagen table participates in regulation of water and electrolyte transport from the intercellular compartment of the epithelium to the vascular compartment.

2. Well-developed GALT, which is continuous with that of the terminal ileum. In the large intestine GALT is more extensively developed; large lymphatic nodules distort the regular spacing of the intestinal glands and extend into the submucosa. The extensive development of the immune system in the colon probably reflects the large number and variety of microorganisms and noxious end products of metabolism normally present in the lumen.

3. A well-developed pericryptal fibroblast sheath, which constitutes a fibroblast population of regularly replicating cells. They divide immediately beneath the base of the intestinal gland, adjacent to the stem cells found in the epithelium (in both the large and small intestines). The fibroblasts then differentiate and migrate upward in parallel and synchrony with the epithelial cells. Although the ultimate fate of the pericryptal fibroblast is unknown, most of these cells, after they reach the level of the luminal surface, take on the morphologic and histochemical characteristics of macrophages. Some evidence suggests that the macrophages of the core of the lamina propria in the large intestine may arise as a terminal differentiation of the pericryptal fibroblasts.

4. Absence of lymphatic vessels in the lamina propria. There are no lymphatic vessels in the core of the lamina propria between the intestinal glands. Lymphatic vessels form a network around the muscularis mucosae, as they do in the small intestine, but no vessels or associated smooth muscle cells extend toward the free surface from that layer. The absence of lymphatic vessels from the lamina propria is important to understanding the slow rate of metastasis from certain colon cancers. Cancers that develop in large adenomatous colonic polyps may grow extensively within the epithelium and lamina propria before they even have access to the lymphatic vessels at the level of the muscularis mucosae. Lymphatic vessels are found in the submucosa and as a network around the muscularis externa.

As noted, in the cecum and colon (the ascending, transverse, descending and sigmoid colon), the outer layer of the ***muscularis externa*** is, in part, condensed into prominent longitudinal bands of muscle, called teniae coli,

which may be seen at the gross level. Between these bands, the longitudinal layer forms an extremely thin sheet. In the rectum, anal canal, and vermiform appendix, the outer longitudinal layer of smooth muscle is a uniformly thick layer, as in the small intestine. Bundles of muscle from the teniae coli penetrate the inner, circular layer of the muscle at irregular intervals along the length and circumference of the colon. These apparent discontinuities in the muscularis externa allow segments of the colon to contract independently, leading to the formation of saccules (haustra) in the colon wall. The muscularis externa of the large intestine produces two major types of contraction: segmentation and peristaltsis. Segmentation is local and does not result in the propulsion of contents. Peristalsis results in the distal mass movement of the colonic contents. Mass peristaltic movements occur infrequently; in healthy persons, they usually occur once a day to empty the distal colon.

The ***submucosa*** of the large intestine corresponds to the general description already given. Where the large intestine is directly in contact with other structures (as on much of its posterior surface), its outer layer is the adventitia; elsewhere, the outer layer is a typical serosa.

Cecum and appendix. The cecum forms a blind pouch just distal to the ileocecal valve; the appendix is a thin, fingerlike extension of this pouch. The histology of the cecum closely resembles that of the rest of the colon; the appendix differs from it in having a uniform layer of longitudinal muscle in the muscularis externa. The most conspicuous feature of the appendix is the large number of lymphatic nodules that extend into the submucosa. In many adults, the normal structure of the appendix is lost, and the appendage is filled with fibrous scar tissue.

Rectum and anal canal. The rectum is the dilated distal portion of the alimentary canal. Its upper part is distinguished from the rest of the large intestine by the presence of folds called transverse rectal folds. The mucosa of the rectum is similar to that of the rest of the distal colon, having straight, tubular intestinal glands with many goblet cells.

The most distal portion of the alimentary canal is the anal canal. It has an average length of 4 cm and extends from the upper aspect of the pelvic diaphragm to the anus. The upper part of the anal canal has longitudinal folds called anal columns. Depressions between the anal columns are called anal sinuses. The anal canal is divided into three zones according to the character of the epithelial lining:

1. Colorectal zone, which is found in the upper third of the anal canal and contains simple columnar epithelium with characteristics identical to that in the rectum.

2. Anal transitional zone (ATZ), which occupies the middle third of the anal canal. It represents a transition between the simple columnar epithelium of the rectal mucosa and the stratified squamous epithelium of the perianal skin. The ATZ possesses a stratified columnar epithelium interposed between the simple columnar epithelium and the stratified squamous epithelium, which extends to the cutaneous zone of the anal canal.

3. Squamous zone, which is found in the lower third of the anal canal. This zone is lined with stratified squamous epithelium that is continuous with the perianal skin.

In the anal canal, the anal glands extend into the submucosa and even into the muscularis externa. These branched, straight tubular glands secrete mucus onto the anal surface through the ducts lined with stratified columnar epithelium. Sometimes the anal glands are surrounded by diffuse lymphatic tissue. They often lead to the formation of pathologic fistulas (a false opening between the anal canal and the perianal skin).

The large apocrine glands, the circumanal glands are found in the skin surrounding the anal orifice. In some animals, the secretion of these glands acts as a sex attractant. Hair follicles and sebaceous glands are also found at this site.

The submucosa of the anal columns contains the terminal ramifications of the superior rectal artery and the rectal venous plexus. Enlargements of these submucosal veins constitute internal hemorrhoids, which are related to elevated venous pressure in the portal circulation (portal hypertension). There are no teniae coli at the level of the rectum; the longitudinal layer of the muscularis externa forms a uniform sheet. The muscularis mucosa disappears at about the level of the anal transitional zone (ATZ), where the circular layer of the muscularis externa thickens to form the internal anal sphincter. The external anal sphincter is formed by striated muscle of the pelvic floor.

Lecture 16

GLANDS OF THE DIGESTIVE SYSTEM

LIVER

The liver is the largest mass of glandular tissue in the body and the largest internal organ. The liver is enclosed in a capsule of fibrous connective tissue; a serous covering (visceral peritoneum) surrounds the capsule, except where the liver adheres directly to the diaphragm or the organs.

Liver Physiology

Many circulating plasma proteins are produced and secreted by the liver. The liver plays an important role in the uptake, storage, and distribution of both nutrients and vitamins from the bloodstream. It also maintains the blood glucose level and regulates circulating levels of very low density lipoproteins (VLDLs). In addition, the liver degrades or conjugates numerous toxic substances and drugs, but it can be overwhelmed by such substances and damaged. The liver is also an exocrine organ; it produces bile secretion that contains bile salts, phospholipids, and cholesterol. Finally, the liver performs important endocrine-like functions.

The liver produces most of the body's circulating plasma proteins:

— albumins, which are involved in regulating plasma volume and tissue fluid balance by maintaining the plasma colloid osmotic pressure;

— lipoproteins, in particular, VLDLs. The liver synthesizes most VLDLs, which participate in the transport of triglycerides from the liver to other organs. The liver also produces small amounts of other plasma lipoproteins, such as low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs). LDLs transport cholesterol esters from the liver to other

tissues. HPLs remove cholesterol from the peripheral tissues and transport it to the liver;

— glycoproteins, which include proteins involved in iron transport such as haptoglobin, transferrin, and hemopexin;

— prothrombin and fibrinogen, important components of the blood clotting cascade;

— nonimmune α- and ß-globulins, which also help maintain plasma colloid osmotic pressure and serve as carrier proteins for various substances.

Several vitamins are taken up from the bloodstream and are then stored or biochemically modified by the liver.

The liver plays a major role in the uptake, storage, and maintenance of circulating levels of **vitamin A** (retinol), an important vitamin of vision Retionol-binding protein is also synthesized by the liver. Night blindness and multiple skin disorders are related to vitamin A deficiency.

Vitamin D (cholecalciferol), an important vitamin in calcium and phosphate metabolism. Vitamin D is acquired from dietary vitamin D3 and is also produced in the skin during exposure to ultraviolet light by conversion of 7-dehydrocholesterol. Unlike vitamin A, vitamin D is not stored in the liver but is distributed to skeletal muscles and adipose tissue. The liver plays an important role in vitamin D metabolism by converting vitamin D; to 25-hydroxycholecalciferol, the predominant form of circulating vitamin D. Further conversion takes place in the kidney to 1.25-hydroxycholecalciferol, which is 10 times more active than vitamin D. Vitamin D5 is essential for development and growth of the skeletal system and teeth. Deficiency of vitamin D is associated with rickets and disorders of bone mineralization.

Vitamin K is important in hepatic synthesis of prothrombin and several other clotting factors. Like vitamin D, it is derived from two sources: dietary vitamin K and synthesis in the small intestine by intestinal bacterial flora. Vitamin K is transported to the liver with chylomicrons, where it is rapidly absorbed, partially used, and then partially secreted with the VLDL fraction. Vitamin K deficiency is associated with hypoprothrombinemia and bleeding disorders.

In addition, the liver functions in the storage, metabolism, and homeostasis of iron. It synthesizes almost all of the proteins involved in iron transport and metabolism, including transferrin, haptoglobin, and hemopexin. Recent studies indicate that hepatocytes are the major sites of long-term storage of iron. Iron overload (as in multiple blood transfusions) may lead

to hemochromatosis, a form of liver damage characterized by excessive amounts of hemosiderin in hepatocytes.

Hepatocytes are involved in degradation of drugs, toxins, and other proteins foreign to the body (xenobiotics). Many drugs and toxins are not hydrophilic; therefore, they cannot be eliminated effectively from the circulation by the kidneys. The liver converts these substances into more soluble forms.

The liver is involved in many other important metabolic pathways. It is important in carbohydrate metabolism as it maintains an adequate supply of nutrients for cell processes. In glucose metabolism, the liver phosphorylates absorbed glucose from the gastrointestinal tract to glucose-6-phosphate. Depending on energy requirements, glucose-6-phosphate is either stored in the liver in the form of glycogen or used in the glycolytic pathways. During fasting, glycogen is broken down by glycogenolysis, and glucose is released into the bloodstream. In addition, the liver functions in lipid metabolism. Fatty acids derived from plasma are consumed by hepatocytes using ß-oxidation to provide energy. The liver also produces ketone bodies that are used as a fuel by other organs (the liver cannot use them as an energy source). The involvement in cholesterol metabolism (synthesis and uptake from the blood) is also an important function of the liver. Cholesterol is used in formation of bile salts, synthesis of VLDLs and biosynthesis of organelles. The liver synthesizes most or the urea in the body from ammonium ions derived from protein and nucleic acid degradation. Finally, the liver is involved in the synthesis and conversion of nonessential amino acids.

Bile production is an exocrine function of the liver. The endocrine-like functions of the liver are represented by its ability to modify the structure and function of many hormones: thyroxine is converted to biologically active triiodothyronine; growth hormone action is modified by liver-produced growth hormone-releasing hormone; insulin and glucagon are degraded mostly in the liver (and the kidney); vitamin D is converted to 25-hydrocalciferol.

The liver has a unique dual **blood supply** consisting of a venous (portal) supply via the hepatic portal vein and the arterial supply via the hepatic artery. Both vessels enter the liver at hilum or porta hepatis, the same site of which the common bile duct, carring the bile secreted by the liver, and the lymphatic vessels leave the liver.

The liver is a unique among organs because it recieves its major blood supply (about 75%) from the hepatic portal vein, which carries largely depleted of oxygen venous blood that is delivered from the digestive tract and

the major abdominal organs, such as the pancreas and spleen. The portal blood contains nutrients and toxic materials absorbed in the intestine, blood cells and breakdown products of blood cells from the spleen, endocrine secretions of the pancreas and enteroendocrine cells of the gastrointestinal tract. Thus, the liver stands directly on the pathway of blood vessels that convey substances absorbed from the digestive tract. While the liver is the first organ to receive metabolic substrates and nutrients, it is also the first exposed to toxic substances that have been absorbed.

The hepatic artery, a branch of the celiac trunk, carries oxygenated blood to the liver, providing the remaining 25% of its blood supply. Because blood from the two sources is mixed just before it perfuses the hepatocytes of the liver parenchyma, the liver cells are never exposed to fully oxygenated blood.

Within the liver, the distributing branches of the portal vein and hepatic artery, which supply the sinusoidal capillaries (sinusoids) that wash the hepatocytes, and the draining branches of the bile duct system, which lead to the common hepatic duct, course together in a relationship termed the portal triad. Although a convenient term, it is a misnomer because one or more vessels of the lymphatic drainage system of the liver always travel with the vein, the artery, and the bile duct.

The sinusoids are in a close contact with the hepatocytes and provide for the exchange of substances between the blood and liver cells. The sinusoids lead to the central vein that in turn empties into the sublobular veins. The blood leaves the liver through the hepatic veins, which empty into the inferior vena cava.

Structural Organization of the Liver

Structural organization of the liver consists of:

1. Parenchyma, consisting of organized plates of hepatocytes, which in the adult are normally one cell thick and are separated by sinusoidal capillaries. In young individuals up to 6 years of age, the liver cells are arranged in plates two cells thick.

2. Connective tissue stroma that is continuous with the fibrous Glisson's capsule. Blood vessels, nerves, lymphatic vessels, and bile ducts travel within the connective tissue stroma.

3. Sinusoidal capillaries (sinusoids), the vascular channels between the plates of hepatocytes.

4. Pensinusoidal spaces (Disse's spaces), which lie between the sinusoidal endothelium and the hepatocytes.

Liver Lobules

There are three ways to describe the structure of the liver in terms of a functional unit: the classic lobule, the portal lobule, and the liver acinus. The classic lobule is the traditional way to describe the organization of the liver parenhyma and it is relatively easy to visualize. It is based on the distribution of the branches of the portal vein and hepatic artery within the organ and the pathway that blood from them follows as it ultimately perfuses the liver cells.

The ***classic hepatic lobule*** is a roughly hexagonal mass of tissue. It consists of stacks of anastomosing plates of hepatocytes, one cell thick, separated by the anastomosing system of sinusoids that perfuse the cells with the mixed portal and arterial blood. Each lobule measures about 2.0× 0.7 mm. At the center of the lobule there is a relatively large venule, the terminal hepatic venule (central vein), into which the sinusoids drain. The plates of cells radiate from the central vein to the periphery of the lobule, as do the sinusoids. At the angles of the hexagon are the portal areas (portal canals), loose stromal connective tissue characterized by the presence of the portal triads. This connective tissue is ultimately continuous with the fibrous capsule of the liver. The portal canal is bordered by the outermost hepatocytes of the lobule. At the edges of the portal canal, between the connective tissue stroma and the hepatocytes, is a small space called the *Mall's space*. This space is thought to be one of the sites where lymph originates in the liver. The classic lobule is easily recognized because the portal areas are connected by relatively thick layers of the connective tissue. In humans, however, there is normally very little interlobular connective tissue, and it is necessary, when examining histologic sections of the liver, to draw imaginary lines between portal areas surrounding the central vein to get some sense of the size of the classic lobule.

The **portal lobule** emphasizes the exocrine functions of the liver. The major exocrine function of the liver is bile secretion. Thus, the morphologic axis of the portal lobule is the interlobular bile duct of the portal triad of the "classic" lobule. Its outer margins are imaginary lines drawn between the three central veins that are closest to that portal triad. These lines define a roughly triangular block of tissue that includes those portions of three clas-

sic lobules that secrete the bile that drains into its axial bile duct. This concept allows a description of hepatic parenchymal structure comparable to that of other exocrine glands.

The **liver acinus** is the structural unit that provides the best correlation between blood perfusion, metabolic activity, and liver pathology. The liver acinus is of hexagonal shape and represents the smallest functional unit of the hepatic parenchyma. The short axis of the acinus is defined by the terminal branches of the portal triad that lie along the border between two classic lobules. The long axis is a line drawn between the two central veins closest to the short axis. Therefore, in a two-dimensional view the liver acinus occupies parts of adjacent classic lobules. This concept allows a description of the exocrine secretory function of the liver comparable to that of the portal lobule.

The hepatocytes in each liver acinus are described as being arranged in three concentric elliptical zones surrounding the short axis.

Zone 1 is the closest to the short axis and the blood supply from penetrating branches of the portal vein and hepatic artery. This zone corresponds to the periphery of the classic lobules.

Zone 3 is the farthest from the short axis and closest to the terminal hepatic vein (the central vein). This zone corresponds to the most central part of the classic lobule that surrounds the terminal hepatic vein.

Zone 2 lies between zones 1 and 3 but has no sharp boundaries.

The zonation is important in the description and interpretation of patterns of degeneration, regeneration, and specific toxic effects in the liver parenchyma relative to the degree or quality of vascular perfusion of the hepatic cells. As a result of the sinusoidal blood flow, the oxygen gradient, the metabolic activity of the hepatocytes, and the distribution of hepatic enzymes varies across the three zones. The distribution of liver damage due to ischemia and exposure to toxic substances can be explained using this zonal interpretation.

The cells in zone 1 are the first to receive oxygen, nutrients, and toxins from the sinusoidal blood and are the first to show morphologic changes following bile duct occlusion (bile stasis). These cells are also the last to die if circulation is impaired and the first to regenerate. On the other hand, the cells in zone 3 are the first to show ischemic necrosis (centrilobular necrosis) in situations of reduced perfusion and the first to show fat accumulation. They are the last to respond to toxic substances and bile stasis. Normal

variations in enzyme activity, the number and size of cytoplasmic organelles, and the size of cytoplasmic glycogen deposits are also seen between zones 1 and 3. The cells in zone 2 have functional and morphologic characteristics and responses intermediate to those of zones 1 and 3.

Blood Vessels of the Parenchyma

The blood vessels that occupy the portal canals are called interlobular vessels. Only the interlobular vessels that form the smallest portal triads send blood into the sinusoids. The larger interlobular vessels branch into the distributing vessels that are located at the periphery of the lobule. These distributing vessels send the inlet vessels to the sinusoids. In the sinusoids, the blood flows centripetally toward the central vein. The central vein courses through the central axis of the classic liver lobule, becoming larger as it progresses through the lobule and empties into the sublobular vein. Several sublobular veins converge to form larger hepatic veins that empty into the inferior vena cava.

The structure of the portal vein and its branches within the liver is typical of veins in general. The lumen is much larger than that of the artery associated with it. The structure of the hepatic artery is like that of other arteries, i.e. it has a thick muscular wall. In addition to providing arterial blood directly to the sinusoids, the hepatic artery provides arterial blood to the connective tissue and other structures in the larger portal canals. Capillaries in these larger portal canals return the blood to the interlobular veins before they empty into the sinusoid.

The central vein is a thin-walled vessel receiving blood from the hepatic sinusoids. The endothelial lining of the central vein is surrounded by small amounts of spirally arranged connective tissue fibers. The central vein, named because of its central position in the classic lobule, is actually the terminal venule of the system of veins and, thus, is more properly called the terminal hepatic venule. The sublobular vein, the vessel that recives blood from the terminal hepatic venules, has a distinct layer of connective tissue fibers, both collagenous and elastic, just external to the endothelium. The sublobular veins as well as the hepatic veins into which they drain, travel alone. Because they are the solitary vessels, they can be readily distinguished in the histologic section from the portal veins that are members of a triad. There are no valves in the hepatic veins.

Hepatic sinusoids are lined with a thin discontinuous endothelium which has a discontinuous basal lamina that is absent over large areas. The discontinuity of the endothelium is evident in two ways: 1) large fenestrae, without diaphragms, are present within the endothelial cells; 2) large gaps are present between neighboring endothelial cells. Hepatic sinusoids differ from other sinusoids with a second cell type, the stellate sinusoidal macrophages, or *Kupffer cell*, as a regular part of the vessel lining. Kupffer cells do not form junctions with neighboring endothelial cells. Processes of Kupffer cells often seem to span the sinusoidal lumen and may even partially occlude it. The presence of red cell fragments and iron in the form of ferritin in the cytoplasm of Kupffer cells suggests that they may be involved in the final breakdown of some damaged or senile red blood cells that reach the liver from the spleen. Some of the ferritin iron may be converted to hemosiderin granules and stored in the cells. This function is greatly increased after splenectomy and is then essential for red blood cell disposal.

Perisinusoidal space (Disse's space) is the site of exchange of materials between blood and liver cells. The perisinusoidal space (Disse's space) lies between the basal surfaces of hepatocytes and the basal surfaces of endothelial cells and Kupffer cells that line the sinusoids. Small, irregular microvilli project into this space from the basal surface of the hepatocytes. The microvilli increase the surface area available for exchange of materials between hepatocytes and plasma by as much as 6 times. Because of the large gaps in the endothelial layer and the absence of the continuous basal lamina, no significant barrier exists between the blood plasma in the sinusoid and the hepatocyte plasma membrane. Proteins and lipoproteins synthesized by hepatocyte are transferred into the blood in the perisinusoidal space; this pathway is for liver secretions other than bile. In the fetal liver, the space between blood vessels and hepatocytes contains islands of blood-forming cells. In cases of chronic anemia in the adult, blood-forming cells may again appear in the perisinusoidal space.

The other cell type found in the perisinusoidal space is the *hepatic stellate cell (commonly called an Ito cell)*. These cells of mesenchymal origin are the primary storage site for hepatic vitamin A within cytoplasmic lipid droplets. Vitamin A is released from the hepatic stellate cell as retinol (alcohol form) bound to RBC. For many years, fish liver oils were medically and economically important nutritional sources of vitamin A.

In certain pathologic conditions, such as chronic inflammation or cirrhosis, hepatic stellate cells lose their lipid and vitamin A storage capability and differentiate into cells with characteristics of myofibroblasts. These cells appear to play a significant role in hepatic fibrogenesis; they synthesize and deposit type I and type III collagen within the perisinusoidal space, resulting in liver fibrosis. This collagen is continuous with the connective tissue of the portal space and the connective tissue surrounding the central vein. An increased amount of perisinusoidal fibrous stroma is an early sign of liver response to toxic substances. The cytoplasm or hepatic stellate cells contains contractile elements, such as desmin and smooth muscle α-actin filaments. During cell contraction, they increase the vascular resistance within the sinusoids by constricting the vascular channels, leading to portal hypertension. In addition, hepatic stellate cells play a role in remodeling the extracellular matrix during recovery from liver injury.

One more type of cells is connected with sinusoidal capillaries — so named *pit-cells*. They are situated in sinusoidal lumen and fixed by processes to endothelial cells, rarely in Disse-space. Their nuclei are dark, cytoplasm containing characteristic granules with dence center resembling pit. According to many features pit cells are natural killers possessing high anticancer activity. They are assumed regulating balance of hepatocytes regeneration and apoptosis.

Lymphatic Pathway. Plasma that remains in the perisinusoidal space drains to the periportal connective tissue where a small Mall's space, is described between the stroma of the portal canal and the outermost hepatocytes. From this collecting site, the fluid then enters lymphatic capillaries that travel with the other components of the portal triad.

The lymph moves to progressively larger vessels, to the same direction as the bile, i.e. from the level of the hepatocytes, toward the portal canals and eventually to the hilum of the liver. About 80% of the hepatic lymph follows this pathway and drains into the thoracic duct, forming the major portion of the thoracic duct lymph.

Hepatocytes are large, polygonal cells measuring between 20 and 30 pm in each dimension. They constitute about 80% of the cell population of the liver and make up the anastomosing cell plates of the liver lobule. Hepatocyte nuclei are large and spherical and occupy the center of the cell. Many cells in the adult liver are binucleate; most cells in the adult liver are tetraploid. Heterochromatin is present as scattered clumps in the nucleo-

plasm and as a distinct band under the nuclear envelope. Two or more well-developed nucleoli are present in each nucleus.

Hepatocytes are relatively long-lived for cells associated with the digestive system; their average lifespan is about 5 months. In addition, liver cells are capable of considerable regeneration when liver substance is lost to hepatotoxic processes, disease, or surgery.

The hepatocyte cytoplasm is generally acidophilic. But basophilic regions represent rough endoplasmic reticulum (rER) and free ribosomes. Numerous mitochondria; as many as 800 to 1000 mitochondria per cell can be demonstrated. Multiple small Golgi complexes, large numbers of peroxisomes are seen in each cell. Deposits of glycogen, lipid droplets of various sizes, lipofuscin pigment within lysosomes are present.

Hepatocytes have as many as 200 to 300 peroxisomes per cell. They are relatively large and vary in diameter from 0.2 to 1.0 pm. Peroxisomes are a major site of oxygen use and in this way perform a function similar to that of mitochondria. They contain large amount of oxidase that generates toxic hydrogen peroxide — H_2O_2. The enzyme catalase, also residing within peroxisomes, degrades hydrogen peroxide to oxygen and water. These types of reactions are involved in many detoxification processes occurring in the liver, e.g., detoxification of alcohol. In fact, about one half of the ethanol that is ingested is converted to acetaldehyde by enzymes contained in liver peroxisomes. In humans, catalase and D-amino acid oxidase, as well as alcohol dehydrogenase, are found in peroxisomes. In addition, peroxisomes are also involved in breakdown of fatty acids (ß-oxidation) as well as gluconeogenesis and metabolism of purines.

The sER in hepatocytes may be extensive but varies with metabolic activity. The sER contains enzymes involved in degradation and conjugation of toxins and drugs as well as enzymes responsible for synthesizing cholesterol and the lipid portion of lipoproteins. Under conditions of hepatocyte challenge by drugs, toxins, metabolic stimulants, the sER may become the predominant organelle in the cell. In addition to stimulating sER activity, certain drugs and hormones induce synthesis of new sER membranes and their associated enzymes. The sER undergoes hypertrophy following administration of alcohol, drugs (i.e, phenobarbital, anabolic steroids, and progesterone), and certain chemotherapeutic agents used to treat cancer.

Stimulation of the sER by ethanol enhances its ability to detoxify other drugs, certain carcinogens, and some pesticides. On the other hand, metabolism

by the sER can actually increase the hepatocyte-damaging effects of some toxic compounds, such as carbon tetrachloride ($CC1_4$) and 3,4-benzpyrene.

The large Golgi apparatus in hepatocytes consists of as many as 50 Golgi units, each consisting of three to five closely stacked cisternae, plus many large and small vesicles, are found in hepatocytes. These "units" are actually branches of the tortuous Golgi apparatus seen in heavy-metal preparations. Elements of the Golgi apparatus concentrated near the bile canaliculus are believed to be associated with the exocrine secretion of bile. Golgi cisternae and vesicles near the sinusoidal surfaces of the cell, however, contain electron-dense granules 25 to 80 nm in diameter that are believed to be VLDL and other lipoprotein precursors. These substances are subsequently released into the circulation as part of the endocrine secretory function of the hepatocytes. Similar dense globules are seen in dilated portions of the sER and, occasionally, in the dilated ends of rER cisternae where they are synthesized.

The liver cell is polyhedral; for convenience, it is described as having six surfaces, although there may be more. Two of its surfaces face the perisinusoidal space. The plasma membrane of two surfaces faces a neighboring hepatocyte and a bile canaliculus. Assuming that the cell is cuboidal, the remaining two surfaces would also face neighboring cells and bile canaliculi. The surfaces that face the perisinusoidal space correspond to the basal surface of other epithelial cells; the surfaces that face neighboring cells and bile canaliculi correspond to the lateral and apical surfaces, respectively, of other epithelial cells.

The ductule collects bile from the bile canaliculi. It is close to the hepatocytes, but the actual connection between bile canaliculi and the intrahepatic ductule is composed of cuboidal epithelium surrounded by a complete basal lamina. The narrow space (asterisks) into which microvilli of hepatocytes project is the periportal space (Mall's) not the perisinusoidal space (Disse's).

The adult human liver secretes, on average, about 1 L of bile a day many components of the bile are recycled via the portal circulation.

About 90% of the bile salts, a component of bile are reabsorbed by the gut and transported back to the liver in the portal blood. The bile salts are then reabsorbed and resecreted by hepatocytes. Hepatocytes also synthesize new bile salts to replace those that are lost.

Cholesterol and phospholipid lecithin, as well as most of the electrolytes and water delivered to the gut with the bile, are also reabsorbed and recycled.

Bilirubin glucuronide, the detoxified end product of hemoglobin breakdown, is not recycled. It is ultimately excreted with the feces and gives them their color. Failure to absorb bilirubin or failure to conjugate it or secrete glucuronide can produce jaundice.

Bile flow from the liver is regulated by hormonal and neural control. The rate of blood flow to the liver and the concentration of bile salts in the blood exert regulatory effects on the bile flow. Bile flow is increased when hormones such as cholecystokinin (CCK), gastrin, and motilin are released by enteroendocrine cells during digestion. Steroid hormones (i.e., estrogen during pregnancy) decrease bile secretion from the liver. In addition, parasympathetic stimulation increases bile flow by prompting contraction of the gallbladder and relaxation of the Oddi's sphincter. Bile that leaves the liver via the common hepatic duct flows through the cystic duct to the gallbladder. The gallbladder stores and can increase the concentration of bile up to 10-fold. Following stimulation, the gallbladder contracts and delivers the bile to the duodenum via the common bile duct.

The liver (and gallbladder) receives nerves from both sympathetic and parasympathetic divisions of the autonomic nervous system. The nerves enter the liver at the porta hepatis and ramify through the liver in the portal canals along with the members of the portal triad. Sympathetic fibers are believed to innervate blood vessels; parasympathetic fibers are assumed to innervate the large ducts (those that contain smooth muscle in their walls) and possibly blood vessels. The cell bodies of parasympathetic neurons are often present near the porta hepatis.

Gallbladder is a pear-shaped, distensible sac with a volume of about 50 ml in humans. It is attached to the visceral surface of the liver. The gallbladder is a secondary derivative of the embryonic foregut, arising as an evagination of the primitive bile duct that connects the embryonic liver to the developing intestine.

The gallbladder is a blind pouch that leads, via the neck to the cystic duct. Through this duct it receives dilute bile from the hepatic duct. Hormones secreted by the enteroendocrine cells of the small intestine in response to the presence of fat in the proximal duodenum, stimulate contractions of the smooth muscle of the gallbladder. As a result of these contractions, concentrated bile is discharged into the common bile duct, which carries the bile to the duodenum.

Mucosa of the gallbladder has several characteristic features. The empty or partially filled gallbladder has numerous deep mucosal folds. The mucosal surface consists of simple columnar epithelium. The tall epithelial cells exhibit the following features:

— numerous well-developed apical microvilli;

— apical functional complexes that join adjacent cells and form a barrier between the lumen and the intercellular compartment;

— localized concentrations of mitochondria in the apical and basal cytoplasm;

— complex lateral plications.

These cells closely resemble the absorptive cells of the intestine.

Both cells share the above-mentioned characteristics, as well as localization of Na^+/K^+-activated ATPase on their lateral plasma membranes and secretory vesicles filled with glycoproteins in their apical cytoplasm.

The lamina propria of the mucosa is particularly rich in fenestrated capillaries and small venules, but there are no lymphatic vessels in this layer. The lamina propria is also very cellular, containing large numbers of lymphocytes and plasma cells. The characteristics of the lamina propria resemble those of the colon, another organ specialized for the absorption of electrolytes and water.

Mucin-secreting glands are sometimes present in the lamina propria in the normal human gallbladder, especially near the neck of the organ, but they are more commonly found in an inflamed gallbladder.

The wall of the gallbladder lacks muscularis mucosae and submucosa. External to the lamina propria is a muscularis extena that has numerous collagen and elastic fibers among the bundles of smooth muscle cells. Despite its origin from a foregut-derived tube, the gallbladder does not have muscularis mucosae or submucosa. The smooth muscle bundles are somewhat randomly oriented, unlike the layered organization of the intestine. Contraction of the smooth muscle reduces the volume of the bladder, forcing its contents out through the cystic duct.

External to the muscularis externa is a thick layer of dense connective tissue. This layer contains large blood vessels, an extensive lymphatic network, and the autonomic nerves that innervate the muscularis externa and the blood vessels (cell bodies of parasympathetic neurons are found in the wall of the cystic duct). The connective tissue is also rich in elastic fibers and adipose tissue. Where the gallbladder attaches to the liver surface, this

layer is referred to as the adventitia. The unattached surface is covered by a serosa or visceral peritoneum consisting of a layer of mesothelium and a thin layer of loose connective tissue.

In addition, deep diverticula of the mucosa, called Rokitansky — Aschoff sinuses, sometimes extend through the muscularis externa. They are thought to presage pathologic changes. Also, bacteria may accumulate in these sinuses, causing chronic inflammation.

Concentration of the bile requires the coupled transport of salt and water. The epithelial cells of the gallbladder actively transport both Na^+ and Cl^- (and HCO_3^-) from the cytoplasm the intercellular compartment of the epithelium. ATPase is located in the lateral plasma membranes of the epithelial cells. This active transport mechanism is essentially identical to that described for the enterocytes of the small intestine and the absorptive cells of the colon.

Active transport of Na^+, Cl^-, and HCO_3^- across the lateral plasma membrane into the intercellular (paracellular) compartment causes the concentration of electrolytes in the intercellular space to increase. The increased electrolyte concentration creates an osmotic gradient between the intercellular space and the cytoplasm and between the intercellular space and the lumen. Water moves from the cytoplasm and from the lumen into the intercellular space because of the osmotic gradient, i.e., it moves down its concentration gradient. Although the intercellular space can distend to a degree often visible with the light microscope, this ability is limited. The movement of electrolytes and water into the space creates hydrostatic pressure that forces a nearly isotonic fluid out of the intercellular compartment into the subepithelial connective tissue (the lamina propria). The fluid that enters the lamina propria quickly passes into the numerous fenestrated capillaries and the venules that closely underlie the epithelium. Studies of fluid transport in the gallbladder first demonstrated the essential role of the intercellular compartment in transepithelial transport of an isotonic fluid from the lumen to the vasculature.

PANCREAS

The pancreas is an elongate gland described as having the head, the body, and the tail. The pancreatic duct (Wirsung's) extends through the length of the gland and empties into the duodenum at the hepatopancreatic

ampulla (Vater's) through which the common bile duct from the liver and gallbladder also enters the duodenum.

A thin layer of loose connective tissue forms a capsule around the gland. From this capsule, septa extend into the gland, dividing it into ill-defined lobules. Within the lobules, a stroma of loose connective tissue surrounds the parenchymal units. Between the lobules, larger amounts of the connective tissue surround the larger ducts, blood vessels, and nerves. Moreover, in the connective tissue surrounding the pancreatic duct, there are small mucous glands that empty into the duct.

The pancreas is the exocrine and endocrine gland. Unlike the liver, in which the exocrine and secretory (endocrine) functions reside in the same cell, the dual functions of the pancreas are relegated to two structurally distinct components.

1. The exocrine component synthesizes and secretes enzymes into the duodenum that are essential for digestion in the intestine.

2. The endocrine component synthesizes and secretes the hormones insulin and glucagon into the blood. These hormones regulate glucose, lipid, and protein metabolism in the body.

The exocrine pancreas is found throughout the organ; within the exocrine pancreas, distinct cell masses called islets of Langerhans are dispersed and constitute the endocrine pancreas.

Exocrine pancreas is a serous gland and closely resembles the parotid gland, with which it can be confused. The secretory units are acinar or tubuloacinar in shape and are formed by a simple epithelium of pyramidal serous cells. The cells have a narrow free (luminal) surface and a broad basal surface. Periacinar connective tissue is minimal.

The serous secretory cells of the acinus produce the digestive enzyme precursors secreted by the pancreas. Pancreatic acini are unique among glandular acini; the initial duct that leads from the acinus, the intercalated duct, actually begins within the acinus. The duct cells located inside the acinus are referred to as centroacinar cells.

The acinar cells are characterized by distinct basophilia in the basal cytoplasm and by acidophilic zymogen granules in the apical cytoplasm. Zymogen granules are most numerous in the pancreas of fasting individuals. The squamous centroacinar cells lack both ergastoplasm and secretory granules; thus, they stain very lightly with eosin. This weak staining helps identify them in routine histologic sections.

Pancreatic enzymes can digest most food substances. The inactive enzymes, or proenzymes, contained in pancreatic zymogen granules are listed below along with the specific substances they digest when activated.

1. Proteolytic endopeptidases (trypsinogen, chymotrypsinogen) and proteolytic exopeptidases procarboxypeptidase, proaminopeptidase) digest proteins by cleaving their internal peptide bonds (endopeptidases) or by cleaving amino acids from the carboxyl or amino end of the peptide.

2. Amylolytic enzymes (α-amylase) digest carbohydrates by cleaving the glycosidic linkages of glucose polymers.

3. Lipases digest lipids by cleaving ester bonds of triglycerides, producing free fatty acids.

4. Nucleolytic enzymes (deoxyribonuclease and ribonuclease) digest nucleic acids, producing mononucleotides.

The pancreatic digestive enzymes are activated only after they reach the lumen of the small intestine. Initially, the proteolytic activity of enzymes (enterokinases) in the glycocalyx of the microvilli of the intestinal absorptive cells converts trypsinogen to trypsin, a potent proteolytic enzyme. Trypsin then catalyzes the conversion of the other inactive enzymes as well as the digestion of proteins in the chyme.

The cytoplasmic basophilia of the pancreatic acinar cells when observed with the TEM appears as an extensive ray of rER and free ribosomes. The presence of these numerous organelles correlates with the high level of synthetic activity of the acinar cells. A well-developed Golgi apparatus is present in the apical cytoplasm and is involved in concentration and packaging of the secretory products. Mitochondria are small and, although found throughout the cell, are concentrated among the rER cisternae. Acinar cells are joined to one another by junctional complexes at their apical poles, thus forming an isolated lumen in which small microvilli extend from the apical surface of the acinar cells and into which the zymogen granules are released by exocytosis.

Duct System of the Exocrine Pancreas

The centroacinar cells are the beginning of the duct system of the exocrine pancreas. They have a centrally placed, flattened nucleus and attenuated cytoplasm, which is typical of a squamous cell.

Centroacinar cells are intercalated duct cells located in the acinus. Centroacinar cells are continuous with the cells of the short intercalated duct that lies outside the acinus. The structural unit of the acinus and centroacinar cells resembles a small balloon (the acinus) into which a drinking straw (the intercalated duct) has been pushed. The intercalated ducts are short and drain into intralobular collecting ducts. There are no striated (secretory) ducts in the pancreas.

The complex, branching network of intralobular ducts drains into the larger interlobular ducts, which are lined with a low columnar epithelium in which enteroendocrine cells and occasional goblet cells may be found. The interlobular ducts, in turn, drain directly into the main pancreatic duct, which runs the length of the gland parallel to its long axis, giving this portion of the duct system a herringbone-like appearance. A second large duct, the accessory pancreatic duct, arises in the head of the pancreas.

Although the acini secrete a small volume of protein-rich fluid, the intercalated duct cells secrete a large volume of fluid rich in sodium and bicarbonate. The bicarbonate serves to neutralize the acidity of the chime that enters the duodenum from the stomach and establish optimal pH for the activity of the major pancreatic enzymes.

Two hormones secreted by the enteroendocrine cells of duodenum, secretin and cholecystokinin, are the principal regulators of the exocrine pancreas.

Endocrine pancreas is a diffuse organ that secretes hormones that regulate blood glucose levels. The islets of Langerhans, the endocrine component of the pancreas, are scattered throughout the organ in cell groupings of varying size. The islets constitute about 1 to 2% of the volume of the pancreas but are most numerous in the tail. Individual islets may contain only a few cells or many hundreds of cells. Their polygonal cells are arranged in short, irregular cords that are profusely invested with a network of fenestrated capillaries.

In H&E-stained sections, the islets of Langerhans appear as clusters of pale-staining cells surrounded by more intensely staining pancreatic acini. It is not practical to attempt to identify the several cell types found in the islets in routinely prepared specimens. After Zenker-formol fixation and staining by the Mallory—Azan method, however, it is possible to identify three principal cell types designated ***A (alpha), B (beta), and D (delta) cells***. With this method, the A-cells stain red, the B-cells stain brownish orange, and the D-cells stain blue. About 5% of the cells appear to be unstained

after this procedure. TEM allows identification of the principal cell types by the size and density of their secretory granules.

In addition to the three principal islet cells, three minor islet cell types have also been identified by using a combination of the TEM and immunocytochemistry. Each cell type can be correlated with a specific hormone, and each has a specific location in the islet.

B-cells constitute about 70% of the total islet cells in humans and are generally located in its central portion. They secrete insulin. B-cells contain numerous secretory granules about 300 nm in diameter with a dense polyhedral core and a pale matrix. The polyhedral core is believed to be crystallized insulin.

Insulin, the major hormone secreted by the islet tissue, decreases blood glucose levels. Insulin is the most abundant endocrine secretion. Its principal effects are on the liver, skeletal muscle, and adipose tissue. Insulin has multiple individual actions in each of these tissues. In general, insulin stimulates:

— uptake of glucose from the circulation. Specific cell membrane glucose transporters are involved in this process;

— storage of glucose by activation of glycogen synthase and subsequent glycogen synthesis;

— phosphorylation and use of glucose by promoting its glycolysis within cells.

Absence or inadequate amounts of insulin lead to elevated blood glucose levels and the presence of glucose in the urine, a condition known as diabetes mellitus.

In addition to its effects on glucose metabolism, insulin stimulates glycerol synthesis and inhibits lipase activity in adipose cells. Circulating insulin also increases the amount of amino acids taken up by cells (which may involve cotransport with glucose) and inhibits protein catabolism. Insulin appears to be essential for normal cell growth and function, as demonstrated in tissue culture systems.

A-cells constitute about 15 to 20% of the human islet population and are generally located peripherally in the islets. They secrete glucagon. A-cells contain secretory granules about 250 nm in diameter that are more uniform in size and more densely packed in the cytoplasm than the granules of B cells. The granule is the site of stored glucagon.

Glucagon, secreted in amounts second only to insulin, increases blood glucose levels. The actions of glucagon are essentially reciprocal to those of insulin. It stimulates release of glucose into the bloodstream, and stimulates

gluconeogenesis (synthesis of glucose from metabolites of amino acids) and glycogenolysis (breakdown of glycogen) in the liver. Glucagon also stimulates proteolysis to promote gluconeogenesis, mobilizes fats from adipose-cells, and stimulates hepatic lipase.

D-cells constitute about 5 to 10% of the total pancreatic endocrine tissue and are also located peripherally in the islets. D-cells secrete somatostatin, which is contained in secretory granules that are larger than those of the A- and B-cells (300 to 350 nm) and contain material of low to medium electron density.

Somatostatin inhibits insulin and glucagon secretion. Somatostatin is secreted by the D-cells of the islets. It is identical with the hormone secreted by the hypothalamus that regulates somatotropin (growth hormone) releasing from the anterior pituitary gland. Although the precise role of somatostatin in the islets is unclear, it has been showing to inhibit both insulin and glucagon secretion.

The minor islet cells constitute about 5% of the islet tissue and may be equivalent to the pale cells seen after Mallory — Azan staining.

PP cells (F cells) secrete pancreatic polipeptide stimulating gastric chief cells, inhibiting bile secretion and intestinal motility nd also inhibiting pancreatic enzymes and HCO_3^- secretion.

D_1-cells produce vasoactive intestinal peptide (VIP) similar in action to those of glucagon, but also affects activity and motility in gut, stimulates pancreatic exocrine secretion.

EC cells produce secretin (stimulates production of HCO_3^-), motilin (increases gastric and intestinal motility), substance P (neurotransmitter properties).

Evidence suggests that some cells may secrete more than one hormone. Immunocytochemical staining has localized several hormones in addition to glucagon in the A cell cytoplasrm. These include gastric inhibitory peptide (GIP), cholecystohinin (CCK), and adrenocorticotropic hormone (ACTH) — endorphin. Although there is no clear morphologic evidence for the presence of G cells (gastrin cells) in the islets, gastrin may also be secreted by one or more of the islet cells. Certain pancreatic islet cell tumors secrete large amounts of gastrin, thereby producing excessive acid secretion in the stomach (Zollinger — Ellison syndrome).

All of the hormones secreted by the endocrine pancreas regulate metabolic functions either systemically, regionally (in the gastrointestinal tract), or locally (in the islet itself).

Lecture 17

THE INTEGUMENTARY SYSTEM. THE RASPIRATORY SYSTEM

SKIN

The skin (integument, cutis) and its derivatives constitute the integumentary system. It forms the external covering of the body and is the largest organ of the body, constituting 15–20% of its total mass. The skin consists of two main 1ayers:

Epidermis is composed of a keratinized stratified squamous epithelium that grows continuously but maintains its normal thickness by the process of desquamation. Epidermis is derived from ectoderm.

Dermis is composed of a dense connective tissue that imparts mechanical support, strength, and thickness to the skin. Dermis is derived from the mesoderm.

Hypodermis, or subcutaneous adipose tissue, contains variable amounts of the adipose tissue arranged into the lobules separated by connective tissue septa. It lies deep to the dermis, and is the equivalent of the superficial fascia described in gross anatomy. In well-nourished individuals and in individuals living in very cold climates, the thickness of the adipose tissue can be quite thick.

Epidermal derivatives of the skin (epithelial skin appendages) include the following organ structures and integumentary products:

— hair follicles and hair;
— sweat (sudoriferous) glands;
— nails;
— sebaceus glands;
— mammary glands.

The integumentary system performs a number of essential functions related to its location on the external surface of the body. 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. The skin executes a number of functions. The major of them are the following:

a) ***a barrier function***, that protects against physical, chemical, and biologic agents in the external environment;

b) ***a homeostatic***, that helps preserving the constancy of the internal environment by regulating body temperature and water loss;

c) ***a sensory function***, that brings information about the external environment to the nervous system;

d) ***an endocrine function***, that occurs by secreting hormones, cytokines, and growth factors and converting molecules into ***hormonally active molecules (vitamin D)***;

e) ***an excretory function***, that occurs through exocrine secretion of sweat, apocrine glands.

An addition, certain lipid-solub1e substances may be absorbed through the skin. Although this is not a function of skin, it is a property that can be taken advantage of in delivery of therapeutic agents.

The thickness of the skin varies over the surface of the body, from less than 1 to more than 5 mm. There are two 1ocations, however, where the skin is obviously different at both gross and histological levels. The palms of the hands and the soles of the feet, which are subject to the most abrasion, are hairless and have a much thicker epidermal layer than skin in any other location. This skin is referred to as ***thick skin***. Elsewhere, the skin possesses a much thinner epidermis and is called ***thin skin***. It contains hair follicles in all but a few locations.

Epidermis. The epidermis is composed of stratified squamous keratinized epithelium in which four distinct layers can be identified. In the case of thick skin, a fifth layer is observed. Beginning with the deepest layer, these are:

1. ***Stratum basale***, also called *stratum germinativum* because of the presence of mitotically active cells, the stem cells of the epidermis.

2. ***Stratum spinosum***, also called the spinous or prickle cell layer because of short processes extending from cell to cell. By some histologists stratum spinosum refer also to stratum germinativum.

3. ***Stratum granulosum***, which cells contain numerous intensely staining granules.

4. ***Stratum lucidum***, limited to thick skin and considered a subdivision of the stratum corneum.

5. ***Stratum corneum***, composed of keratinized cells.

The stratum basale is represented by a single layer of cells that rests on the basal lamina. It contains the stem cells from which new cells, the keratinocytes, arise by mitotic division. For this reason, the stratum basale is also called the stratum germinativum. The cells are small and are cuboidal to low columnar. They have less cytoplasm than the cells in the layer above; consequently, their nuclei are more closely spaced. This, in combination with the basophilic cytoplasm of these cells, imparts a noticeable basophilia to the stratum basale. The basal cells also contain various amount of melanin in their cytoplasm that is transferred from neighboring melanocytes interspersed in this layer. Basal cells exhibit extensive cell junctions. They are connected to each other and to keratinocytes by desmosomes and to the underlying basal lamina by hemidesmosomes. As new keratinocytes arise in this layer by mitotic division, they move into the next layer, thus beginning the process of upward migration. This process terminates when the cell becomes a mature keratinized cell, eventually to be sloughed off at the skin surface.

The keratinocyte is the predominate cell type of the epidermis. On leaving the basal epidermal layer, keratinocytes assume two essential activities in order to accomplish its functional role:

1. They produce keratin, the major structural protein of the epidermis. Keratin constitutes almost 85% of fully differentiated keratinocytes.

2. They participate in the formation of the epidermal water barrier.

The keratinocytes in the basal layer contain numerous free ribosomes, scatterd 7 to 9 nm intermediate (keratin) filaments, a small Golgi apparatus, mitochondria, and rough ER. The cytoplasm appears basophilic because of the large number of free ribosomes, most of which are engaged in the synthesis of intermediate keratin filaments. These filaments are called tonofilaments.

As the cells enter and are moved through the stratum spinosum, the synthesis of tonofilaments continues, and the filaments become grouped into bundles that are called tonofibrils. The cytoplasm becomes eosinophilic due to the staining reaction of the tonofibrils that fill more and more of the cytoplasm.

In the upper part of the stratum spinosum, the free ribosomes within the keratinocytes begin to synthesize ***keratohyalin granules*** and lamellar bodies (membrane-coating granules). Keratohyalin granules contain the two major intermediate filament-associated proteins, *filaggrin* and *trichohyalin*. As the number of granules increase, the contents of the granules are released into the keratinocytes cytoplasm. Filaggrin and trichohyalin function as promoters in the aggregation of keratin filaments into tonofibrils, thus initiating the conversation of granular cells into cornified cells. This process is called keratinization and occurs in 2 to 6 hrs, the time it takes for the cells to leave the stratum granulosum and to enter the stratum corneum. The keratin formed in this process is called *soft keratin* in contrast to the *hard keratin* of hair and nails.

The transformation of a granular cell to a cornified cell also involves breakdown of the nucleus and other organelles and thickening of the plasma membrane. Finally, cells are regularly exfoliated (desquamated) from the surface of the stratum corneum. The cells that will desquamate accumulate acid phosphatase, which is thought to participate in the exfoliation of these keratinized cells.

During the embryonic life, melanocyte precursor cells migrate from the neural crest and enter the developing epidermis.

The ***epidermal melanocyte*** is a dendrite cell found scattered among the basal cells of the stratum basale. They are called dendritic cells because the rounded cell body resides in the basal layer and extends long processes between the keratinocytes of the stratum spinosum.

Neither the processes nor the cell body form desmosomal attachments with neighboring keratinocytes. However, melanocytes that reside close to the basal lamina have structures that resemble hemidesmosomes. The ratio of melanocyte to keratinocyte or their precursors in the basal layer rangers from 1:4 to 1:10 in different parts of the body. Melanacytes are pigment-producing cells. Melanin is produced by the oxidation of tyrosine to 3,4-dehydroxypheny1alanine (DOPA) by tyrosinase and the subsequent transformation of DOPA into melanin. These reactions occur initially in membrane-bounded structures, called premelanocytes that are derived from the Golgi comp1ex. Premelanosomes are concentrated near the Golgi complex, nearly mature melanosomes are concentrated at the basal of the cell processes, and mature melanosomes are most commonly found in ends of the processes. Developing melanosomes are transferred to neighboring kerati-

nocytes by phagocytosis of the tips of the melanocyte processes by the keratinocytes. This process is a type of *cytocrine secretion* because a small amount of cytoplasm surrounding the melanosomes is also phagocytosed. Melanocytes maintain the capacity to replicate throughout their life, although at a much slower rate than keratinocytes, thus maintaining the epidermal-melanin unit.

Langerhans's cell plays a role in the immune response by presenting antigens to T-cells. They are dendritic-appearing cells in the epidermis. They encounter and present antigens entering through the skin. Like the melanocyte, the Langerhans's cell doesn't form desmosomes with neighboring keratinocytes. The nucleus staining heavily with hematoxylin, and the cytoplasm is clear. Langerhans's cells can be readily seen in the stratum spinosum. They possess dendritic processes resembling those of the melanocyte. Also, it possesses characteristic, tennis racket-shaped *Birbeck granules.* They represent relatively small vesicles, which appear as rods with a bulbous expansion at their end. As an antigen-presenting cell, the Langerhan's cell is involved in the initiation of cutaneous contact hypersensitivity reactions (e.g., contact allergic dermatitis, and other cell-mediated immune responses in the skin) through the uptake of antigen in the skin and its transport to the lymph nodes. Langerhans's cells are of mesenhymal origin and are derived from the stem cells in the bone marrow. Therefore, they constitute part of the mononuclear phagocytotic system.

Merkel's cells are modified epidermal cells located in the stratum basale. They are most abundant in the skin where sensory perception is acute, such as the fingertips. Merkel's cells are bound to adjoining keratinocytes by desmosomes and contain intermediate (keratin) filaments in their cytoplasm. The nucleus is lobed, and the cytoplasm is somewhat denser that of melanocytes and Langerhans's cells. They may contain some melanosomes in their cytoplasm. Merkel's cells are closely associated with the expanded terminal bulb of afferent myelinated nerve fibers. The neuron terminal loses its Schwann cell covering and immediately penetrates the basal lamina, where it expands into a disk or plate-like endings that lie in close apposition to the base of the Merkel's cell. The combination of the neuron and epidermal cell, called a ***Merkel's corpuscle***, is a sensitive mechanoreceptor.

The ***stratum spinosum*** is at least several cells thick. The cells are larger than those of stratum basale. They exhibit numerous cytoplasmic processes or spines, which gives this layer its name. The cells are also called prickle

cells. As the cells mature and are moved more superficially, they increase in size and become flattened in a plane parallel to the most superficial spinous cells, where the nuclei also become elongate instead of ovoid, matching the acquired squamous shape of the cells.

The ***stratum granulosum*** is the most superficial layer of the nonkeratinized portion of the epidermis. This layer varies from one to a few cells thick. The cells contain numerous *keratohyalin granules*, hence the name of the layer. The granules contain cystine-rich and histidine-rich proteins, which are precursors of the protein *filagrin* that aggregates the keratin filaments present within the cornified cells of the stratum corneum. Keratohyalin granules are variable in size and shape, and because of their intense basophilic staining are readily seen in routine histologic sections.

The ***stratum lucidum***, considered a subdivision of the stratum corneum by some histologists, is found only in thick skin. In the light microscope, it often has a refracting appearance and may stain poorly. This highly refracting layer contains eosinophilic cells in which the process of keratinization is well advanced. The nucleus and cytoplasmic organelles become disrupted and disappear as the cell gradually fills with the intracellular protein keratin. Corneal flakes accumulate air bubbles on the place of broken nucleus.

Usually, an abrupt transition occurs between the nucleated cells of the stratum granulosum and the flattened, desiccated, anuclear cells of the ***stratum corneum***. The cells of the stratum corneum are the most differentiated cells in the skin. They lose their nucleus and cytoplasmic organelles and become filled almost entirely with keratin filaments. Keratinocytes synthesize here protein involucrin possessing ability of plasma membrane proteins immobilisation making it as lipid envelope of the cell. The thick plasma membrane of these cornified, keratinized cells is coated from the outside, in the deeper portion of these layer, with the extracellular layer of lipids that form the major constituent of the water barrier in the epidermis.

The stratum corneum is the layer that varies most in thickness, being the thickest in thick skin. The thickness of this layer constitutes the principal difference between the epidermis of thick and thin skin. This cornified layer will become even thicker at sites subjected to unusual amounts of friction, as in the formation of calluses on the palms of the hand and on the fingertips.

Water barrier in the epidermis. An epidermal water barrier is essential for mammalian "dry" epithelia and is responsible for maintaining body home-

ostasis. The barrier is established primarily by two factors in terminally differentiating keratinocytes: 1) deposition of insoluble proteins on the inner surface of the plasma membrane and 2) a lipid layer that is attached to the outer surface of the plasma membrane.

As the keratinocytes in the stratum spinosum begin to produce keratohyalin granules, they also produce membrane-bounded *lamellar bodies (membrane-coating granules)*. Spinous and granular cells synthesize a heterogenous mixture of *glycosphingolipids, phospholipids, and ceramides*. This mixture is assembled into lamellar bodies in the Golgi apparatus. The contents of the granules is then secreted by exocytosis into the intercellular space between the stratum granulosum and stratum corneum. The organization of these intercellular lipid lamellae is responsible for the formation of the epidermal water barrier.

The epidermal water barrier thus consists of two structural elements:

1. *The cell envelope*, a 15 nm-thick-layer of insoluble proteins deposited on the inner surface of the plasma membrane that contributes to the strong mechanical properties of the barrier. The cell envelope is formed by cross-linking small praline containing proteins and larger structural proteins. The structural proteins include *desmosomal proteins, envoplakin, filaggrin*, five different *keratin* chains, *loricrin*. Loricrin is the major structural protein. This insoluble protein has the highest glycine content of any known protein in the body.

2. *The lipid envelope*, a 5 nm-thick layer of lipids attached to the cell surface by ester bunds. The major lipid components of the lipid envelope are *ceramides,* which belong to the class of sphingolipids; *cholesterol* and *free fatty acids*. However, the most important component is the ceramide, which provides a "teflon-like" coating on the cell surface. Ceramides also play an important role in the cell signaling and are partially responsible for inducing cell differentiation, triggering apoptosis, and reducing cell proliferation.

Dermis. Sections of skin cut perpendicular to the surface reveal numerous finger-like connective tissue protrusions, *derma1 papilla*, that project into the undersurface of the epidermis. The papillae are complemented by what appear to be similar epidermal protrusions, called *epidermal ridges* or *rete ridges*, that project into the dermis. If the plane of section is parallel to the surface of the epidermis and passes at a level to include the dermal papillae, however, the epidermal tissue appears as a continuous sheet of epithelium, containing circular islands of connective tissue within it. The islands are cross sections of true finger-like dermal papillae that project into

the epidermis. At sites where there is increased mechanical stress on the skin, the epidermal ridges are much deeper (the epithelium is thicker), and the dermal papillae are much longer and more closely spaced. This creates a more extensive interface between the dermis and epidermis. Dermal ridges tend to have a parallel arrangement, with the dermal papillae located between them. These ridges form a distinctive pattern that is genetically unique for each individual and is reflected in the appearance of epidermal grooves and ridges on the surface of the skin. This is the basis of the science of dermatoglyphics, or fingerprint and footprint identification. The dermal ridges and papillae are most prominent in the thick skin of the palmar and plantar surfaces.

The basal surface of the basal epidermal cells exhibits a pattern of irregular cytoplasmic protrusions that increase the attachment surface between the epithelial cells and subadjacent basal lamina. A series of hemidesmosomes link the intermediate filaments of the cytoskeleton into the basal lamina. The hemidesmosome is structurally similar to the desmosomal components on other parts of the basal cell membrane except that there is no paired structure linked to it on an adjacent cell. It looks like one-half of a desmosomal junction, hence the name.

The dermis is composed of two layers: a papillary layer and a reticular layer.

The ***papillary layer*** is the more superficial 1ayer and consists of loose connective tissue immediately beneath the epidermis. The collagen fibers located in this part of the dermis are not as thick as those in the deeper portion. This delicate collagen network contains predominantly type I and type III collagen molecules. Similar1y, the elastic fibers here are thread-like and form an irregular network. The papillary layer is relatively thin and includes the substance of the dermal papillae and dermal ridges.It also contains nerve processes, some of which penetrate the basal lamina to enter the epithelial compartment. A lot of blood vessels of microcirculatory bed travel in papillary layer supplying epidermis. Bundles of smooth muscle cells situated here can perform heat lossing by spasm of vessels.

The reticular layer is formed by dense irregular connective tissue, lies deep to the papillary layer; it varies in different parts of the body but is always considerably thicker and less cellular than the papillary layer. It is characterized by thick, irregular bundles of mostly type I collagen and by the presence of more coarse elastic fibers. The collagen and elastic fibers are not randomly oriented but form regular lines of tension in the skin, called *Langer's lines***.**

In the skin of the areole, penis, scrotum, and perineum, smooth muscle cells form a loose plexus in the deepest parts of the reticular layer. This accounts for the puckering of the skin at these sites, particular1y in the erectile organs.

Deep to the reticular layer is a layer of adipose tissue, the *panniculus adiposus*, which varies in thickness. This layer serves as a major energy storage site and also provides insulation. This layer and its associated loose connective tissue constitute the ***hypodermis*** or ***subcutaneous layer***. Individual smooth muscle cells or small bundles of smooth muscle cells that originate in this layer form the *arrector pili muscles* that connect the deep part of hair follicles to the more superficial dermis. Contraction of these muscles in humans produce the erection of hairs and puckering of skin called "goose flesh".

Epidermal Skin Appendages

Skin appendages are deprived from downgrowths of epidermal epithelium during development. They include:

— ***hair follicles*** and their product, ***hair***;

— ***sebaceous glands*** and their product, ***sebum***;

— ***eccrine sweat glands*** and their product, ***sweat***;

— ***apocrine sweat glands*** and their mixed product.

Both hairs and sweat glands have specific roles in regulation of body temperature. Sebaceous glands secrete an oily material that may have protective functions. Apocrine sweat glands produce a serous secretion containing pheromones that acts as a sex attractant in animals and, possibly, humans. The epithelium of the skin appendages can serve as a source of new epithelial cells for skin wound repair.

Hair follicles and hairs are present over almost the entire body; they are absent only from the sides and palmar surfaces of the hands, sides and plantar surfaces of the feet, the lips, and the region around the urogenital orifices.

The hair follicle is responsible for the production and growth of the hair. Coloration of hairs is due to the content and type of melanin that the hair contents. The hair follicle is divided into three segments:

1. ***Infundibulum***, which extends from the surface opening of the follicle to the level of the opening of its sebaceous gland. The infundibulum is a part of the pilosebaceous canal that is used as a route for the discharge of sebum.

2. ***Isthmus***, which extends from the infundibulum to the level of insertion of the arrector pili muscle.

3. ***Inferior segment***, which in the growing follicle is of nearly uniform diameter except at its base, where it expends to form the ***bulb***. The base of the bulb is invaginated by a tuft of vascularized loose connective tissue called a ***dermal papilla***.

Other cells forming the bulb are collectively referred to as the matrix, which consists simply of matrix epithelial (epidermal) cells. Matrix cells immediately adjacent to the dermal papilla represent the germinative layer of the follicle. Division and proliferation of these cells accounts for the growth of the hair. Scattered melanocytes are also present in this germinative layer. They contribute melanosomes to the developing hair cells in a manner analogous to that in stratum germinativum of the epidermis. The dividing matrix cells in the germinative layer differentiate into the keratin-producing cells of the hair and ***internal root sheath***. The internal root sheath is a multilayered cellular covering that surround the deep part of the hair.

The internal root sheath has three layers:

1. ***The cuticle***, which consists of squamous cells whose outer free surface covers the hair shaft.

2. ***Huxley's layer***, which consists of a single or double layer of flattened cells that form the middle plate of internal root sheath.

3. ***Henle's layer***, which consists of an outer single layer of cuboidal cells. These cells are in direct contact with the outermost part of the hair follicle, which represents a downgrowth of the epidermis and is designated the ***external (outer) root sheath***.

Keratinization of the hair and internal root sheath occurs shortly after the cells leave the matrix, in a region called the *keratogenous zone*. By the time the hair emerges from the follicle, it is entirely keratinized as *hard keratin*. The internal root sheath, consisting of *soft keratin*, does not emerge from the follicle with the hair but is broken down at about the isthmus level where sebaceous secretions enter the follicle. A thick basal lamina, called the *glassy membrane*, separates the hair follicle from the dermis. Surrounding the follicle is a dense irregular connective tissue sheath to which the arrector pili muscle is attached.

Hairs are elongated filamentous structures that project from the hair follicles. They consist of three layers:

1. ***Medulla***, which forms the central part of the shaft and contain large vacuolated cells. The medulla is present only in thick hairs.

2. ***Cortex,*** which is located peripheral to the medulla and contains cuboidal cells. These cells undergo differentiation into keratin-filled cells.

3. ***Cuticle of the hair shaft***, which contains squamous cells that form the outermost layer of the hair.

Hair shaft contains melanin pigment produced by melanocytes present in the germinative layer of the hair bulb.

The ***sebaceous*** **glands** are simple alveolar brunched and secrete sebum that coats the hair and skin surface. The sebaceous glands develop as outgrowths of the external root sheath of the hair follicle, usually producing several glands per follicle. The oily substance, sebum, is the product of holocrine secretion. The entire cell produces and become filled with the fatty product while it simultaneously undergoes programmed cell death (apoptosis) as the product fills the cell. Ultimately, both the secretory product and cell debris are discharged from the gland as sebum into the infundibulum of a hair follicle. New cells are produced by mitosis of the basal cells at the periphery of the gland. The basal lamina of these cells is continuous with that of the epidermis and the hair follicle. The process of sebum production from the time of basal cell mitosis to the secretion of the sebum takes about 8 days.

The basal cells of the sebaceous gland contain smooth and rough endoplasmic reticulum, free ribosomes, mitochondria, glycogen, and well-developed Golgi apparatus. As the cells move away from the basal layer and begin to produce the lipid secretory product, the amount of sER increases. The cells gradually become filled with numerous lipid droplets separated by thin strands of the cytoplasm.

The **sweat glands** are classified on the basis of their structure and the nature of their secretion. Two types of sweat glands are recognized:

The *eccrine sweat glands*, which are distributed over the entire body surface except for the lips and part of the external genitalia.

The *apocrine sweat glands*, which are limited to the axilla, areola and nipple of the mammary gland, skin around the anus, and the external genitalia. The *ceruminous glands* of the external acustic meatus canal and the apocrine glands of eyelashes (Moll's glands) are also apocrine-type glands.

The ***eccrine sweat glands*** are simple coiled tubular glands that regulate body temperature. They are independent structures, not associated with

hair follicle that arises as a downgrowth from the fetal epidermis. Each eccrine gland consists of two segments: *secretory segment* located deep in the dermis or in the upper part of the hypodermis and a directly continuous, less coiled *duct segment* that leads to the epidermal surface. The secretory portion of the glands produces a secretion similar in composition to an ultrafiltrate of the blood. Resorption of some of sodium and water in the duct results in the release of a hypotonic sweat at the skin surface. This hypotonic watery solution is low in protein and contains varying amounts of sodium chloride, urea, uric acid, and ammonia. Thus, the eccrine sweat gland also serves, in part, as an excretory organ.

Excessive sweating can lead to loss of other electrolytes, such as potassium and magnesium, and to significant water loss. Normally, the body loses about 600 ml of water a day through evaporation from the lungs and skin. Control of thermoregulatory sweating is cholinergic, while emotional sweating may be stimulated by adrenergic portions of the sympathetic division of the autonomic nervous system.

Three cell types are present in the secretory segment of the gland:

1. *Clear cells* are characterized by abundant glycogen. The cytoplasm of clear cells stains poor. Membranous organelles include numerous mitochondria, sER, and a relatively small Golgi apparatus. The morphology of these cells indicates that they produce the watery component of sweat.

2. *Dark cells* are characterized by abundant rER and secretory granules. The Golgi apparatus is relatively large, a feature consistent with the glycoprotein secretion of these cells. The apical cytoplasm contains mature secretory granules and occupies most of the luminal surface.

3. *Myoepithelial cells* are limited to the basal aspect of the secretory segment. They lie between the secretory cells, with their processes oriented transversally to the tubule. The cytoplasm contains numerous contractile actin filaments. Contraction of these cells is responsible for rapid expression of sweat from the gland.

The duct segment of the gland continues from the secretory portion with coiling. The duct is composed of stratified cuboidal epithelium, consisting of a basal cell layer and a luminal cell layer. The duct cells are smaller and appear darker than the cells of the secretory portion of the gland. The duct portion does not possess myoepithelial cells. These features are useful in distinguishing the duct from the secretory portion in a histologic section.

The ***apocrine sweat glands*** develop from the same downgrowths of epidermis that give rise to hair follicles. Like the eccrine glands, apocrine glands are coiled tubular glands. They are sometimes branched. The secretory portion of apocrine glands differs in several respects from that of the eccrine glands. The most obvious difference is its very wide lumen. Unlike the eccrine glands, the apocrine glands store their secretory product in the lumen. The secretory portion of the gland is composed of simple epithelium. Only one cell type is present. The apical cytoplasm contains numerous small granules, the secretory component within the cell which is discharged by exocytosis. Other features of the cell include numerous lysosomes and lipofuscin pigment granules. Mitochondria are also numerous.

The myoepithelial cells are present in the secretory portion of the gland and are situated between the secretory cells and the adjacent basal lamina.

The duct of the apocrine gland is similar to that of the eccrine duct, it has a narrow lumen. However, it continues from the secretory portion of the gland in a relatively straight path to empty into the follicle canal.

The apocrine glands produce a secretion that contains proteins, carbohydrate, ammonia, lipid, and certain organic compounds that may color the secretion. The apocrine glands become functional at puberty, as with axillary and pubic hair, their development depends on sex hormones. In the female, both axillary and areolar apocrine glands undergo morphologic and secretory changes that parallel the menstrual cycle. Male pheromones (androstenol/androstenone) in the secretion of apocrine ghlands have a direct impact on the female menstruation cycle. Furthermore, female pheromones (copulins) influence male perception of females and may also induce hormonal changes in males.

Nails. The slightly arched fingernails and toenails, more properly referred to as *nail plates*, rest on *nail beds*. The nail bed consists of epithelial cells that are continuous with the stratum basale and stratum spinosum of the epidermis.

The proximal part of the nail, the *nail root*, is buried in a fold of the epidermis and covers the cells of the germinative zone, or *matrix*. The matrix contains a variety of cells including stem cells, epithelial cells, melanocytes, Merkel's cells, and Langerhans's cells. The stem cells of the matrix regularly divide, migrate toward the root of the nail, and there differentiate and produce the hard keratin of the nail. Unlike the soft keratin of the epidermis, it does not desquamate. It consists of densely packed keratin

filaments embedded in a matrix of amorphous keratin with a high sulfur content, which is responsible for the hardness of the nail.

As the nail plate grows, it moves over the nail bed. The nail plate contains closely packed interdigitating *corneocytes* lacking nuclei and organelles.

The crescent-shaped white area near the root of the nail, the *lunula,* derives its color from the thick, opaque layer of partially keratinized matrix cells in this region. The edge of skin folds covering the root of the nail is the *eponychium*, or *cuticle*. The cuticle is also composed of hard keratin, and for this reason it does not desquamate. A thickened epidermal layer, the *hyponychium*, secures the free edge of the nail plate at the fingertip.

RESPIRATORY SYSTEM

The respiratory system consists of the paired lungs and a series of air passages that lead to and from the lungs. As the air passages continue within the lung, they branch into increasingly smaller tubes until the very smallest air spaces, cal1ed alveoli. Three principal functions are performed by this system: *air conduction, air fi1tration*, and *gas exchange (respiration)*. The latter occurs in the alveoli. In addition, air passing through the larynx is used to produce speech, and air passing over the olfactory mucosa in the nasal cavities carries stimuli for the sense of smel1. The respiratory system also participates to a lesser degree in endocrine functions, as well as regulation of immune responses to inhaled antigens.

The air passages consist of a conducting portion and a respiratory portion. The ***conducting portion*** consists of those air passages that lead to the sites of respiration within the lung where gas exchange takes place. The conducting passages include those located outside as well as within the lungs. The passages external to the lungs consist of:

— nasal cavities (and, during forced breathing, the oral cavity);
— nasopharynx and oropharynx;
— larynx;
— trachea;
— paired (main) primary bronchi.

Within the lungs, the main bronchi undergo extensive branching to give rise to the distributing ***bronchioles.*** They represent the terminal part of the

conducting passages. Collectively, the internal bronchi and the bronchioles constitute the ***bronchial tree.***

The ***respiratory portion*** is that portion of the respiratory tract in which gas exchange occurs. It sequentially includes:

— respiratory bronchioles;
— alveolar ducts;
— alveolar sacs;
— alveoli.

Air passing through the respiratory passages must be conditioned before reaching the terminal respiratory units. Conditioning of the air occurs in the conducting portion of the system and includes *warming, moistening* and *removal of particulate materials*. Mucous and serous secretions play a mayor role in the conditioning process. Not only do they moisten the air, but they also trap particles that were not trapped by special short thick hairs, called *vibrissae,* in the nasal cavities. Mucus also prevents the dehydration of the underlying epithelium by the moving air. Mucus covers almost the entire luminal surface of the conducting passages and is continuously produced by goblet cells and mucus-secreting glands in the wall of passages.

The ***nasal cavities*** are paired chambers separated by a bony and cartilaginous septum. The chambers are divided into three regions:

— vestibule;
— respiratory segment;
— olfactory segment.

Vestibule is lined with stratified squamous epithelium, a continuation of the skin of the face, and contains a variable number of stiff hairs that filter out large particulate matter, before it is carried in the air stream to the rest of the cavity. Sebaceous glands are also present, and their secretions assist in the entrapment of particulate matter. Posterior1y, where the vestibule ends, the stratified squamous epithelium becomes thinner and undergoes a transition to the pseudostratified columnar epithelium that characterizes the respiratory segment. The underlying lamina propria is attached to the periosteum of the adjacent bone.

The ciliated, pseudostratified columnar epithelium of the ***respiratory segment*** is composed of five cell types:

1) ***ciliated cells***, tall columnar cells with cilia that project into the mucus covering the surface of the epithelium;

2) ***goblet cells*** that synthesize and secret mucus;

3) ***brush cells***, a general name for those cells in the respiratory tract that bear short, blunt microvilli;

4) ***small granule cells*** that resemble the basal cell but contain secretory granules;

5) ***basal cells***, stem cells from which the other cell types arise.

The ***olfactory segment*** is located in part of the dome of each nasal cavity. It is lined with a specialized *olfactory mucosa*. The olfactory epithelium, like the epithelium of the respiratory segments, is also pseudostratified, but it contains very different cell types:

1. ***Olfactory cells, bipolar neurons*** that provide the thickness of the epithelium. The apical pole of each olfactory cell is dendritic process that project above the epithelial surface as a knob-like structure called the *olfactory vesicle*. The cilia with typical basal bodies arise from the olfactory vesicle and extend radially in a plane parallel to the epithelial surface. The plasma membrane of the cilia contains *odorant-binding proteins* that act as olfactory receptors. Incoming odorant molecules are solubilized in the olfactory mucus and interact with the olfactory receptors to generate an action potential. The basal pole of the cell gives rise to an axonal process that leaves the epithelium to enter the connective tissue, where it joins with axons from other olfactory cells to form the *olfactory nerve (cranial nerve I)*.

2. ***Supporting or sustentacular cells***, columnar cells that provide mechanical and metabolic support to the olfactory cells. Supporting cells are the most numerous cells in the olfactory epithelium. Their nuclei occupy the apical position. These cells have numerous microvilli on their apical surface, and abundant mitochondria. Smooth and rough endoplasmic reticulums are observed in the cytoplasm. They also possess lipofuscin granules. The supporting cells function in a manner comparable to that of glial cells, providing both metabolic and physical support to the olfactory cells.

3. ***Basal cells***, stem cells from which new olfactory cells and supporting cells differentiate. They are small rounded cells located close to the basal lamina. Their nuclei are frequently invaginated. The cytoplasm contains few organelles, a feature consistent with their role as a reserve or stem cell.

4. ***Brush cells***, the same cell type that occurs in the respiratory epithelium. Brush cells exhibit large, blunt microvilli at their apical surface, a feature that gives them their name. The basal surface of a brush cell is in synaptic contact with nerve fibers that penetrate the basal lamina. The nerve

fibers are terminal branches of the *trigeminal nerve (cranial nerve V)* that function in general sensation rather than olfaction. Brush cells appear to be involved in transduction of general sensory stimulation of the mucosa.

Pharynx

The pharynx connects nasal and oral cavities to the larynx and esophagus. It serves as a passageway for air and food and acts as a resonating chamber for speech. The pharynx is divided regionally into the nasopharynx and oropharynx. The auditory (Eustachian) tubes connect the nasopharynx to each middle ear. Diffuse lymphatic tissue and lymphatic nodules are present in the wall of the nasopharynx. The concentration of lymphatic nodules at the junction between the superior and posterior walls of the pharynx is called the *pharyngeal tonsil*.

Larynx

The passageway for air between the oropharynx and trachea is the larynx. This complex tubular segment of the respiratory system is formed by hyaline and elastic cartilage. Larynx serves as the organ for speech (phonation).

The *vocal folds*, also referred to as *vocal cords*, are two folds of mucosa that project into the lumen of the larynx. They are oriented in an anteroposterior direction and define the lateral boundaries of the opening of the larynx, the *rima glottis*. A supporting ligament and *skeletal muscle* is contained within each vocal fold.

The luminal surface of the vocal cords is covered with *stratified squamous non-keratinized epithelium*, as is most of the epiglottis. This serves to protect the mucosa from abrasion caused by the rapidly moving air stream. The other parts of the larynx are lined with the ciliated, pseudostratified columnar epithelium that characterizes the respiratory tract. The connective tissue of the larynx, excepting vocal cords, contains mixed mucoserous glands that secrete through ducts onto the laryngeal surface.

Expelled air passing through the glottis can be induced to cause the vocal folds to vibrate. The vibrations are altered by modulating the tension on the vocal folds and by changing the degree of glottal opening. This is regulated by the skeletal muscle that forms the basis of the vocal folds.

Trachea

The trachea extends from the larynx to about the middle of the thorax, where it divides into the two main (primary) bronchi. The lumen of the trachea stays open because of the arrangement of the series of cartilaginous rings. The wall of trachea consists of four definable layers:

1) ***mucosa***, composed of ci1iated, pseudostratified epithelium and an elastic fiber-rich lamina propria;

2) ***submucosa***, composed of a slightly denser connective tissue than lamina propria;

3) ***cartilaginous 1ayer***, composed of C-shaped hyaline cartilages;

4) ***adventitia***, composed of connective tissue that binds the trachea to adjacent structures.

A unique feature of the trachea is the presence of a series of C-shaped hyaline cartilages that are stacked one on the top of each other to form a supporting structure. These cartilages prevent collapse of the tracheal lumen, particularly during expiration. Fibroelastic tissue and smooth muscle bridge the gap between the free ends of the C-shaped cartilages at the posterior border of the trachea, adjacent to the esophagus.

Ciliated columnar cells, mucous (goblet) cells, and basal cells are the principle cell types in the tracheal epithelium. Brush cells are also present but in small numbers, as are the small granule cells.

Ciliated cells, the most numerous of the cell types, extend through the full thickness of the epithelium. Cilia appear as short hair-like projections from the apical surface. The cilia provide a coordinated sweeping motion of the mucous coat from the farthest reaches of the air passages toward the pharynx. In effect, the ciliated cells provide a "ciliary escalator" that serves as an important projective mechanism for removing small inhaled particles from the lungs.

Mucous cells are similar in appearance to the intestinal goblet cells and are, thus, often referred to by the same name. They are interspersed among the ciliated cells and also extend through the full thickness of the epithelium. They are readily seen in the light microscope after accumulating mucinogen granules in their cytoplasm. In contrast to ciliated cells, they lack of cilia at the apical surface, and the number of mucous cells increases during chronic irritation of the air passages.

Brush cells have the same general features as those described in the respiratory epithelium of the nasal cavity. They are columnar cells that bear blunt microvilli. The basal surface of the cells is in synaptic contact with an afferent nerve ending (epitheliodendritis synapse). Thus, the brush cell is regarded as a receptor cell.

Small granule cells are respiratory representatives of the general class of enteroendocrine cells of the gut and gut derivatives. These cells usually occur singly in the trachea, dispersed sparsely among the other cell types. The nucleus is located near the basement membrane. The cytoplasm exhibits numerous, membrane-bounded, dense-core granules. In one type of small granules cell the secretion is a catecholamine. A second cell type produces the polypeptide hormones such as serotonin, calcitonin, and gastrin-releasing peptide (bombesin). The function of these cells is not well understood. Some cells have been found in groups in association with nerve fibers, forming what is described as neuroepithelial bodies that are thought to function in reflexes regulating the airway or vascular caliber.

Basal cells serve as a reverse population by maintaining individual cell replacement in the epithelium. Basal cells tend to be prominent because their nuclei form a row in close proximity to the basal lamina. Although nuclei of other cells reside at this same general level within the epithelium, they are relatively sparse.

The basement membrane of the tracheal epithelium consists of densely packed collagenous fibers that lie immediately under the epithelial basal lamina. Structurally, it can be regarded as an unusually thick and dense reticular lamina and, as such, is a part of the lamina propria. In smokers, particularly those who experience chronic coughing, this layer may be considerably thicker, a response to irritation of the mucosa.

The lamina propria appears as a typical loose connective tissue. It is very cellular, containing numerous 1ymphocytes, many of which infiltrate the epithelium. Plasma cells, mast cells, eosinophi1s, and fibroblasts are the other cell types readily observed in this layer. Lymphatic tissue, in both diffuse and nodular forms, has a constant presence in the lamina propria and submucosa of the trachea wall. This lymphatic tissue is the developmental and functional equivalent of the ***bronchus-associated lymphatic tissue (BALT).***

The submucosa in trachea is a relatively loose connective tissue similar in appearance to the lamina propria, which makes it difficult to determine

where it begins. Diffuse lymphatic tissue and lymphatic nodules characteristically extend into this layer from the lamina propria. The submucosa contains the larger distributing vessels and lymphatics of the tracheal wall.

Submucosal glands composed of mucus-secreting acini with serous demilunes. Their ducts, consisting of a simple cuboidal epithelium and extend through the lamina propria to deliver the product, largely glycoproteins, on the apical surface.

The tracheal cartilages, which number is about 16 to 20 in humans, represent the next layer of the tracheal wall. The C-shaped cartilages sometimes anastomose with adjacent cartilages, but their arrangement provides flexibility to the tracheal pipe and also maintains patency of the lumen. With the age, the hyaline cartilage may be partially replaced by bone tissue, causing it to lose much of its flexibility. A band of smooth muscles fills the gap between end of C-shaped tracheal cartilages and serves to separate the trachea from esophagus.

The adventitia, the outer layer, contains the largest blood vessels and nerves that supply the tracheal wall, as well as the larger lymphatics that drain the wall.

Bronchi

The trachea divides into two branches forming the ***main (primary) bronchi***. On entering the hilum of the lung, each main bronchus divides into the ***lobar bronchi of large diameter (secondary bronchi).*** Into the lungs the lobar bronchi divides into ***segmental bronchi of middle diameter***. Segmental bronchi, branching continues into bronchi of ***small diameter***.

The bronchi initially have the same general histologic structure as the trachea. At the point where the bronchi enter the lungs to become intrapulmonary bronchi, the structure of the bronchial wall changes. The cartilage rings are replaced by cartilage plates of irregular shape (bronchi of large diameter). The plates are distributed in the linear array around the entire circumference of the wall, giving the bronchi a circular or cylindrical shape in contrast to the ovoid shape with a flattened posterior wall of the trachea. If the bronchi decrease in size because of branching, the cartilage plates become smaller and less numerous, called cartilage islands (characteristic of

middle diameter bronchi). The islands ultimately disappear, at the point where the airway reaches a diameter of about 1 mm the bronchi continue into *bronchiole* (bronchi of small diameter).

The second feature of the intrapulmonary bronchus is the addition of smooth muscle becomes an increasingly conspicuous layer as the amount of cartilage diminishes. Initially, the smooth muscle is in the form of interlacing bundles forming a continuous layer. In the smaller bronchi, the smooth muscle may appear discontinuous. As the smooth muscle forms a separate layer, namely, a muscularis, the wall of the bronchus can be regarded as having five layers.

Mucosa is composed of a pseudostratified epithelium with the same cellular composition as the trachea. The height of the cells decreases as the bronchi decrease in diameter. The basement membrane is conspicuous in the primary bronchi but quickly diminishes in the thickness and disappears as a discrete structure. The lamina propria is similar to that of the trachea but is reduced in amount is proportion to the diameter of the bronchi.

Muscularis, a continuous layer of smooth muscle in the larger bronchi. It is more attenuated and loosely organized in smaller bronchi, where it may appear discontinuous because of its spiral course.

Submucosa remains as a relatively loose connective tissue. Glands are present as well as adipose tissue in the larger bronchi. Amount of glands is reduced in the middle bronchi and disappear in the small bronchi.

Cartilage layer consists of discontinuous cartilage plates that become smaller as the bronchial diameter diminishes.

Adventitia is moderately dense connective tissue that is continuous with that of adjacent structures, such as the pulmonary artery and lung parenchyma.

Bronchioles

Pulmonary acini are smaller units of structure that make up the lobules. Each acinus consists of the ***terminal bronchiole***, the ***respiratory bronchioles*** — alveolar ducts, alveolar sacs and ***alveoli*** aerated by the terminal bronchiole. The smallest functional unit of pulmonary structure is, thus, the respiratory bronchiolar unit. It consists of a single respiratory bronchiole and the alveoli that it supplies.

Bronchioles are air-conducting ducts that measure 1 mm or less in diameter. The larger bronchioles represent branches of the segmental bronchi. These ducts branch repeatedly, giving rise to the smaller terminal bronchioles that also branch. They finally give rise to the respiratory bronchioles.

The larger-diameter bronchioles initially have a ciliated, pseudostratified columnar epithelium that gradually transforms into a simple ciliated columnar epithelium as the duct narrows. Gobbet cells are still present in the largest bronchioles but are not found in the terminal bronchioles that follow. There are no subepithelial glands in bronchioles. Cartilage plates are absent in bronchioles. Instead, a relatively thick layer of smooth muscle is present in the wall of all bronchioles.

Small bronchioles have a simple cuboidal epithelium. The *terminal-bronchioles* are lined with a simple cuboidal epithelium in which ***Clara cells*** are found among the ciliated cells. Clara cells increase in number as the ciliated cells decrease along the length of the bronchioles. Occasional brush cells and small, dense-core granule cells are also present. A sma1l amount of connective tissue underlies the epithelium, and a circumferential layer of smooth muscle underlies the connective tissue in the conducting portion.

Clara cells are nonciliated cells that have a characteristic rounded or dome-shaped apical surface projection. They have a well-developed basal rER, a lateral or supranuclear Golgi comple*x*, secretory granules that stain for protein, and numerous cisternae of sER in the apical cytoplasm. Clara cells secrete a surface-active agent, a lipoprotein. This agent prevents luminal adhesion should the wall of the airway collapse on itself, particularly during expiration. In addition, Clara cells produce Clara cell protein, which is abundant component of the airway secretion and is used as a measurable pulmonary marker in bronchoalveolar lavage fluid and serum.

Respiratory bronchioles constitute a transitional zone in the respiratory system concerned with both air conduction and gas exchange between air and blood. They have a narrow diameter and are lined by a cuboidal epithelium that contains both ciliated cells and Clara cells. Distally, the Clara cells predominate. Occasional brush cells and dense-core granule cells are also found along the length of the respiratory bronchiole. Scattered, thin-walled alveoli extend from the lumen of the respiratory bronchioles.

Alveoli

Alveoli are the terminal air spaces of respiratory system and are the actual site of gas exchange. Each alveolus is a thin-walled polyhedral chamber approximately 0.2 mm in diameter that is confluent with an alveolar sac. Each alveolus is surrounded by a network of capillaries. Alveolus is confluent with:

— ***aveolar ducts***, which elongate airways that have almost no walls, only alveoli, as their peripheral boundary. Rings of smooth muscle are present in the knob-like interalveolar septa;

— ***alveolar sacs***, are spaces surrounded by clusters of alveoli. The surrounding alveoli open into these spaces.

Alveolar sacs usually occur at the termination of the alveolar duct but may occur anywhere along its length. Alveoli are separated from one another by a thin connective tissue layer that contains numerous blood capillaries. The tissue between adjacent alveolar air spaces is called the ***alveolar septum*** or ***septal wall***.

The alveolar surface forms a vulnerable biologic interface that is subject to many destabilizing surface forces and to continuous exposure to inhaled particles, pathogens, and toxins. The alveolar epithelium is composed of several specialized cells and their products, some of which play defensive and protective roles.

Type I alveolar cells or type I pneumocytes, are extremely thin squamous cells that line most (95%) of the surface of the a1veoli. These cells are joined to one another and to the other cells of the alveolar epithelium by occluding junctions. The junctions form an effective barrier between the air space and the components of the septal wall. Type I alveolar cells are not capable of cell division.

Type II alveolar cells, also called type II pneumocytes or septal cells, are secretory cells. They are cuboidal cells interspersed among the type I cells but tend to congregate at septal junctions. Type II cells are as numerous as type I cells, but because of their different shape they cover only about 5% of the alveolar air surface. Like Clara cells, type II cells tend to bulge into the air space. Their apical cytoplasm is filled with granules that are stacks of parallel membrane lamellae, the *lamellar bodies*. They are rich in phospholipids, neutral lipids, and proteins that is secreted by exocytosis to form an alveolar lining, surface-active agent called surfactant. The

lamellar bodies are released into the alveolar space by endocytosis. In addition to secretion of surfactant, type II alveolar cells are progenitor cells for type I alveolar cells.

Brush cells are also present in the alveolar wall, but they are extremely few in number. They may serve as receptors that monitor air quality in the lung.

The surfactant layer produced by type II alveolar cells reduced the surface tension at the air-epithelium interface. The most critical agent for air space stability is specific phospholipids which accounts for almost all surface tension-reducing properties of surfactant. Surfactant synthesis in the fetus occurs after 35th week of gestation and is modulated by a variety of hormones, including cortisol, insulin, prolactin, and thyroxine. Without adequate secretion of surfactant, the alveoli would collapse on each successive exhalation.

In addition to phospholipids, hydrophobic proteins are necessary for the structure and function of surfactant. They are responsible for surfactant homeostasis (regulating synthesis and secretion of surfactant by type II alveolar cells), modulate immune responses for virus, bacteria, and fungi. It also responsible for absorption and spreading of surfactant onto the surface of the alveolar epithelium, it modulates an allergic response to various inhaled antigen.

The alveolar septum is the site of the ***air-blood barrier***. The air-blood barrier refers to the cells and cell products across which gases must diffuse between the alveolar and capillary compartments. The thinnest air-blood barrier consists of: 1) surfactant, covered inner surface of alveoli, 2) a type I alveolar epithelial cells and basal lamina of the alveolar epithelium 3) a capillary endothelium cell and its basal lamina. Often, these two basal laminae are fused. Connective tissue cells and fibers that may be present between the two basal laminae widen the air-blood barrier.

Alveolar macrophages are unusual in that they function both in the connective tissue of the septum and in the air space of the alveolus. In air spaces, they scavenge the surface to remove inhaled particulate matter. Alveolar macrophages are derived from blood monocytes and belong to the mononuclear phagocytotic system. Some engorged macrophages pass up the bronchial tree in the mucus and are disposed of by swallowing or expectoration when they rich the pharynx. Other macrophages return to or remain in the septal connective tissue, where filled with accumulated phago-

cytized material, they may remain for much of an individual's life. Alveolar macrophages also phagocytose infectious organisms.

Some openings in the alveolar septa are present. These opening allow circulation of air from one alveolus to another and are called ***alveolar pores (Kobn's)***. These alveolar pores can be of great significance in some pathologic conditions in which obstructive lung disease blocks the normal pathway of air to the alveoli. The alveoli distal to the blockage may continue to be aerated via the pores from an adjacent lobule or acinus.

Lecture 18

THE URINARY SYSTEM

The urinary system consists of the paired ***kidneys***, which are urinific organs; paired ***ureters***, which lead from the kidney to the ***bladder***; and the ***urethra***, which leads from the bladder to the exterior of the body.

Like the lungs and liver, the kidneys retrieve essential materials and dispose of wastes. They conserve water, essential electrolytes, and metabolites, and they remove certain waste products of metabolism from the body. The kidneys play an important role in regulating and maintaining the composition and volume of extracellular fluid. They also are essential in maintaining acid-base balance by excreting H^+ when bodily fluids become to acidic or excreting bicarbonate when bodily fluids become too basic.

The kidneys are highly vascular organs. They receive approximately 25% of the cardiac output. The kidneys produce *urine*, initially an *ultrafiltrate* of the blood that is then modified by selective resorption and specific secretion by the cells of the kidney. The final urine contains water and electrolytes as well as waste products, such as urea, uric acid, and creatinine, and breakdown products of various substances.

The endocrine activities of the kidneys include:

1. Synthesis and secretion of glucoprotein hormone ***erythropoietin***, which is a growth factor regulating red blood cell formation. Erythropoietin acts on specific receptors expressed on the surface of colony-forming units for erythrocytes (CFU-E) cells in the bone marrow.

2. Synthesis and secretion of the acid protease ***renin***, an enzyme involved in control of blood pressure and blood volume. Renin cleaves circulating angiotensinogen to release ***angiotensin I***.

3. Hydroxylation of vitamin D_3, a steroid precursor produced in the liver, to hormonally active vitamin D_3. This step is regulated primarily by parathyroid hormone (PTH).

The **kidneys** are large, reddish, bean-shaped organs. On the upper pole of each kidney, embedded within the renal fascia and a thick protective layer of perirenal adipose tissue, lies an adrenal gland. The medial border of the kidney is concave and contains a deep vertical fissure, called the *hilum*, through which the renal vessels and nerves passed and through which the expanded, funnel-shaped origin of the ureter, called the *renal pelvis*, exits.

The kidney surface is covered by a thin but tough connective tissue ***capsule.*** The capsule consists of an outer layer of fibroblasts and collagen fibers and an inner layer of myofibroblasts.

Kidney substance can be divided into two distinct regions:

— ***cortex***, the outer reddish brown part;

— ***medulla***, the much lighter-colored inner part.

Approximately 90–95% of the blood passing through the kidney is in the cortex; 5–10% is in the medulla.

The **cortex** consists of *renal corpuscles*, along with the *convoluted and straight tubules* of the *nephron*, the *collecting tubules, collecting ducts*, and an extensive vascular supply. The nephron is the basic functional unit of the kidney. The renal corpuscles are spherical structures and compose the beginning segment of the nephron and contain a unique capillary network called a *glomerulus*. Vertical striations that appear to emanate from the medulla are called *medullary rays* (or Ferrein). Each medullary ray contains straight tubules of the nephrons and collecting ducts. Each nephron and its collecting tubule form the *uriniferous tubule*. The regions between medullary rays contain the renal corpuscles, the convoluted tubules of the nephrons, and the collecting tubules. These areas are referred to as *cortical labyrinths.*

The straight tubular segments of the nephrons and the collecting ducts continue from the cortex into the medulla. They are accompanied by a capillary network, the *vasa recta*, that runs in parallel with the various tubules. These vessels represent the vascular part of the *countercurrent exchange system* that regulates the concentration of the urine.

The tubules in the medulla collectively form a number of conical structures called *pyramids*. Usually 8 to12, but as many as 18 pyramids may by present in the human kidney. The caps of cortical tissue that lie over the pyramids are sufficiently extensive that they extend peripherally around the lateral portion of the pyramid, forming the *renal columns* (Bertin's). The apical portion of each pyramid, which is known as the *papilla*, projects into

a *minor calyx,* a cup-shaped structure that represents an extension of the renal pelvis. The tip of the papilla (*area cribrosa*) is perforated by the openings of the collecting ducts. The *minor calyces* are branches of the two or three major calyces that in turn are major divisions of the renal pelvis.

Each medullary pyramid and the associated cortical tissue at its base and sides (one half of each adjacent renal column) constitutes a *lobe* of the kidney. The lobes are further subdivided into *lobules* consisting of a central medullary ray and the surrounding cortical material. The medullary ray contains the collecting duct for a group of nephrons that drain into that duct constitutes the renal secretory unit.

Nephron

The nephron is the fundamental structural and functional unit of the kidney. Each human kidney contains approximately 2 mln nephrons. Nephrons are responsible for the production of urine and correspond to the secretory part of other glands. The collecting ducts are responsible for the final concentration of the urine.

The renal corpuscle represents the beginning of the nephron. It consists of the glomerulus, a tuft of capillaries composed of 10–20 capillary loops, surrounded by a double-layered epithelial cup, the ***renal*** or ***Bowman's capsule***. Bowman's capsule is the initial portion of the nephron where blood flowing through the glomerular capillaries undergoes a filtration to produce the initial *urine filtrate* (or *glomerular ultrafiltrate*).

The glomerular capillaries are supplied by an *afferent arteriole* and are drained by an *efferent arteriole* that then branches, forming a new capillary network to supply the kidney tubules. This is then an arterial portal system. The site where the afferent and efferent arterioles penetrate and exit from the parietal layer of Bowman's capsule is called the *vascular pole*. Opposite this site is the *urinary pole* of the renal corpuscle, where the proximal convoluted tubule begins.

Continuing from Bowman's capsule, the remaining parts of the nephron are:

— *proximal thick segment*, consisting of the *proximal convoluted tubule (pars convolute)* and the *proximal straight tubule (pars recta)*;

— *thin segment*, which constitutes the limb of the Henle's loop;

— *distal thick segment*, consisting of the *distal straight tubule (pars recta*) and the *distal convoluted tubule (pars convolute*).

The distal segment ascends through the pyramid and medullary ray as the ascending distal straight tubule to the vicinity of its renal corpuscle of origin. The distal tubule then leaves the medullary ray and makes contact with the vascular pole of its wall renal corpuscle. At this place, the epithelial cells of the tubule adjacent to the afferent arteriole of the glomerulus and modified to form the macula densa.

The descending limb of the proximal thick segment, the thin segment and its hairpin turn, and the adjacent limb of the distal thick segment are collectively called the loop of Henle.

Several types of nephrons are identified, based on the location of their renal corpuscles in the cortex:

1. ***Cortical*** or ***subcapsular nephrons*** have their renal corpuscles located in the outer part of the cortex. They have short loops of Henle, extending only into the outer medulla.

2. ***Juxtamedullary nephrons*** make up about one-eighth of the total nephron count. Their renal corpuscles occur in proximity to the base of a medullary pyramid. They have long loops of Henle and long ascending thin segments that extend well into the inner region of the pyramid. These structural features are essential to the urine concentraiting mechanism.

3. ***Intermediate*** or ***midcortical nephrons*** have their renal corpuscles in the midregion of the cortex. Their loops of Henle are of intermediate length.

Blood Supply

Each kidney receives a large branch from the abdominal aorta, called the ***renal artery***. The renal artery branches and sends ***interlobar*** branches into the substance of the kidney. The interlobar arteries travel between the pyramids as far as the cortex and then turn to follow an arched course along the base of the pyramid between the medulla and cortex. Thus, these interlobar arteries are designated ***arcuate arteries*** at the border between the medulla and cortex.

Interlobular arteries branch from the arcuate arteries and ascend through the *cortex* toward the capsule, giving off branches of ***afferent arterioles*** one to each glomerulus. Afferent arterioles give rise to the capillaries that form the glomerulus. The glomerular capillaries reunite to form an ***efferent***

arteriole that, in turn, gives rise to a second network of capillaries, the ***peritubular capillaries***. Summerized diameter of glomerular capillaries is more than diameter of efferent arterioles that leads to high pressure (more 50 mmHg) into glomerular capillaries. This is important necessary conditions to the first phase of uropoiesis — filtration. Blood pressure into peritubular capilaries is low (about 10–12 mm of mercury) that promotes the second phase of uropoiesis — reabsorption. The arrengement of these capillaries differs according to whether they originate from cortical or juxtamedullary glomeruli.

Efferent arterioles from cortical glomeruli lead into the peritubular capillary network that surrounds the local uriniferous tubules.

Efferent arterioles from juxtamedullary glomeruli descend into the *medulla* alongside the Henle's loop. They break up into smaller vessels that continue toward the apex of the pyramid but make hairpin turns at vatious levels to return again as straight vessels toward the base of the pyramid. Thus, the efferent arterioles from the juxtamedullary glomeruli give rise to ***vasa recta*** involved in the countercurrent exchange system and their peritubular capillary network.

Generally, venous flow in the kidney follows a reverse course of arterial flow, with the veins running in parallel with the corresponding arteries.

Peritubular cortical capillaries drain into the ***interlobular veins***, which in turn drain into the ***arcuate veins***, ***interlobar veins***, and the ***renal veins***.

The medullary vascular network drains into ***arcuate veins*** and so forth.

Peritubular capillaries near the kidney surface and *capillaries of the capsule* drain into the ***stellate veins***, which drain into the ***interlobular veins***, and so forth.

As a result of described differencies the juxtamedullary nephrons participation in the process of urine formation is not such an active as those at cortical nephrons, but juxtamedullary nephrons act as shunts, providing fast blood flow through kidney during intensive blood circulation.

Renal (Malpighian) Corpuscle

The renal corpuscle consists of the glomerular capillary tuft and the surrounding visceral and parietal epithelial layers of Bowman's capsule. The filtration apparatus, enclosed by the parietal layer of Bowman's capsule consists of:

1. ***Endothelium of the glomerular capillaries***, which possesses numerous fenestrations. These fenestrations are larger (70 to 90 nm in diameter), more numerous, and more irregular in outline than fenestrations in other capillaries. Endothelial cells of glomerular capillaries possess a large number of water channels that allow the fast movement of water through the epithelium.

2. ***Glomerular basement membrane (GBM)***, a thick basal lamina (300–350 nm) that is the joint product of the endothelium and the podocytes, the cells of the visceral layer of Bowman's capsule. The GBM is the principal component of the filtration barrier.

3. ***Visceral layer of Bowman's capsule***, which contains specialized cells called ***podocytes*** or ***visceral epithelial cells***. These cells extend processes (cytotrabeculae) around the glomerular capillaries. Each process has numerous secondary processes called ***pedicels*** or ***foot processes***. The foot processes interdigitate with foot processes of neighboring podocytes. The elongated spaces between the interdigitating foot processes, called ***filtration slits***, are about 25 nm wide and allow the ultrafiltrate from the blood to enter the Bowman's space. The foot processes contain numerous microfilaments (actin) that are thought to have a role in regulating the size and patency of the filtration slits. An additional factor that may influence the passage of substances through the filtration slits is the presence of a thin membrane. This membrane, the ***filtration slit membrane***, spans the slits. The filtration apparatus may thus be described as a semipermeable barrier having two discontinuous cellular layers applied to either side of a continuous extracellular layer, the basal lamina.

The GBM acts as a physical barrier and an ion-selective filter. The GBM contains type IV collagen, sialoglycoprotein, and other noncollagenous glycoproteins, as well as proteaglycans and glycosaminoglycans, particularly heparan sulfate. The components are localized in particular portions of the GBM:

— ***the lamina rara interna***, adjacent to the capillary endothelium. It is rich in heparin sulfate, which specifically impede the passage of the positively charged molecules;

— ***the lamina rara externa***, adjacent to the podocyte processes. Its molecules features are similar to those of the lamina rara interna;

— ***the lamina densa***, the fused portion of the basal laminae, sandwiched between the laminae rarae. It contains type IV collagen, which is

organized into a network that acts as a physical filter. The sialoglycoproteins are involved in the attachment of the endothelial cells and podocytes to the GBM.

The GBM restricts the movement of particles, usually proteins, larger than approximately 3.6 nm radius, e.g., albumin or hemoglobin. Despite the ability of the filtration barrier to restrict protein, several grams of protein do pass through the barrier each day. This protein is reabsorbed by endocytosis in the proximal convoluted tubule. The presence of significant amounts of albumin or hemoglobin in the urine (albuminuria or hematuria) indicates physical or functional damage to the GBM.

The narrow slit pores formed by the pedicles and the filtration slit membranes also act as physical barriers to bulk flow and free diffusion. The fenestrae of the capillary endothelium restrict the movement of blood cells and other formed elements of the blood from the capillaries. In addition to the structural barriers, the flow rate and the pressure of the blood in the glomerular capillaries also have an effect on the filtration function of the renal corpuscle.

The outer, parietal layer of Bowman's capsule contains parietal epithelial cells and forms a simple squamous epithelium. At the urinary pole of the renal corpuscle, it is continuous with the cuboidal epithelium of the proximal convoluted tubule. The space between the visceral and parietal layers of Bowman's capsule is called the *urinary or Bowman's space*. It is the receptacle for the plasma filtrate produced by the filtration apparatus of the renal corpuscle. At the urinary pole of the renal corpuscle, the urinary space is continuous with the lumen of the proximal convoluted tubule.

Mesangium

The renal corpuscle contains an additional group of cells and extracellular matrix which constitutes the ***mesangium***. It is most obvious at the vascular stalk of the glomerulus and at interstices of adjoining glomerular capillaries. ***Mesangia1 cells*** are positioned much the same as pericytes, in that they are enclosed by the basal lamina of the glomerular capillaries. The mesangial cells are not entirely confined to the renal corpuscle, some are located outside of the corpuscle along the vascular pole and form part of what is called the ***juxtaglomerular apparatus***. Although all of the functions of mesangial cells are not yet fully understood, the following functions have been demonstrated:

— ***Phagocytosis.*** Mesangial cells can remove trapped residues and aggregated proteins from the GBM, thus keeping the glomerular filter free of debris.

— ***Structural support***. Mesangial cells and their matrix provide structural support for the podocytes where the epithelial basement membrane is absent or incomplete.

— ***Secretion.*** Mesangial cells synthesize and secrete a variety of molecules such as interleukin-1 and platelet-derived growth factor, which play a central role in response to glomerular injury.

The primary function of the mesangial cells is to clean the GBM. Clinically, it has been observed that mesangial cells proliferate in certain kidney diseases in which abnormal amounts of protein and protein complexes are trapped in the basement membrane. Mesangial cells are contractile. Thus, they may also play a role in regulating glomerular blood flow.

Juxtaglomerular Apparatus

The juxtaglomerular apparatus includes the macula densa, the juxtaglomerular cells, and the extraglomerular mesangial cells. Lying directly adjacent to the afferent and efferent arterioles and adjacent to some mesangial cells at the vascular pole of the renal corpuscle is the terminal portion of the distal thick segment of the nephron. At this site the wall of tubule contains cells that are collectively referred to as the ***macula densa***. These cells are usually narrower and taller than other distal tubule cells. The nuclei of these cells appear crowded, even to the extent that they appear partially superimposed over one another, thus the name "macula densa".

In this same region, there the smooth muscle cells of the adjacent afferent arteriole (and, sometimes, the efferent arteriole) are modified. They contain secretory granules and their nuclei are spherical, as opposed to the typical elongate smooth muscle cells nucleus.

Juxtaglomerular cells are responsible for activating the ***renin-angiotensin-aldosterone system***. This system plays an important role in maintaining sodium homeostasis and renal hemodynamics. Juxtaglomerular cells are located under epithelial cells in the wall of the afferent and efferent arterioles. The granules of the juxtaglomerular cells contain an aspartil protease, called ***renin***, which is sinthesized, stored, and released into the bloodstream. In the blood, rennin catalyzes the hydrolysis of circulating α-globin, ***angiotensinogen***, to produce the decapeptide ***angiotensin I***.

Angiotensin I is converted to the active octapeptide ***angiotensin II*** by ***angiotensin-converting enzyme*** present on the endothelial cells of lung capillaries.

Angiotensin II stimulates the synthesis and release of the hormone ***aldosterone*** from the zona glomerulosa of the adrenal gland.

Aldosterone, in turn, acts on collecting ducts to increase reabsorption of water, thereby raising blood volume and pressure.

Angiotensin II is also a potent vasoconstrictor that has a regulatory role in the control of renal and systemic vascular resistance.

Extravascular or ***juxtavascular cells*** are located in triangular space between afferent and efferent arterioles and macula densa. They have oval or irregular shape and form the long processes contacting with mesangial cells. Their cytoplasm contains fibrillar structures.

The juxtaglomerular apparatus functions both as an endocrine organ that helps to regulate blood composition and volume and as a sensor of blood composition and volume. Decreased blood volume or decreased sodium concentration in the blood are believed to be stimuli for the release of renin by juxtaglomerular cells. The cells of the macula densa monitor NaCL concentration in the afferent arteriole and regulate the release of renin by the juxtaglomerular cells. An increase in blood volume sufficient to cause stretching of the juxtaglomerular cells in the afferent arteriole may be stimulus that closes the feedback loop and stops secretion of rennin.

Interstitial Cells

The connective tissue of the kidney parenchyma, called interstitial tissue surrounds the nephrons, ducts, blood and lymphatic vessels.

In the cortex, two types of interstitial cells are recognized: cells that resemble fibroblasts, found between the basement membrane of the tubules and the adjacent peritubular capillaries, and occasional macrophages. Fibroblasts synthesize and secrete the collagen and glycosaminoglycans of the extracellular matrix of the interstitium.

In the medulla, the principal interstitial cells resemble myofibroblasts. They are oriented to the long axes of the tubular structures and may have a role in compressing these structures. The cells contain prominent bundles of actin filaments, abundant rough endoplasmic reticulum, a well-developed Golgi complex, and lysosomes. Prominent lipid droplets in the cytoplasm appear to increase and decrease in relation to the diuretic state. These

cells may secrete a hormone-like material that reduces blood pressure, but this substance has been neither isolated nor characterized. In addition prostaglandins and prostacyclin may also be synthesized in the interstitium.

KIDNEY TUBULE FUNCTION

As the glomerular filtrate passes through the uriniferous and collecting tubules of the kidney, it undergoes changes that involve both active and passive absorption, as well as secretion.

Certain substances within the ultrafiltrate are reabsorbed, some partially (water, sodium, and bicarbonate) and some completely (glucose).

Other substances (creatinine and organic acids and bases) are added to the ultrafiltrate (the primary urine) by secretory activity of the tubule cells.

The volume of the filtrate is reduced substantially, and the urine is made hyperosmotic. The long *Henle's loops* and the *collecting tubules* that pass parallel to similarly arranged blood vessels, the vasa recta, serve as the basis of the ***countercurrent multiplayer mechanism*** that is instrumental in concentrating the urine, thereby making it hyperosmotic.

The proximal convoluted tubule receives the primary filtrate from the urinary space of Bowman's capsule. The cuboidal cells of the proximal convoluted tubule have the elaborate surface specializations associated with cells engaged in absorption and fluid transport. They exhibit the following features:

— *a brush border* composed of relatively long, closely packed, and straight microvilli;

— *a junctional complex*, consisting of a narrow, tight junction that seals off the intercellular space from the lumen of the tubule and a zonula adherens that maintains the adhesion between the neighboring cells;

— *plicae* or *folds* located on the lateral surfaces of the cells, which are large flattened processes, alternating with similar processes of adjacent cells;

— extensive *interdigitation of basal processes* of adjacent cells;

— *basal striations*, consisting of elongate mitochondria concentrated in the basal processes and oriented normal to the basal surface.

The basal striations and the apical brush border help to distinguish the cells of the proximal convoluted tubule from those of the other tubules. The actin filaments are present at the base of proximal convoluted tubule cell and may play a role in regulating the movement of fluid from the basolateral

extracellular space across the tubule basal lamina toward the adjacent peritubular capillary.

The proximal convoluted tubule reabsorbs about 120 L of fluid per day or about 65% of the ultrafiltrate. Two major proteins are responsible for fluid reabsorption in the proximal convoluted tubule:

— *Na^+ / K^+ — ATPaze pumps*, transmembrane proteins that are localized in the lateral folds of the plasma membrane. They are responsible for the reabsorption of Na^+, which is the major driving force for reabsorption of water in the proximal convoluted tubule. The active transport of Na^+ is followed by passive diffusion of Cl^- to maintain electrochemical neutrality. The accumulation of NaCl in the lateral intercellular spaces creates an osmotic gradient that draws water from the lumen into the intercellular compartment. This compartment distends as the amount of fluid in it increases; the lateral folds separate to allow this distension.

— *AQP-1*, a small transmembrane protein that functions as a molecular water channel in the cell membrane of proximal convoluted tubule. Movement of water through these membrane channels does not require the high energy of pumps (passive transport). The fluid is reabsorbed into the vessels of the peritubular capillary network.

The microvilli of the proximal convoluted tubule cell are covered with a well-developed glycocalyx that contains several ATPases, peptidases, and high concentrations of disaccharidases. In addition to amino acids and monosacharides, the ultrafiltrate also contains small peptides and disaccharides.

Deep tubular invaginations are present between the microvilli of the proximal convoluted tubule cells. Proteins and large peptides are reabsorbed by endocytosis in the proximal tubule. Proteins in the ultrafiltrate, on reaching the tubule lumen, bind to the glycocalyx that covers the plasma membrane of the invaginations. Then endocytotic vesicles containing the bound protein bud from the invaginations and fuse in the apical cytoplasm to form large protein-containing endosomes. These endosomes are destined to become lysosomes, and the endocytosed proteins are degraded by acid hydrolyses.

Also, the pH of the primary filtrate is modified in the proximal convoluted tubule by the reabsorption of bicarbonate and by the specific secretion into the lumen of exogenous organic acid and organic bases derived from the peritubular capillary circulation.

The cells of the ***proximal straight segment*** are not as specialized for absorption as are those of the proximal convoluted tubule. They are shorter, with a less developed brush border and with fewer and less complex lateral processes. There are fewer apical invaginations and endocytotic vesicles, as well as fewer lysosomes.

Thin segment of Henle's loop. In the light microscope it is possible to detect at least two kinds of thin segment tubules, one with a more squamous epithelium than the other. The cells of thin segment are following.

Type I epithelium is found in the thin segment of the Henle's loop of short-looped nephrons. It consists of a thin, simple epithelium. The cells have almost no interdigitations with neighboring cells and have few organelles.

Type II epithelium, found in the thin descending limb of long-looped nephrons, consists of taller epithelium. These cells possess abundant organelles and have many small, blunt microvilli. The extent of lateral interdigitations with neighboring cells shows species variation.

Type III epithelium, found in the thin descending limb in the inner medulla, consists of a thinner epithelium. The cells have a simple structure and fewer microvilli than cells of the type II epithelium. Lateral interdigitations are absent.

Type IV epithelium, found at the bend of long-looped nephrons and through the entire thin ascending limb, consists of 1ow, flattened epithelium without microvilli. The cells possess few organelles.

The specific functional roles of the four cell types in the thin segment are not yet clear, although this segment is part of the countercurrent exchange system that functions in concentrating the urine.

The two limbs of the Henle's loop have different permeabilities and thus different functions:

1. *The thin descending limb* of the Henle's loop is permeable, permitting free passage or equilibration of salt and water between the lumen of the nephron and the peritubular connective tissue. Because the interstitial fluid in the medulla is hyperosmotic, water diffuses out of, and salt diffuses into, the nephron at this site. The cells of this limb do not actively transport significant amount of ions; thus changes in osmolarity are the result of passive movement of water into the peritubular connective tissue and of salt and urea into the thin descending limb.

2. *The thin ascending limb* of the Henle's loop allows passive diffusion of NaCl into the interstitium. The ascending portion of the thin segment is largely impermeable to water, at this site, as the salt concentration increases in the interstitium. The interstitium becomes hyperosmotic and the fluid in the lumen of the nephron becomes hypoosmotic.

Distal straight tubule. The straight segment of the distal tubule, like the ascendic thin limb, transports ions from the tubular lumen to the interstitium. Na^+ is actively transported across the extensive basal-lateral placations by the Na^+/K^+-ATPasa pumps. Cl^- and K^+ diffuse out from the intercellular space by the Cl^- and K^+ channels, causing the tubular lumen to be positively charged in respect to the interstitium. This positive gradient provides the driving force for the reabsorption of many other ions such as Ca^+ and Mg^+. Note that this significant movement of ions occurs without the movement of water through the wall of the distal straight tubule, resulting in separation of water from its solutes.

The large cuboidal cells of the distal straight tubule have extensive basal-lateral plications and numerous large mitochondria associated with these basal folds. They have considerably fewer and less well developed microvilli than proximal straight tubule cells. The nucleus is located in the apical portion of the cell.

The ***distal convoluted tubule***, located in the cortical labyrinth, is only about one third as long as the proximal convoluted tubule. This short tubule is responsible for:

— reabsorption of Na^+ and secretion of K^+ into the ultrafiltrate to conserve Na^+.

— continued reabsorption of bicarbonate ion, with concomitant secretion of hydrogen ion, leading to further acidification of the urine.

— *conversion of ammonia to ammonium ion* that then can enter the urea cycle that counteracts the toxic effects of ammonia.

Aldosterone, secreted by the adrenal gland and released under stimulation by angiotensin II, increases the reabsorption of Na^+ and secretion of K^+. These effects increase blood volume and blood pressure in response to increased blood Na^+ concentration. Antidiuretic hormone (ADH) released by the posterior pituitary gland, can act on the terminal portion of the distal convoluted tubule to increase the permeability of the tubule to water, thereby producing a more concentrated urine.

Collecting tubules and collecting ducts. Both the collecting tubules and

collecting ducts are composed of a simple epithelium. There are flattened cells, somewhat squamous to cuboidal in shape. The medullary collecting ducts have cuboidal cells, with a transition to columnar cells as the ducts increase in size. Two distinct types of cells are present in the collecting tubules and collecting ducts:

1. *Light cells,* also called *collecting duct or CD cells,* are the principal cells of the system. They are pale-staining cells with true basal infoldings rather than processes that inderdigitate with those of adjacent cells. They possess a single cilium and have relatively few short microviili. They contain small spherical mitochondria. These cells are responsible for water permeability of the collecting ducts.

2. *Dark cells*, also called *intercalated (IC) cells*, occur in considerably smaller numbers. They have many mitochondria and their cytoplasm appears denser. Microplicae, cytoplasmic folds, are present on their apical surface, as well as microvilli. The cells do not show basal infoldings but have basally located interdigitations with neighboring cells. Numerous vesicles are present in the apical cytoplasm. The intercalated cels are involved in the secretion of H^+ or bicarbonate depending on whether the kidney need to excrete acid or alkali.

The cells of the collecting duct gradually become taller as the ducts pass from the outer to the inner medulla and become columnar in the region of the renal papilla.

Histophysiology of the Kidney

The term "countercurrent" indicates a flow of fluid in adjacent structures in opposite directions. The ability to excrete hyperosmotic urine depends on the countercurrent multiplier system that involves three structures:

1. *Loop of Henle* acts as countercurrent multiplier. The ultrafiltrate moves within the descending limb of the thin segment of the loop toward the renal papilla and moves back toward the corticomedullary junction within the ascending limb of the thin segment. The osmotic gradients of the medulla are established along the axis of the loop of Henle.

2. *Vasa recta* form loops parallel to the loop of Henle. They act as countercurrent exchangers of water and solutes between the descending

part (arteriolae recta) and ascending part (venulae recta) of the vasa recta. The vasa recta help to maintain the osmotic gradient of the medulla.

3. *Collecting duct* in the medulla acts as an osmotic equilibrating device. Modified ultrafiltrate in the collecting ducts can be further equilibrated with the hyperosmotic medullary interstitium.

EXCRETORY PASSAGES

All excretory passages, except the urethra, have the same general organization:

— mucosa (lined by transitional epithelium),

— muscularis,

— adventitia (or, in some regions, serosa).

Transitional epithelium (urothelium) of mucosa lines the excretory passages leading from the kidney. This stratified epithelium is essentially impermeable to salts and water. The epithelium begins in the minor calyces as two cell layers and increase to an apparent four to five layers in the ureter and as many as six or more layers in the empty bladder. However, when the bladder is distended, as few as three layers are seen. This change reflects an ability of the cells to accommodate to distention. A dense collagenous lamina propria underlies the epithelium throughout the excretory passages. Neither the muscularis mucosae nor the submuscular layers are present in their walls.

In the tubular portions (ureters and urethra), usually two layers of smooth muscle lie beneath the lamina propria:

1) the *inner layer* is arranged in a loose spiral described as a *longitudinal layer.*

2) the *outer layer* is arranged in a tight spiral described as *a circular layer*.

The smooth muscle of the urinary passages is mixed with connective tissue, so that it forms parallel bundles rather than pure muscular sheets. Peristaltic muscle contractions move the urine from the minor calyces through the ureter to bladder.

Ureters. Each ureter conducts urine from the renal pelvis to the urinary bladder. Transitional epithelium lines the luminal surface of the wall of the ureter. The ureters possess the expressed ability to a stretching thanks to presence in them of deep longitudinal folds of a mucosa. In the inner part of ureter the tunica propria consists of small alveolar-tubular glands.

The smooth muscle is arranged in three layers: an *inner longitudinal* layer, a *middle circular* layer, and an *outer longitudinal* layer. The outer longitudinal layer is present only at the distal end of the ureter, particularly in the portion of the ureter that passes through the bladder wall. Usually the ureter is embadded in the retroperitoneal adipose tissue, forming with vessels and nerves the adventitia of ureter.

Urinary bladder. The bladder is a distensible reservoir for urine. It contains three openings, two for the ureters and one for the urethra. The triangular region defined by these three openings, the *trigone*, is relatively smooth and constant in thickness, whereas the rest of the bladder wall is thick and folded when the bladder is empty and thin and smooth when the bladder is distended. This peculiarity reflects 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. In an empty bladder the mucous forms great number of folds, but they are absent in trigone. In this area the mucous membrane contains glands similar to glands of the inner part of the ureter and is densely connected to the muscularis.

The muscularis of the urinary bladder includes three layers: inner and outer with the longitudinal direction of smooth muscle fibers and the most prominent middle with the circular direction. The muscle and collagen bundles are randomly mixed. Towards the opening of the urethra, the muscle fibers form the involuntary *internal urethral sphincter*.

Outer layer of urinary bladder is formed by combination of adventitia and partially serous layers (on the upper-dorsal and side surfaces).

Urethra. The size, structure, and functions of the urethra differ in males and females. In the male, the urethra serves as the terminal duct for both the urinary and genital systems. It's about 20 cm long and has three distinct segments: prostatic urethra, membranous and penile (spongy) urethra.

Prostatic urethra is lined with transitional epithelium. The ejaculatory ducts of the genital system enter the posterior wall of this segment, and many small prostatic ducts also empty into this segment.

Membranous urethra passes through the urogenital diaphragm of the pelvic floor as it enters the perineum. The skeletal muscle of the urogenital diaphragm surrounding the membranous urethra forms the external (voluntary) sphincter of the urethra. Transitional epithelium ends in the membranous urethra. This segment is usually described as lined with a stratified or

pseudostratified columnar epithelium resembling epithelium of the genital duct system.

The penile urethra is lined with pseudostratified columnar epithelium except at its distal end, where it is lined with stratified squamous epithelium continuous with that of the skin of the penis. Ducts of the bulbourethral glands and of the mucus-secreting urethral glands (Littre's glands) empty into the penile urethra.

In the female, the urethra is short, measuring 3 to 5 cm in length. The mucosa is described as having longitudinal folds. The lining is initially transitional epithelium, but this change to stratified squamous epithelium before its termination. Numerous small *urethral glands,* particularly in the proximal part of the urethra, open into the urethral lumen. Other glands, the *paraurethral glands*, which are homologous to the prostate gland in the male, open into the urethra. They produce an alkaline secretion. The lamina propria is a highly vascularized layer of connective tissue that resembles the corpus spongiosum in the male. Where the urethra penetrates the urogenital diaphragm, the striated muscle of this structure forms the external (voluntary) urethral sphincter.

Lecture 19

THE MALE REPRODUCTIVE SYSTEM

The male reproductive system consists of the testes, genital excurrent ducts, accessory sex glands (seminal vesicles, prostate and bulbourethral glands), and the penis. The two primary functions of the testis are the production of sperm, male gametes (spermatogenesis) and the synthesis of androgens, or sex hormones (steroidogenesis). Androgens, mainly testosteron, are essential for spermatogenesis, play an important role in embryonic development of the male embryo into the phenotypic male fetus, and are responsible for sexual demorphism (male physical and behavioral characteristics).

The essential constituents of the seminal fluid, the spermatozoa, are not products of cellular secretion but are themselves cellular elements that are formed in the tubules of the testis and leave the body through the genital ducts. For this reason, the testes, and the ovaries as well, are known as cytogenic organs. The other constituents of the semen are not formed in the testis but are secreted by the genital ducts, seminal vesicles, prostate and bulbourethral glands.

TESTIS

The adult testes are paired ovoid bodies that have the organization of compound tubular glands. Each testis is enclosed in an unusually thick dense fibrous connective tissue capsule, the ***tunica albuginea***. The inner part of this capsule, the ***tunica vasculosa***, is a loose connective tissue layer that contains blood vessels. Each testis is divided into approximately 250 lobules by incomplete connective tissue septa that project from the capsule.

The tunica albuginea of the posterior portion of the testis is greatly thickened to form the ***mediastinum testis***, from which connective tissue septa radiate into the organ. In this way, the interior of the testis is subdivided into a number of pyramidal lobules. Blood vessels, lymphatic vessels and genital excurrent ducts pass through the mediastinum entering or leaving the testis.

Behind the testis and outside of its tunica albuginea there is an elongated body, the ***epididymis.***

Each lobule of the testis contains 1 to 4 ***seminiferous tubules***, surrounded and supported by intertubular connective tissue. Each tubule within the lobule forms a loop and, because of its considerable length, is highly convoluted. The ends of the loop are located near the mediastinum of the testis, where they assume a short straight course. This part of the seminiferous tubule is called the ***straight tubule (tubuli recti)***. The straight tubules pass into the mediastinum and there empty into an irregular network of thin walled channels, the ***rete testis***.

From the rete testis 8 to 15 tubules arise, the ***efferent ductules***, which pass into the head of the epididymis and there converge to form the ***duct of the epididymis***. It begins in the head, where it receives the ductuli efferentes. At the caudal pole it turns sharply upon itself and passes without any definite demarcation into the ***ductus deferens***.

The seminiferous tubules consist of ***seminiferous epithelium***, located on a basal membrane. The wall of a seminiferous tubule forms the tunica propria, consisting of *basal layer* (stratum basale), *myoid layer* (stratum myoideum), and *fibrous layer* (stratum fibrosum).

The basal layer is formed by the network of collagen fibers and is located between two basal membranes (membrane of spermatogenic epithelium and membrane of myoid layer).

The myoid layer is formed by peculiar myoid cells which demonstrate features associated with smooth muscle cells, including the basal membrane and large numbers of actin filaments. They also exhibit a significant amount of rough endoplasmic reticulum, a feature indicating their role in collagen synthesis in the absence of typical fibroblasts. Rhythmic contractions of the myoid cells create peristaltic waves that help moving spermatozoa and testicular fluid through the seminiferous tubules to the excurrent duct system.

Fibrous layer consists of two parts: 1) inner non-cellular layer, formed by basal membrane of myoid cells and collagen fibers, and 2) outer layer, formed by atypical fibroblasts.

The seminiferous epithelium consists of two basic cell populations:

1. The ***Sertoli cells***, also known as ***supporting***, or ***sustenticular cells***. These cells do not replicate after puberty. The Sertoli cells are tall columnar cells with extensive lateral and apical processes surrounding the adjacent spermatogenic cells and occupying the spaces between them. The Sertoli cells are the only ones that extend from the basal lamina to the lumen, and thus they give structural organization to the tubule.

The Sertoli cells contain an extensive sER, a well-developed rER numerous spherical and elongated mitochondria, a well developed Golgi apparatus and varying numbers of microtubules, lysosomes, vesicles, lipid droplets, glycogen granules and filaments. The euchromatic Sertoli cell nucleus, a reflection of these very active cells, is generally ovoid or triangular and may have deep infoldings.

In addition to secreting fluid that facilitates passage of the maturing sperm along the seminiferous tubules, the Sertoli cells are responsible for *phagocytosis* of residual cytoplasm cast off during maturation of spermatocytes and for the synthesis of *androgen-binging protein (ABP)* that helps to maintain the high androgen levels within the seminiferous tubule, where high concentrations of testosterone are essential for normal maturation of the developing sperm.

The Sertoli cells also secrete several endocrine substances such as *inhibin*, a glycoprotein hormone involved in the feedback loop that inhibits FSH release from the anterior pituitary gland. The Sertoli cells, themselves, are stimulated by both FSH and testosterone. The Sertoli cells also synthesize *plasminogen activator*, which converts plasminogen to the active proteolytic hormone *plasmin* and *transferrin* (an iron transporting protein). FSH receptors are believed to be present only on Sertoli cells and are essential for the secretion of ABH, inhibin, and plasminogen activator.

2. ***Spermatogenic cells*** lie between the cells of Sertoli in an orderly manner, with four to eight layers occupying the space between the basal lamina and the lumen. Spermatogenic cells regularly replicate and differentiate into mature sperm. These cells are derived from primordial germ cells originating in the yolk sac. Spermatogenic cells are organized in poorly defined layers of progressive development between adjacent Sertoli cells. The most immature spermatogenic cells, called *spermatogonia*, rest on the basal lamina. The most mature cells, called *spermatids*, are attached to the apical portion of the Sertoli cell, where they border the lumen of the tubule.

The primitive germ cells, or spermatogonia, from which all of the spermatozoa are ultimately derived, are located directly next to the basal lamina. Human spermatogonia are irregular spherical or cuboidal cells of nuclear type classified into three types on the basis of their nuclei appearance:

1. *Type A dark (Ad) spermatogonia* have ovoid nuclei with intensely basophilic, finely granular chromatin. These spermatogonia are thought to be the stem cells of the seminiferous epithelium giving rise to Ad or Ap spermstogonia.

2. *Type A pale (Ap) spermatogonia* have ovoid nuclei with lightly staining, finely granular chromatin. They are committed to the differentiation process that produces the sperm. They undergo several successive mitotic divisions, thereby increasing their number.

3. *Type B spermatogonia* have generally spherical nuclei with chromatin that is condensed into large clumps along the nuclear envelope and around a central nucleolus.

An unusual feature of the division of a type Ad spermatogonium into two type Ap spermatogonia is that the daughter cells remain connected by a thin cytoplasmic bridge. These cytoplasmic connections remain intact to the last stages of spermatid maturation and are essential for the synchronous development of each clone from an original pair of type Ap cells. After several division, type A spermatogonia differentiate into type B spermatogonia. The appearance of type B spermatogonia represents the last event in the spermatogonial phase.

Spermatogenesis is the process by which spermatogonia develop into sperm.

Spermatogonesis is the sequence of events by which spermatogonia are transformed into mature sperms or spermatozoa. This maturation process begins at puberty (13 to 16 years) and continues into old age (Fig. 1).

Spermatogonia begin to increase in number at puberty. All spermatogonia contain diploid number of chromosomes and after several ordinary mitotic divisions ***(phase of proliferation)*** the last generation of spermatogonia enters an interdivisional ***period or phase of growth***. Spermatogonia undergo changes that transform them into primary spermatocytes, the largest germ cells in the seminiferous tubules containing twice the normal chromosomal number (4n) and double the amount of DNA (2d). Then the ***maturation phase*** represented by two reduction miotic division follows. Prophase of the first meiotic division ensures genetic diversity thanks to

pared homologous chromosomes, consisting of four chromatids and called tetrads, exchange genetic material in a process called crossing-over. At the first meiotic division each primary spermatocyte subsequently undergoes a reduction division to form two secondary spermatocytes, which are about half of the size of primary spermatocytes and have a reduced number of chromosomes to 1n which is represented by 22 autosomes and an X or Y chromosomes. Each of these chromosomes consists of two sister chromatids. The secondary spermatocyte has the 2d (diploid) amount of DNA. Subsequently the secondary spermatocytes undergo a second miotic division without synthesizing new DNA to form four haploid (23 single-stranded chromosomes (1n) and 1d amount of DNA) spermatids, which are about half of size of secondary spermatocytes and through genetic exchange differ from each other and from every other spermatid.

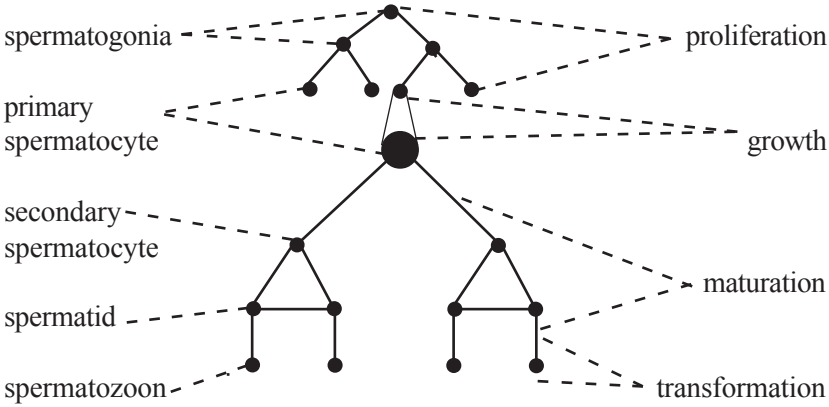

Fig. 1. Spermatogenesis

Spermiogenesis

The spermatids do not divide but mature to form the spermatozoa by a process known as spermiogenesis ***(phase of transformation)***. During most of their maturation the spermatids are enveloped by the cytoplasmic process of the Sertoli cells. A specialized attachment region occurs at the surface of the Sertoli cell where the spermatids are present.

The spermatids are small cells, about half the size of the secondary spermatocytes. They have round and rather dark staining nuclei. One of the earliest changes is in the Golgi complex where small granules, known as proacrosomal granules, appear in some of the Golgi vacuoles. Fusion of these leads to the formation of a larger vacuole with a relatively large granule, the acrosomal granule, visible with the light microscope. Continued growth of the acrosomal complex occurs by incorporation of other newly formed vesicles and granules. In the meantime, the acrosomal system of the Golgi complex moves closer to the nucleus, thereby marking the anterior pole of the cell. The acrosomal vesicle increases its zone of contact with the nucleus, and then the vesicle, with its enclosed acrosomal material, forms a cuplike structure over the anterior two-thirds of the nucleus that is known as the acrosomal cap.

The centrioles move toward the caudal portion of the cells, where the distal centriole functions as a basal body for the development of the flagellum. Then the centrioles and the base of the flagellum move back toward the nucleus. The proximal centriole becomes closely applied to the caudal pole of the nucleus and maintains its ultrastructural characteristics in the mature sperm. While these changes have been occurring, a ring appears around the nucleus at about the level of the caudal end of the acrosomal cap. Microtubules project caudally from the ring, forming a cylindrical caudal sheath. This is followed by a rapid elongation of the cell and the mitochondria move to a position in the proximal portion of the developing tail of the sperm.

The Sertoli-cell-to-Sertoli cell junctional complex is the site of blood testis barrier.

Sertoli cells are bound to one another by an unusual junctional complex. This complex is characterized, in part, by an exceedingly tight junction (zonula occludens) that includes more than 50 parallel fusion lines in the adjacent membranes. A similar-appearing junctional complex in the Sertoli cell is also present at the site where the spermatids are attached. Other junctional specializations of the Sertoli cells include gap junctions between Sertoli cells, desmosome-like junctions between Sertoli cells and early-stage spermatogenic cells, and hemidesmosomes at the Sertoli cell-basal lamina interface.

The Sertoli cell-to-Sertoli cell junctions establish two epithelial compartments, a *basal epithelial compartment* and a *luminal compartment*. Spermatogonia and early primary spermatocytes are restricted to the basal com-

partment, i.e., between the Sertoli cell-to-Sertoli cell junctions and the basal lamina. More mature spermatocytes and spermatids are restricted to the luminal side of the Sertoli cell to Sertoli cell junctions. Early spermatocytes produced by mitotic division of type B spermatogonia must pass through the junctional complex to move from the basal compartment to the luminal compartment. This movement occurs via the formation of a new junctional complex between Sertoli cells processes that extend between the newly formed spermatocytes, followed by the breakdown of the junction above them. Thus, in the differentiation of the spermatogenic cells, the processes of meiosis and spermatogenesis occur in the luminal compartment.

The function of Sertoli cells is the exchange of metabolic substances and wastes between the developing spermatogenic cells and the circulatory system. Sertoli cells phagocytose and break down the residual bodies formed in the last stage of spermiogenesis. They also phagocytose any spermatogenic cells that fail to differentiate completely.

The Seroli cell to Sertoli cell junctional complex also creates a permeability barrier called the ***blood-testis barrier***. Blood-testis barrier structures are situated between the lumen of capillaries and the lumen of seminiferous tubules. This barrier is essential in creating a physiologic compartmentalization within the seminiferous epithelium with respect to ionic, amino acid, carbohydrate, and protein composition. Therefore, the composition of the fluid in the seminiferous tubules and excretory ducts differs considerably from composition of the blood plasma and testicular lymph.

The blood-testis barrier isolated the genetically different and therefore antigenic haploid germ cells (secondary spermatocytes, spermatids, and sperm) from the immune system of the adult male. Antigens produced by, or specific to, the sperm are prevented from reaching the systemic circulation. Conversely, γ-globulins and specific sperm antibodies found in some individuals are prevented from reaching the developing spermatogenic cells in the seminiferous tubule. Therefore, the blood-testis barrier serves an essential role in isolating the spermatogenic cells from the immune system.

Internal Secretion of the Testes

It has been known for a long time that the testes are in some way responsible for the appearance of the secondary sex characters. When the testes are removed before puberty, these characters remain in an infantile

condition or are entierly suppressed. The penis and prostate gland are small, the male type of chest and pelvis fails to develop, the face, chest and limbs are hairless, the larynx remains small and, as a result, the voice maintains its infantile pitch. Sometimes there is considerable deposition of fat, its distribution characteristic of the feminine type.

The most profound and constant effects of castration in mammals, as shown experimentally, are upon the genital ducts and accessory glands. The epithelium of these structures fails to develop to the normal height and does not show secretory activity if the testes are removed before puberty or, if they are removed after maturity, the epithelium of these structures will involute.

The effect of the testes on these accessory reproductive structures appears to be due solely to a hormone secreted by the testes called testosterone. There are other male sex hormones that are found in the urine, but presumably they are not secreted by the testes but are transformation products of the testis hormone or are secreted by other organs (adrenals), although not in sufficient amounts to substitute for the testis hormone.

The evidence is quite conclusive that the interstitial cells of the testes secrete the male sex hormone. Histochemical studies have shown that compounds with the chemical properties of testosterone are present in the interstitial cells but not elsewhere in the testis. There is a correlation between the testosterone content and amount of interstitial tissue.

Estrogen, the female sex hormone, also occurs in the male. It has been shown that about 80% of the estrogen arises from the Leydig cells and about 20% from the adrenal gland.

Hormones secreted by cells of the anterior hypophysis are essential for the endocrine and spermatogenic function of the testis. The activity of the interstitial cells of Leydig is dependent on the interstitial cell-stimulating hormone (luteinizing hormone), and the development of germ cells is dependent on the follicle-stimulating hormone. The anterior hypophysis has an indirect influence on the accessory reproductive organs through its action on the testis.

Interstitial Cells

Besides the usual connective tissue elements, the stroma contains characteristic cells known as the interstitial cells or ***Leydig cells***. These cells form the internal secretion known as hormone ***testosterone.***

The interstitial cells occur in groups of various sizes and are quite distinct in the human testis. Small blood vessels are usually present in the groups. The interstitial cells are large, ovoid or polygonal in shape. They have a large nucleus which is frequently eccentrically located. The cytoplasm of the interstitial cells is eosinophilic that typically contains lipid droplets, and fairly dense near the nucleus but peripherally it is vacuolated, and in usual preparations it stains quite lightly. Most of the endoplasmic reticulum is of the smooth-surfaced type, and the mitochondria have tubular rather than shelf-like cristae. These are characteristic but not universal features of cells that secrete steroid hormones. The interstitial cells also contain lipochrome pigment granules and crystalloids. The pigment granules increase in number in older men. The crystalloids are rod-shaped structures and in cross section are oval or round. Their number and size vary greatly.

Electron micrographs show two types of Leydig cells: fusiform cells with relatively few organelles, and large cells that have the usual organelles plus many small membrane-bounded vesicles, granules, lipid droplets, osmiophilic pigment and large protein crystals. The fusiform cell is probably a precursor stage of the mature interstitial cell.

The Leydig cell arises from fibroblasts and may revert to cells that are indistinguishable from fibroblasts. There are also numerous macrophages in the interstitial tissue interposed among the Leydig cells.

The Leydig cells are active in the early differentiation of the male fetus and then undergo a period of inactivity beginning at about 5 months of fetal life. When Leydig cells are exposed to gonadotropic stimulation at puberty, they again become androgen-secreting cells and remain active throughout life.

INTRATESTICULAR AND EXCURRENT DUCT SYSTEM

The ***straight tubules*** **are** varying in length of a short terminal section of the seminiferous tubules and are lined only by Sertoli cells, which form simple columnar type of epithelium. The Sertoli cells change somewhat in their structure: cytoplasm becoms more vacuolated and nucleus is more dense. They increase in number and finally form a continuous epithelial

layer. Near the termination, the straight tubules narrow, and their lining changes to a simple cuboidal epithelium.

The straight tubules empty into the *rete testis*, a complex series of wide anastomosing channels lined by a simple epithelium that varies somewhat in height but is characteristically cuboidal or low columnar type. These cells have a single apical cilium and relatively few short apical microvilli. The tubules of the rete testis have no definite lamina propria that is distinct from the connective tissue comprising the mediastinum and no smooth muscle cells.

Efferent ductules connect the rete testis with the duct of epididymis. As the efferent ductules exit the testis, they become highly coiled and form 6 to 10 conical masses, *coni vasculosi*, whoses bases form part of the epididymis head. The epithelium of the efferent ductules is a pseudostritified columnar which consists mainly of clumps of high columnar cells alternating with groups of cuboidal cells. This gives inner surface of the tubule a characteristic irregular contour of a sawtooth appearence. Interspersed among the columnar cells are occasional basal cells that serve as epithelial stem cells.

The tall columnar cells are ciliated. The short nonciliated cells have numerous microvilli and canalicular invaginations of the apical surface as well as numerous pinocytotic vesicles, membrane-bounded dense bodies, lysosomes, and other cytoplasmic structures associated with endocytotic activity. In addition to the usual organelles, the cytoplasm often contains fat droplets and pigment granules. Most of the fluid secreted in the seminiferous tubules is reabsorbed in the efferent ductules. Beating of cilia, chiefly of the tall columnar cells, aids in the transport of sperm.

The epithelium rests on a distinct basement membrane, surrounded by lamina propria of connective tissue containing many capillaries and some circular smooth muscle fibers.

A smooth muscle layer in the excurrent ducts first appears at the beginning of the efferent ductules. The smooth muscle cells form a layer of several cells in which the cells are arrayed as a circular sheath in the wall of the ductule to facilitate transport of sperm. Interspersed among the muscle cells are elastic fibers.

Epididymis. The epididymis lies along the superior and posterior surface of the testis. It consists of the efferent ductules and the duct of epididymis. The duct of epididymis is a highly coiled tube. The epididymis is divided into a head, a body, and a tail. The efferent ductules occupy the head, and the duct of epididymis occupies the body and tail. Newly pro-

duced sperm, which enter the epididymis from the testis, mature during their passage through the duct of the epididymis, acquiring motility and the ability to fertilize an oocyte. During this androgen-depending maturation process, the head of the sperm is modified by the addition of *surface-associated decapacitation factor* containing epididymal fluid glycoconjugates. This process, called *decapacitation*, inhibits the fertilizing ability of the sperm in a reversible manner. The surface-associated decapacitation factor is later released during the *capacitation* process that occurs in the female reproductive tract just before fertilization.

Like most of the excurrent duct system, the duct of the epididymis is also lined with a pseudostritified columnar epithelium. It consists of two types of cells: *principal* are very narrow, tall columnar cells and *basal* — rounded short cells. The principal cells bear processes called stereocilia. Electron micrographs show that these structures lack the axial complex characteristic of cilia and that they are merely long, branching cell processes. They differ from typical microvilli by their greater length and by their branching. Nevertheless, they are usually regarded as modified microvilli. The cytoplasm of principal cells contains lysosomes and some pigment granules. Their nuclei are elongate and lie at somewhat different levels.

The principal cells secrete glycerophosphocholine, sialic acid, and glycoproteins, which, in addition to the glycocalyx and steroids, aid in the maturation of the sperm.

The basal cells are similar to those in the efferent tubules but are much more numerous. The small round basal cells rest on the basal lamina. They are the stem cells of the duct epithelium. In addition, intraepithelial lymphocytes (halocells) are found in the epithelium.

The smooth muscle is circular but is small in amount except near the junction with the ductus deferens where it increases in amount and longitudinal bundles appear.

Ductus deferens. Its proximal portion which runs along the epididymis is coiled. Then it straightens out and, as a part of the spermatic cord, passes into the abdominal cavity to terminate in the prostatic portion of the urethra. Shortly before reaching the prostate, the ductus deferens shows a spindle-shaped dilation, the ampulla, which gradually narrows to form the thin ejaculatory duct. The two ejaculatory ducts penetrate the prostate gland and empty into the urethra on either side of prostatic utricle.

The wall of ductus deferens consists of three coats: mucosa, muscularis and fibrosa.

The mucosa lined by a pseudostratified columnar epithelium somewhat similar to that of the duct of the epididymis. The surface cells are lower, however, and the stereocilia show a variable distribution, being absent on some cells and present on other. Cytoplasmic granules are not as numerous as they are in the epithelium of the epididymis. The epithelium is surrounded by a connective tissue exceedingly rich in elastic fibers and in the deeper portion, numerous blood vessels.

The muscularis is the thickest coat and consists of three smooth muscular layers: an inner longitudinal, a middle circular and an outer longitudinal.

The fibrosa consists of fibroelastic tissue containing numerous blood vessels, nerves and often scattered bundles of smooth muscle fibers.

In the ampulla, the mucosa shows numerous folds forming crypts or recesses, many of which extend as tubular structures into the underlying connective tissue.

The ***ejaculatory ducts*** have a thin mucous membrane thrown into numerous fine folds, with glandular recesses like those of the ampulla. The epithelium is simple columnar or pseudostratified and becomes transitional near the urethral opening. Beneath the epithelium there is a rich network of elastic fibers. A distinct muscularis is present only at the begining. In the prostatic portion, the muscularis disappears and is replaced by the fibromuscular tissue of the prostate gland.

ACCESSORY SEX GLANDS

The ***seminal vesicles*** are elongated, convoluted sacs which lie closely apposed to the ampulla and open into the ductus deferens at the junction of ampulla and ejaculatory duct. The mucosa is folded in a complicated manner, forming numerous irregular chambers or crypts. The epithelium varies somewhat but is usually pseudostratified, being composed of cuboidal or columnar cells that reach the surface. The borders of the surface cells are very distinct. The cytoplasm of those cells contains secretory granules and a yellowish lipochrome pigment. The pigment makes its appearance at sexual maturity and increases with age.

The lamina propria is rich in elastic fibers and forms a continuous layer around the vesicle. The connective tissue of the folds is likewise rich in elastic fibers and contains some smooth muscle cells. Outside of the lamina propria there is smooth muscle, which may display indistinct inner circular and other longitudinal layers, both layers being thinner than in ductus deferens. The secretion of the seminal vesicles is a whitish yellow, viscous material. It contains fructose which serves as an energy source for the sperms and is the principal metabolic substrate for them, along with other simple sugars, amino acids, ascorbic acid, and prostaglandins. The secretory function and morphology of the seminal vesicles are under the control of testosterone.

The ***prostate gland*** is in reality an aggregation of many branched tubuloalveolar glands with wide ducts and terminal tubules, which open into the urethra.

The gland is surrounded by a vascular capsule of fibroelastic tissue containing numerous smooth muscle fibers in its inner layer. From the capsule, broad septa penetrate into the interior and become continuous with an unusually abundant fibroelastic supportive tissue, which separates scattered tubules or alveoli.

The epithelium shows a great variation in different glands and alveoli and even in a single alveolus. It is usually a simple cuboidal or columnar type. Basal cells may be present. The cytoplasm contains secretion granules and lipid droplets. The epithelium and subjacent connective tissue form folds that project into the cavities of the glands. The ducts are lined by a simple columnar epithelium that changes, near the terminations of the ducts, to the transitional epithelium of the urethra. The epithelium depends on testosterone for normal morphology and function.

Prostate gland consists of 30 to 50 tubuloalveolar glands arranged in the three concentric layers: an inner *mucosal* layer, an intermediate *submucosal* layer, and a *peripheral* layer containing the *main prostatic glands.*

The alveoli of the prostatic glands, especially those in older men, often contain *prostatic concretions (corpora amylacea)*. Corpora amylacea (concretions) occur normally in some of the alveoli of most prostate glands. Typically, they are spherical bodies about 2 mm in diameter, but there is considerable variation in size. In the fresh condition, they are fairly soft and light yellowish brown in color. They are composed of protein and carbohydrates. Corpora increase in number with age. They

may become calcified and then are known as calculi, some of which are very large.

Prostatic secretion is rich in enzymes, citric acid and contains lipids and also contains large amounts of acid phosphates as well.

Within the prostate the *vesicula prostatica (utriculus prostaticus, uterus masculinus)* is found, the remains of the fetal Mullerian duct. It consists of a blind tubule with a folded mucous membrane lined by a simple or pseudostratified columnar epithelium.

The *bulbourethral glands* or Cowper's glands are two small glandular structures placed close to the bulb of the urethra. They are compound tubuloalveolar glands whose tubules and ducts have a very irregular diameter. The terminal portions may be tubular or alveolar or in the form of cyst-like dilations. They are lined by a simple epithelium whose height varies from columnar to low cuboidal and which may even be flat in distended alveoli. Most of the columnar cells are of the mucous type, with the nuclei basally placed, and the cytoplasm containing mucinogen droplets. Other cells stain darker with eosin and have a granular appearance, often containing fibrillar or spindle-shaped inclusions.

The smaller ducts are lined by a simple epithelium and seem to be secretory in character. The main ducts have a stratified columnar epithelium.

The connective tissue between the tubules consists of fibroelastic tissue with only a few muscle fibers. Smooth and skeletal muscle fibers are quite numerous in the septa between the lobules. Externally, the glands are enclosed by a layer of skeletal muscle fibers from the deep perineal and bulbocavernosus muscles.

During erotic stimulation the gland secretes a glairy substance resembling mucus into the urethra. This preseminal fluid probably serves as a lubricant for the epithelium.

Semen

Semen consists of seminal plasma, spermatozoa and usually some cells cast from the lining of the reproductive ducts and glands. Seminal plasma consists of the secretion of the prostate, seminal vesicles, bulbourethral glands and epididymis, the chief contribution being from the prostate and seminal vesicles. It is alkaline and may help to neutralize the acid environ-

ment of the urethra and the vagina. Semen also contains prostaglandins that may influence sperm transit in both the male and female reproductive ducts and that may have a role in implantation of the fertilized ovum.

Testicular or epididymal sperm are inactive but quickly become active in seminal plasma. They carry little of the foodstuff for metabolism but acquire this from the carbohydrates, chiefly fructose, in seminal plasma. The sugar is reduced to lactic acid, glycolysis being best carried out under nearly anaerobic conditions.

Lecture 20

THE FEMALE REPRODUCTIVE SYSTEM

The female reproductive organs are comprised of the ovaries responsible for production of gametes and steroid hormones, a system of genital ducts — the uterine (Fallopian) tubes, uterus and vagina, — and the external genitalia.

Ovary

The ovaries are somewhat flattened, ovoid bodies, measuring about 4 cm in length, 2 cm in width and 1cm in thickness. The ovaries have two interrelated functions: the production of gametes *(oogenesis)* and production of steroids (*steroidogenesis)*. Developing gametes are called *oocytes*, mature gametes are called *ova.*

Two major groups of steroid hormones, estrogens and progestogens, are secreted by the ovaries:

Estrogens promote growth and maturation of internal and external sex organs and are responsible for the female sex characteristics that develop at puberty. Estrogens also act on mammary glands to promote breast development by stimulating ductal and stromal growth and accumulation of adipose tissue.

Progestogens prepare the internal sex organs, mainly the uterus, for pregnancy by promoting secretory changes in the endometrium. Progestogens also prepare the mammary gland lactation by promoting lobular proliferation.

Both hormones play an important role in the menstrual cycle by preparing the uterus for implantation of a fertilized ovum. If implantation does not

occur, the endometrium of the uterus degenerates and menstruation follows.

The surface of the ovary is covered by a single layer of cuboidal and, in some part, almost squamous *germinal epithelium*, which is continuous with the mesothelium that covers the mesovarium. A dense connective tissue layer, the ***tunica albuginea***, composed of fewer cells and more closely packed fibers, lies between the germinal epithelium and underlying cortex. At the hilus, the connective tissue of the mesovarium and ovarian ligament passes into the ovary and becomes continuous with the ovarian connective tissue.

In section of the ovary, two zones may be distinguished, a central deeper portion, the ***medulla*** or ***zona vasculosa***, and a broad outer layer, the ***cortex***. The two zones blend into each other without any distinct demarcation.

The medulla is composed of a framework of loose connective tissue rich in elastic fibers and containing numerous large blood vessels, lymphatic vessels and nerves. Bundles of smooth muscle fibers are found near the hilus.

The cortex is a broad peripheral layer interrupted at the hilus where the medulla becomes continuous with the tissues of the mesovarium. It consists of a compact, richly cellular connective tissue in which the characteristic epithelial structures of the ovary, the ***ovarian follicles*** are scattered. The connective tissue cells are fusiform or spindle-shaped, with elongated vesicular nuclei. They are placed in a felt work of delicate collagenous fibrils. Elastic tissue, except in the walls of the blood vessels, is practically absent.

Follicle Development

Histologically, three basic types of ovarian follicles can be identified on the basis of developmental state:

— primordial follicles;

— growing follicles which are subcategorized as primary and secondary (or antral) follicles;

— mature or graafian follicles.

In the cycling ovary, follicles are found at all stages of development, but primordial follicles predominate. Each ovarian follicle consists of an ovocyte surrounded by epithelial cells.

Primordial follicles first appear in the wall of the yolk sac during the 3rd week of human fetal development, and they migrate to the germinal ridges (embryonic gonads) by the 5th week. The primordial germ cells become intermingled with the surface epithelial cells, and cordlike projections of cells extend from the surface into the underlying gonadal tissue during the 2nd and 3rd months. These masses of cells proliferate actively and become subdivided into clusters, each composed of several primordial germ cells and numerous follicular cells. It was once thought that the germ cells as well as the follicular cells by differentiation of ovarian surface cells. The evidence is now convincing that the germ cells arise in the yolk sac and it is generally held that the endoderm is the germ cell layer of origin.

After a relatively short period, the clusters of cells that have invaded the gonadal tissue become subdivided into smaller bodies known as primordial and primary follicles, each consisting of an oocyte surrounded by a single layer of follicular cells. A primordial follicle was originally defined as one in which the oocyte is surrounded by a single layer of squamous follicle cells. The outer surface of the follicle cells is bounded by a basal lamina. At this stage, the oocyte and the surrounding follicle cells are closely apposed to one another.

As a primordial follicle develops into a growing follicle, changes occur in the oocyte, in the follicle cells, and in the adjacent stroma. Initially, the oocyte enlarges and the surrounding flattened follicle cells proliferate and become cuboidal. At this stage the follicle is identified as a ***primary follicle***. The primary follicles are spheroid bodies measuring 30 nm. The central oocyte, about 20 nm in diameter, has a large, vesicular nucleus with deeply staining chromatin and a rather indistinct nucleolus. The cytoplasm is finely granular.

Most of the follicles in the ovary of the newborn are of the primordial and primary types, with the former predominating. Counts made on serial sections show that at least 600,000 to 800,000 primordial and primary follicles are present in the ovaries of the newborn infant. And only about 400 of this large number, relatively few are destined to reach full maturity. The reproductive life of woman, from puberty to menopause, lasts about 45 to 55 years. During this period, even if no disturbing factors appear, one ovum matures normally each month. All the other ultimately degenerate and from birth the follicles progressively diminish in number through *atresia*, the spontaneous death and subsequent resorption of immature oocytes.

As the oocyte grows, a homogenous, deeply staining acidophilic refractile layer called the *zona pellucida* appears between the oocyte and the adjacent follicle cells. The growing oocyte secretes the gel-like zona pellucida, which is rich in glycosaminoglycans and glycoproteins.

Through rapid mitotic proliferation, the single layer of follicular cells gives rise to a stratified epithelium, the *stratum granulosum*, surrounding the oocyte. The basal lamina retains its position between the outermost layer of the follicle cells, which become columnar, and the connective tissue stroma.

As the stratum granulosum cells proliferate, stromal cells immediately surrounding the follicle form a sheath of connective tissue cells, known as the *theca folliculi*, which differentiates into two layers:

1. The *theca interna* is the inner, highly vascularized layer of cuboidal secretory cells. Cells of theca interna possess a large number of luteinizing hormone (LH) receptors. In response to LH stimulation, they synthesize and secrete the androgens that are the precursors of estrogens. In addition to secretory cells, the theca interna contain fibroblasts, collagen bundles, and a rich network of small vessels typical of endocrine organs.

2. The *theca externa* is the outer layer of connective tissue cells. It contains mainly smooth muscle cells and bundles of collagen fibers.

The distribution of organelles changes as the oocyte matures. The number of free ribosomes, mitochondria, small vesicles, and the amount of rough endoplasmic reticulum and Golgi apparatus increase. Occasional lipid droplets and masses of lipochrome pigment may be seen. The oocytes exhibit specialized secretory vesicles known as *cortical granules*. The granules contain proteases that are released by the exocytosis when the ovum is activated by the sperm.

Numerous irregular microvilli project from the oocyte into the *perivitelline space* between the oocyte and the surrounding zona granulose as the zona pellucida is deposited. At the same time, slender processes from the granulosa cells develop and project toward the oocyte and may contact the plasma membrane.

The primary follicle initially moves deeper into the cortical stroma as it increases in size. Several factors are required for oocyte and follicular growth:

— follicle-stimulating hormone (FSH);

— growth factors;

— calcium ions (Ca^{+}).

When the stratum granulosum reaches a thickness of 6 to 12 cell layers, fluid-filled cavities appear among the granulosa cells. As the hyaluronic acid-rich fluid called *liquor folliculi* continues to accumulate among the granulose cells, the cavities begin to coalesce forming a single cavity called the *antrum*. The follicle is now identified as a ***secondary follicle*** or ***vesicular follicle***.

As the secondary follicle increase in size, the antrum, lined by several layers of granulosa cells, also enlarges. The *stratum granulosum* has a relatively uniform thickness except for the region associated with the oocyte. Here, the granulose cells form a thickened accentrically positioned mound, the *cumulus oophorus*, which project into the antrum. The cells of the cumulus oophorus that immediately surround the oocyte and remain with it at ovulation are reffered to as the *corona radiata*. The *corona radiata* is composed of cumulus cells that send penetrating microvilli throughout the zona pellucida to communicate via gap junctions with microvilli of the oocyte. During follicular maturations, the number of surface microvilli of granulosa cells increases and is correlated with an increased number of LH receptors on the free antral surface.

The ***mature follicle***, also known as a ***Graafian follicle***, has a diameter of 10 mm or more. Because of its large size, it extends through the full thickness of the ovarian cortex and causes a bulge on the surface of the ovary. As the follicle reach its maximum size, the mitotic activity of the granulosa cells decreases. The stratum granulosum appears to become thinner as the antrum increases in size. The oocyte and cumulus cells are gradually loosened from the rest of the granulosa cells. The cumulus cells immediately surrounding the oocyte now form a single layer of cells of the corona radiata. These cells and loosely attached cumulus cells remain with the oocyte at ovulation.

During this period of follicle maturation, lipid droplets appear in the cytoplasm of the theca interna cells, and the cells demonstrate ultrastructural features associated with steroid-producing cells.

In the mature ovary, there are always several antrum containing follicles of various sizes. Most of these are destined to undergo atresia at some stage in their growth. As a rule, only one follicle matures each month, and the process of transformation from a growing to a mature follicle is accomplished in about 2 weeks. In rare instances, two or even several follicles may reach maturity at the same time.

Oogenesis

Oogenesis is the sequence of events by which oogonia are transformed into mature oocytes. This maturation process begins before birth (prenatal maturation) and is completed after puberty (postnatal maturation) and continues to menopause (Fig. 2).

During early fetal life oogonia ***proliferate*** by ordinary mitotic division and daughter cells contain the full (diploid) number of chromosomes. Then oogonia enlarge to form primary oocytes before birth. This period is known as the ***period of slow growth*** and implies reduplication of DNA (homologous chromosomes become paired), and RNA, tubulins, ATP synthesis. As a primary oocyte forms, it is enclosed by single layer of flattened follicular epithelial cells constituting togather a primordial follicle. The primary oocytes within primordial follicles begin the first meiotic division, but the completion of this process is arrested at the diplotene stage of the 1st meiotic prophase until just before ovulation. Thus, primary oocytes remain arrested in the first meiotic prophase for 12 to 50 years. The follicular cells surrounding the primary oocyte are believed to secrete an oocyte maturation inhibitor, which keeps the miotic process of the oocyte arrested.

Beginning during puberty, as a follicle mature, the primary oocytes encrease in size providing cytoplasm with yolk inclusions and organelles for future zygote. This period is known as ***intensive or fast growth***, which shortly

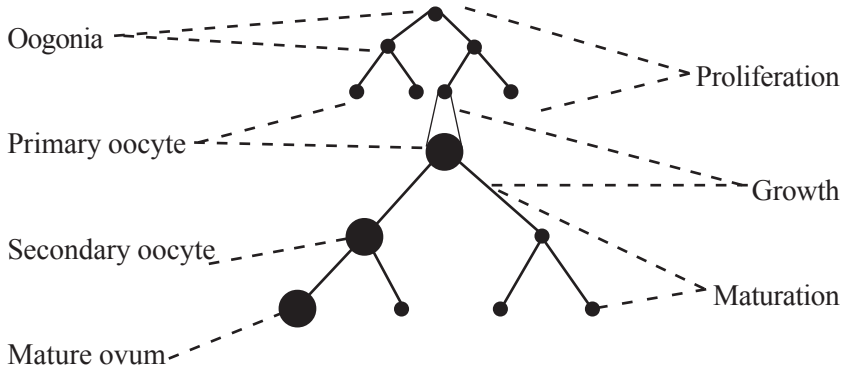

Fig. 2. Oogenesis

before ovulation is completed by the first reduction meiotic division of ***maturation***. Unlike the corresponding stage of spermatogenesis, however, the division of cytoplasm is dramatically unequal. The secondary oocyte receives almost all cytoplasm, and the first polar body receives very little. The polar body is a small, nonfunctional cell that soon degenerates. At ovulation, the nucleus of the secondary oocyte begins the second miotic division of matutarion, but progresses only to metaphase, when division is arrested. If a sperm penetrates the secondary oocyte, the second miotic division is completed, and most cytoplasm is again retained by one cell, the fertilised oocyte. The other cell, the second polar body, also a small nonfunctional cell, soon degenerates. As soon as the polar body is extrusded, maturation of the oocyte is complete.

Thus, of the three–four cells formed from the primary oocyte, only one reaches functional maturity, retaining practically all of the cytoplasm with the nutritive contents. The volume of the mature ovum is about 250,000 times greater than that of the spermatozoon.

Ovulation

Ovulation is the process by which a secondary oocyte is released from the Graafian follicle. During ovulation, the oocyte traverses the entire follicular wall, including the germinal epithelium. Ovulation is stimulated by action of LH and oxytocin. A combination of hormonal changes and enzymatic effects is responsible for the actual release of the secondary oocyte in the middle of the menstrual cycle (on the 14th day of a 28 day of the cycle). These factors include:

a) increase in the volum and pressure of the follicular fluid;

b) enzymatic proteolysis of the follicular wall by activated plasminogen;

c) hormonally directed deposition of glycosaminoglycans between the oocyte-cumulus complex and the stratum granulosum;

d) contraction of the smooth muscle fibers in the theca externa layer, triggered by prostaglandins.

The oocyte, surrounded by corona radiata and cells of cumulus oophorus, is expelled from the ruptured follicle. The oocyte is then transported into the uterine tube. At the time of ovulation, the fimbriae of the uterine tube become closely apposed to the surface of the ovary and direct the oocyte into the uterine tube, preventing its passage into the peritoneal cavity. After ovulation, the secondary oocyte remains viable for approximately

24 hrs. If fertilization does not occur during this period, the secondary oocyte degenerates as it passes through the uterine tube.

Normally, only one follicle completes maturation in each cycle and ruptures to release its secondary oocyte. Rarely, oocytes are released from other follicle having reached full maturity during the same cycle, leading to the possibility of multiply zygotes.

Corpus Luteum

After ovulation, the ruptured follicle does not degenerate at once, it is transformed temporally into a glandular structure, the *corpus luteum (yellow body)*, or *luteal gland*. The follicular cavity closes over by healing of the wound and becomes filled with a serous, fibrin-containing fluid which usually contains some blood and forms the *corpus hemorrhagicum* with a central clot. This first stage of corpus luteum formation is called as the ***period of proliferation and vascularization***. Connective tissue from the stroma then invades the former follicular cavity. The granulosa cells of the follicle do not proliferate to any significant degree but increase greatly in size. Both granulosa and theca interna cells increase in size and become epithelioid in their characteristics, filling with lipid droplets. A lipid-soluble pigment, lipochrome, in the cytoplasm of the cells gives them a yellow appearance in fresh preparations. This period is called as ***period of glandular metamorphosis***.

At the ultrastructural level, the cells demonstrate features associated with steroid-secreting cells, namely, abundant sER some free ribosomes, and mitochondria with tubular cristae. Two types of luteal cells are identified:

— *granulosa lutein cells*, large, centrally located, derived from the granulosa cells;

— *theca lutein cells*, smaller, more deeply staining, peripherally located cells, derived from the cells of theca interna.

Connective tissue from the theca externa penetrates the lutein mass and forms delicate interlacing septa, in which there are numerous capillaries. This highly vascularized structure located in the cortex secretes progesterone and estrogens in the ***period of secretion***. These hormones stimulate the growth and secretory activity of the lining of the uterus, the *endometrium*, to prepare it for the implantation.

If fertilization and implantation do not occur, the corpus luteum remains active for 14 days. This is the *corpus luteum of menstruation*. The cells of

such a corpus luteum gradually decrease in size, show increasing vacuolization and are finally resorbed. The connective tissue between the lutein cells increases in amount, and loose, gelatinous type of connective tissue, often containing brownish pigment of the blood, fills the central cavity. The gland becomes progressively smaller and, several weeks after beginning of involution, is transformed into the *corpus albicans*. Formation of corpus albicans characterizes the ***period of involution***.

If the ovum is fertilized, the corpus increases in size for a time and is known as the *corpus luteum of pregnancy.* The existence and function of the corpus luteum depends on a combination of paracrine and endocrine secretions, collectively described as *luteotropin*. Paracrine luteotropins are locally produced by the ovary (estrogens, insulin-like growth factor (IGF). Endocrine luteotropins are produced at a distance from their target organ, the corpus luteum. They include LH and prolactin (secreted by the pituitary gland), human chorionic gonadotropin HCG (secreted by the trophoblast of the embryo), and insulin (produced by the pancreas).

High levels of progesterone, produced by the corpus luteum, block the cyclic development of ovarian follicles.

Atresia

Very few of the ovarian follicles that begin their differentiation in the embryonic ovary are destined to complete their maturation. Most of the follicles degenerate and disappear through a process called ***ovarian follicular atresia***. Atresia is mediated by apoptosis of granulose cells. Large numbers of follicles undergo atresia during fetal development, early postnatal life and puberty. After puberty, groups of follicles begin to mature during each menstrual cycle. Normally only one follicle completes its maturation.

In atresia of primordial and small growing follicles, the immature oocyte becomes smaller and degenerates. Similar changes occur in the granulosa cells. Atretic follicles shrink and eventually disappear from the stroma of the ovary as a result of repeated apoptosis and phagocytosis by granulosa cells. The oocyte undergoes typical changes associated with degeneration and autolysis. The zona pellucida, which is resistant to the autolytic changes occurring in the cell associated with it, becomes folded and collapses. The basement membrane between the follicle cells from the theca interna may separate from the follicle cells and increase in thickness, forming a wavy hyaline layer called *glassy membrane*.

Enlargement of the cells of the theca interna occurs in some atretic follicles. These cells are similar to theca lutein cells and become organized into radially arranged strands separated by connective tissue. These atretic follicles are called *corpora lutea atretica*.

As atretic follicles continue to degenerate, a scar with hyaline streaks develops in the center of cell mass, giving it appearance of a small *corpus albicans*. This structure eventually disappears as the ovarian stroma invades the degenerating follicle.

Interstitial Cells

The cortical stroma contains clusters or strands of epithelioid connective tissue cells whose cytoplasm contains fine lipid granules. These form from the theca interna and are known as interstitial cells. The interstitial cells are an important source of the estrogens that influence growth and development of the secondary sex organs during the early phases of puberty.

In the human ovary, there are relatively few interstitial cells. They occur in the largest numbers in the first years of life and during the early phases of puberty, corresponding the times of increased follicular atresia.

Hormones of Ovary

The ovary is under the direct influence of hormones of the anterior hypophysis. These hormones, the gonadotropins, control the maturation of follicles and the formation of corpora lutea.

The ovarian hormones are steroids. One of them, estrogen (principally estradiol), is secreted by the growing follicles and to a lesser degree by the corpus luteum. The other, progesterone, is produced mainly by the corpus luteum but also to some extent by the mature follicle just prior to ovulation. Because there is a wave of follicular maturation during the first half of each month, followed by formation of a corpus luteum immediately after ovulation, the levels of the two hormones normally show regular cyclic fluctuation. Estrogen secretion is high during the preovulatory period and reaches a peak at about the time of ovulation. Progesterone secretion increases rapidly as the ruptured follicle becomes luteinized, and it remains at a high level until regression of the corpus luteum.

THE UTERINE TUBE

The ***Fallopian (uterine) tubes*** are paired structures, each of which is about 10–12 cm long and 6 to 8 mm in diameter. One end of a tube opens into the peritoneal cavity near the ovary; the other end opens into the superior lateral part of the uterine cavity. The tubes conduct the ova that are discharged at ovulation in the uterine cavity.

The wall of the Fallopian tube consists of three coats: mucosa, muscularis and serosa.

The ***mucosa***, the inner lining of the uterine tube exhibits relatively thin longitudinal folds that project into the lumen.

The ***muscularis***, throughout most of its length, is organized into an inner, relatively thick circular layer and an outer, thiner longitudinal layer. The boundary between these layers is often indistinct.

The ***serosa*** is the outermost layer of the uterine tube and is composed of mesothelium and a thin layer of connective tissue.

The epithelium lining the Fallopian tubes is a simple columnar type, some cells of which are ciliated, whereas other are narrow, peg-shaped and nonciliated.

Ciliated cells are more numerous in the infundibulum and ampulla. The wave of the cilia is directed toward the uterus.

Nonciliated, peg cells are secretory cells produce the fluid that provides nutritive material for the ovum.

The height of the epithelium and the proportion of ciliated to nonciliated secretory cells show changes that correlate with the stages of the menstrual cycle. The epithelium during the first half (follicular phase) of the cycle is taller than it is in the second half, which is under the influence of the corpus luteum. During pregnancy, the epithelium is quite low and there are an increased number of "peg" cells. Cyclic changes also occur in numbers and size of cilia on the ciliated cells. The epithelium secretes mucus and probably other substances necessary for the maintenance of the ovum during its journey down the tube. The cilia beat toward the uterus. The beating of the cilia and the waves of muscular contraction transport the ovum through the tubes. The connective tissue of the lamina propria is richly cellular. No submucosa and no glands are present in the Fallopian tubes.

THE UTERUS

The uterus is a thick walled, pear-shaped organ. It varies considerably in size, averaging about 7–7.5 cm in length, 5 cm in width and 2.5 cm in thickness.

The wall of the uterus consists of three layers. From the lumen outward they are:

— *endometrium*, the mucosa of the uterus.

— *myometrium*, the thick muscular layer.

— *perimetrium*, the outer serous layer or visceral peritoneal covering of the uterus, which covers the corpus and a portion of the cervix. It is firmly attached to the underlying muscularis and has the usual structure of a serous membrane.

The *myometrium* is a massive muscular coat, about 15 mm in thickness, consisting of bundles of smooth muscle fibers held together by connective tissue. The disposition of the muscle fibers is quite complicated but, in a general way, three layers may be distinguished. The *inner*, muscular layer, the *stratum subvasculare*, consists of fibers running longitudinally. The *middle layer, stratum vasculare*, forms the bulk of the muscularis and is composed mainly of fibers running circularly or spirally. In the interstinal tissue there are numerous large blood vessels, especially veins. The *outer layer*, *stratum supravasculare*, is relatively thin and is composed of both circular and longitudinal fibers. The latter predominate and form a fairly distinct subserous layer which becomes continuous with the longitudinal muscle coat of the vagina. In the cervix, the inner longitudinal layer is absent. During pregnancy, the muscle tissue of the uterus is greatly increased.

The interstitial tissue contains numerous blood vessels and consists of loosely arranged collagenous fibers and relatively few connective tissue cells. Elastic fibers are found in considerable amounts in the outer layer of the muscularis and in the subserous connective tissue. The inner portions of the myometrium are relatively poor in elastic tissue. In the cervix elastic tissue is abundant.

The ***endometrium*** is lined by simple collumnar epithelium with a mixture of secretoty and ciliated cells. Throughout the reproductive lifespan, the endometrium undergoes cyclic changes each month and varies from 1 to 6 mm in thickness, 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 two layers or zones that differ in structure and function:

— ***Stratum functionale*** or ***functional layer*** is a thick inner part of the endometrium, which is sloughed off at menstruation.

— ***Stratum basale*** or ***basal layer*** is retained during menstruation and serves as the source for regeneration of the stratum functionale.

The surface epithelium invaginates into the underlying lamina propria, the endometrial stroma, forming the *uterine glands*. These simple tubular glands, containing fewer ciliated cells, occasionally branch in the deeper aspect of the endometrium. The endometrial stroma is highly cellular and contains abundant intercellular ground substance. No submucosa separates the endometrium from myometrium.

The endometrium contains a unique system of blood vessels. The uterine artery gives off 6 to 10 arcuate arteries that anastomose in the myometrium. Branches from these arteries, the *radial arteries*, enter the basal layer of the endometrium where they give off small *straight arteries* that supply this region of the endometrium. The main branch of the radial artery continues upward and becomes highly coiled *spiral artery*. Spiral arteries give off numerous arterioles that often anastomose as the supply of a rich capillary bed. The capillary bed includes thin-walled dilated segments called *lacunae*. The straight arteries and the proximal part of the spiral arteries do not change during the menstrual cycle. The distal portions of the spiral arteries, under the influence of estrogens and progesterone, undergo degeneration and regeneration with each menstrual cycle.

The endometrium of the uterine cervix differs from the rest of the uterus. It measures about 2 to 6 mm in thickness and contains large, branched glands. It also lacks spiral arteries, which undergo important functional changes during each menstrual cycle that are related to the transport of spermatozoa within the cervical canal. Hormonal mechanism facilitates favourable environment for sperm megration at midcycle and restricts this passage at other times. The portion of the cervix that projects into the vagina is covered with a stratified squamous epithelium. An abrupt transition between this squamous epithelium and the mucus-secreting columnar epithelium of the cervical canal occurs in the transformation zone that during the reproductive age of women is located just outside the external os.

OVARIAN MENSTRUAL CYCLE

The menstrual cycle is a continuum of developmental stages in the functional layer of the endometrium. It is ultimately controlled by gonadotropins secreted by the parts distalis of the pituitary gland that regulate the steroid secretions of the ovary. The cycle normally repeats every 28 days, during which the endometrium passes through a sequence of morphologic and functional changes.

Four stages, each of which has characteristic structural features, are distinguishable in the endometrium during an ovarian menstrual cycle. The indicated duration of each stage is based on a 28-day cycle.

I. The ***menstrual stage*** continues for the first 3 to 5 days of the cycle and accompanied by bleeding from mucousal vessels and discharge of tissue and blood from vagina.

II. The ***proliferative stage*** (estrogenic) begins with the termination of menstruation and extends untill the 1st day after ovulation, which occurs at about 13th or 14th day.

III. The ***secretory stage*** (progesterone) extends from the middle of the cycle to the 26th or 27th day.

IV. The ***premenstrual stage*** is 1 or 2 days in length and is terminated by the appearance of external bleeding.

The *menstrual stage* is characterized by the menstrual flow. In the first day of menstrual cycle ovarian hormones are at the lowest level.

The *proliferative stage* of the cycle is characterized by rapid regeneration of the endometrium from the narrow basal layer remaining after menstruation. Epithelial cells from the remaining bottom portions of the glands reconstitute the glands and cover the denuded surface. As the gland cells increase in number, they become tall and closely packed together, and the glands increase in length. The glands remain relatively straight and uniform in diameter. Numerous mitoses occur in cells of the connective tissue. The connective tissue cells secrete collagen and ground substance. Blood vessels traverse the regenerating tissue.

In the *secretory stage* of the cycle, the endometrium hypertrophies. The increase is due not to mitotic activity but to hypertrophy of the gland cells and to an increase in edema and vascularity. The gland cells remain about the same in height but become broader.

With the development of the secretory stage, two layers can be distinguished clearly in the endometrium. The basal layer is the deepest layer and stays relatively narrow. Its glands undergo little or no change. The functional layer undergoes dramatic changes beginning a day or two after ovulation. It becomes edematous. The gland cells undergo certain progressive secretory changes. Both glycogen and mucin increase rapidly in the gland cells of the functional layer, and these secretions are localized at first in the basal portions of the cells. During the latter half of the secretory stage, the secretion moves to the apical zone of the gland cells. The secretion, composed of glycogen, mucin and some fat, then appears in the lumen of the glands. Hypertrophy of the epithelial cells is accompanied by an increase in vascularity and edema. The spiral arteries lengthen and become more coiled. They extend nearly to the surface of the endometrium.

In the *premenstrual phase* of the cycle, important changes occur in the spiral, coiled arteries, leading to a breakdown of the functional layer. A constriction of the coiled arteries and vascular stasis occur during the premenstrual period, producing a condition of anemia and anoxia.

Finally, blood escaped from the vessels. In the premenstrual stage there is also a decrease in edema and an infiltration of the stroma with leukocytes. The glands fragment, the surface of the endometrium breaks down and blood and tissue debris appear in the uterine lumen.

During menstruation, the functional layer is lost. The spiral arteries undergo necrosis and some blood may spurt from them. The secretion of the glands and the broken down tissue of the functional layer are added to this blood .

Summing up, in the first period, a new functional layer is built up, with its glands and interstitial tissue. In the second, the functional layer is transformed into a swollen nutritive compartment ready for the implantation of the fertilized ovum. If the egg is not implanted, desquamation of the functional layer occurs, rapidly followed by regeneration of the epithelial surface.

The cyclic uterine changes are closely related to the ovarian cycles associated with ovulation. From the available evidence, it is possible to relate, temporally, the specific phases of the two cycles. At the end of menstrual phase, the endometrium consists of a thin connective tissue, about 1 mm thick, containing a basal portion of the uterine glands and the lower portion of the spiral arteries. This layer is the stratum basale. Under the influence of

estrogens, the proliferative phase is initiated. The postmenstrual proliferative changes correspond to the preovulatory period of maturation of the follicle. At the end of the proliferative stage, the endometrium has reached a thickness of about 3 mm. The glands have narrow lumina and are relatively straight. Accumulations of glycogen are present in the basal portion of the epithelial cells.

The secretory stage is always associated with the formation and growth of the corpus luteum, and it lasts as long as the latter retains its full function. Under the influence of progesterone, changes occur in the stratum functionale, beginning a day or two after ovulation. The endometrium becomes edematous and may eventually reach a thickness of 5 to 6 mm. The glands enlarge and become corkscrew shaped, and their lumina fill with secretory products. The mucoid fluid produced by the gland epithelium is rich in nutrients.

The beginning involution of the corpus luteum always marks the onset of menstruation. Ovulation occurs normally at the end of the proliferative period. The corpus luteum actively produces hormones for about 10 days if fertilization does not occur. As hormone levels rapidly decline, changes occur in the blood supply to the stratum functionale, which become ischemic. The glands stop secreting. After about 2 days the extended periods of arterial contraction cause disruption of the surface epithelium and rupture of the blood vessels.

VAGINA

The vagina is a fibromuscular tube that joins internal reproductive organs to the external environment. The wall of the vagina consists of three layers:

1. ***Inner mucosal layer***, which has numerous transverse folds and is lined with stratified squamous nonkeratinized epithelium. Epithelium rests on a basement membrane and an underlying lamina propria increases with the administration of estrogen. The epithelium of the vagina undergoes cyclic changes during the menstrual cycle. Under the influence of estrogens, during the follicular phase, the epithelial cells synthesize and accumulate glycogen as they migrate toward the surface. Connective tissue papillae from the lamina propria project into the epithelial layer. Keratohyalin gran-

ules may be present in the epithelial cells, but under normal conditions, keratinization does not occur.

The lamina propria consists of loose connective tissue especially rich in elastic fibers immediately below the epithelium. It also contains polymorphonuclear leukocytes and lymphocytes and it occasionally has aggregations of lymphocytes resembling solitary. Leukocytes invade the epithelium especially just before, during and just after menstruation, and they appear as free cells in the lumen of the vagina. The lamina propria exhibits two distinct regions. The outer region immediately below the epithelium is a highly cellular loose tissue. The deeper region, adjacent to the muscular layer, is denser and may be considered a submucosa.

The vaginal wall is entirely devoid of glands, and the mucus found in the lumen is derived from the glands of the cervix. In the estrogen phase of the cycle the vaginal fluid has a lower pH than at other times. The vagina has few general sensory nerve endings.

2. ***Intermediate muscular layer***, which is organized into two intermingling smooth muscle layers, an outer longitudinal layer and an inner circular layer. The outer layer is continuous with the corresponding layer in the myometrium of the uterus. The muscle bundles are separated by connective tissue rich in elastic fibers.

3. ***Outer adventitial layer***, which is organized into an inner dense connective tissue layer adjacent to the muscularis and an outer loose connective tissue layer that blends with the adventitia of the surrounding structures. The inner layer contains numerous elastic fibers.

The portion of the cervix that projects into the vagina, the vaginal part, the *ectocervix*, is covered with a stratified squamous epithelium.

Lecture 21

EARLY HUMAN EMBRYOGENESIS

Human development begins at fertilization of oocyte by sperm in the ampulla of the uterine tube. Chemical signals (attractants), secreted by the oocyte and surrounding follicular cells, guide the capacitated sperms (sperm chemotaxis) to oocyte. Several hundred sperms pass through the uterus and enter the uterine tube, many of them surround the secondary ovocyte. When it is contacted by a sperm, oocyte completes the second miotic division and its nucleus constitutes female pronucleus. After the sperm enters the mature oocyte, its head separates from the tail and enlarges to become the male pronucleus. Fertilization is complete when the pronuclei unite and the maternal and paternal chromosomes intermingle during metaphase of the first mitotic division of the ***zygote***. As it passes along the uterine tube toward the uterus, the zygote undergoes ***cleavage*** (a series of mitotic cell divisions) into a number of smaller cells — ***blastomeres***. About three days after fertilization, a ball of 12 or more blastomeres — a ***morula*** — enters the uterus. A cavity soon forms in the morula, converting it into a ***blastocyst,*** consisting of the ***embryoblast***, an inner cell mass, which gives rise to the embryo and some extraembryonic tissues, a ***blastocystic cavity***, a fluid-filled space, and the ***trophoblast***, a thin outer layer of cells. The trophoblast encloses the embryoblast and blastocystic cavity and later forms extraembryonic structures and the embryionic part of the placenta. While floating in the uterus, embryo derives nourishment from secretions of uterine glands. Four to five days after fertilization, the zona pellucida is shed allowing rapid increase of early embryo and the trophoblast adjacent to the embyoblast attaches to the endometrial epithelium about 6 days after fertilization. The trophoblast at the embrionic pole proliferates rapidly and differentiates into two layers, an inner layer of cells, the *cytotrophoblast,* and an outer thicker

layer consisting of a multinucleated protoplasmic mass, the *syncytiotrophoblast.* The fingerlike processes of syncytiotrophoblast produce enzymes and extend through the endometrial epithelium and envade the connective tissue. By the end of the first week, the blastocyst is superficially implanted in the endometrium and is deriving its nourishment from the eroded maternal tissue (histiotrophic type)

Villi on the deeply embedded surface of the blastocyst grow rapidly and form the fetal component of the placenta, the *chorion frondosum.* Villi of the chorion frondosum are attached to a firm portion of the chorion, the *chorionic plate.* Villi on the surface of the chorion facing the uterine cavity do not grow as rapidly as on the deeply embedded surface, and they degenerate by the end of the third month of pregnancy. This portion of the chorion is known as the *chorion laeve.*

The endometrium also shows important structural changes during pregnancy. Since all the endometrium, except the deepest layer, is destined to be shed at parturition, the endometrium in a pregnant uterus is referred to as *decidua.* Three regions of the decidua are distinguished:

1. Overlying the blastocyst is the *decidua capsularis.*
2. Underlying it is the *decidua basalis.*
3. All the remaining mucosa of the body of the uterus is the *decidua parietalis.*

It is the decidua basalis which becomes the maternal component of the placenta. In the early part of pregnancy, the endometrium increases in thickness. A characteristic feature is the presence of *decidual celts,* which are enlarged stromal cells. The cytoplasm is vesicular or finely granular and contains large amounts of glycogen. They provide a favorable environment for the nourishment of the embryo and create a specialized layer that facilitates the separation of placenta from the uterine wall at the termination of pregnancy.

As chorionic villi grow into the decidua basalis, they destroy and erode endometrium, leaving spaces, or *lacunae.* With further enlargement of villi, lacunae become interconnecting and contain blood liberated by penetration of the maternal vessels by the trophoblast. Diffusion of dissolved substances now can occur between the maternal blood in the lacunae and fetal blood in the capillaries of the villi (hematotrophic type).

Concurrently various changes occur in the embryoblast at the second week of development: the primary yolk sac forms and extraembryonic mes-

oderm develops, the extraembryonic coelom later becomes the chorionic cavity, the amniotic cavity appears as a space between the cytotrophoblast and the embryoblast. The embryoblast differentiates into bilaminar embryonic disc consisting of epiblast, related to amniotic cavity, and hypoblast, adjacent to blastocyst cavity (first stage of gastrulation). The precordal plate, an important organizer of the head region of the embryo, develops as a localized thickening of the hypoblast. In the third week of embryogenesis the second stage of gastrulation convertes bilaminar embryonic disc into trilaminar due to mesoderm formation.

Notochord, neural tube, neural crests form and mesoderm passes trasformation into dorsal segmented (somites), lateral (nephrogenotoms and metanephrogenic cord) and ventral (splanchnotomic layers including coelom) portions.

PLACENTA

The placenta consists of a fetal portion, formed by the chorion, and a vaternal portion formed by the decidue basalis. Both parts are involved in physiologic exchange of substances between maternal and fetal circulations.

Proliferation of the cytotrophoblast, growth of chorionic mesoderm, and blood vessels development successively give rise to:

— ***primary chorionic villi***, which are formed by the rapidly proliferating cytotrophoblast. It sends cords or masses of cells into the blood-filled trophoblastic lacunae in the syncytiotrophoblast. The primary villi appear between days 11 and 13 of development.

— ***secondary chorionic villi***, which are composed of a central core of mesenchyme, surrounded by an inner layer of cytotrophoblast and an outer layer of syncytiotrophoblast. They develop at about day 16, when the primary chorionic villi become invaded by loose connective tissue from chorionic mesenhyme. The secondary villi cover the entire surface of the chorionic sac.

— ***tertiary chorionic villi*** are formed by the end of the third week as the secondary villi become vascularized by blood vessels that have developed in their connective tissue cores.

As the tertiary villi are forming, cytotrophoblastic cells in the villi continue to grow out through the syncytiotrophoblast. When they meet the maternal endometrium, they grow laterally and meet similar processes growing from

neighboring villi. Thus, a thin layer of cytotrophoblastic cells, called the *trophoblastic shell*, is formed around the syncytiotrophoblast. The trophoblastic shell is interrupted only at sites where maternal vessels communicate with the intervillous spaces. Future growth of the placenta is accomplished by interstitial growth of the trophoblastic shell.

The *uteroplacental circulatory system* begins to develop around day 9, with development of vascular spaces called *trophoblastic lacunae* within the sycytiotrophoblast. Maternal sinusoids, which develop from capillaries of the maternal side, anastomose with the trophoblastic lacunae. Numerous pinocytotic vessels present in the syncytiotrophoblast indicate the transfer of nutrients from the maternal vessels to the embryo.

The placenta consists of two components, a ***fetal portion*** and a ***maternal portion***, formed by deciduas basalis. The fetal component consists of a chorionic plate and branching processes or villi which arise from the chorionic plate and lie in the spaces through which the maternal blood circulates.

The *chorionic villi* are usually classified into two types, *anchoring* and *free* or *floating* villi, the structure of the two being similar. The anchoring villi pass from the chorionic plate to the decidua basalis; thus one of their functions is the anchoring of the chorionic plate to the decidua. They give origin throughout their length to branches that float in the blood-filled lacunar spaces between the fetal portion of the placenta and the decidua basalis, and these are known as the free or floating villi. As development proceeds, the villi become very numerous. They also became increasingly irregular in shape, with many protuberances which correspond to the loops and coils of the capillaries that they contain. They are covered by trophoblasts, as are also the chorionic plate and the chorionic surface of the deciduas basalis. The outer (maternal) border of the villus often has adherent fibrin and fibrinoid material, particularly in the later months of pregnancy (*Langhans's fibrinoid*).

During the 4th and 5th month, the deciduas form a number of septa, the *decidual septa*, which project into intervillous space but do not reach the chorionic plate. These septa have a core of maternal tissue, but their surface is covered by a layer of syncytial cells, so that at all times a syncytial layer separates maternal blood in intervillous lakes from fetal tissue of the villi. As a result of this septum formation the placenta is divided into a number of compartments or ***cotyledons***. Cotyledon is a structural functional unit of the finally developed fetal placenta, which is formed by anchoring

villi and its branchings. The total amount of cotyledons in a placenta reaches 200.

The maternal component of the placenta is formed by the decidua basalis. This comprises all of the endometrium beneath the fetal portion of the placenta except the deepest part, which is destined to remain after parturition as in normal menstruation. In the decidua basalis, and also in the decidua parietalis, many of the connective tissue cells undergo a pronounced change. They hypertrophy, forming large, ovoid cells of somewhat irregular shape, and are named *decidual cells*. They are one of the most striking features of the endometrium in the first half of pregnancy. Some of them contain two or more nuclei. The cytoplasm is vesicular or finely granular. Especially the smaller decidual cells contain large amount of glycogen. Decidual cells regress and by the end of pregnancy are rarely present. The *amorphous substance (Ror's fibrinoid)* is on a surface of decidua basalis turned to the chorionic plate. Trophoblast together with the fibrinoid provides immunologic homeostasis in the system mother — fetus.

Separation of the fetal and maternal blood, referred to as the ***placental barrier***, is maintained primarily by the layers of fetal tissue. Starting at the fourth month, these layers become very thin to facilitate the exchange of products across the placental barrier. The thinning of the wall of the villus is caused in part by degeneration of the inner cytotrophoblast layer. At its thinnest, the placental barrier consists of the:

— syncytiotrophoblast;
— discontinuous inner cytotrophoblast layer;
— basal lamina of the trophoblast;
— connective (mesenhymal) tissue of the villus;
— basal lamina of the endothelium;
— endothelium of the fetal placental capillary in the tertiary villus.

This barrier exchanges the oxygen and carbon dioxide between the maternal blood and the fetal blood. The fetal blood enters the placenta through a pair of *umbilical arteries*. The maternal blood is supplied to the placenta through broken spiral endometrial arteries that penetrate the basal plate. From the spiral arteries the blood flows into the base of the intervillous spaces, which contain about 150 ml of the maternal blood. The maternal blood is exchanged 3 to 4 times per a minute.

Exchange of gases and metabolic products occurs as the blood passes over the villi. Normally, water, carbon dioxide, metabolic waste products,

and hormones are transferred from the fetal blood to the maternal blood. Water, oxygen, metabolites, electrolytes, vitamins, hormones, and some antibodies pass in the opposite direction. The placental barrier does not exclude many potentially dangerous agents, such as alcohol, nicotine, viruses, drugs, exogenous hormones, and heavy metals.

The placenta also functions as an endocrine organ, producting steroid and peptide hormones as well as prostaglandins that play an important role in the onset of labor. Immunocytochemical studies indicate that the syncytiotrophoblast is the site of synthesis of these hormones.

The steroid hormones (progesterone and estrogen) have essential roles in the maintenance of pregnancy. As pregnancy proceeds, the placenta takes over the major role in the secretion of steroids from the corpus luteum. The placenta produces enough *progesterone* by the end of the eighth week to maintain pregnancy. In the production of placental *estrogen,* the fetal adrenal cortex plays an essential role, providing the precursors needed for estrogen synthesis.

The following peptide hormones are secreted by the placenta:

1. *Human chorionic gonadotropin (HCG)*, the synthesis of which begins near the 6th day, even before syncytiotrophoblast formation. HCG exhibits marked homology to pituitary thyroid-stimulating hormone and stimulates the maternal thyroid gland to increase secretion of thyroxin. It also maintains the corpus luteum during early pregnancy.

2. *Human chorionic somatomammotropin (HCS),* also known as human placental lactogen, is closely related to human growth hormone. Synthesized in the syncytiotrophoblast, it promotes general growth, regulates glucose metabolism, and stimulates mammary duct proliferation in the maternal breast. Its role in fetal development remains inknown.

3. *Endothelial growth factor (EGF*), exhibits an age-dependent dual action on the early placenta. It's synthesized by the cytotrophoblast in the 4- to 5-week old placenta and stimulates proliferation of the trophoblast.

4. *Relaxin* is synthesized by decidual cells and is involved in the "softening" of the cervix and the pelvic ligaments in preparation for parturition.

5. *Leptin* is synthesized by syncytiotrophoblast, particularly during the last month of gestation. Leptin appears to regulate maternal nutrient storage to the nutrient requirements of the fetus. It is also involved in transporting nutrients across the placental barrier from mother to the fetus.

6. *Other growth factors* (fibroblast growth factor, colony-stimulating factor, platelet-derived growth factor, and interleukins) stimulate cytotrophoblastic

growth or inhibit trophoblast growth and proliferation (e.g., tumor necrosis factor).

MAMMARY GLANDS

The mammary glands are cutaneous in origin, developing within the superficial fascia. Each gland consists of 15 to 20 irregular lobes, each of which is a compound branched tubulo-alveolar gland with a separate lobar duct opening at the apex of the nipple. The lobes, separated by fibrous connective tissue bands, radiate from the mammary papilla, or nipple. Abundant adipose tissue is present in the dense connective tissue of the interlobular spaces. Fat is also present within the gland, the amount varying with the functional state.

The epidermis of the adult nipple and areola is highly pigmented. It is covered by stratified squamous keratinized epithelium. The pigmentation of the nipple increases at puberty, and the nipple becomes more prominent.

The areola contains sebaceous glands, sweat glands, and modified mammary glands (Montgomery's glands). These glands have a structure intermediate between sweat glands and the mammary glands and produce small elevations on the surface of the areola. The tubulo-alveolar glands, derived from modified sweat glands in the epidermis, lie in the subcutaneous tissue. Each gland ends in a *lactiferous duct* that opens into the nipple. Each duct has a dilated portion, the *lactiferous sinus.* The epithelial lining of the duct shows a gradual transition from stratified squamous to two layers of cuboidal cells in the lactiferous sinus and finally to a single layer of columnar or cuboidal cells through the remainder of the duct system. Myoepithelial cells of ectodermal origin lie within the surface of the epithelial cells and the basal lamina.

In the inactive gland, the glandular component is sparse and consists chiefly of duct elements. During the menstrual cycle, the inactive breast undergoes slight cyclic changes. Early in the cycle, the ducts appear as cords with little or no lumen. Under estrogen stimulation, at about the time of ovulation, the secretory cells increase in height, empty spaces appear in the ducts as small amounts of secretions accumulate, and fluid accumulates in the connective tissue.

The mammary glands exhibit a number of changes in preparation for lactation. The changes in the glandular tissue are accompanied by a de-

crease in the amount of connective tissue and adipose tissue. The development of the glandular tissue is not uniform, and variation in the degree of development is seen even within a single lobule. The cells vary in shape from flattened to low columnar. As the cells proliferate by mitotic division, the ducts branch and alveoli begin to develop.

Milk production occurs in alveolae. The secreting cells contain abundant granular endoplasmic reticulum, a moderate number of large mitochondria, the Golgi apparatus, and a number of lysosomes. Depending on the secretory state, large lipid droplets and secretory vesicles may be present in the apical cytoplasm. The secretory cells produce two distinct products that are released by different mechanisms:

1. *Merocrine secretion*. The protein component of the milk is synthesized in the rER, packaged into membrane-limited secretory vesicles for transport in the Golgi apparatus, and released from the cell by fusion of the vesicle's limiting membrane with the plasma membrane.

2. *Apocrine secretion.* The fatty or lipid component of the milk arises as lipid droplets free in the cytoplasm. The lipid droplets pass to the apical region of the cell and project into the lumen of the acinus. The droplets are invested with an envelope of plasma membrane as they are released. A thin layer of cytoplasm is trapped between the plasma membrane and lipid droplets and is released with the lipid.

The secretion released in the first few days after childbirth is known as *colostrum*. This premilk is an alkaline, yellowish secretion with a higher protein, vitamin A, sodium, and chloride content and a lower lipid, carbohydrate, and potassium content than milk. It contains considerable amount of antibodies that provide the newborn with some degree of passive immunity.

The initial growth and development of the mammary gland at puberty occur under the influence of estrogens and progesterone produced by the maturing ovary. During pregnancy, the corpus luteum and placenta continuously produce estrogens and progesterone. Estrogen present in the circulation stimulates proliferation of the lactiferous duct components, and progesterone stimulates growth of alveoli. Immediately after birth, the sudden loss of estrogen and progesterone secretion from the placenta and corpus luteum allows the prolactin to assume its lactogenic role. Production of milk also requires adequate secretion of growth hormone, adrenal glucocorticoids, and parathyroid hormones.

UMBILICAL CORD

The development of the umbilical cord begins with the formation of the extraembryonic coelom which almost surrounds the early embryo and which remains attached to the chorion by the connective stalk of mesenchyme. With further embryonic development, the site of attachment of the connecting stalk becomes located ventrally, just caudal to the point where the vitello-intestinal duct connects the yolk sac to the mid gut. As the embryo grows, the amniotic sac expands greatly, filling the extraembryonic coelom and compressing the vitello-intestinal duct and yolk sac remnant up against the connective stalk. These structures ultimately fuse to form the umbilical cord which now is surrounded by the amniotic epithelium and amniotic cavity.

By the middle of the 5th month, the remnants of the vitello-intestinal duct, yolk sac and sheath of extraembryonic coelom atrophy and disappear. All that remains are two umbilical arteries and single umbilical vein surrounded by mucoid connective tissue, often called *Wharton's jelly*. This tissue is rich in proteoglycans 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. The umbilical arteries convey deoxygenated fetal blood to the placenta whilst the umbilical vein conveys oxygenated blood back to the fetus.

The attachment of the umbilical cord is usually near the center of the placenta.

The umbilical cord is usually 1 to 2 cm in diameter and 30 to 90 cm in length. Excessively long or short cords are uncommon. Long cords have a tendency to prolapse and/or to entangle around the fetus. The cord entanglement around the fetus's neck may make the cord relatively short, interfering with delivery of the baby.

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Навчальне видання

Серія «Бібліотека студента-медика»

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УЛЬЯНЦЕВА Олена Аркадіївна

ГІСТОЛОГІЯ

Курс лекцій
Навчальний посібник
(Англійською мовою)

Провідний редактор ***В. М. Попов***
Редактор ***Р. В. Мерешко***
Художній редактор ***О. А. Шамшуріна***
Технічний редактор ***А. В. Попов***
Коректор ***О. В. Титова***
Поліграфічні роботи ***І. К. Каневський***

Формат 60x84/16. Ум. друк. арк. 20,31. Тираж 500. Зам. 1425.

Видавець і виготовлювач Одеський національний медичний університет.
65026, Одеса, Валіховський пров., 2.
Свідоцтво суб'єкта видавничої справи ДК № 688 від 13.11.2001